Effect of variations in concentration of algae and silt on filtration and growth of the razor clam (*Ensis directus*, Conrad)

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Summary

As part of a collaboration between the research programme Knowledge for Primary Processes Silt of Rijkswaterstaat Waterdienst NWOB (department of Infrastructure and Environment, MinIenM, RWS) and the Monitoring programme Sand extraction RWS and the LaMER Foundation, RWS-WD NWOB requested further research into the relation between food availability and *Ensis* production. The aim is to better understand the effect of different algae and silt concentrations on filtration and growth rates and improve prediction of effects. Laboratory experiments were carried out with *Ensis directus* to estimate food intake rate and growth rate as a function of food density and clam size. Growth experiments carried out in 2010 showed that the species seems to be very fragile as shown by the low growth rates and high mortality rates. Improvements designed to optimize the experimental conditions, survival rates and experimental set-up were implemented in 2011. These were: experimental animals were collected with a box corer instead of a suction dredge; animals were kept in cylinders without sediment, but their shells were closed with elastic bands during the filtration experiments; circular tanks were used with increased water movement; the diet during the growth experiment consisted of two species of algae.

Two food levels were tested: low food availability (6.5 µg Chla/l) and high food availability (16.5 µg Chla/l) at four silt concentrations (0, 50, 150 and 300 mg/l). Only the highest silt concentration induced a reduction in filtration rate. Food level did not influence filtration rate of *Ensis,* but intake rate is higher at the high food concentration, because more algal cells are present in a certain volume of water. Long-term (10 weeks) exposure to silt concentrations of 300 mg/l showed significantly higher growth than the 150 mg/l treatment indicating that exposure to a high silt concentration did not induce a reduction in growth. Long-term (10 weeks) exposure to a food level of 6.5 ug chla per liter reduced shell growth of *Ensis* compared to growth at 16.5 ug chla per liter. The filtration and growth rate results are used in a modelling study on growth and condition of *Ensis* during sand extraction 2013-2017 (Schellekens, in prep).

The conclusions of this study give more notion of the effects of sand extraction in the coastal zone of the North Sea on the viability of the razor clam *Ensis directus*. Sand extraction always goes together with an increase of silt concentration in the water column. This reduces the light conditions for algal growth which reduces the food availability for *Ensis*. The laboratory experiments suggest that *Ensis* is more sensitive to a reduction in algal concentration than to an increase in silt concentration. Some discussion is given on the implications of the results for the management of sand extraction.

Project name:	Effect of variations in concentration of algae and silt on filtration and growth of the razor clam (Ensis directus, Conrad)
Commissioned by:	Rijkswaterstaat Waterdienst afdeling NWOB
Programme:	Project Landelijke taken B&O Waterbeheren (RWS –WD) Monitoring and Evaluation Programme Sand extraction RWS LaMER, part B4-1 (Ellerbroek et al., 2008).
Partners:	Rijkswaterstaat DNZ, Foundation LaMER
Supervised by:	Charlotte Schmidt (RWS-WD) Marcel Rozemeijer (RWS-WD, Imares afdeling Vis) Saa H. Kabuta (RWS-WD) John de Ronde (Deltares)
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1. Introduction and assignment

Multiple use of, and construction works in the Dutch coastal zone necessitate the extraction of sand from the coastal seafloor. These extractions occur at a water depth of approximately 20 meters. Sand and silt are mobilised with water and the mixture is pumped into the hopper. The excess water with fines (fine sand and silt) flows overboard resulting in an increased suspension of silt in the water column. Silt in this flow separates into three compartments: a small proportion deposits directly with the fine sand, another small proportion (5-15%) forms a plume and floats away, and the majority remains in the form of a density driven current on the sea floor. This will either deposit onto the sea floor at an unknown moment or be resuspended by currents and waves (Fig. 1). Because of this, direct turbidity resulting from the overflow of the dredger is negligible. The impact of silt discharged with the overflow by hoppers causes a typically far field effect (Aarninkhof et al. 2010, Spearman et al. 2011).

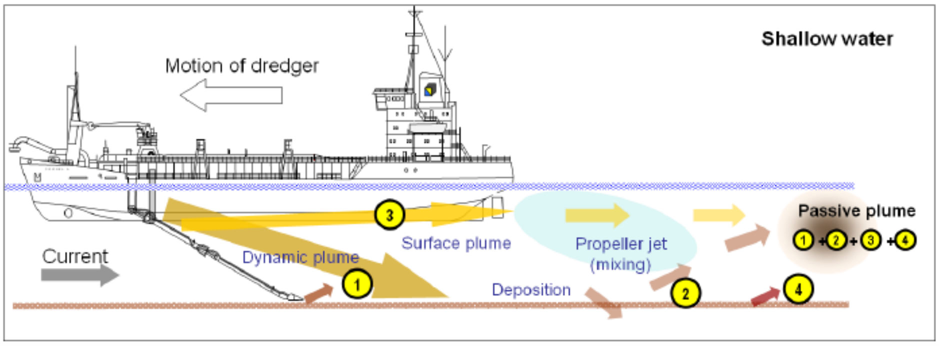


Figure 3. Resuspension processes near Trailing Suction Hopper Dredger. Release of fine sediments occurs from draghead interaction with the sea bed (Process 1), propeller impacts on local sediments (Process 2), formation of the surface plume due to interaction with the boundary layer underneath the vessel (Process 3) and mixing of near-bed density current with ambient waters (Process 4). The combined effect of these four processes yields the passive plume.

Fig. 1 The mechanism of overflow (Aarninkhof et al. 2010, Spearman et al. 2011).

As a direct consequence, light penetration in the water column is reduced over a larger area, which can negatively affect phytoplankton growth. Phytoplankton constitutes the basis of the food web, thus a decreased availability can affect higher trophic levels that live on these microscopic plants, such as filter-feeding shellfish. In addition to a reduced phytoplankton abundance in the water column, the elevated silt concentrations may impede the intake of phytoplankton by shellfish, and potentially give additional stress (i.e. higher energetic cost) to these organisms as they need to excrete silt in the form of pseudo-faeces. Shellfish make up an important component of the coastal food web, for example for shellfish-eating birds such as the Common Scoter in the Dutch Coastal zone. Therefore, insights into potential effects of sand extraction on shellfish populations are desirable for regulating agencies and engineering companies that wish to evaluate whether sand extraction operations are in violation with (inter-)national environmental regulations. Experimental work on the potential environmental impacts is best assessed for organisms that represent an important component of coastal ecosystems.
Since its introduction to European waters in the 1970's, *Ensis directus* has become a widely distributed and most dominant shellfish species in the Dutch coastal zone. As such, it can be considered a representative filter-feeding shellfish species for the monitoring of the effects of sand extraction on shellfish populations in the Dutch coastal waters (Ellerbroek et al., 2008).
Research questions of the Monitoring programme RWS LaMER are: (1) What are the effects of reduced food conditions on the growth of *E. directus* and (2) When does food limitation occur during declining

food conditions? The approach taken is the development and use of a Dynamic Energy Budget model (DEB) for *E. directus*, (Wijsman et al., 2011, Kamermans et al. 2010, Cardoso et al. 2011) which can be is linked to water-quality-models like Delft 3D, Ecowasp and ERSEM (Schellekens 2012).

During previous projects carried out in the context of the Monitoring programme RWS LaMER, the impact of silt concentration and food supply on filtration, respiration and growth rates have been studied (Witbaard and Kamermans, 2009; Kamermans et al., 2011). However, several questions regarding the physiological response to increased silt concentrations remained, which hampered evaluating the environmental implications for this abundant species. Therefore, as part of a collaboration between the project Landelijke Taken B&O Waterbeheren of Rijkswaterstaat Waterdienst NWOB (department of Infrastructure and Environment, MinIenM, RWS) and the Monitoring programme Sandmining RWS and the LaMER Foundation, RWS-WD NWOB requested further research into the relation between food availability and Ensis production through field measurements, laboratory measurements and modelling. The aim is to better understand these relations and to improve the prediction of the effects on the ecosystem.

Therefore research questions of this study are:

1. How can the long-term growth laboratory experiment with *Ensis directus* be optimised, in such a way that the survival rates are comparable to or higher than the field situation?
2. What is the algal-silt coefficient in the physiological growth model DEB (Wijsman e.a. 2011) of *Ensis directus,* based on the filtration rate at different algae: silt ratio's*?* This parameter indicates the amount with which silt reduces food intake. *is* How does the algal-silt coefficient for *Ensis* relate to other shellfish?
3. What is the growth of *Ensis directus* under different algae and silt concentrations? And how do the growth rates compare to other field- and laboratory results?

As a follow-up of the studies done in years 2009 (Witbaard & Kamermans, 2009) and 2010 (Kamermans et al, 2011), this report covers the results of experimental work done in 2011 (April- October), on the effects of different food levels and silt concentrations on filtration and growth rates of *Ensis directus*. The experiments performed in 2010 (Kamermans et al, 2011) showed that the species are very sensitive to experimental conditions, shown by low growth and low survival rates.
Improvements designed to optimise the experimental set-up in order to increase growth and survival rates are incorporated and discussed in the sections 2.1 and 4.1 of this report. The effect of different algae and silt concentrations on filtration rates was used to determine the functional response in the Dynamic Energy Budget (DEB) model (Schellekens, 2012).

2. Materials and Methods

2.1 Improvement of handling and experimental set-up

The first experiments in 2009 and 2010 in small cylinders showed that animals crawled out of the sediment, were unable to dig into it again and died a few days later. Table 1 shows a list of possible causes for this behaviour and the related improvements in the test set-up.

Table 1. Causes of mortality during experiments in 2010.

Possible cause	**Possible improvement**
Stress because animals were fished with a commercial suction dredge.	Sampling with box corer (2010 and 2011).
Stress because clams are sensitive to vibrations and changes in light.	No other activities in climate room and continuous low light (2010 and 2011).
Movement in sediment is hindered by lack of space in cylinder.	For the filtration experiment animals were kept in cylinders without sediment. In 2011 shells were closed with elastic bands during the filtration experiments . For the growth experiment animals were kept in large containers during the growth experiment (400 liter in 2010; 300 liter in 2011).
Stress as a result of a bad water quality in the corners of the cubicle tanks (local accumulation of toxic compounds).	More water movement and using circular tanks in 2011.
Low growth as a result of malnutrition, caused by feeding with a single algal species.	Diet of two species of algae in 2011.
Low growth because food didn't reach all animals as a result of low water movement.	More water movement and using circular tanks in 2011.

2.2 Collection and storage of *Ensis* prior to experiments

Razor clams were collected during two cruises (April and July) of the southwest coast of the Netherlands (Table 2) using a box corer, following the protocol described in Kamermans et al. (2011). Due to a low number of large *Ensis* and some mortality of the large specimens, new large *Ensis* were collected separately using a commercial suction dredge in August. On the day of collection, the *Ensis* were transported to IMARES Yerseke, where they were kept in an outdoor basin in buckets filled with sand. The basins were continuously supplied with fresh seawater directly from the Oosterschelde. Oxygen levels were kept high by aeration. To prevent excessive growth of weeds, the basins were sheltered from direct sunlight using a cloth cover and the grazing periwinkle *Litorina litorea* was placed in the basins.

Table 2. Origin of different Ensis batches used for the experiments

Batch number	**Number of individuals**	**Period of outdoor storage**	**Collection date**	**Start experiment**	**Sample area**	**Collection method**	**Transport method**
1	Approx. 260	Approx. 2-3 months	19 April 2011	7 June (filtration)	51^0 46′ 3^0 47′	Box core	In sand
2	Approx. 500	Approx. 1 month	6 July 2011	10 August (growth)	51^0 50′ 3^0 46′	Box core	In sand
3	Approx. 60	Approx. 2 months	8 June 2011	10 August (growth)	51^0 38′ 3^0 47′	Suction dredge	Dry in cooler

Prior to the experiments, special attention was paid to the condition of the animals. Basins were checked regularly for dead specimens, which were removed. Temperature was monitored continuously using a HOBO Outdoor/Industrial 4-channel External Datalogger, in order to be able to correlate mortality rates to temperature fluctuations. The average water temperature in the outdoor basins varied from 16.3 to 20.1 °C (see Annex 3). Clams were fed daily with Shellfish Diet 1800 (Reed Mariculture Inc.) or phytoplankton (*Chaetoceros muelleri*) cultured at IMARES, until the start of the growth experiment. The collected *Ensis* specimens were divided in three size classes: small *Ensis*, ranging from 30 to 75 mm, a middle-sized group, ranging from 75 to 100 mm, and a large specimens groups, which covers shell sizes >100 mm.

2.3 Selected algae for nutrition, culture conditions and treatments

During the 2010 experiments the *Ensis* were fed with a single phytoplankton species (the flagellate *Pavlova lutheri*). This may have resulted in an insufficient intake of particular nutritional components (i.e. fatty acids), which resulted in a decrease in weight. As a means of improving the nutritional value of the food supplied to the *Ensis* during the experiments, a 50/50% dry-weight based diet of the diatom *Chaetoceros muelleri* and flagellate *Pavlova lutheri* was given. The fatty acid composition of a combination of these algae should provide a better-balanced diet that is more representative for a natural mixed phytoplankton assemblage (Helm et al., 2004). Both phytoplankton species were grown in batch cultures at 19°C under a 24h light regime using Walne medium (Walne, 1970). The size of the used phytoplankton species ranges from 4 to 6 µm diameter. For silt, we used Kaolonite (Keramicos b.v.), which is used in other studies on the relation between silt and filtration by filter feeders (Sornin, 1988; Tracey, 1988; Roper & Hickey 1995; Gremare & Amouroux, 1998; Barille et al 2006).

During the experiments, two different food concentrations were given to the *Ensis* (6.5 and 16.5 µg Chla/l). These levels are based on the relationship between food concentrations (x) and uptake rates (y) (y=86200000*ln(x)-848600000) from Witbaard & Kamermans (2009), in which the 6 µg Chla/l represents the food concentration at 20% of the maximum uptake rate, and 15 µg Chla/l resembles food concentrations at 60% of the maximum uptake rate. The maximum silt concentration used in the experiments was 300 mg/l. This represents a concentration that can occur during sand extraction in windy periods (M. Rozemeijer, pers. comm., Witbaard et al., 2012). Annex 1 explains how the levels were determined.

2.4 Set-up and implementation of the filtration experiment

Laboratory experiments were carried out with *Ensis directus* to estimate food intake rate (J_w) as a function of food density (Food), silt density (Y) and clam size (L) (Equation 1).

$$\dot{J}_W = \{\dot{J}_{Wm}\}(\delta_M L)^2 \left(\frac{Food}{X_K \left(1 + \frac{Y}{Y_k}\right) + Food} \right)$$ Equation 1.

J_W = food intake rate (d^{-1})
J_{Wm} = $L^{-2}d^{-1}$
δ_M = no unit
L = shell length (cm)
Food = food density (µg Chl/l+mg POM/l)
X_K = half saturation value for food intake (µg Chl/l+mg POM/l)
Y_k = half saturation value for inhibition by silt (no unit)

Food intake rate is filtration rate multiplied by algal density. This is shown in Fig. 2. As the silt content of the water increases, the filtration rate will decrease and, as a result, also the food intake rate. Y_k is the rate in which the half saturation constant for food intake changes with the silt concentration. The larger Y_k, the smaller the effect of silt on the half saturation constant. The ratio X_k/Y_k indicates the amount in which silt reduces food intake.

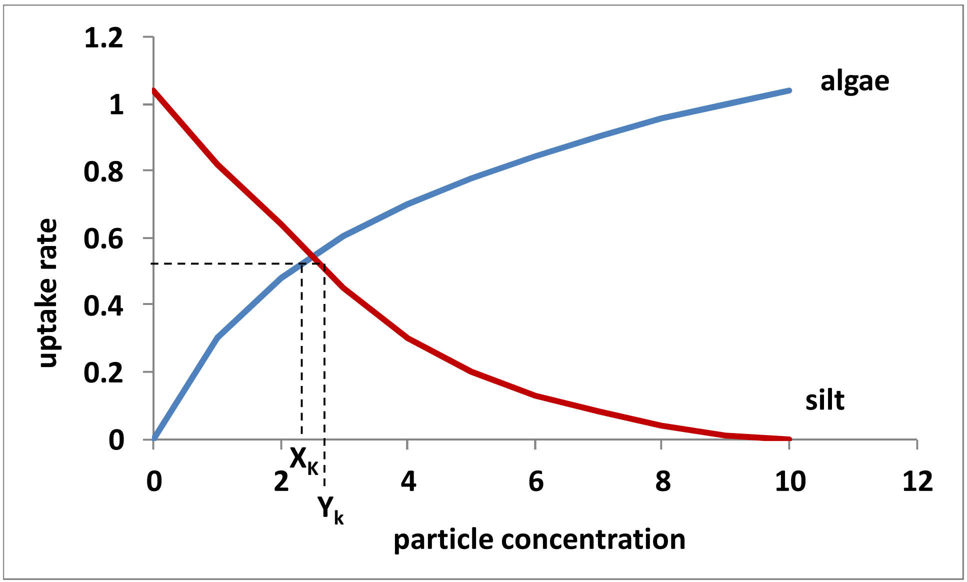


Fig. 2. Food intake rate (j_W theoretical d^{-1}*) as a function of particle density (*theoretical µg Chl/l+mg POM/l*). The values on the x-axis is algal cell density for algae and silt concentration for silt.*

To determine filtration rates, we measured clearance rates. Clearance rate is the rate in which a certain volume of water is cleared from all particles. Clearance rate equals filtration rate if the particles are 100% efficiently retained by the bivalve gills (Smaal, 1997). By only studying the clearance rate on particles in the size range 4 to 10 µm, which are 100% efficiently retained (Cranford, 2011), filtration rates are directly determined.

Prior to the experiment, a basic test was done to test the vitality of the selected specimens, by allowing them to dig themselves back in sand. The specimens that remained on top of the sediment were excluded from the experiment. For this year's experiment, an experimental flow-through system with slightly tilted cylinders without sediment was chosen (Fig. 3). This was done to prevent distortion of the measurements as a result of resuspension of particles caused by vertical movements of *Ensis*. The cylinder volume for small *Ensis* was 21 ml, for the middle and large *Ensis* cylinders of 110 ml were used. Initial tests on the feasibility and impact of experimental conditions on the functioning of the *Ensis* specimens were performed. These tests showed that *Ensis* of all size classes demonstrated filtration rates similar to filtration rates found during other studies (van Duren & Troost, in prep.), indicating that this set-up did not result in a bias to the experimental outcome (see Annex 2). Moreover, after a day in this experimental set-up, the *Ensis* quickly dug themselves back into the sand, further suggesting that this experimental set-up did not negatively affect the functioning of the *Ensis*.

Five specimens were individually placed in cylinders (without sediment) through which water was pumped using a peristaltic pump (Fig. 3). The large- and middle-sized specimens were bound up with elastic bands that function as a counter-pressure, similar to what *Ensis* experience in sediment. The small-sized individuals were used without elastic bands. Flow rates were adjusted such that a significant difference in particle concentration (approx. 30%) was detected between the water flowing into the cylinders and the water flowing out of the cylinders (Pascoe et al, 2009). The *Ensis* were given time to adapt (approx. 1 hour), after which the filtration rates under reference conditions (0 mg silt /l) were measured. Silt concentrations were then increased to 50 mg/l, 150 mg/l and 300 mg/l. After the desired silt concentrations were attained, and *Ensis* was given 0.5 -1 hour to acclimatise, 5 to 8 measurements on filtration rates were performed in a time span of one hour. For this, samples from the outflowing water were sampled for each cylinder and the concentration of particles ranging between 4 and 10 µm were analysed using a Z2 particle counter (Beckman coulter). Silt was kept in suspension by keeping water in circulation using an Eheim pump. To correct for particle settlement inside the cylinders we measured the particle concentration in the water flowing out of a blanco treatment, a cylinder containing an *Ensis* replica composed of shells cemented together. The clearance rate was determined by measuring the number of particles in the outflow and comparing these data with the outflow of the blanco. In case, despite our pre-selection, an individual *Ensis* was inactive during the experiment, the specimen was replaced by another. The results of the inactive specimens were excluded from the data. Pseudo-faeces production events, visible as a sudden increase in particle concentration, was recorded and expressed as number of production events per total number of filtration measurements for that treatment.

Fig. 3. Set-up to determine filtration rates with tilted cylinders holding Ensis on the left, peristaltic pump in the middle and container holding mixture of silt and algae on the right.

For the calculation of the clearance rate the following formula (Widdows, 1985) was used:

$$CR=((C\_in - C\_out)/C\_out)*Q$$

Where:

CR = clearance rate in L/h per individual

C_in = particle concentration of the outflow of the blanco (number/L)

C_out = particle concentration of the outflow (number/L)

Q = flow rate in L/h

After the filtration experiments, the *Ensis* specimens length, wet weight (WW) and ash-free dry weight (ADW) were determined. Ash-free dry weight was determined by first measuring the dry weight (DW) of the flesh after at least two days of drying at 70°C and cooling to room temperature in a dessicator. Ash-weight (AW) was analysed by ashing at 560°C and afterwards cooling down in a dessicator. The DW and AW were used to calculate the ADW by subtracted AW from DW (DW-AW).

2.5 Set-up of growth experiment

Six circular tanks were filled with 20 cm medium coarse sand (median grain size 442 µm) obtained from a supplier and 300 liter seawater was added (Fig. 4). Prior to the experiment, the sand was washed to remove organic material and larger particles. Containers were placed in a climate room at 18°C. *Ensis* stored in the outside basins were collected, individually marked using nail polish, and length and WW was measured. The *Ensis* were divided over the six tanks: 15 specimens per size class per container. In addition, five specimens of each size class (the initial sample) were analysed for WW, ADW and shell length. The growth of *Ensis* over 10 weeks was tested under two different food concentrations (6.5 and 16 µg Chla/L), and three different silt concentrations (0 mg/l, 150 mg/l and 300 mg/l).

Fig. 4. Circular tanks holding sediment, individually marked Ensis and water. Each container had a different algae and silt mixture. Silt and algae were kept in suspension using an overhead stirrer motor with propeller.

The algae aqua feed regulator used during the 2010 experiment was unable to differentiate between silt particles and phytoplankton cells. As a result, it could not be used for adding algae to the tanks. Therefore, algae were supplied continuously by pumping diluted algae cultures in the tanks with a dosing pump. Algal cell concentrations in the containers were measured once a day using an Accuri flow cytometer (FCM). The amount of phytoplankton cells needed to obtain the aimed food levels were calculated based on the filtration experiment data, and adjusted daily based on the FCM counts. Silt concentration was monitored daily using a Beckman Coulter Counter. First, a regression line between particle counts and actual silt concentration was determined by consecutively filtering the silt suspension on pre-weighed filters, drying the filters for 2 days and weighing the filters again (see Annex 5). Then, the particle concentrations in the tanks were measured with the Coulter Counter. The particle concentrations measured in the tanks with a 0 mg/l silt concentration were used as background values.

These values were subtracted from the total particle concentrations in the treatments where silt was added.. The silt concentration was adjusted if necessary, by adding more silt. A preliminary test to keep the silt in suspension showed that air bubbling was not sufficient, even at extreme airflow rates (Fig. 5). Stirring of the water with a RW 20 digital overhead stirrer motor with a propeller at approx. 500 rpm resulted in low settling rates of the sediment. A direct benefit of this circulation was a better exchange at the water-sediment interface, which prevented the forming of an isolated water layer low in oxygen just above the sediment. Low oxygen levels are thought to have a significant negative effect on *Ensis* (Witbaard, pers. Comm).

Fig. 5. Test to keep silt suspended with an air pump.

Throughout the experiment, parameters indicative of water quality were monitored daily (see Annex 4). The pH, O_2 concentrations and temperature was measured using a Hach HQd Field case Cat. No. 58357-000. Salinity was measured with a Bellingham & Stanley Ltd. Eclipse refractometer. The NO_2 and NH_4 concentrations were determined using TETRA Tests. In addition, mortality was checked daily, and dead specimens were removed. After 10 weeks, the *Ensis* were taken out of the containers and WW, ADW and shell length of all surviving *Ensis* specimens were measured individually.

2.6 Statistical analysis

Effects of food and silt concentration on filtration, growth and mortality rates were tested with ANOVA. Linearity of the data was examined with residual plots. The homogeneity of variances was tested with a Levene test. Since the variances were not distributed homogeneously, the data were transformed (square root for counts or Poisson data; arc-sin for percentages and proportions; log or 1/x for rates, ratios, concentrations and other data). After transformation of the data, the results indicated that the assumptions were still violated. However, large balanced ANOVA designs are considered robust to departures from variance assumptions (Underwood, 1997). Thus, the analysis was conducted on untransformed data. To reduce the likelihood of type-I error, a more conservative significance level of

P<0.01 was used. Significant effects were examined using posthoc Bonferroni tests. Statistical analyses were performed using IBM SPSS 19.

3.Results

3.1 Collection, storage and survival

Mortality rates were high during the first week after collections, and rapidly decreased during the following weeks. No significant correlation was found between temperature and mortality (Annex 3). This implies that other factors, such as inability to adapt or damage to the shell during sampling and transport may have been the primary cause of mortality.

3.2 Filtration experiments

Clearance rates varied between size classes, increasing significantly with increasing clam size (Annex 7). The clearance rates decreased with increasing silt concentrations (Fig. 6). The 300 mg/l treatment differed significantly from the other treatments (Annex 7). Differences in clearance rates between size classes in relation to food levels were observed, but the effect of food levels was not significant (*see* Table 3a and Annex 7). Under high food levels, the clearance rates by the small size class were too low to detect any further effect of silt concentration (Fig. 5).

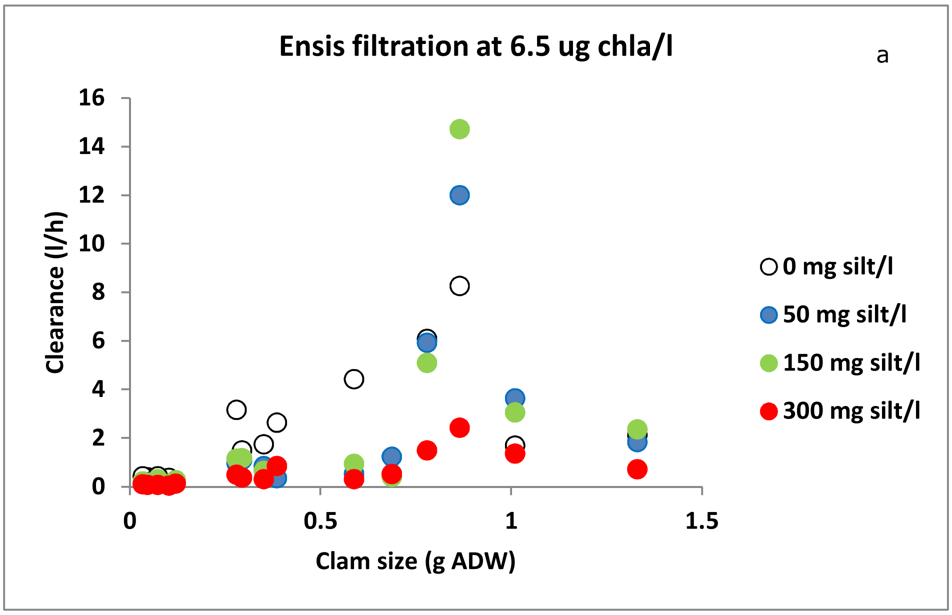
Table 3a. Average clearance rates (CR) per size class ± sd (N=5) under different food levels (Low = 6.5 µg Chla/l and High = 16.5 µg Chla/l and silt concentrations (0,50,150 and 300 mg/l). (individuals are measured 5 to 9 times).

Size class (mm)	CR ± *sd* (l/h) Silt=0; Chla=6.5	CR ± *sd* (l/h) Silt=50; Chla=6.5	CR ± *sd* (l/h) Silt=150; Chla=6.5	CR ± *sd* (l/h) Silt=300; Chla=6.5
33-75	0.36 ±0.13	0.15 ±0.04	0.20 ±0.06	0.07 ±0.02
75-100	2.68 ±0.76	0.75 ±0.22	0.95 ±0.39	0.46 ±0.12
>100	3.73 ±1.24	4.92 ±0.78	5.12 ±1.84	1.30 ±0.5
	CR ± *sd* (l/h) Silt=0; Chla=16.5	CR± *sd* (l/h) Silt=50; Chla=16.5	CR ± *sd* (l/h) Silt=150; Chla=16.5	CR ± *sd* (l/h) Silt=300; Chla=16.5
33-75	0.1 ±0.01	No data	No data	No data
75-100	1.74 ±0.78	0.70 ±0.24	0.52 ±0.08	0.29 ±0.07
>100	4.51 ±1.58	4.76 ±1.26	3.29 ±0.54	2.55 ±1.36

Table 3b. The frequency of excretion of pseudo-faeces (% of all observations) per size class (N=5) under different food levels (Low = 6.5 µg Chla/l and High = 16.5 µg Chla/l and silt concentrations (0,50,150 and 300 mg/l). Average clearance rate of five individuals is presented (measured 5 to 9 times on each individual).

Size class (mm)	*frequency* (%) Silt=0; Chla=6.5	*frequency* (%) Silt=50; Chla=6.5	*frequency* (%) Silt=150; Chla=6.5	*frequency* (%) Silt=300; Chla=6.5
33-75	0	14	9	16
75-100	7	18	13	10
>100	0	4	28	33
	frequency (%) Silt=0; Chla=16	*frequency* (%) Silt=50; Chla=16	*frequency* (%) Silt=150; Chla=16	*frequency* (%) Silt=300; Chla=16
33-75	0	No data	No data	No data
75-100	40	40	28	12
>100	0	24	28	43

All size classes of *Ensis* produced pseudo-faeces. Significant effects of silt concentration, food level and clam size were observed (Annex 7). The frequency of pseudo-faeces production was higher at the high food concentration. This frequency was lower for small clams compared to middle and large size clams. The effect of not adding silt on the production of pseudofaeces was significantly different from the production by adding silt. Middle sized clams produced pseudo-faeces more frequently when no silt was added in contrary to the other two size classes. An increase in pseudo-faeces production along with increasing silt concentration was recorded for large sized clams (Table 3b). This size class managed to keep up reasonable stable filtration rates under higher silt concentration, compared to the other size classes. The difference in clearance rate (l/h) measured just before and after the excretion of pseudo-faeces varies from 50% (n=3) for small *Ensis* and approx. 50% (n=18) for middle-sized *Ensis* to approx. 40% for large *Ensis* (n=9).



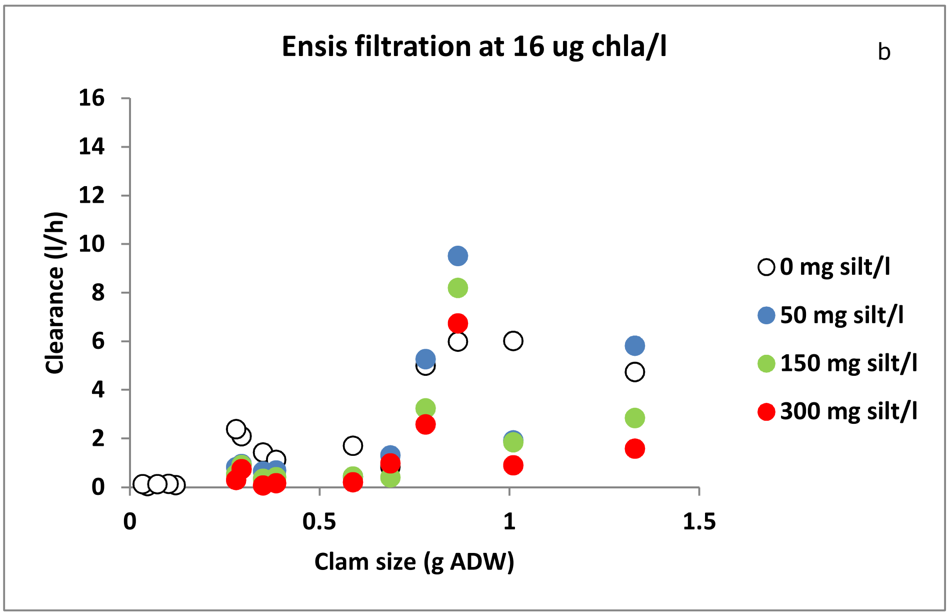


Fig. 6. Clearance rate in relation to the clam size at a food level of (a) 6.5 µg Chla/l and of (b) 16 µg Chla/l.

The data from Fig. 6 were used by Schellekens (in prep.) to determine the half saturation constants Y_k and X_k (see page 10). For each individual, the food intake rate was calculated by multiplying the chlorophyll concentration with the filtration rate. The same was done for the intake of silt. The uptake rate of the sum of silt and chla is the uptake rate of chla multiplied by the silt: chlorophyll ratio. The uptake rate (including chla and silt) is lowered due the negative effect of silt. The change in intake rate was measured by subtracting the uptake rate without silt from the uptake rate with silt for each silt concentration. The relation between change in uptake rate and silt concentration is exponential and has a similar exponent for each size class and at each chlorophyll level (average $y=a*e^{-0.011x}$, s.d.=0.002, n=15). Because the relative change in intake rate is the same for all individuals the decrease in intake rate from silt is described by a constant factor of 0.011. In the functional response this factor is X_k/Y_k (see paragraph 2.4 and equation 1). Wijsman (2011) determined that X_k=0.75, then Y_k=68.18.

3.3 Growth experiment

A substantial difference in cell concentrations between the low and high food treatment was achieved (Table 4; Annex 5). Nevertheless the flow cytometric analyses revealed a substantial variation in algal cell concentration in the tanks. The cell concentrations in the different tanks may be biased by variable sinking rates of the cells, potentially through adhesion to silt particles. Therefore, an additional calibration of the amount of food given to the different tanks was done, using the counted amount of phytoplankton cells added to the tanks during the course of the experiment. These calculations show that the amount of phytoplankton cells supplied to the tanks differed considerably between the low and high food treatment (Table 4). Silt concentrations were relatively stable throughout the experiment (Table 4; Annex 5).

Table 4. Average algal cell concentrations (in µg Chl*a*/l and cells per ml*) measured daily by flow cytometry, total supplied algal cells and average silt concentration over the course of the growth experiment.*

Treatment	Silt=0, Chla=6.5	Silt=150,Chla =6.5	Silt=300,Chla =6.5	Silt=0, Chla=16	Silt =150, Chla=16	Silt=300, Chla=16
FCM daily	5.25 µg Chl*a*/l 13886 ±5395 Cells/ml	5.5 µg Chl*a*/l 14531 ± 5326 Cells/ml	6.5 µg Chl*a*/l 17165 ± 6780 Cells/ml	15.5 µg Chl*a*/l 35427 ±15408 Cells/ml	15.1 µg Chl*a*/l 34512 ± 20676 Cells/ml	16.7 µg Chl*a*/l 37984 ± 14161 Cells/ml
Total cells added	$3.2*10^{12}$	$2.5*10^{12}$	$1.9*10^{12}$	$6.6*10^{12}$	$6.7*10^{12}$	$6.5*10^{12}$
Silt (mg/l)	0	138 ±22	284 ±39	0	153 ±23	296 ±35

Within the first 2 weeks, a substantial mortality of *Ensis* was observed in all tanks. Initially, the water in the tanks was refreshed (approximately 60%) when threshold values for NO_2 (0.5 mg/l) and NH_4 (1mg/l) were reached. Because of the mortality, the refreshing was intensified to twice a week. This coincided with a substantial decrease of dead specimens. Another unforeseen cause of mortality was that *Ensis* that got out of the sediment may have been hit by the propeller. Mortality was highest among large *Ensis* (Table 5; Annex 6).

Table 5. Mortality during growth experiment.

Treatment	Total mortality (%)	Mortality small *Ensis* (%)	Mortality middle-sized *Ensis* (%)	Mortality large *Ensis* (%)
0Low	**40.00**	**20.00**	**40.00**	**53.00**
150Low	**42.22**	**20.00**	**6.67**	**60.00**
300Low	**51.11**	**20.00**	**6.67**	**86.67**
0High	**48.89**	**6.67**	**6.67**	**66.67**
150High	**26.67**	**13.33**	**13.33**	**40.00**
300High	**35.56**	**20.00**	**26.67**	**53.33**

Other parameters indicative of water quality showed little variability over the course of the experiment. Oxygen levels remained between 7.5 and 8.5 mg/l, temperature between 16.5 and 20°C, and salinity between 32 and 35‰. A slight difference in daily temperature (up to 0.5°C) was recorded between tank 1 and 6, which is linked to the location in the climate room (see Annex 4).

The growth as indicated by shell length was significantly related to size class. Small *Ensis* showed the largest increase in shell length, whereas shell size of large *Ensis* was overall similar to the size measured before the experiment (Fig. 7 and Annex 7). Significantly larger increase in shell size was recorded for specimens grown under higher food levels, with several individuals demonstrating a growth higher than assumed to be theoretically possible according to the von Bertalanffy growth curve (Cardoso et a., 2011) (Fig. 7, Annex 7). Shell growth was significantly larger at 300 mg/l compared to 150 mg/l and 0 mg/l (Annex 7).

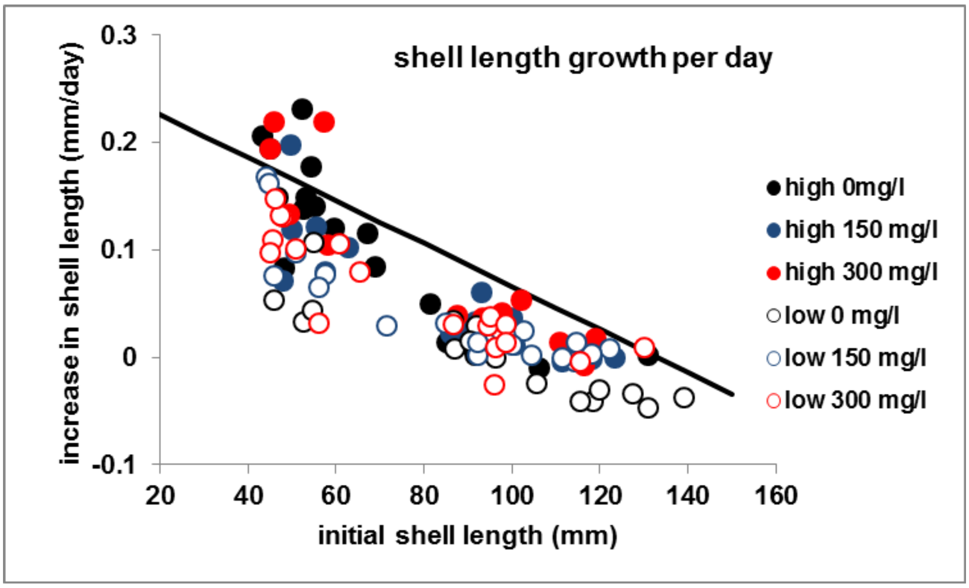
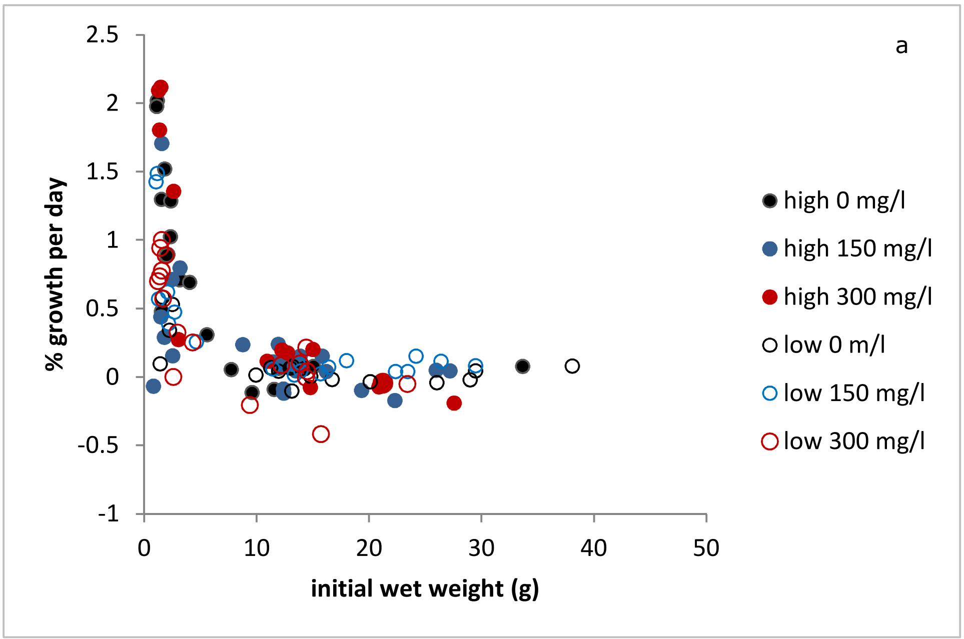


Fig. 7. Shell length growth in mm per day in relation to food availability. Solid line indicates average growth expected according to the Von Bertalanffy equation $dL/dt= 0.002 * k(L_\infty - L_x)$, based on literature from Cardoso et al. (2011). Silt concentrations are 0 mg/l, 150 mg/l and 300 mg/l. High= 16 µg Chla/L and Low = 6.5 µg Chla/L.

Relative growth, indicated as increase in % wet weight, demonstrates a significant effect of size on growth with highest growth in small *Ensis* (Fig. 8, Annex 7). In addition, most pronounced increases were measured for the *Ensis* grown under high food levels. Larger *Ensis* (>10 gr) show no, or only a slight, increase in wet weight and differed significantly from the other two groups (Fig. 8, Annex 7). Silt concentration did not affect relative growth (Annex 7).



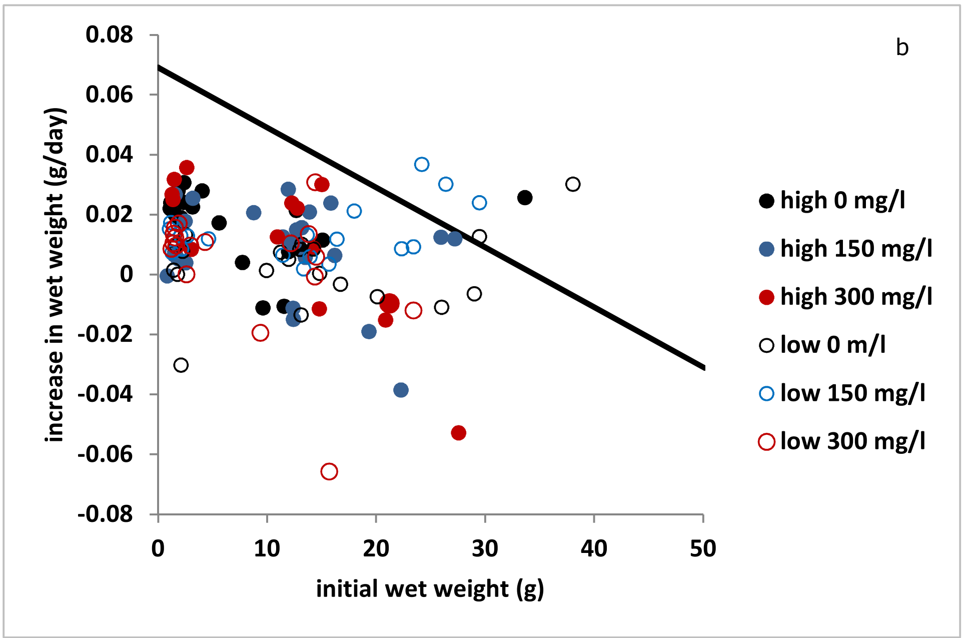


Fig. 8. The relation between growth rate and the different treatments (food and silt concentration), expressed as increase in wet weight in (a) % and (b) gram per day. Solid line indicates average growth expected according to the Von Bertalanffy equation $dL/dt= 0.002 * k(W_\infty - W_x)$, based on literature from Cardoso et al. (2011). Silt concentrations are 0 mg/l, 150 mg/l and 300 mg/l. High= 16 µg Chla/L and Low = 6.5 µg Chla/L.

Food concentration had a significant effect on the individual ash free dry weight (ADW) (Fig. 9, Annex 7). The ADW in middle-sized *Ensis* decreases under the low food availability, whereas ADW in the high food treatment under elevated silt concentrations increases. The ADW in small *Ensis* is higher in all treatments when compared to the initial sample (Fig. 9). However, only the increase in the high food no silt treatment was significant for the small clams (Annex 7). In medium sized *Ensis* the high food and 300 mg/l silt treatment was the only group that showed a significant increase in comparison with the initial value. Silt concentration did not affect ash-free dry weight (Annex 7). The individual ash-free dry weight (ADW) of large *Ensis* at the end of the experiment did not significantly differ from the initial ADW.

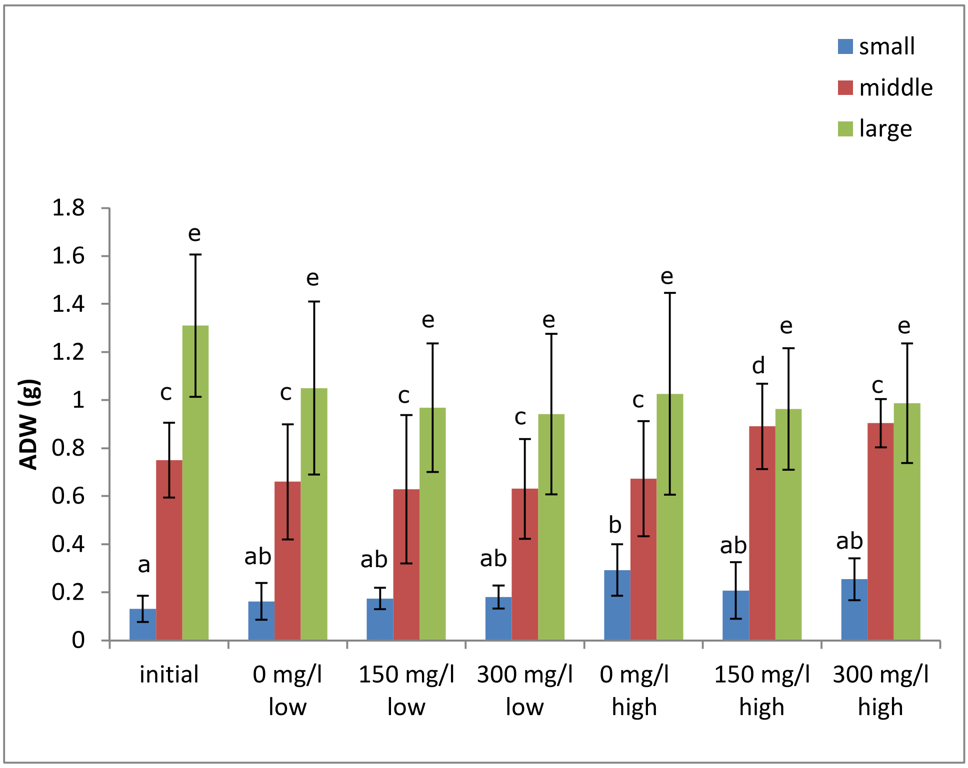


Fig. 9. Ash-free dry weight (ADW), average in g with sd, of an initial sample and the ADW of the Ensis after the growth experiment. Letters that are not the same indicate significant differences (p<0.01) between treatments within size groups.

4. Discussion and conclusions

4.1 Improvements of the laboratory experiments

<u>Survival</u>

Our monitoring of mortality rates in the storage basin showed that mortality was especially high in the first week after collection. This is likely to be the result of several factors, notably possible damage to the shell during collection, and stress during adaptation to a new environment. As such, the storage of the collected *Ensis* for a certain period of time may provide a good selection for vitality prior to experimental work. During the growth experiment, mortality was highest in the large *Ensis*. This group already showed some mortality after collection with a box corer and extra individuals were collected with a dredge instead of a box corer. This indicates that large *Ensis* is more sensitive and that collection with a box corer yields better survival than collection with a suction dredge.

<u>Food availability for growth</u>

Algae concentrations fluctuated significantly during the growth experiment, which could have several reasons. First of all, shellfish do not filter feed at a constant rate, therefore, variations in food uptake may have been unnoticed, resulting in the observed unstable phytoplankton cell concentrations. Another possibility may be coagulation of silt particles and phytoplankton cells, thereby increasing sedimentation/settling rates of the cells, particularly under higher silt concentrations. Nevertheless, in 2011 higher growth rates were obtained than in 2010, which shows that using a diet composed of a flagellate (*Pavlova lutheri*) and a diatom (*Chaetoceros mulleri*) and increasing water movement with a stirrer improved the conditions for growth. The growth was most pronounced in the small size class, where growth rates exceeded rates expected according to the von Bertalanffy curve. Perhaps the quality or quantity of the provided algal diet was less suitable for larger clams. It should be noted however, that in the experiments of 2010 the larger animals lost weight. This was not the case in 2011, suggesting a better experimental set-up.

<u>Shell growth versus meat growth</u>

In the growth experiment, an increase in shell length and wet weight was more visible than an increase in ash-free dry weight. Wet weight is the weight of the clam including shell, meat and water contained within the shell. It is a measure of size more than a measure of weight. Furthermore, wet weight is difficult to measure, because above the water the clams can spit out water and in that way reduce their weight. Therefore, it is not the best measure for growth. Ash-free dry weight has the disadvantage that it cannot be measured on the same individuals before and after the experiment, because you need to sacrifice the animal to determine ash-free dry weight. Therefore, the parameter is less accurate. However, the only treatments that did show an increase in ash-free dry weight, were the high food treatments. This suggests that reserves were used for an increase in shell length, but in most treatments the amount of food given was not enough for an increase in ash-free dry weight. Data on *Ensis* in the North Sea coastal zone of Witbaard (in prep) show first an increase in meat content (ADW) and then an increase in shell length (Fig. 9). In addition, compared to the von Bertalanffy curve, the shell growth of the larger animals is low.

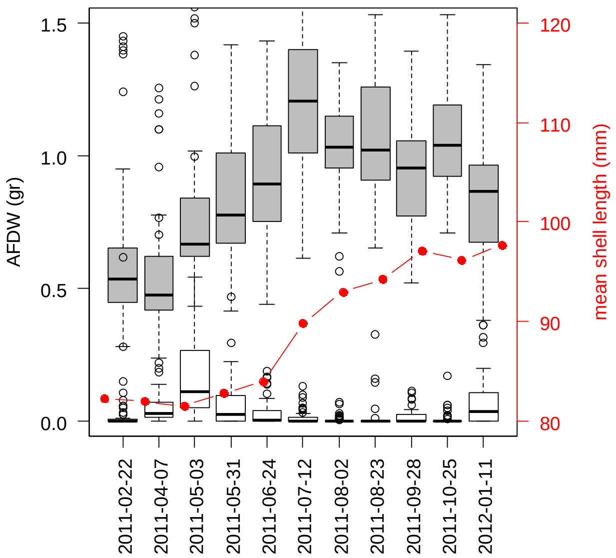


Fig. 9. A combination plot of the seasonal change in ash-free dry weight of the somatic tissue (grey box plots), gonadal tissue (white box plots), and the average shell length (red line) of animals smaller than 110 mm. From Witbaard et al. (2012).

4.2 Filtration rates of Ensis related to other shellfish

Our 2011 study confirmed the observations made in 2010 that filtration rates increased with clam size. This positive relation has been observed for other bivalve species as well. Mohlenberg & Riisgard (1979) measured positive relations between filtration rate and bivalve weight in *Cardium echinatum , Cerastoderma edule , Mytilus edulis , Modiolus modiolus Arctica islandica, Spisula subtruncata*, *Hiatella striata*, *Cultellus pellucidus*, *Mya arenaria, Venerupis pullastra, Pecten furtivus* and *P. opercularis.* Shumway et al (1985) measured an average filtration rate of 0.93 l/h/g DW in *Ensis directus*. Van Duren & Troost (in prep) measured 2.9 l/h/g DW, and in a previous study Witbaard & Kamermans (2009) measured 0.2-3.1 l/h/g DW. These values are comparable with the 2010 values of 0.1-3.9 l/h/g DW (Kamermans et al, 2011). The filtration rates measured in 2011 varied from the lowest average of 0.7 l/h/g DW to the highest average of 5.9 l/h/g DW. A possible explanation for the higher value can be the moment of measurement in relation to the moment of collection in the field. The filtration measurements of 2010 were carried out with clams at the end of the growth experiment. The low growth rates during that growth experiment suggest that these clams were stressed. In 2011, the clams in the filtration measurements were collected freshly.

In our 2011 measurements, filtration rates of *Ensis directus* were significantly lower at 300 mg/l compared to the rates measured at 150 mg/l, 50 mg/l and 0 mg/. In 2009, reduced filtration rates started at 200 mg/l (Witbaard & Kamermans, 2009). This corresponds with the 2011 results. In 2011, silt addition from 0 mg/l to 300 mg/l reduced filtration rates with 16-19 % for the small and medium sized *Ensis* and 34-56% for the large sized *Ensis*. The filtration rate of 50-60 mm *Solen cylindraceus*, a species related to *Ensis directus*, showed a reduction in filtration rate of 45% between silt concentrations of 100 en 250 mg/l (Table 6, De Villiers & Hodgson, 1993).

Pseudo-faeces were produced by all size classes of *Ensis* and at all silt concentrations (50, 150 and 300 mg/l). Production increased with silt concentration and clam size. Pseudo-faeces production has been observed at particle concentrations as low as 3 mg/l for other bivalve species (Table 7). Information on pseudo-faeces production in *Ensis directus* is not available in the literature. Pseudo-faeces production of *Solen cylindraceus* was minimal at seston concentrations below 50 mg/l, but between 100 and 500 mg/l the amount of pseudo-faeces produced increased significantly (De Villiers & Hodgson, 1993).

Table 6. Literature overview of measured reductions in filtration rates as a result of increase in particle load for bivalve shellfish.

Particle increase	Clearance rate change	Species	Reference
1.0 to 3.0 mg/l	5.0 to 2.0 l/h	*Placopecten magellanicus*	Bacon et al., 1998
1.0 to 3.0 mg/l	3.0 to 1.5 l/h	*Mya arenaria*	Bacon et al., 1998
1 to 15 mg/l	19 to 6 l/h	*Placopecten magellanicus*	Cranford and Gordon, 1992
3 to 20 mg/l	2 to 1 l/h	*Saccostrea commercialis*	Kesarcodi-Watson et al., 2001
2 to 6 mg/l	0.5 to 1.2 l/h	*Meretrix meretrix*	Zhuang and Wang, 2004
6 to 10 mg/l	1.2 l/h	*Meretrix meretrix*	Zhuang and Wang, 2004
10 to 18 mg/l	1.2 to 0.2 l/h	*Meretrix meretrix*	Zhuang and Wang, 2004
100 to 250 mg/l	85% to 40%	*Solen cylindraceus*	De Villiers and Hodgson, 1993
1 to 2 mg/l	2 to 1 mg/h	*Cerastoderma edule*	Navarro et al., 1992
200 to 633 mg/l	0.042 to 0.014 l/h/g	*Meretrix lusoria*	Chien and Hsu, 2006
8 to 25 mg/l	10.8-12.2 l/h/g	*Spisula subtruncata*	Mohlenberg & Kiorboe, 1981

Table 7. Literature overview of pseudo-faeces production rates and thresholds for bivalve shellfish.

Particle increase	Pseudo-faeces production rate (mg/h)	Species	Reference
1.0 to 3.0 mg/l	0.1 to 0.3 mg/h/gr	*Placopecten magellanicus*	Bacon et al., 1998
7.0 to 14.0 mg/l	2.0 mg/h/gr	*Placopecten magellanicus*	Bacon et al., 1998
1.0 to 14.0 mg/l	0.1 to 0.4 mg/h/l	*Mya arenaria*	Bacon et al., 1998
2, 5, 10 and 15 mg/l bentonite	0.2, 4.5, 12.0 and 16.2 mg/h	*Placopecten magellanicus*	Cranford and Gordon, 1992
20 to 90 mg/l	10-20 to 150 mg/h	*Cerastoderma edule*	Hawkins et al., 1998
20 to 90 mg/l	10-20 to 130 mg/h	*Crassostrea gigas*	Hawkins et al., 1998
20 to 90 mg/l	10-20 to 180 mg/h	*Mytilus edulis*	Hawkins et al., 1998
25 to 50 mg/l	< 5 to 10-50 mg/h	*Solen cylindraceus*	De Villiers and Hodgson, 1993
<3 mg/l food concentration	<0.3 proportion	*Saccostrea commercialis*	Kesarcodi-Watson et al., 2001
From 10 mg/l	From 0.02 g/h	*Mytilus edulis*	Kooijman, 2006

4.3 *Ensis* growth and mortality under long-term stress by high concentrations of silt

Freudendahl et al (2010) studied survival and growth of 60-70 mm *Ensis americanus* in the Wadden Sea in the period August to November. The shell growth was 5.9 mm in 9 weeks which corresponds to 0.09 mm increase in shell length per day. Witbaard (in prep) observed a shell increase from 83 mm to 96 mm in 3 months or 0.14 mm per day. Our growth rates for this size class were in the same range (0.08-0.12 mm per day). This is a little lower than the average growth of 0.21 mm expected according to the Von

Bertalanffy equation based on literature from Cardoso et al. (2011). The average survival rate recorded by Freudendahl et al (2010) was 57% in 9 weeks. We observed comparable survival rates from 73-51% in a period of 10 weeks. Overall both shell growth and survival seem to be comparable to the observations in the field and to theoretical considerations.

Ecological significance

In our experiments we found a positive effect of increased food availability and of increased silt concentration on growth. There is no information in the literature on the relation between *Ensis directus* growth or mortality and exposure to silt. Mohlenberg & Kiorboe (1981) studied the effect of suspended sediments on growth and energetics in *Spisula subtruncata* (Da Costa) and also found a positive correlation. They explained this by a higher assimilation efficiency of the algae when silt was added. Mussels (*Mytilus edulis*) react differently to silt exposure. Essink et al. (1990) observed reduces growth in mussels at a high suspended silt concentrations. The capacity to optimally use food in mussels starts to decline at a suspended silt concentration of 50 mg/l and at concentrations higher than 100 mg/l weight loss occurs (Prins & Smaal, 1989). The reaction of a species seems to fit to the general ecology and niche of a shellfish. Typical animals of the turbulent coastal zone seem to be able to adapt themselves to high silt concentrations whereas the mussel, more a tidal flat animal seems more vulnerable.

4.4. Potential implications for sand extraction

The project Landelijke Taken B&O Waterbeheren of Rijkswaterstaat Waterdienst NWOB (department of Infrastructure and Environment, MinIenM, RWS) and the Monitoring programme Sandmining of RWS and the LaMER Foundation, are working on a method to estimate effects of sand extraction on marine benthic fauna in the coastal zone. Part of this method involves the DEB model that will be integrated with water quality models such as Ecowasp, ERSEM and Delft 3D. The present study delivers data on functional responses and parameters for the DEB model. Schellekens (2012) used these data in the MER Zandwinning 2013-2017.

Sand extraction always goes together with an increase of silt concentration in the water column. This reduces light conditions for algal growth. The laboratory experiments show that the razor clam *Ensis directus* is more sensitive to a reduction in algal concentration than to an increase in silt concentration (see also Schellekens, 2012). Filtration rate was reduced above a silt concentration of 300 mg/l, but we found a positive effect of increased silt concentration on growth. Witbaard et al (2012) measured silt concentrations in the North Sea near Egmond. They observed that silt concentrations above 300 mg/l do not occur very often (Fig. 10 and Fig. 11). The measurements were carried out in 2011 and include effects of sand extraction activities (previous sandmining activities like suppletion sand of RWS, Zwakke Schakels, sand for construction, Maasvlakte 2, Sand Engine). Average concentrations are well below the 300 mg/l where impact on Ensis is expected. Thus, we expect that the effect of sand extraction on *Ensis* will mostly be through the reduction in algal concentration and primary production (by light reduction of silt) and not through the direct impact of extra silt on filtration.

Harezlak et al. (2012a,b) predicted that effects of increased silt concentrations caused by sand extraction will be spread over large areas and long time periods (it dilutes in time and space). From Schellekens (2012) and Brinkman (2012) it becomes apparent that sand extraction could be a problem on populations of *Ensis* and benthic communities in the Wadden Sea when large quantities are mined in the entire coastal zone. Thus, the management and mitigation of impact should be focussed on management of the total volumina mined by all stakeholders (e.g. quota) rather than on each sand extraction project apart.

It should be noted that the response of other marine benthic fauna may not be the same, e.g. be more sensitive to silt concentration as well as to the reduction of algae and primary production. It seems wise to determine the appropriate functional response (filtration and growth under different concentrations of algae and silt) for at least those species for which already a first, more basic, DEB model has been constructed (cockles (*Cerastoderma edule*), mussel (*Mytilus edulis*), *Macoma balthica*, soft shell clam (*Mya arenaria*), Pacific oyster (*Crassostrea gigas*) (van der Veer et al, 2006; Wijsman & Smaal, 2011). In addition, it is advisable to generate a DEB model on *Donax vittatus* and *Spisula subtruncata*. These species are particularly important in the N2000 area of the North Sea coastal zone, which has an juridical improvement obligation on the Benthic Habitat 1110B and the shellfish stocks in particular.

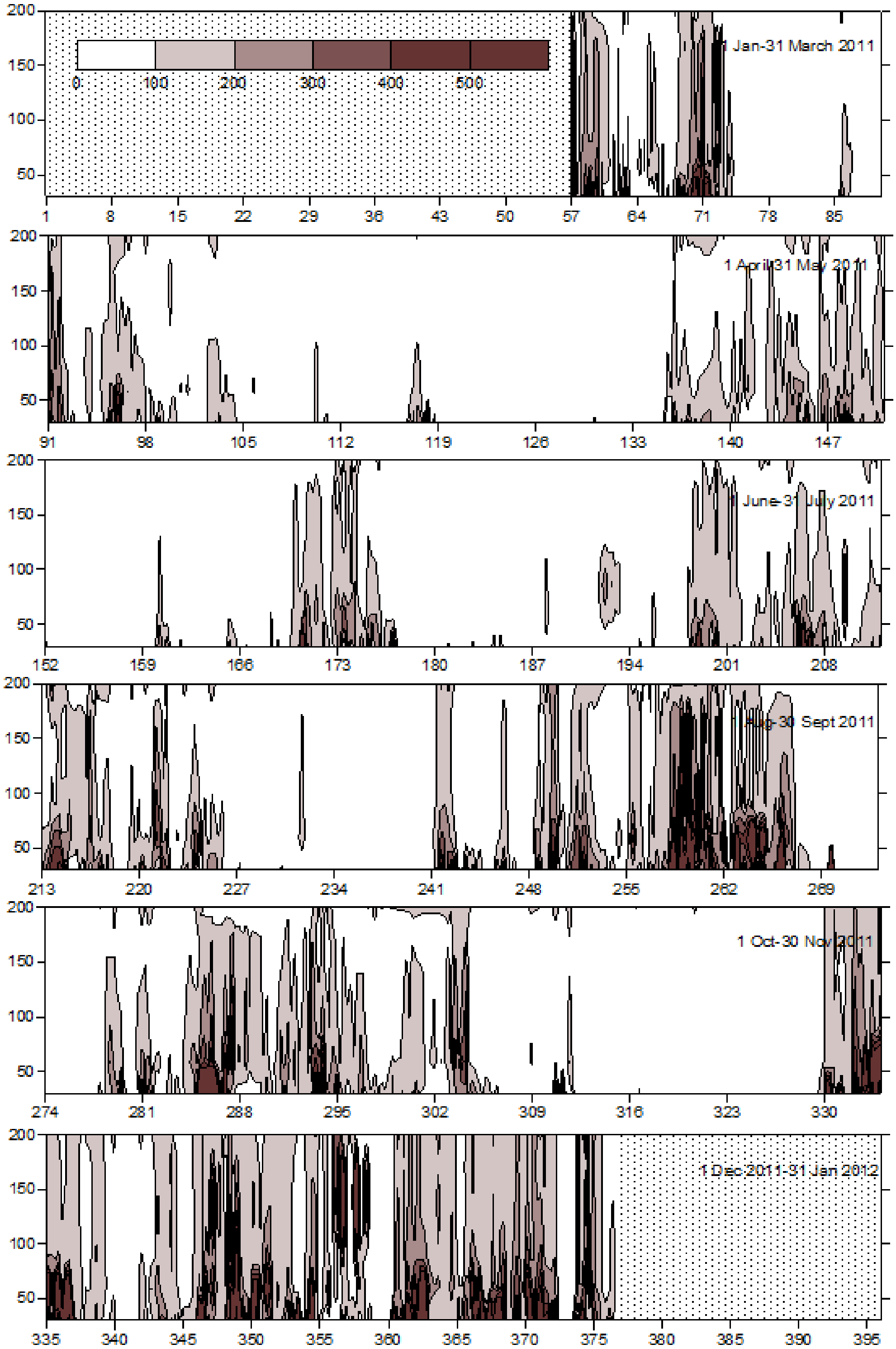


Fig. 10. Contour plots of suspended matter concentrations near Egmond over the measurement period and below 500mg/L. Numbers along x-axis is day number since 1/1/2011. Numbers along y axis denote height above the seafloor. From Witbaard et al. (2012).

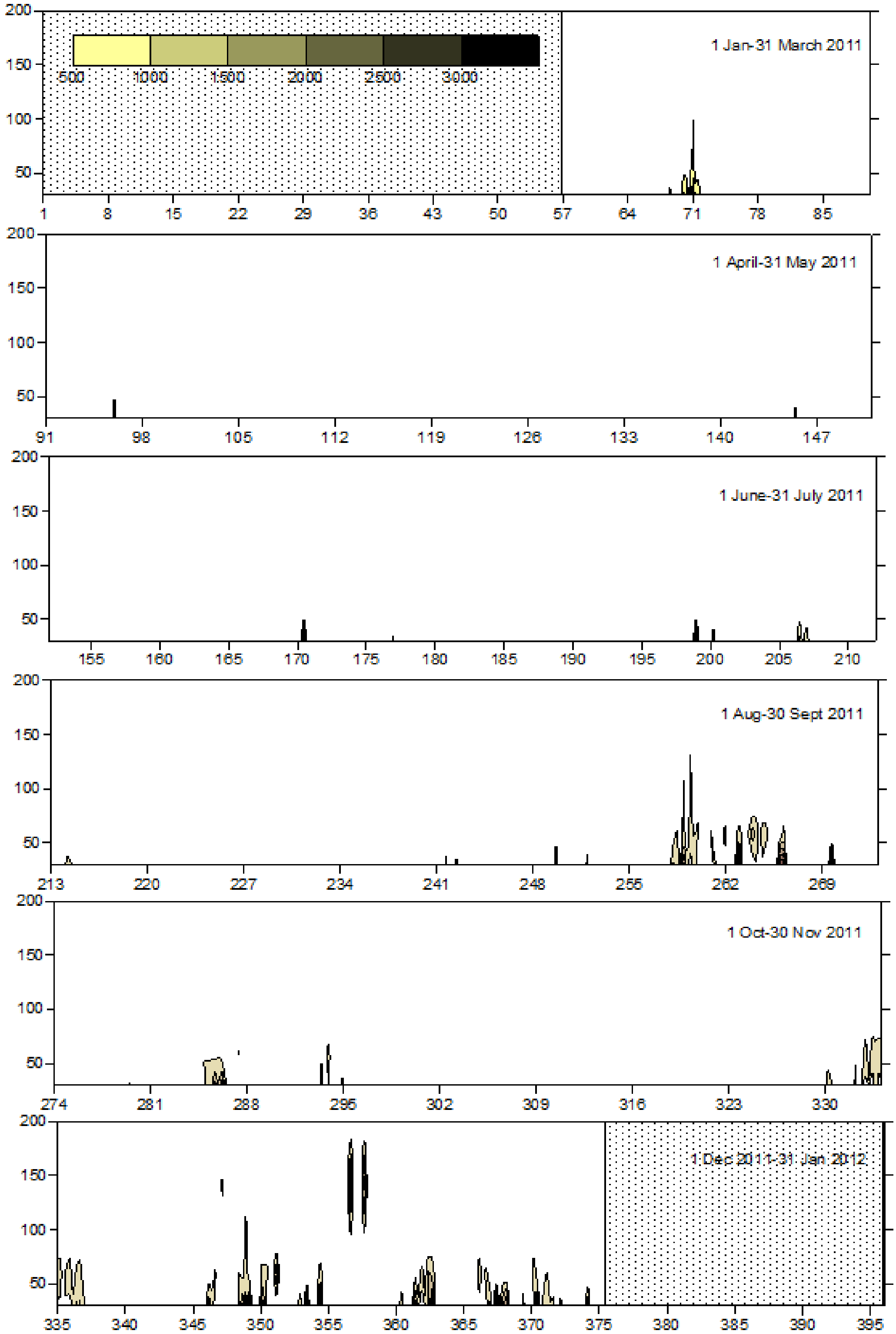


Fig. 11. Contour plots of suspended matter concentrations near Egmond over the measurement period and exceeding 500mg/L. Numbers along x-axis is day number since 1/1/2011. Numbers along y axis denote height above the seafloor. From Witbaard et al. (2012).

4.5. Conclusions

- Improvements of the set-up for laboratory experiments were carried out successfully: collecting *Ensis* with a box corer yields better survival than collection with a suction dredge; using a diet composed of a flagellate (*Pavlova lutheri*) and a diatom (*Chaetoceros muelleri*) and increasing the mixing rate and water movement with a stirrer improved the conditions for growth.
- Filtration rates of *Ensis directus* at 300 mg/l silt differed significantly from rates at 150 mg/l, 50 mg/l and 0 mg/l silt. This indicates that only the highest silt concentration induced a reduction in filtration rate. Silt addition to 300 mg/l reduced filtration rates with 16-19 % for the small and medium sized *Ensis* and 34-56% for the large *Ensis*.
- The tested food levels did not influence filtration rate of Ensis. However, the intake rate is higher at higher food concentration, because more algal cells are present in a certain volume of water.
- Long-term exposure (10 weeks) to silt concentrations of 300 mg/l showed significantly higher growth than the 150 mg/l treatment indicating that exposure to a high silt concentration did not induce a reduction in growth, but stimulated the growth.
- Long-term exposure (10 weeks) to a food level of 6.5 ug chla per liter reduced shell growth of small *Ensis* with 41% compared to a food level of 16 ug chla per liter; shell growth of medium sized clams was reduced with 38%.
- Small and medium sized Ensis showed significant differences in shell length and as-free dry weight between treatments. This was not the case for large *Ensis*. Food and silt concentration dit not influence shell length or AFDW of large Ensis.
- The filtration and growth rate results are used in a modelling study on growth and condition of *Ensis* during sand extraction 2013-2017 (Schellekens, 2012). The relation between change in uptake rate (y) and silt concentration (x) is $y=a*e^{-0.011x}$. Data from the present study were used to determine the half saturation constants Y_k (68.18) and X_k (0.75) and thereby refine the DEB model of Ensis directus that was generated by Wijsman et al. (2011).
- The conclusions of this study give more notion of the effects of sand extraction in the coastal zone of the North Sea on the viability the razor clam of *Ensis directus*. Sand extraction always goes together with an increase of silt concentration in the water column. This reduces the light conditions for algal growth which reduces the food availability for *Ensis*. The laboratory experiments show that *Ensis* is more sensitive to a reduction in algal concentration than to an increase in silt concentration.

Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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Justification

Rapport Number: C017/12
Project Number: 4303102201

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: E.M. Foekema
Researcher

Signature:

Date: 20 April 2012

Approved: B.D. Dauwe
Head Department Delta

Signature:

Date: 20 April 2012

Annex 1. Calculation of cell density and silt concentration for Ensis experiments

CELL DENSITY

We used PASW Statistics 17.0 to fit a logaritmic curve on data of the filtration measurements at different algal densities in 2009. We used data of the Ensis Batches I en II that were fed *Pavlova lutheri*. The R^2 value was 0.455 and the p value 0.016 for Batch I en II together, R^2 0.578 and p 0.080 for Batch I and R^2 0.667 and p 0.047 for Batch II. The results are presented below.

cell density Batch I en II together

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH celdens
  /CONSTANT
  /MODEL=LOGARITHMIC
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_1
Dependent Variable	1	celuptake
Equation	1	Logarithmic
Independent Variable		celdens
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

Case Processing Summary

	N
Total Cases	12
Excluded Cases[a]	0
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

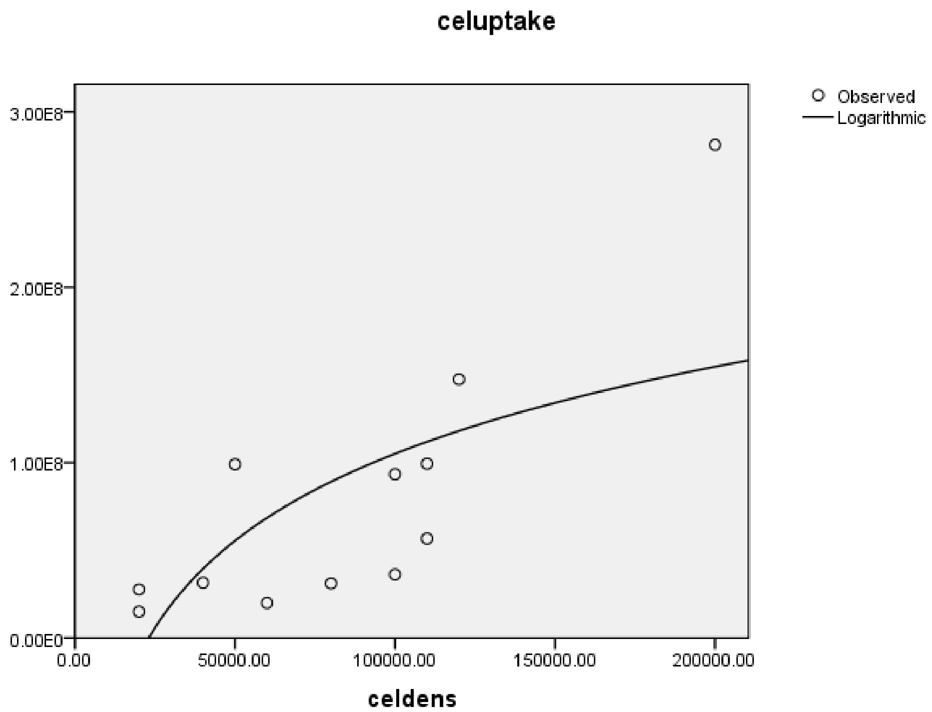
		Variables	
		Dependent	Independent
		celuptake	celdens
Number of Positive Values		12	12
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	0	0

Model Summary and Parameter Estimates

Dependent Variable:celuptake

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Logarithmic	.455	8.358	1	10	.016	-7.193E8	7.160E7

The independent variable is celdens.



cell density Batch I

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH celdens
  /CONSTANT
  /MODEL=LOGARITHMIC
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_2
Dependent Variable	1	celuptake
Equation	1	Logarithmic
Independent Variable		celdens
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

Case Processing Summary

	N
Total Cases	6
Excluded Cases[a]	0
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

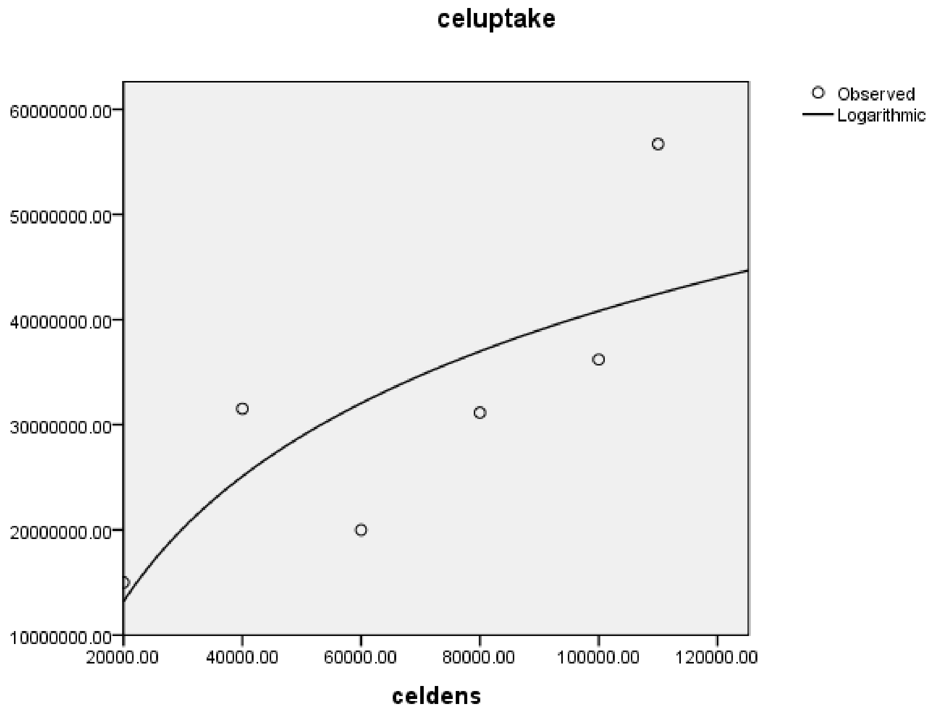
		Variables	
		Dependent	Independent
		celuptake	celdens
Number of Positive Values		6	6
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	0	0

Model Summary and Parameter Estimates

Dependent Variable:celuptake

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Logarithmic	.578	5.468	1	4	.080	-1.568E8	1.717E7

The independent variable is celdens.



cell density Batch II

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH celdens
  /CONSTANT
  /MODEL=LOGARITHMIC
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_3
Dependent Variable	1	celuptake
Equation	1	Logarithmic
Independent Variable		celdens
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

Case Processing Summary

	N
Total Cases	6
Excluded Cases[a]	0
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

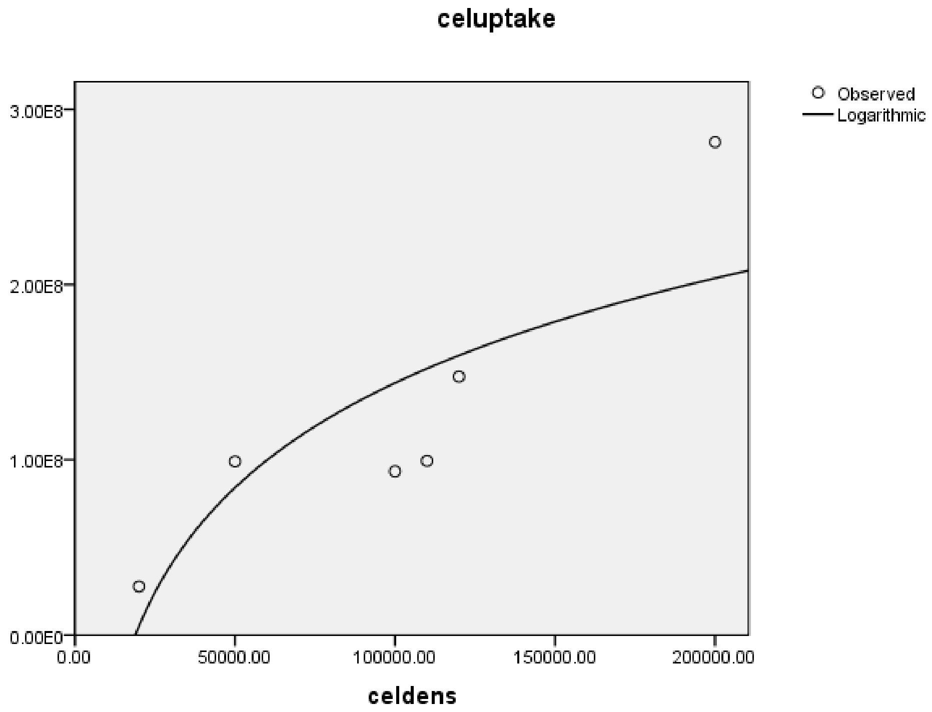
		Variables	
		Dependent	Independent
		celuptake	celdens
Number of Positive Values		6	6
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	0	0

Model Summary and Parameter Estimates

Dependent Variable:celuptake

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Logarithmic	.667	8.012	1	4	.047	-8.486E8	8.620E7

The independent variable is celdens.



The fit of the results of Batch I and II together had the lowest p value. The fit of the results of Batch II had the highest R^2 value, but not the lowest p value. Uptake of Batch I was much lower than Batch II. Possibly, the clams of Batch I were less active as a result of stress. The significance of differences between Batch I and II together and Batch II alone was tested with ANOVA. This was not the case (p=0.258). Therefore, the formula of Batch II was used and not the formula of both groups together. This formula (y=86200000*ln(x)-848600000) is used to determine the cell density at which 20% of the maximum uptake and 60% of the maximum uptake occurs:

algae concentration (75.000 cells per ml = 60% of uptake) = 15 ug/l chla	algae (30.000 cells per ml, = 20% of uptake) = 6 ug/l chla

Next to the fit with PASW Statistics 17.0 we also used the DEB formula to fit the data.

$$\dot{j}_W = \{\dot{j}_{Wm}\}(\delta_M L)^2 \left(\frac{Food}{X_K \left(1 + \frac{Y}{K_Y}\right) + Food} \right)$$

For this the solver of Excel was used. The programme estimates the maximum uptake and the X_K half saturation constant for feed intake. A linear curve was fit, while from a theoretical point of view, one would expect a logarithmic curve. Probably too few data points were available for a better estimation. Thus, the maximum intake was fixed at the measured value of 281301805. Now it was possible to fit a logaritmic curve. Calculation of the cell density at which 20% of the maximum uptake takes place and 60% of the maximum uptake gives the following results:

algae concentration (162.000 cells per ml = 60% of uptake) = 32.4 ug/l chla	algae concentration (27.000 cells per ml, = 20% of uptake) = 5.4 ug/l chla

The low concentration is similar to the previously estimated value (27.000 cells per ml vs 30.000 cells per ml). The high value is much higher (162.000 cells per ml vs 75.000 cells per ml). As this high value is probably above the pseudo-faeces threshold, and the value is influenced by the one fixed uptake value of 281301805 we have chosen to follow the PASW Statistics 17.0 approach.

SLIBCONCENTRATIE

For determination of the silt concentration the same approach is used. The R^2 value was 0.664 and the p value 0.001 for Batch II and III together, R^2 0.304 and p 0.449 for Batch II and R^2 0.559 and p 0.021 for Batch III. The results are presented below.

<u>Silt concentration Batch II and III together</u>

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH siltconc
  /CONSTANT
  /MODEL=EXPONENTIAL
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_4
Dependent Variable	1	celuptake
Equation	1	Exponential[a]
Independent Variable		siltconc
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

a. The model requires all non-missing values to be positive.

Case Processing Summary

	N
Total Cases	13
Excluded Cases[a]	0
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

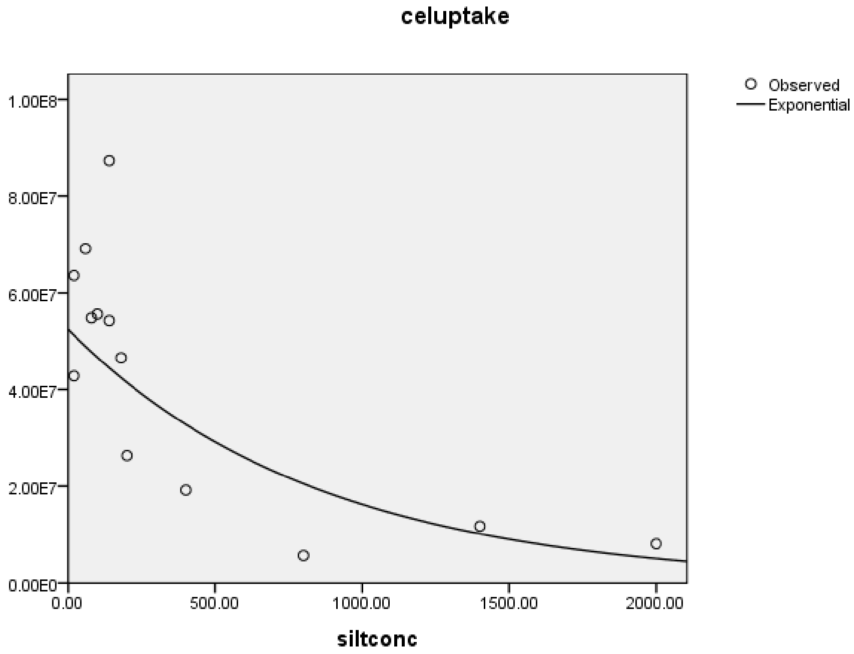
		Variables	
		Dependent	Independent
		celuptake	siltconc
Number of Positive Values		13	13
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	0	0

Model Summary and Parameter Estimates

Dependent Variable:celuptake

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Exponential	.664	21.718	1	11	.001	5.243E7	-.001

The independent variable is siltconc.



Slib concentratie Batch II

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH siltconc
  /CONSTANT
  /MODEL=EXPONENTIAL
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_5
Dependent Variable	1	celuptake
Equation	1	Exponential[a]
Independent Variable		siltconc
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

a. The model requires all non-missing values to be positive.

Case Processing Summary

	N
Total Cases	13
Excluded Cases[a]	9
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

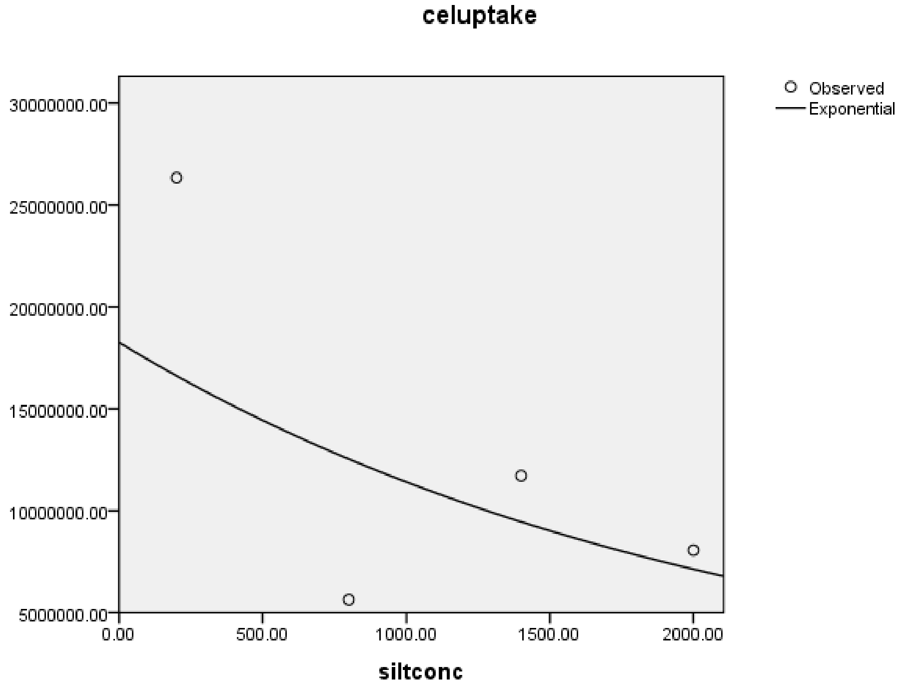
		Variables	
		Dependent	Independent
		celuptake	siltconc
Number of Positive Values		4	4
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	9	9

Model Summary and Parameter Estimates

Dependent Variable:celuptake

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Exponential	.304	.874	1	2	.449	1.826E7	.000

The independent variable is siltconc.



Silt concentration Batch III

```
* Curve Estimation.
TSET NEWVAR=NONE.
CURVEFIT
  /VARIABLES=celuptake WITH siltconc
  /CONSTANT
  /MODEL=EXPONENTIAL
  /PLOT FIT.
```

Curve Fit

[DataSet0]

Model Description

Model Name		MOD_6
Dependent Variable	1	celuptake
Equation	1	Exponential[a]
Independent Variable		siltconc
Constant		Included
Variable Whose Values Label Observations in Plots		Unspecified

a. The model requires all non-missing values to be positive.

Case Processing Summary

	N
Total Cases	13
Excluded Cases[a]	4
Forecasted Cases	0
Newly Created Cases	0

a. Cases with a missing value in any variable are excluded from the analysis.

Variable Processing Summary

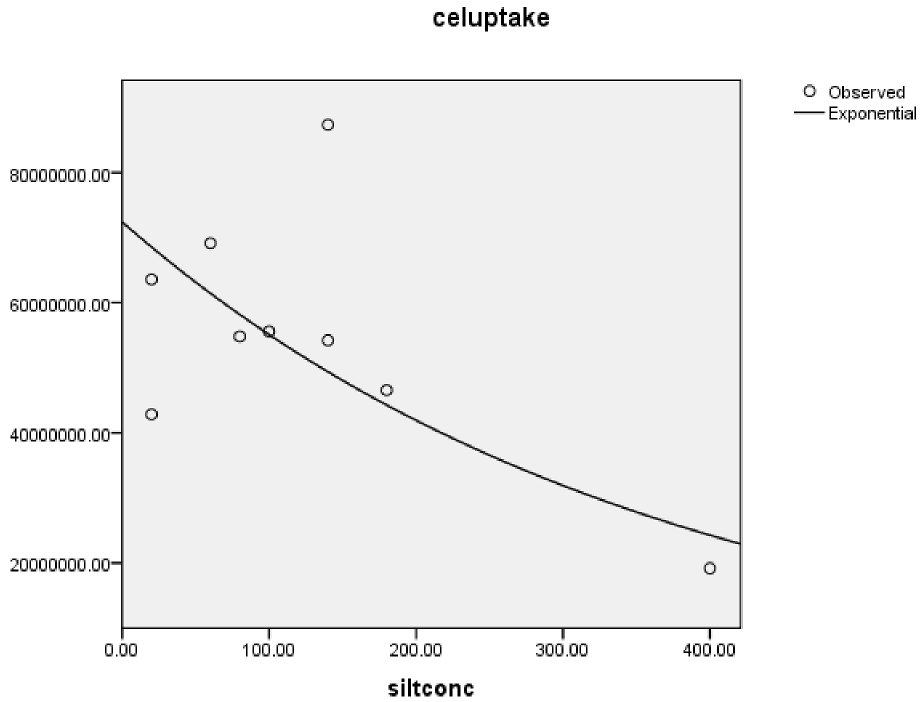
		Variables	
		Dependent	Independent
		celuptake	siltconc
Number of Positive Values		9	9
Number of Zeros		0	0
Number of Negative Values		0	0
Number of Missing Values	User-Missing	0	0
	System-Missing	4	4

Model Summary and Parameter Estimates

Dependent Variable:celuptake

	Model Summary					Parameter Estimates	
Equation	R Square	F	df1	df2	Sig.	Constant	b1
Exponential	.559	8.867	1	7	.021	7.240E7	-.003

The independent variable is siltconc.



In this case the R^2 value was highest and the p value lowest for Batch II and II together. That formula ($y=52430000*e^{-0.001x}$) was used to determine silt concentration at different percentages of maximum uptake:

silt high (300 mg/l, 74% of maximum uptake, expect will cost energy)
silt middle (150 mg/l, 86% of maximum uptake, expect some hinderance)
slib low (50 mg/l, 95% of maximum uptake, expect little hinderance)

The different treatments are as follows:

	algae concentration low 30.000 cells per ml = 6 ug/l chla	algae concentration high 75.000 cells per ml = 15 ug/l chla
silt 0 mg/l	treatment 1	treatment 5
silt 50 mg/l	treatment 2	treatment 6
silt 150 mg/l	treatment 3	treatment 7
silt 300 mg/l	treatment 4	treatment 8

Annex 2. Results set-up tests of filtration experiment

Before the start of the actual filtration experiments, the impact of the set-up on the vitality and filtration was tested. Most importantly, we focused on the reproducibility of the results by monitoring the recovery of filtration rate in relation to silt concentration.

In order to evaluate the potential effect of the duration of the experiments on the filtration rates, we additionally measured the response (i.e. filtration rates) over a >24h period in the middle size class (Fig. 2.1). The results show that despite remaining in the experimental set-up overnight under elevated silt concentrations, the *Ensis* readily recovered, and demonstrate usual patterns of filtration rate to variable silt concentrations the day after (i.e. increasing rate under lower silt and vice versa).

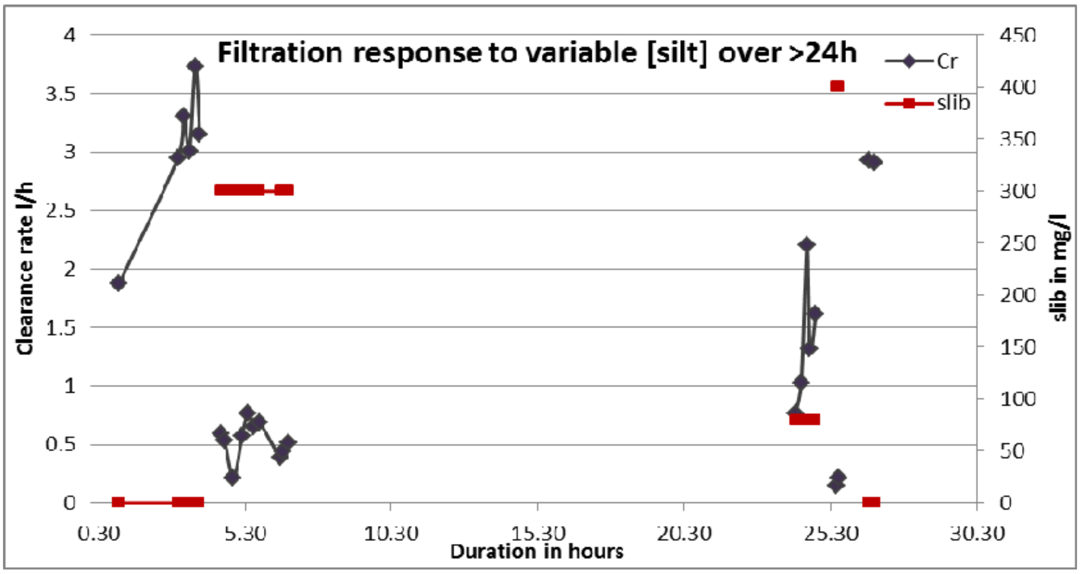


Fig. 2.1. The filtration by middle-sized Ensis over a 30 hour period.

Under natural conditions, *Ensis* experiences counter-pressure from the surrounding sediments. The absence may, under the experimental conditions applied during this study, result in an additional stress factor for particularly larger specimens. We tested the filtration rate by large *Ensis* both with and without elastic bands (Fig. 2.2). Results show that filtration rates were substantially higher when *Ensis* was bound with elastic bands (Fig. 2.2b). Therefore, we used these bands for the middle and large size class.

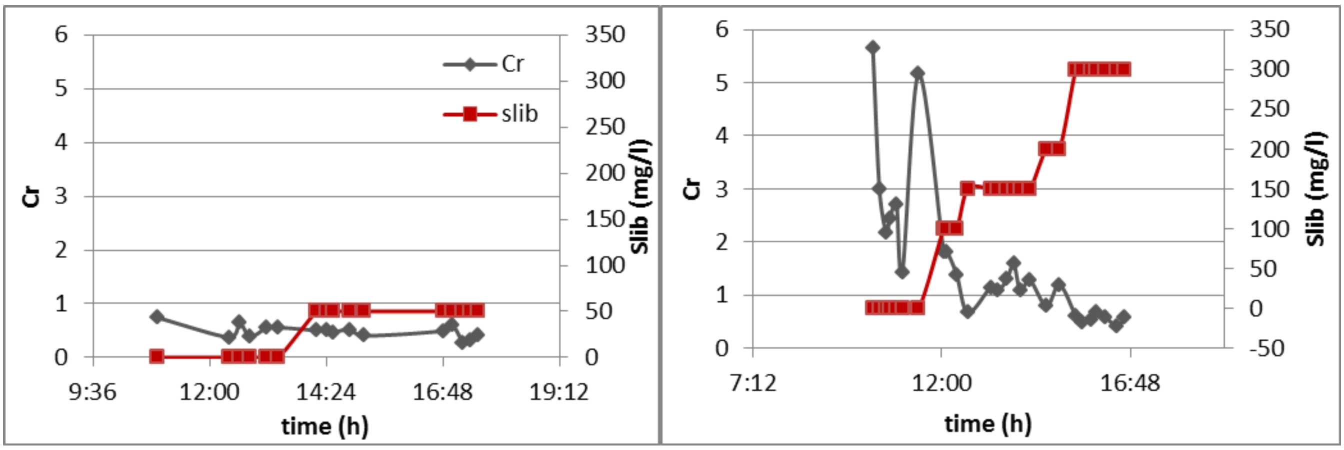


Fig. 2.2 a) Filtration rates (grey line) at different silt concentrations (red line) by large Ensis without elastic bands (a) used as counter pressure, or with elastic bands (b).

Annex 3. Temperature / mortality correlation during outdoor storage

date	temp	mortality	date	temp	mortality	date	temp	mortality
5/30/2011	17.13178		7/6/2011	20.12179		8/12/2011	18.51246	
5/31/2011	16.5596	1	7/7/2011	19.67744	34	8/13/2011	18.53373	
6/1/2011	16.49498		7/8/2011	19.15608	38	8/14/2011	18.48131	
6/2/2011	17.14667		7/9/2011	19.2264	27	8/15/2011	18.5264	
6/3/2011	17.94954		7/10/2011	19.42279	30	8/16/2011	19.5366	
6/4/2011	18.65781		7/11/2011	19.67181				
6/5/2011	18.24096		7/12/2011	19.66213				
6/6/2011	17.84419	3	7/13/2011	18.09881				
6/7/2011	17.80994		7/14/2011	16.65894				
6/8/2011	17.5949		7/15/2011	17.87985				
6/9/2011	17.54033	1	7/16/2011	18.38223				
6/10/2011	17.62954		7/17/2011	18.06948	10			
6/11/2011	16.94571		7/18/2011	17.51856				
6/12/2011	18.02369	5	7/19/2011	17.69742	7			
6/13/2011	17.2945		7/20/2011	18.14383				
6/14/2011	17.92179	1	7/21/2011	18.24146	72			
6/15/2011	18.29206	4	7/22/2011	18.32177				
6/16/2011	17.97498	1	7/23/2011	17.65885				
6/17/2011	16.86783		7/24/2011	17.17025				
6/18/2011	16.52956	12	7/25/2011	17.54638	172			
6/19/2011	16.52794		7/26/2011	17.43446				
6/20/2011	16.30175		7/27/2011	17.62077				
6/21/2011	17.58258	3	7/28/2011	18.05421	5			
6/22/2011	17.08242		7/29/2011	17.98733				
6/23/2011	16.99631		7/30/2011	17.19115				
6/24/2011	17.34013	5	7/31/2011	17.418	4			
6/25/2011	16.40838		8/1/2011	18.21327				
6/26/2011	17.28498		8/2/2011	19.08369				
6/27/2011	18.3241		8/3/2011	19.39221				
6/28/2011	19.10765	2	8/4/2011	19.60858				
6/29/2011	19.54946	3	8/5/2011	19.34017				
6/30/2011	19.11435	1	8/6/2011	18.93777				
7/1/2011	18.78467		8/7/2011	16.49694				
7/2/2011	17.99667		8/8/2011	17.03021	3			
7/3/2011	18.28015		8/9/2011	17.97638				
7/4/2011	18.55415	2	8/10/2011	18.04975				
7/5/2011	19.6399		8/11/2011	18.2911				

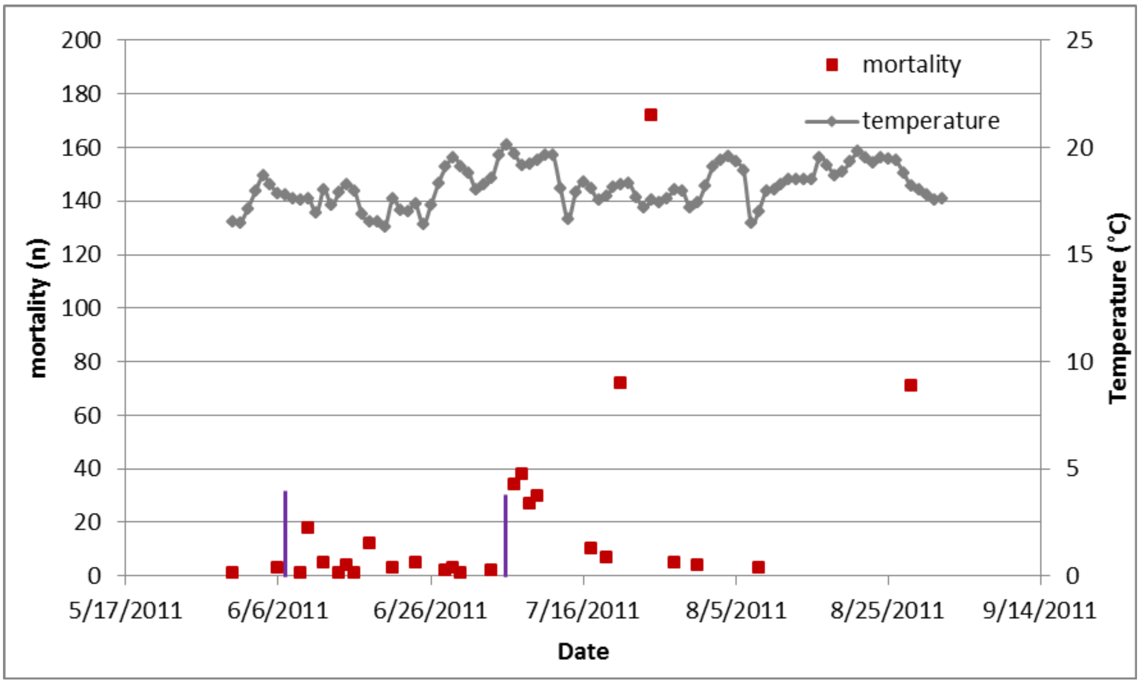


Fig. 3.1. Mortality plotted with the temperature measured in the outdoor basins from the 30th of May until the 1st of August. Purple lines indicate collection of large Ensis (8th of June), and a new batch of Ensis collected using a box corer (6th of July). The high values on the 21th and 25th of July and 28th of August were observed when the basins were cleaned and dead individuals that had jumped out of the buckets were found.

Annex 4. Water quality parameters

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
8/10/2011	1	0.25	0	7.79	7.91	18.1	34
8/10/2011	2	0.25	0	7.99	8.22	17.9	34
8/10/2011	3	0.25	0	8.06	8.26	17.7	34
8/10/2011	4	0.25	0	8.05	8.27	17.7	34
8/10/2011	5	0.25	0	8.04	8.28	17.6	34
8/10/2011	6	0.25	0	8.02	8.27	17.5	34
8/11/2011	1	0.25	0	7.74	7.91	18.7	35
8/11/2011	2	0.25	0	7.96	8	17.9	34
8/11/2011	3	0.25	0	8.03	8.25	17.6	34
8/11/2011	4	0.25	0	8.01	8.28	17.6	34
8/11/2011	5	0.25	0	8.01	8.28	17.5	34
8/11/2011	6	0.25	0	8.01	8.27	17.5	34
8/12/2011	1	0.25	0	7.87	8.24	18.3	34
8/12/2011	2	0.25	0	7.92	8.26	18.1	34
8/12/2011	3	0.25	0	7.95	8.28	18.1	34
8/12/2011	4	0.25	0	7.94	8.33	18.1	34
8/12/2011	5	0.25	0	7.92	8.35	18.1	34
8/12/2011	6	0.25	0	7.98	8.31	18.1	34
8/13/2011	1	0.25	0.2	99.70%	8.14	18.5	35
8/13/2011	2	0.25	0.2	99.40%	8.28	18.4	35
8/13/2011	3	0.25	0.2	99.90%	8.34	18.4	35
8/13/2011	4	0.25	0.2	99.60%	8.34	18.3	35
8/13/2011	5	0.25	0.2	99.30%	8.34	18.3	35
8/13/2011	6	0.25	0.2	99.30%	8.34	18.3	35
8/14/2011	1	0.25	0.2	7.79	8.04	18.9	34
8/14/2011	2	0.25	0.2	7.85	8.29	18.6	34
8/14/2011	3	0.25	0.2	7.92	8.29	18.6	34
8/14/2011	4	0.25	0.2	7.91	8.36	18.5	34
8/14/2011	5	0.25	0.2	7.89	8.37	18.4	34
8/14/2011	6	0.25	0.2	7.88	8.33	18.3	34
8/15/2011	1	0.25	0	7.89	8.14	18.8	34
8/15/2011	2	0.25	0	7.93	8.27	18.5	34
8/15/2011	3	0.25	0	7.98	8.33	18.4	34
8/15/2011	4	0.25	0	7.99	8.34	18.2	34
8/15/2011	5	0.25	0	7.97	8.34	18.2	34
8/15/2011	6	0.25	0	7.96	8.32	18.2	34
8/16/2011	1	0.25	0.25	7.75	7.93	19.3	32
8/16/2011	2	0.25	0.75	7.87	8.18	18.8	33
8/16/2011	3	0.25	0.75	7.94	8.22	18.4	33
8/16/2011	4	0.25	0.75	7.97	8.14	18.2	33
8/16/2011	5	0.25	0.25	7.99	7.94	18.2	33
8/16/2011	6	0.25	0.25	8	8.21	18	33
8/17/2011	1	0.25	0.25	7.75	8.07	19.1	32
8/17/2011	2	0.25	0.75	7.85	8.18	19.8	33
8/17/2011	3	0.25	0.75	7.9	8.22	18.6	32
8/17/2011	4	0.25	0.75	7.95	8.28	18.4	33
8/17/2011	5	0.25	0.25	7.95	8.29	18.3	33
8/17/2011	6	0.25	0.75	7.95	8.3	18.3	32
8/18/2011	1	0.25	0.25	7.95	8.05	18.6	33
8/18/2011	2	0.25	0.75	7.98	8.17	18.5	32
8/18/2011	3	0.25	0.75	7.95	7.91	17.1	33
8/18/2011	4	0.25	0.25	7.99	8.09	17	33
8/18/2011	5	0.25	0.25	8.3	8.2	17.8	33
8/18/2011	6	0.25	0.75	8.04	8.09	17.3	32
8/19/2011	1	0.25	0.25	7.97	7.82	19.5	33
8/19/2011	2	0.25	0.75	8.02	8.25	18.6	33
8/19/2011	3	**0.25**	**0.75**	7.98	8.3	18.6	33
8/19/2011	4	0.25	0.25	8.02	8.33	18.4	33

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
8/19/2011	5	0.25	0.25	8.01	8.34	18.4	33
8/19/2011	6	0.25	0.25	8	8.34	18.4	33
8/20/2011	1	0.25	0.25	7.91	8.02	18.9	32
8/20/2011	2	0.25	0.25	8.01	8.32	18.7	32
8/20/2011	3	0.25	0.25	8.05	8.35	18.3	32
8/20/2011	4	0.25	0.25	8.08	8.4	18.1	32
8/20/2011	5	0.25	0.25	8.06	8.41	18.1	32
8/20/2011	6	0.25	0.25	8.04	8.4	18	32
8/21/2011	1	0.25	0.25	7.84	7.75	18.9	33
8/21/2011	2	0.25	0.25	8.00	8.22	18.6	32
8/21/2011	3	0.25	0.25	8.00	8.3	18.4	32
8/21/2011	4	0.25	0.25	8.03	8.36	18.4	32
8/21/2011	5	0.25	0.25	7.99	8.39	18.4	32
8/21/2011	6	0.25	0.25	8.00	8.38	18.2	32
8/22/2011	1	0.25	0.25	7.89	8.1	19	33
8/22/2011	2	0.25	0.25	8.05	8.29	18.7	33
8/22/2011	3	0.25	0.75	8.02	8.32	18.5	33
8/22/2011	4	0.25	0.25	8.05	8.4	18.4	32
8/22/2011	5	0.25	0.25	8.02	8.41	18.3	33
8/22/2011	6	0.25	0.25	8.03	8.41	18.3	33
8/23/2011	1	0.25	0.25	7.75	8.08	19.4	33
8/23/2011	2	0.25	0.75	7.85	8.26	19.2	32
8/23/2011	3	0.25	0.75	7.89	8.33	18.9	32
8/23/2011	4	0.25	0.75	7.52	8.37	18.7	32
8/23/2011	5	0.25	0.25	7.92	8.4	18.6	32
8/23/2011	6	0.25	0.25	7.91	8.41	18.5	32
8/24/2011	1	0.25	0.25	7.84	7.97	19.2	35
8/24/2011	2	0.25	0.75	7.96	8.21	18.9	34
8/24/2011	3	0.25	0.75	7.96	8.29	18.8	34
8/24/2011	4	0.25	0.25	7.99	8.33	18.6	33
8/24/2011	5	0.25	0.25	7.97	8.35	18.5	33
8/24/2011	6	0.25	0.25	7.97	8.35	18.4	33
8/25/2011	1	0.25	0.25	7.97	7.95	19.3	32
8/25/2011	2	0.25	0.75	7.88	8.15	19.1	32
8/25/2011	3	0.25	0.75	7.92	8.22	18.8	33
8/25/2011	4	0.25	0.25	7.95	8.28	18.6	32
8/25/2011	5	0.25	0.25	7.95	8.32	18.5	32
8/25/2011	6	0.25	0.25	7.96	8.33	18.4	33
8/26/2011	1	0.25	0.5	7.77	8.07	18.8	33
8/26/2011	2	**0.5**	**1**	7.84	8.18	19.1	31
8/26/2011	3	**0.5**	**1**	7.83	8.22	19	31
8/26/2011	4	0.25	0.75	7.89	8.31	18.5	32
8/26/2011	5	0.25	0.75	7.89	8.34	18.5	32
8/26/2011	6	0.25	0.75	7.85	8.32	18.5	32
8/27/2011	1	0.25	0	7.94	8.22	18.7	32
8/27/2011	2	0.25	0.25	8.04	8.37	18.7	32
8/27/2011	3	0.25	0.5	8.05	8.39	18.5	32
8/27/2011	4	0.25	0.5	7.99	8.37	18.3	32
8/27/2011	5	0.25	0	8.01	8.41	18.3	32
8/27/2011	6	0.25	0	7.99	8.39	18.2	32
8/28/2011	1	0.25	0.25	7.91	7.99	18.6	32
8/28/2011	2	0.25	0.25	8.04	8.23	18.6	32
8/28/2011	3	0.25	0.5	8.07	8.31	18.3	32
8/28/2011	4	0.25	0.5	8.02	8.28	18.2	32
8/28/2011	5	0.25	0.25	8.04	8.35	18.1	32
8/28/2011	6	0.25	0.25	8.04	8.35	18	32
8/29/2011	1	**0.8**	0.5	7.97	7.92	18.4	32
8/29/2011	2	0.25	0	8.09	8.14	18.4	32
8/29/2011	3	0.25	0.5	8.09	8.21	18.1	32
8/29/2011	4	**0.7**	**1.5**	8.04	8.22	17.9	32
8/29/2011	5	0.25	0.5	8.04	8.28	17.8	33
8/29/2011	6	0.25	0.75	8.08	8.27	17.7	32
8/30/2011	1	**1.5**	**0.75**	7.96	8.09	18.2	32

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
8/30/2011	2	**0.5**	**0.25**	8.11	8.31	18.3	33
8/30/2011	3	**0.5**	**0.75**	8.13	8.3	18.1	33
8/30/2011	4	**1.5**	**1.5**	8.08	8.3	18.5	32
8/30/2011	5	**0.25**	**1**	8.07	8.33	17.9	33
8/30/2011	6	**1.6**	**0.5**	8.06	8.3	17.8	32
8/31/2011	1	0.25	-	7.90	7.95	18.6	32
8/31/2011	2	0.25	-	8.04	8.2	18.5	32
8/31/2011	3	0.25	-	8.09	8.24	18.3	32
8/31/2011	4	0.25	-	8.04	8.25	18.1	32
8/31/2011	5	0	-	8.03	8.29	18	33
8/31/2011	6	0.25	-	8.00	8.27	18	33
9/1/2011	1	**0.5**	-	7.95	8	18.5	33
9/1/2011	2	**0.25**	-	8.10	8.21	18.3	32
9/1/2011	3	**0.4**	-	8.07	8.24	18.2	33
9/1/2011	4	**0.8**	-	8.02	8.27	18.2	32
9/1/2011	5	**0.25**	-	8.00	8.29	18.1	33
9/1/2011	6	**0.8**	-	8.00	8.21	18.1	32
9/2/2011	1	0.25	0.25	7.92	7.98	18.4	33
9/2/2011	2	0.25	0.25	7.99	8.21	18.4	34
9/2/2011	3	0.25	0.25	8.00	8.24	18.3	33
9/2/2011	4	0.25	0.25	7.98	8.25	18.2	33
9/2/2011	5	0.25	0.25	7.96	8.28	18.2	33
9/2/2011	6	0.25	0.25	7.95	8.27	18.1	33
9/3/2011	1	0.25	0.25	7.90	8.24	18.8	33
9/3/2011	2	0.25	0.25	7.92	8.24	18.8	33
9/3/2011	3	0.25	0.25	7.87	8.21	19	33
9/3/2011	4	0.25	0.25	7.82	8.15	19	33
9/3/2011	5	0.25	0.25	7.74	8.11	19.2	33
9/3/2011	6	0.25	0.25	7.31	7.76	20	33
9/4/2011	1	0.25	0.25	7.88	8.32	18.8	33
9/4/2011	2	0.25	0.25	7.90	8.32	18.8	33
9/4/2011	3	0.25	0.4	7.85	8.28	18.9	33
9/4/2011	4	0.25	0.4	7.77	8.21	19	33
9/4/2011	5	0.25	0.4	7.70	8.13	19.1	33
9/4/2011	6	0.25	0.4	7.54	7.85	19.8	33
9/5/2011	1	0.25	0.25	7.91	7.98	18.8	33
9/5/2011	2	0.25	0.25	8.09	8.2	18.6	33
9/5/2011	3	0.25	0.25	8.03	8.25	18.6	33
9/5/2011	4	**0.8**	0.25	7.97	8.26	18.4	33
9/5/2011	5	0.25	0.25	7.93	8.28	18.5	33
9/5/2011	6	**1.6**	0.25	7.89	8.26	18.5	33
9/6/2011	1	0.25	0.25	8.00	8.02	18.5	33
9/6/2011	2	0.25	0.25	8.05	8.19	18.5	33
9/6/2011	3	0.25	0.25	8.04	8.25	18.4	32
9/6/2011	4	0.4	0.25	8.00	8.23	18.3	33
9/6/2011	5	0.25	0.25	7.93	8.23	18.3	33
9/6/2011	6	0.25	0.25	7.86	8.21	18.4	32
9/7/2011	1	0.25	0.25	7.97	8.06	18.6	33
9/7/2011	2	0.25	0.25	8.05	8.2	18.5	32
9/7/2011	3	0.25	0.25	8.05	8.2	18.5	33
9/7/2011	4	**0.4**	0.25	7.89	8.19	18.8	33
9/7/2011	5	0.25	0.4	7.88	8.25	18.4	33
9/7/2011	6	**0.5**	0.4	7.74	8.17	19	34
9/8/2011	1	0.25	0.25	7.94	8.2	18.6	34
9/8/2011	2	0.25	0.25	8.07	8.34	18.4	34
9/8/2011	3	0.25	0.25	8.04	8.37	18.3	34
9/8/2011	4	0.25	0.25	8.01	8.35	18.1	34
9/8/2011	5	0.25	0.25	7.98	8.34	18.1	33
9/8/2011	6	0.25	0.25	7.98	8.34	18.2	33
9/9/2011	1	0.25	0.25	7.80	7.55	19.6	33
9/9/2011	2	0.25	0.25	7.86	8.14	19.3	33
9/9/2011	3	0.25	0.25	7.90	8.19	19.2	33
9/9/2011	4	0.3	0.25	7.87	8.19	19.1	33

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
9/9/2011	5	0.4	0.25	7.82	8.2	19.1	33
9/9/2011	6	0.25	0.25	7.82	8.2	19.1	33
9/10/2011	1	0.25	0.25	7.93	8.2	19.1	32
9/10/2011	2	0.25	0.25	7.83	8.35	18.7	32
9/10/2011	3	0.25	0.25	7.90	8.4	18.7	32
9/10/2011	4	0.25	0.25	7.78	8.34	18.7	32
9/10/2011	5	0.25	0.25	7.75	8.31	18.8	32
9/10/2011	6	0.25	0.25	7.80	8.26	19	32
9/11/2011	1	0.25	0	7.98	8.36	18.8	32
9/11/2011	2	0.25	0	7.99	8.34	18.7	32
9/11/2011	3	0.25	0	7.94	8.29	18.9	32
9/11/2011	4	0.25	0	7.91	8.23	18.9	32
9/11/2011	5	0.25	0	7.86	8.14	18.9	32
9/11/2011	6	0.25	0	7.84	7.94	19	32
9/12/2011	1	0.25	0.25	7.81	7.99	19.4	32
9/12/2011	2	0.25	0.25	7.91	8.15	19.1	32
9/12/2011	3	0.25	0.25	7.92	8.21	19.1	32
9/12/2011	4	0.25	0.25	7.91	8.25	18.9	32
9/12/2011	5	0.25	0.25	7.90	8.26	18.9	32
9/12/2011	6	0.25	0.25	7.87	8.25	18.9	32
9/13/2011	1	0.25	0	7.90	8.21	19	32
9/13/2011	2	0.25	0	7.85	8.32	18.9	32
9/13/2011	3	0.25	0	7.86	8.38	18.8	32
9/13/2011	4	0.25	0	7.81	8.4	18.8	32
9/13/2011	5	0.25	0	7.76	8.35	18.8	32
9/13/2011	6	0.25	0	7.79	8.27	18.9	32
9/14/2011	1	0.25	0	7.89	7.88	18.7	33
9/14/2011	2	0.25	0	8.14	8.17	18.4	33
9/14/2011	3	0.25	0	8.16	8.23	18.2	33
9/14/2011	4	0.25	0	8.15	8.26	18	33
9/14/2011	5	0.25	0	8.19	8.28	18	33
9/14/2011	6	0.25	0	8.09	8.27	18	33
9/15/2011	1	0.25	0	8.06	7.96	18.6	33
9/15/2011	2	0.25	0	8.16	8.13	18.4	33
9/15/2011	3	0.25	0	8.18	8.19	18.3	33
9/15/2011	4	0.25	0	8.17	8.22	18.1	33
9/15/2011	5	0.25	0	8.16	8.25	18	33
9/15/2011	6	0.25	0	8.12	8.24	18	33
9/16/2011	1	0.25	0	7.89	8.04	19	32
9/16/2011	2	0.25	0	8.00	8.2	18.9	32
9/16/2011	3	0	0	8.02	8.25	18.7	32
9/16/2011	4	0.25	0	8.03	8.28	18.6	32
9/16/2011	5	0.25	0	8.01	8.3	18.5	32
9/16/2011	6	0.25	0	8.00	8.29	18.5	32
9/17/2011	1	0	0.25	8.10	8.19	18.1	32
9/17/2011	2	0	0	8.05	8.36	18.3	32
9/17/2011	3	0	0.25	8.09	8.36	18.4	32
9/17/2011	4	0	0.25	8.06	8.32	18.4	32
9/17/2011	5	0	0.25	8.02	8.26	18.4	32
9/17/2011	6	0	0	7.96	8.19	18.5	32
9/18/2011	1	0	0	8.04	8.2	18	33
9/18/2011	2	0.25	0	8.09	8.2	18.1	33
9/18/2011	3	0	0	8.07	8.2	18.1	33
9/18/2011	4	0.25	0	8.03	8.2	18	33
9/18/2011	5	0.25	0	7.99	8.2	18.1	33
9/18/2011	6	0.25	0	7.90	8.2	18.3	33
9/19/2011	1	0.25	0	7.98	8.09	18.7	33
9/19/2011	2	0	0	8.12	8.27	18.5	33
9/19/2011	3	0.25	0	8.13	8.31	18.3	33
9/19/2011	4	0	0	8.12	8.32	18.1	33
9/19/2011	5	0.25	0	8.09	8.32	18.1	33
9/19/2011	6	0.25	0	8.05	8.31	18	33
9/20/2011	1	0.25	0	7.82	7.95	19.3	32

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
9/20/2011	2	0	0	8.09	8.19	18.6	33
9/20/2011	3	0.25	0	8.11	8.23	18.5	32
9/20/2011	4	0	0	8.11	8.27	18.3	33
9/20/2011	5	0.25	0	8.09	8.29	18.2	33
9/20/2011	6	0.25	0	8.06	8.27	18.1	33
9/21/2011	1	0.25	0	8.02	7.88	18.6	33
9/21/2011	2	0	0	8.18	8.17	18.3	33
9/21/2011	3	0.25	0	8.18	8.23	18.2	33
9/21/2011	4	0	0	8.15	8.27	18.1	33
9/21/2011	5	0.25	0	8.12	8.28	18	33
9/21/2011	6	0.25	0	8.07	8.26	18	33
9/22/2011	1	0.25	0	7.88	8.03	19.1	33
9/22/2011	2	0	0	8.12	8.21	18.7	33
9/22/2011	3	0.25	0	8.13	8.26	18.4	34
9/22/2011	4	0	0	8.12	8.28	18.2	33
9/22/2011	5	0.25	0	8.12	8.3	18.1	34
9/22/2011	6	0.25	0	8.06	8.27	18.1	33
9/23/2011	1	0	0	7.99	8.03	18.8	32
9/23/2011	2	0	0	8.16	8.17	18.5	32
9/23/2011	3	0	0	8.13	8.22	18.4	32
9/23/2011	4	0	0	8.10	8.24	18.3	32
9/23/2011	5	0	0	8.10	8.26	18.2	32
9/23/2011	6	0	0	8.07	8.25	18.2	32
9/24/2011	1	0	0	8.12	8.33	18.2	34
9/24/2011	2	0	0	8.16	8.31	18.3	33
9/24/2011	3	0	0	8.10	8.28	18.3	32
9/24/2011	4	0	0	8.05	8.25	18.3	33
9/24/2011	5	0.25	0	8.00	8.19	18.4	33
9/24/2011	6	0.25	0	7.81	7.99	18.9	33
9/25/2011	1	0.25	0	8.12	8.3	18.3	34
9/25/2011	2	0	0	8.14	8.29	18.4	33
9/25/2011	3	0.25	0	8.10	8.27	18.4	32
9/25/2011	4	0	0	8.01	8.21	18.5	33
9/25/2011	5	0.25	0	7.92	8.15	18.8	33
9/25/2011	6	0.25	0	7.59	7.87	18.6	33
9/26/2011	1	0	0	8.14	8.32	18.5	31
9/26/2011	2	0	0	8.13	8.33	18.5	31
9/26/2011	3	0	0	8.08	8.28	18.6	31
9/26/2011	4	0	0	8.04	8.25	18.7	31
9/26/2011	5	0	0	7.97	8.21	18.9	31
9/26/2011	6	0	0	7.72	8.15	19.5	31
9/27/2011	1	0	0	8.13	8.24	18.7	32
9/27/2011	2	0	0	8.25	8.35	18.5	32
9/27/2011	3	0.25	0	8.23	8.35	18.6	32
9/27/2011	4	0	0	8.21	8.35	18.5	32
9/27/2011	5	0.25	0	8.19	8.36	18.5	32
9/27/2011	6	0.25	0	8.17	8.35	18.4	32
9/28/2011	1	<0.3	0	7.88	8.8	19.5	33
9/28/2011	2	<0.3	0	8.09	8.31	18.9	33
9/28/2011	3	0.3	0	8.12	8.34	18.9	33
9/28/2011	4	0	0	8.12	8.34	18.7	33
9/28/2011	5	0.3	0	8.11	8.36	18.7	33
9/28/2011	6	0.3	0	8.08	8.34	18.6	33
9/29/2011	1	<0.3	0	7.95	8.17	19.4	33
9/29/2011	2	<0.3	0	8.07	8.23	19.1	33
9/29/2011	3	0.3	0	8.11	8.27	19	33
9/29/2011	4	0	0	8.11	8.3	18.8	33
9/29/2011	5	0.3	0	8.1	8.32	18.8	33
9/29/2011	6	0.3	0	8.05	8.3	18.8	33
9/30/2011	1	<0.3	0	8.07	8.27	10.1	30
9/30/2011	2	<0.3	0	8.07	8.27	19.2	30
9/30/2011	3	0.3	0	8.04	8.24	19.3	30
9/30/2011	4	0	0	7.98	8.19	19.4	30

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
9/30/2011	5	0.3	0	7.9	8.13	19.5	30
9/30/2011	6	0.3	0	7.59	7.93	20.3	30
10/1/2011	1	0.3	0	7.60	7.98	20.3	30
10/1/2011	2	0.3	0	8.08	8.22	19	30
10/1/2011	3	0.3	0	0.08	8.3	18.9	30
10/1/2011	4	0.3	0	0.08	8.3	18.8	30
10/1/2011	5	0.3	0	0.08	8.33	18.7	30
10/1/2011	6	0.3	0	0.08	8.32	18.7	30
10/2/2011	1	0.3	0	7.73	8.07	19.9	30
10/2/2011	2	0.3	0	8.07	8.27	19	30
10/2/2011	3	0.3	0	8.09	8.3	18.9	30
10/2/2011	4	0.3	0	8.09	8.29	18.8	30
10/2/2011	5	0.3	0	8.09	8.33	18.7	30
10/2/2011	6	0.3	0	8.04	8.31	18.7	30
10/3/2011	1	0.3	0	8	8	18.9	32
10/3/2011	2	0.3	0	8.08		18.7	32
10/3/2011	3	0.3	0	8.12		18.5	32
10/3/2011	4	0.3	0	8.12		18.5	32
10/3/2011	5	0.3	0	8.14		18.4	32
10/3/2011	6	0.3	0	8.11		18.3	32
10/4/2011	1	<0.3	0	7.96	8.11	19.1	32
10/4/2011	2	<0.3	0	8.08	8.24	18.7	32
10/4/2011	3	<0.3	0	8.11	8.29	18.6	32
10/4/2011	4	<0.3	0	8.11	8.31	18.5	32
10/4/2011	5	0.3	0.1	8.1	8.31	18.4	32
10/4/2011	6	<0.3	0	8.08	8.32	18.4	32
10/5/2011	1	0.3	0	7.87	8.17	19.2	32
10/5/2011	2	0.3	0	8.02	8.25	18.8	32
10/5/2011	3	0.3	0	8.08	8.31	18.7	32
10/5/2011	4	0.3	0	8.08	8.32	18.5	32
10/5/2011	5	0.5	0.1	8.03	8.28	19	32
10/5/2011	6	0.3	0	7.99	8.33	18.5	32
10/6/2011	1	0.3	0	8.09	8.35	18.2	34
10/6/2011	2	0.3	0	8.05	8.31	18.4	32
10/6/2011	3	0.3	0	8.09	8.34	18.3	32
10/6/2011	4	0.3	0	8.06	8.32	18.2	33
10/6/2011	5	0.5	0.25	8.05	8.33	18.1	33
10/6/2011	6	0.3	0	8.12	8.35	18.1	32
10/7/2011	1	0.3	0	8.10	8.19	18.2	33
10/7/2011	2	0.3	0	8.15	8.14	18.2	33
10/7/2011	3	0.3	0	8.17	8.28	18	33
10/7/2011	4	0.3	0	8.16	8.3	17.9	33
10/7/2011	5	0.5	0.2	8.16	8.32	17.8	33
10/7/2011	6	0.3	0	8.19	8.34	17.7	33
10/8/2011	1	0.3	0	8.25	8.2	17.7	34
10/8/2011	2	0.3	0	8.28	8.32	17.8	34
10/8/2011	3	0.3	0	8.27	8.34	17.7	34
10/8/2011	4	0.3	0	8.26	8.35	17.5	34
10/8/2011	5	>0.3	0	8.23	8.36	17.5	34
10/8/2011	6	0.3	0	8.27	8.39	17.5	34
10/9/2011	1	0.3	0	8.27	8.13	17.7	34
10/9/2011	2	0.3	0	8.30	8.29	17.7	34
10/9/2011	3	0.3	0	8.30	8.31	17.7	34
10/9/2011	4	0.3	0	8.27	8.31	17.5	34
10/9/2011	5	>0.3	0	8.25	8.34	17.5	34
10/9/2011	6	0.3	0	8.27	8.36	17.5	34
10/10/2011	1	0	0	7.97	8.06	18.5	33
10/10/2011	2	0	0	8.19	8.19	18	33
10/10/2011	3	0	0	8.20	8.29	18	33
10/10/2011	4	0	0	8.19	8.31	17.9	33
10/10/2011	5	0.3	0	8.24	8.32	17.7	33
10/10/2011	6	0.3	0	8.23	8.36	17.8	33
10/11/2011	1	0	0	8.14	8.18	18.4	34

datum	tank	NO2 (mg/l)	NH4 (mg/l)	O2 (mg/l)	pH	temp (0C)	sal (psu)
10/11/2011	2	0	0	8.18	8.25	18.2	34
10/11/2011	3	0	0	8.19	8.28	18.3	34
10/11/2011	4	0	0	8.15	8.27	18.2	34
10/11/2011	5	0.3	0	8.14	8.29	18.2	34
10/11/2011	6	0.3	0	8.16	8.3	18.1	34
10/12/2011	1	0.3	0	7.95	8	19	32
10/12/2011	2	<0.3	0	8.07	8.2	18.7	32
10/12/2011	3	0.3	0	8.11	8.25	18.6	32
10/12/2011	4	0.3	0	8.09	8.27	18.4	32
10/12/2011	5	0.4	0.1	8.09	8.31	18.3	32
10/12/2011	6	0.3	0	8.13	8.31	18.3	32
10/13/2011	1	0	0	8.29	8.13	18.1	33
10/13/2011	2	0	0	8.32	8.28	18.1	33
10/13/2011	3	0	0	8.30	8.3	18.1	33
10/13/2011	4	0	0	8.25	8.3	18	33
10/13/2011	5	0.3	0	8.25	8.33	18	33
10/13/2011	6	0.3	0	8.29	8.33	18	33
10/14/2011	1	0	0	8.44	8.08	17.5	33
10/14/2011	2	0	0	8.43	8.2	17.8	33
10/14/2011	3	0	0	8.42	8.24	17.7	33
10/14/2011	4	0	0	8.38	8.24	17.5	33
10/14/2011	5	0.3	0	8.37	8.25	17.5	33
10/14/2011	6	0.3	0	8.40	8.27	17.4	33
10/15/2011	1	0	0	8.42	8.28	17.3	33
10/15/2011	2	0	0	8.45	8.26	17.3	33
10/15/2011	3	0	0	8.45	8.24	17.3	33
10/15/2011	4	0	0	8.49	8.19	17.2	33
10/15/2011	5	0.3	0	8.46	8.14	17.1	33
10/15/2011	6	0	0	8.53	8.02	17	33
10/16/2011	1	0	0	8.40	8.26	17.1	35
10/16/2011	2	0	0	8.46	8.22	17.2	35
10/16/2011	3	0	0	8.46	8.23	16.9	35
10/16/2011	4	0	0	8.45	8.18	16.8	35
10/16/2011	5	0.3	0	8.50	8.14	16.7	35
10/16/2011	6	0.3	0	8.64	8.03	16.4	35
10/17/2011	1	0	0	8.10	8.04	18	35
10/17/2011	2	0	0	8.22	8.18	17.9	35
10/17/2011	3	0	0	8.24	8.23	17.7	35
10/17/2011	4	0	0	8.21	8.23	17.9	35
10/17/2011	5	0.3	0	8.20	8.25	17.9	35
10/17/2011	6	0.3	0	8.24	8.26	17.5	35

Annex 5. Silt concentration and phytoplankton cell concentration during the growth experiment

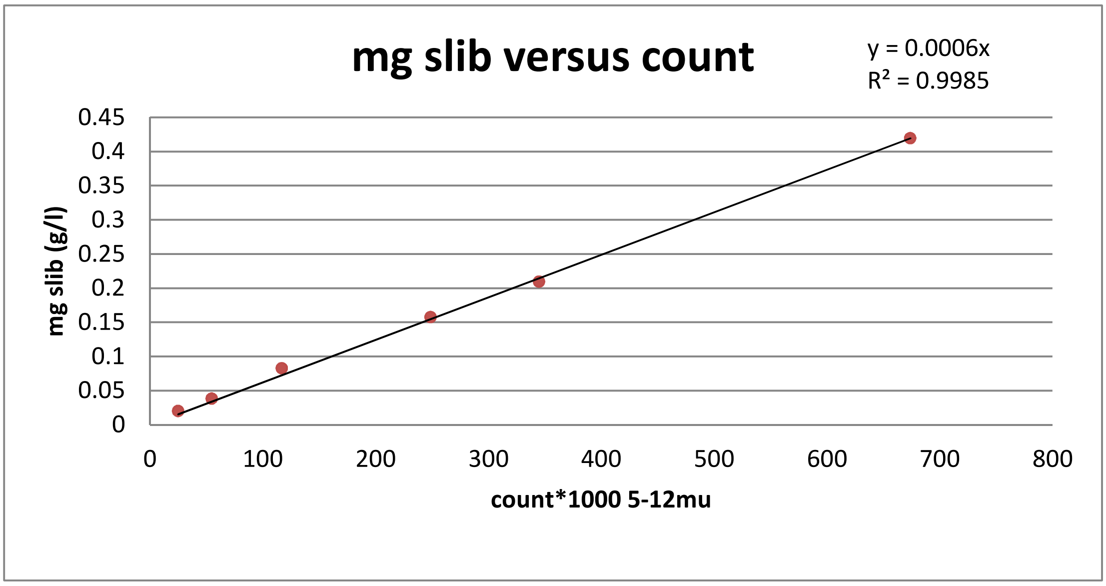


Fig. 5.1. Relation between coulter counter counts and actual silt concentration.

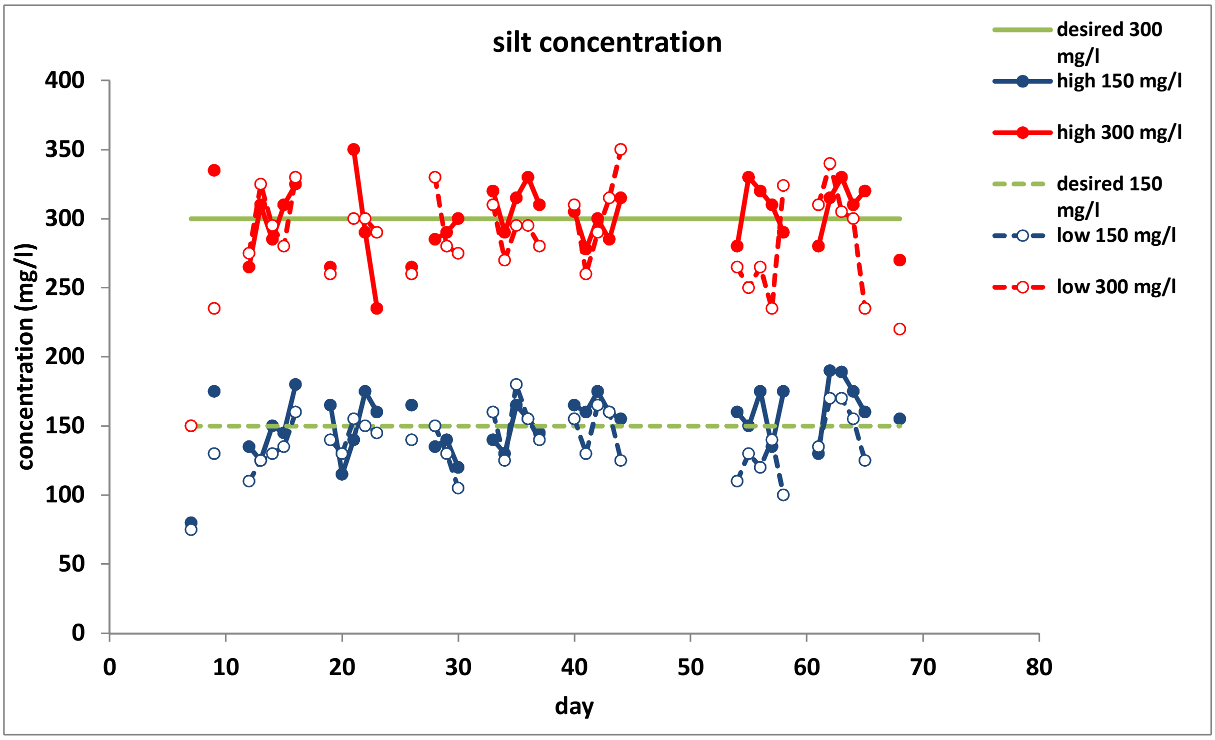


Fig. 5.2. The silt concentration in the tanks during the growth experiment.

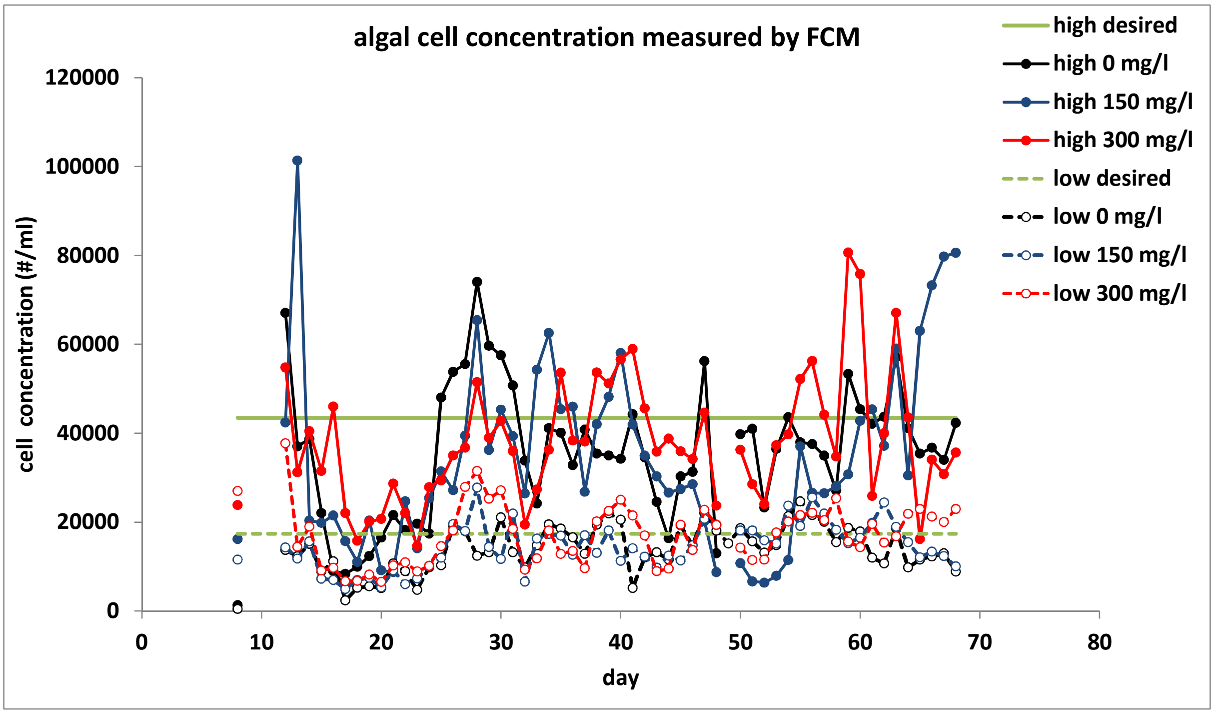


Fig. 5.3. The algal cell concentration in the tanks during the growth experiment.

Annex 6. Survival during growth experiment in the various tanks

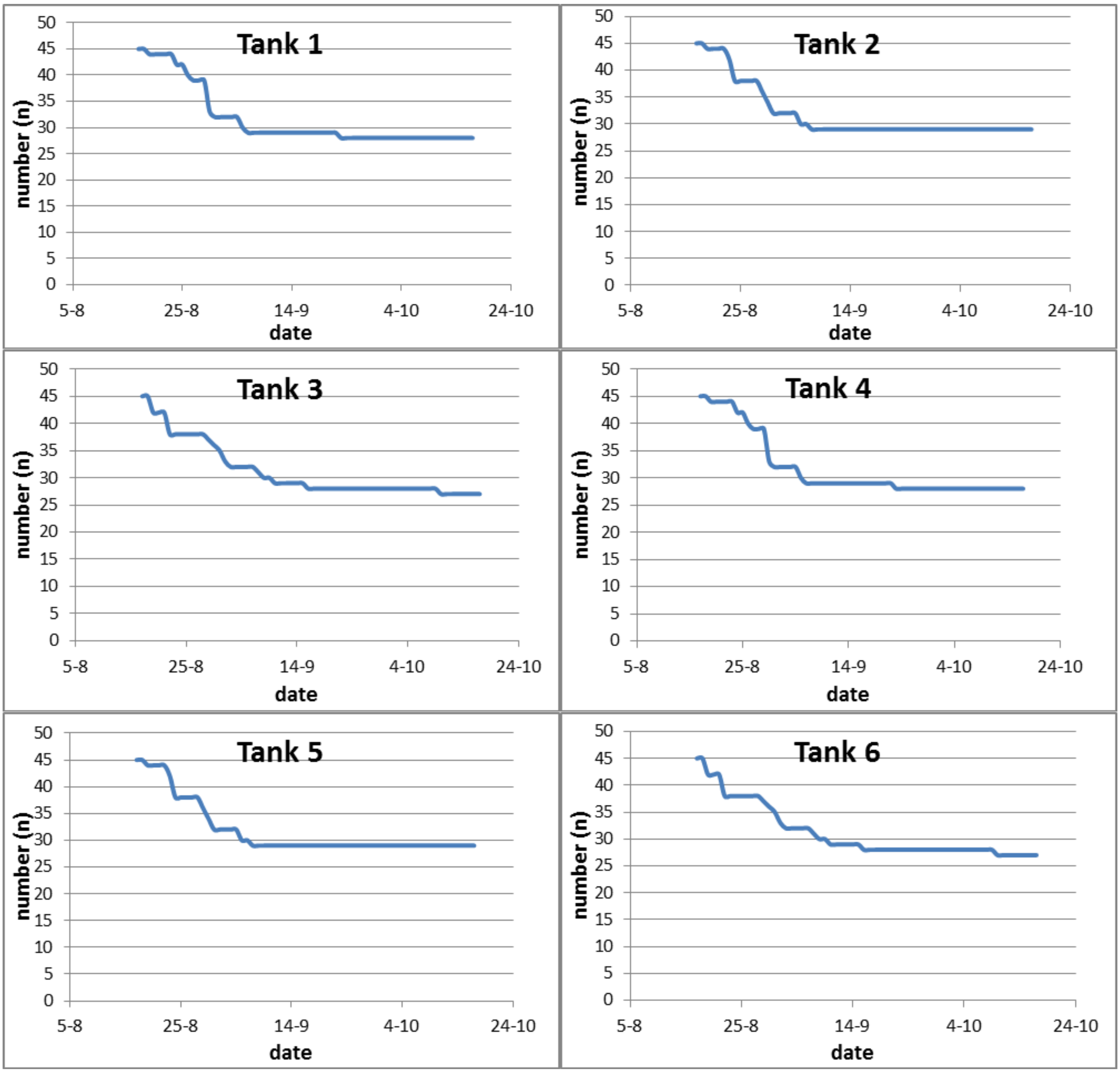


Fig. 6.1. Number of surviving Ensis per tank. Tank 1 = low food without silt, Tank 2 = low food 150 mg/l, Tank 3 = low food 300 mg/l, Tank 4 = high food without silt, Tank 5 = high food 150 mg/l, Tank 6 = high food 300 mg/l.

Annex 7. Output statistical analyses

Filtration experiments

Effect of size class, silt and chlorophyll concentration on clearance rate

Tests of Between-Subjects Effects

Dependent Variable:CR

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3873.798[a]	104	37.248	17.476	.000
Intercept	1400.220	1	1400.220	656.957	.000
silt	127.844	3	42.615	19.994	.000
chla	1.096	1	1.096	.514	.474
adw	2923.412	16	182.713	85.726	.000
silt * chla	40.098	3	13.366	6.271	.000
silt * adw	462.858	43	10.764	5.050	.000
chla * adw	93.501	14	6.679	3.133	.000
silt * chla * adw	254.399	24	10.600	4.973	.000
Error	1148.810	539	2.131		
Total	7093.989	644			
Corrected Total	5022.608	643			

a. R Squared = .771 (Adjusted R Squared = .727)

Bonferroni Post-hoc silt concentration

(I) silt	(J) silt	Mean Difference (I-J)	Std. Error	Sig.
1.00	2.00	.3413	.15614	.175
	3.00	.4746*	.16322	.023
	4.00	1.2345*	.16140	.000
2.00	1.00	-.3413	.15614	.175
	3.00	.1333	.16534	1.000
	4.00	.8932*	.16354	.000
3.00	1.00	-.4746*	.16322	.023
	2.00	-.1333	.16534	1.000
	4.00	.7599*	.17032	.000
4.00	1.00	-1.2345*	.16140	.000
	2.00	-.8932*	.16354	.000
	3.00	-.7599*	.17032	.000

1=0 mg/l, 2=50 mg/l, 3=150 mg/l, 4=300 mg/l

<u>Effect of size class, silt and chlorophyll concentration on pseudofeces production</u>

Tests of Between-Subjects Effects

Dependent Variable:pseudo

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	67.533[a]	20	3.377	5.046	.000
Intercept	85.578	1	85.578	127.887	.000
silt	6.847	3	2.282	3.411	.020
food	4.801	1	4.801	7.175	.008
size	5.108	2	2.554	3.817	.025
silt * food	1.750	3	.583	.872	.458
silt * size	25.368	6	4.228	6.318	.000
food * size	3.532	2	1.766	2.639	.076
silt * food * size	3.511	3	1.170	1.749	.161
Error	78.293	117	.669		
Total	240.000	138			
Corrected Total	145.826	137			

a. R Squared = .463 (Adjusted R Squared = .371)

Bonferroni Post-hoc silt concentration

(I) silt	(J) silt	Mean Difference (I-J)	Std. Error	Sig.
.00	50.00	-.6889*	.18588	.002
	150.00	-.6889*	.19690	.004
	300.00	-.9147*	.19094	.000
50.00	.00	.6889*	.18588	.002
	150.00	.0000	.20876	1.000
	300.00	-.2258	.20314	1.000
150.00	.00	.6889*	.19690	.004
	50.00	.0000	.20876	1.000
	300.00	-.2258	.21327	1.000
300.00	.00	.9147*	.19094	.000
	50.00	.2258	.20314	1.000
	150.00	.2258	.21327	1.000

Bonferroni Post-hoc clam size

(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.
L	M	-.0392	.16530	1.000
	S	.7208*	.17513	.000
M	L	.0392	.16530	1.000
	S	.7600*	.17353	.000
S	L	-.7208*	.17513	.000
	M	-.7600*	.17353	.000

Growth experiments

Effect of size class, silt and chlorophyll concentration on growth in mm per day

Tests of Between-Subjects Effects

Dependent Variable:growthmm

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.386[a]	17	.023	25.649	.000
Intercept	.212	1	.212	238.774	.000
size	.256	2	.128	144.421	.000
chla	.019	1	.019	20.951	.000
silt	.007	2	.003	3.693	.029
size * chla	.012	2	.006	6.694	.002
size* silt	.005	4	.001	1.370	.250
chla * silt	.009	2	.005	5.258	.007
size * chla * silt	.006	4	.001	1.692	.158
Error	.085	96	.001		
Total	.792	114			
Corrected Total	.471	113			

a. R Squared = .820 (Adjusted R Squared = .788)

Bonferroni Post-hoc initial size

(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.
g	k	-.1314*	.00754	.000
	m	-.0329*	.00722	.000
k	g	.1314*	.00754	.000
	m	.0985*	.00639	.000
m	g	.0329*	.00722	.000
	k	-.0985*	.00639	.000

g=large, m=middle, k=small.

Bonferroni Post-hoc silt concentration

(I) silt	(J) silt	Mean Difference (I-J)	Std. Error	Sig.
1.00	2.00	.0072	.00658	.822
	3.00	-.0172	.00706	.050
2.00	1.00	-.0072	.00658	.822
	3.00	-.0244*	.00699	.002
3.00	1.00	.0172	.00706	.050
	2.00	.0244*	.00699	.002

1=0 mg/l, 2=150 mg/l, 3=300 mg/l

Effect of size class, silt and chlorophyll concentration on growth % wet weight per day

Tests of Between-Subjects Effects

Dependent Variable:growthww

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.350[a]	17	1.374	12.863	.000
Intercept	7.092	1	7.092	66.411	.000
size	14.757	2	7.378	69.096	.000
silt	.117	2	.059	.548	.580
chla	.581	1	.581	5.444	.022
size * silt	.790	4	.197	1.848	.126
size * chla	1.048	2	.524	4.906	.009
silt2 * chla	1.006	2	.503	4.711	.011
size * silt * chla	1.337	4	.334	3.130	.018
Error	10.145	95	.107		
Total	47.736	113			
Corrected Total	33.495	112			

a. R Squared = .697 (Adjusted R Squared = .643)

Bonferroni Post-hoc size class

(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.
g	k	-.868490*	.0851318	.000
	m	-.060370	.0825966	1.000
k	g	.868490*	.0851318	.000
	m	.808119*	.0691654	.000
m	g	.060370	.0825966	1.000
	k	-.808119*	.0691654	.000

g=large, m=middle, k=small.

Differences between initial and final ash-free dry weight

Tests of Between-Subjects Effects

Dependent Variable:adw small

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.188[a]	6	.031	4.895	.000
Intercept	2.186	1	2.186	341.355	.000
treat	.188	6	.031	4.895	.000
Error	.346	54	.006		
Total	2.867	61			
Corrected Total	.534	60			

a. R Squared = .352 (Adjusted R Squared = .280)

Bonferroni Post hoc treatment small

(I) treat	(J) treat	Mean Difference (I-J)	Std. Error	Sig.
.00	1.00	-.0318	.04133	1.000
	2.00	-.0428	.03663	1.000
	3.00	-.0490	.03374	1.000
	4.00	-.1611*	.03267	.000
	5.00	-.0760	.03374	.594
	6.00	-.1228	.03866	.052
1.00	.00	.0318	.04133	1.000
	2.00	-.0110	.04686	1.000
	3.00	-.0171	.04464	1.000
	4.00	-.1293	.04383	.099
	5.00	-.0442	.04464	1.000
	6.00	-.0910	.04846	1.000

2.00	.00	.0428	.03663	1.000
	1.00	.0110	.04686	1.000
	3.00	-.0062	.04033	1.000
	4.00	-.1183	.03944	.086
	5.00	-.0332	.04033	1.000
	6.00	-.0800	.04452	1.000
3.00	.00	.0490	.03374	1.000
	1.00	.0171	.04464	1.000
	2.00	.0062	.04033	1.000
	4.00	-.1121	.03677	.075
	5.00	-.0271	.03773	1.000
	6.00	-.0739	.04218	1.000
4.00	.00	.1611*	.03267	.000
	1.00	.1293	.04383	.099
	2.00	.1183	.03944	.086
	3.00	.1121	.03677	.075
	5.00	.0850	.03677	.516
	6.00	.0383	.04133	1.000
5.00	.00	.0760	.03374	.594
	1.00	.0442	.04464	1.000
	2.00	.0332	.04033	1.000
	3.00	.0271	.03773	1.000
	4.00	-.0850	.03677	.516
	6.00	-.0468	.04218	1.000
6.00	.00	.1228	.03866	.052
	1.00	.0910	.04846	1.000
	2.00	.0800	.04452	1.000
	3.00	.0739	.04218	1.000
	4.00	-.0383	.04133	1.000
	5.00	.0468	.04218	1.000

1=low 0mg/l, 2=low 150mg/l, 3=low 300mg/l, 4=high 0 mg/l, 5=high 150mg/l, 6=high 300mg/l, 7=initial

Tests of Between-Subjects Effects

Dependent Variable:adw middle

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.197[a]	6	.366	8.013	.000
Intercept	30.460	1	30.460	666.642	.000
Treat	2.197	6	.366	8.013	.000
Error	3.198	70	.046		
Total	40.282	77			
Corrected Total	5.395	76			

a. R Squared = .407 (Adjusted R Squared = .356)

Bonferroni Post hoc treatment middle

(I) treat	(J) treat	Mean Difference (I-J)	Std. Error	Sig.
1.00	2.00	.0351	.08923	1.000
	3.00	.0337	.09153	1.000
	4.00	-.0475	.08923	1.000
	5.00	-.2270	.08557	.207
	6.00	.4463*	.10166	.001
	7.00	-.0830	.08557	1.000
2.00	1.00	-.0351	.08923	1.000
	3.00	-.0014	.09340	1.000
	4.00	-.0827	.09115	1.000
	5.00	-.2621	.08757	.080
	6.00	.4111*	.10335	.004
	7.00	-.1182	.08757	1.000
3.00	1.00	-.0337	.09153	1.000
	2.00	.0014	.09340	1.000
	4.00	-.0813	.09340	1.000
	5.00	-.2607	.08991	.105
	6.00	.4125*	.10534	.004
	7.00	-.1168	.08991	1.000
4.00	1.00	.0475	.08923	1.000
	2.00	.0827	.09115	1.000
	3.00	.0813	.09340	1.000
	5.00	-.1794	.08757	.928
	6.00	.4938*	.10335	.000
	7.00	-.0355	.08757	1.000

5.00	1.00	.2270	.08557	.207
	2.00	.2621	.08757	.080
	3.00	.2607	.08991	.105
	4.00	.1794	.08757	.928
	6.00	.6732*	.10021	.000
	7.00	.1440	.08384	1.000
6.00	1.00	-.4463*	.10166	.001
	2.00	-.4111*	.10335	.004
	3.00	-.4125*	.10534	.004
	4.00	-.4938*	.10335	.000
	5.00	-.6732*	.10021	.000
	7.00	-.5293*	.10021	.000
7.00	1.00	.0830	.08557	1.000
	2.00	.1182	.08757	1.000
	3.00	.1168	.08991	1.000
	4.00	.0355	.08757	1.000
	5.00	-.1440	.08384	1.000
	6.00	.5293*	.10021	.000

1=low 0mg/l, 2=low 150mg/l, 3=low 300mg/l, 4=high 0 mg/l, 5=high 150mg/l, 6=high 300mg/l, 7=initial

Tests of Between-Subjects Effects

Dependent Variable:adw large

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.282[a]	6	.214	2.201	.054
Intercept	70.722	1	70.722	728.614	.000
treatl	1.282	6	.214	2.201	.054
Error	6.115	63	.097		
Total	85.704	70			
Corrected Total	7.397	69			

a. R Squared = .173 (Adjusted R Squared = .095)

Effect of size class, silt and chlorophyll concentration on ash-free dry weight

Tests of Between-Subjects Effects

Dependent Variable:adw

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.765[a]	17	.869	11.577	.000
Intercept	61.518	1	61.518	820.022	.000
size	13.543	2	6.771	90.262	.000
silt	.056	2	.028	.376	.687
chla	.513	1	.513	6.837	.010
size * silt	.084	4	.021	.281	.890
size * chla	.092	2	.046	.612	.544
silt * chla	.008	2	.004	.053	.949
size * silt * chla	.384	4	.096	1.281	.280
Error	11.253	150	.075		
Total	98.203	168			
Corrected Total	26.018	167			

a. R Squared = .567 (Adjusted R Squared = .518)

(I) size	(J) size	Mean Difference (I-J)	Std. Error	Sig.
G	k	.7125*	.05408	.000
	m	.2062*	.04966	.000
K	g	-.7125*	.05408	.000
	m	-.5063*	.05294	.000
M	g	-.2062*	.04966	.000
	k	.5063*	.05294	.000

g=large, m=middle, k=small