First pioneering laboratory experiments on filtration, respiration and growth of the razor clam (*Ensis directus*, Conrad)

Pauline Kamermans, Emiel Brummelhuis & Jeroen Wijsman

Rapport C115/11

IMARES Wageningen UR

Institute for Marine Resources & Ecosystem Studies

Client:	Rijkswaterstaat Waterdienst MEP Zandwinning RWS LaMER Postbus 17 8200 AA Lelystad
Publication date:	October 7, 2011

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P.O. Box 68
1970 AB IJmuiden
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 26
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 77
4400 AB Yerseke
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 59
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 57
1780 AB Den Helder
Phone: +31 (0)317 48 09 00
Fax: +31 (0)223 63 06 87
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 167
1790 AD Den Burg Texel
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 62
E-Mail: imares@wur.nl
www.imares.wur.nl

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1 Summary

In Dutch marine circumstances, sand extraction releases silt into the water column. The extra silt can reduce light penetration into the water and consequently algal growth. To predict potential effects of an expansion of sand extraction activities it is necessary to know possible impacts on the environment. *Ensis directus*, a dominant species web of the North Sea coastal zone, has a key position in the food web. Therefore, it was selected as model species in this study to predict the effects of the reduced food conditions due to sand extraction on the growth of *E. directus*. A DEB (Dynamic Energy Budget) model is in development. This study describes the basic experiments that have been done to determine empirical relations between clam size or food concentration and filtration, respiration and growth rates necessary for the DEB modelling. Also, the basic values on physiology itself have their value because little is known on this species.

Filtration and respiration rates were measured at four food levels (2, 5, 20 and 40 µg chlorophyll a/l). Clam shell length varied from 42 to 135 mm. Filtration rate decreased with an increase in clam size from maximally 3.3 lh^{-1} g^{-1} ash-free dry weight (ADW) to 0. lh^{-1} g^{-1} ADW. There was no relation between food concentration on filtration rate. Respiration rates showed a similar decrease with clam size from maximally 5000 mg O_2 lh^{-1} g^{-1} ADW to 1500 mg O_2 lh^{-1} g^{-1} ADW. In addition, an increase in respiration rate was found with an increase in food concentration. In the growth experiment five food levels were tested (0, 2, 5, 20 and 40 µg chlorophyll a/l).Clams smaller than 75 mm shell length showed more growth (up to 1% increase in wet weight (WW) per day or 0.3% shell length per day) than larger clams (maximally 0.16% increase in WW per day or 0.01% shell length per day). Growth rates showed an increase with increased food concentration.

Programme: Monitoring and Evaluation Programme Sandmining from RWS and LaMER
Penvoerder: RWS-WD
Begeleidingscommissie: dr. M.J.C. Rozemeijer (RWS-WD)
dr. S. Kabuta (RWS-WD)
dr. J. de Kok (Deltares)

2 Introduction

Sand extraction is being carried out in the North Sea coastal zone, at a depth of -20 m NAP and more. The effects of this activity will be evaluated by the Monitoring and Evaluation Programme Sandmining of the National Institute for Waterways and Public Works of the Department of Infrastructure and Environment (RWS) and the LaMER Foundation, an organisation of “waterbouwers” (marine engineers). An effect of sand extraction is the release of silt into the water column. The extra silt can reduce light penetration into the water and consequently phytoplankton growth. Phytoplankton is at the basis of the food web. Therefore, reduced levels of phytoplankton can have effects on other species such as algae-eating shellfish and consequently shellfish-eating birds. In addition, the extra silt can hamper food intake by filter feeding organisms causing e.g. reduced growth. To predict potential effects of (an expansion of) sand extraction activities it is necessary to know possible impacts on the filter feeding shellfish populations. For this purpose the use of a dominant species (*Ensis directus*) that has a key position in the food web of the North Sea coastal zone was proposed as a major topic of investigation of an extensive monitoring programme of RWS and LaMER (Ellerbroek e.a. 2008).

The American razor clam *Ensis directus* is an exotic species that was first observed in the European waters near the German North Sea coast in 1979 and it is thought to have been introduced in Europe shortly before by larval transport in ballast waters of ships that crossed the Atlantic (Von Cosel et al. 1982). In Dutch waters, the first well identified *E. directus* specimen was found in the Ems estuary in 1981 (Essink 1985). The species has managed to build up a strong population in Dutch waters, including the North Sea coastal zone, over the last decades (Dekker and Waasdorp 2007, Perdon and Goudswaard 2007). At present, it is by far the most dominant shellfish species in Dutch coastal zone, which potentially makes it a good candidate to monitor and predict effects of sand extraction.

Research questions of the Monitoring programme RWS LaMER are: (1) What are the effects of the reduced food conditions on the growth of *E. directus* and (2) When does food limitation occur as a result of these changed conditions? The approach taken is the development and use of a DEB (Dynamic Energy Budget) model (Kooijman, 2000) for *E. directus*. This DEB model will then be integrated in the algae module of a larger system model (Delft3D).

In order to estimate the species specific model parameters experiments have been done in 2010 and 2011 to derive empirical relations between clam size and filtration, respiration and growth rate. In addition, the effect of food concentration on these processes is studied. In this report the results of the 2010 experiments are presented. Growth rates are compared to literature data collected by Cardoso et al (2011). The relations between respiration rate, respiration rate or growth rate and clam size or food level are used in the DEB model, which is reported by Wijsman (2011). Besides the use of the generated data in the DEB modelling, the physiological data achieved have their value as well since little is known on this species.

3 Materials & Methods

3.1 Razor clams

The clams used in the experiment were collected at different dates in 2010 and locations in the North Sea coastal zone, resulting in 7 batches (Table 1). Collection was done with a commercial suction dredge and with a box corer (sample area 0.077 m2). Transport took place the same day. Batch 1, 4 and 6 were placed in containers with sand covered by a water layer. Batch 1 and 6 had extra oxygen added. Batch 2, 3 and 5 were bundled with a rubber band and transported dry in a cool box with cooling elements. On arrival the clams were stored in an outdoor basin with running seawater where they were kept until use in the experiments (Table 1). A protocol for collection, transport and holding of the clams was developed and is given in Annex 1.

Table 1. Origin of different clam batches used in the experiments.

Code	**Batch number**	**Number of individuals**	**Period in outdoor basin before use (days)**	**Collection date**	**Start experiment**	**Sample location**	**Collection method**	**Transport method**
1 - 30	1	30	4	24-Jun 2010	28-Jun 2010	Egmond N52 38 481 E4 36 012 (several locations in this area)	box core	in sand with extra oxygen
33 - 36	2	4	98	23-Mar 2010	29-Jun 2010	Voordelta N51 37 893 E3 37 466	suction dredge	dry in cooler
37 - 45	3	9	96	25-Mar 2010	29-Jun 2010	Voordelta N51 37 928 E3 37 356 and N51 38 400 E3 37 400	suction dredge	dry in cooler
46 - 48	4	3	26	3-Jun 2010	29-Jun 2010	Egmond N52 38 481 E4 36 012 (several locations in this area)	box core	in sand
49 - 68	5	20	14	1-Jul 2010	15-Jul 2010	Voordelta N51 38 650 E3 37 400	special Ensis dredge	dry in cooler
no code	6	see table 3	37	1-Sep 2010	28 Sep 2010	Egmond N52 38 481 E4 36 012 (several locations in this area)	box core	Part in sand with extra oxygen, part between wet towels in cooler
no code	7	see table 3	31	7-Sept 2010	28 Sep 2010	Vlakte van Raan. N51 31 500 E3 17 000	suction dredge	dry in cooler

3.2 Food

The flagellate *Pavlova lutheri* (Droop) was cultured in batch with Walne medium (Walne, 1970), at 19˚C and 24 h light. Cultures were scaled up from 30 ml to 100 ml in tissue flasks to 3 l in glass Erlenmeyer's and finally to 25 l in plastic bags. The size of *P. lutheri* is 4-6 um.

3.3 Growth setup

Five plastic 600-liter containers were placed in a climate room at 18˚C. The light was dimmed. The containers were filled with a 15-20 cm layer of medium coarse river sand obtained from a supplier. The sand was sieved over a 1 mm sieve before use to remove particles other than sand. After filling the tanks, the clams were collected from their outdoor storage tank and individual length was measured with a calliper to the nearest 0.01 mm. Wet weight (WW) was measured on an analytic scale to the nearest 0.01 g. Before weighing whole clams were dried by dipping with a tissue. All clams were given an individual code with different colours of nail polish on their shell. The different clam batches where evenly distributed over the tanks on the 28th of June (batch 1-4) and 15th of July (batch 5) 2010 (Table 2). At the start of the experiment the length and wet weight of an extra set of sixty five clams was measured. This set was used to determine the initial dry weight and ash-free dry weight (AW) of the meat. The meat was separated from the shell and dry weight (DW) of the meat was measured after at least 2 days drying at 70 ˚C and cooling to room temperature in an dessicator, on an analytic balance to the nearest 0.0001g. Previous experience at IMARES has shown that further weight reduction does not occur after 2 days. After ashing at 560 ˚C for 4 hours and cooling down in a dessicator the ash-weight (AW) was measured. The ash-free dry weight (ADW) is DW-AW. At the end of the experiment, on the 16th of September 2010, the WW, ADW and shell length of all clams used was determined.

Five different food concentrations were offered. Based on experience with *Mytilus edulis*, *Cerastoderma edule* L. and *Venerupis pullastra* (Foster-Smith, 1975), it was assumed that 2 mg dry weight (DW) l^{-1} represents the optimal particle concentration just below the pseudofeces threshold for *Ensis directus*. This is four times higher than the normally occurring concentrations in the Dutch Coastal Zone (pers. comm. Johan de Kok). Concentrations of 0% to 200% of optimal particle concentration were calculated based on the DW of *P. lutheri*. The used concentrations were: 0% (0 mg DW/l or 0 µg chla/l), 10% (0.2 mg DW/l or 2 µg chla/l), 25% (0.5 mg DW/l or 5 µg chla/l), 100% (2 mg DW/l or 20 µg chla/l) and 200% (4 mg DW/l or 40 µg chla/l), in containers 1 to 5 respectively. The concentrations were held constant with an algae aqua feed regulator (Kamermans in prep). The regulator measures the algal concentration in the water and has a feed-back mechanism that operates a pump. The pump adds more algae when the algal level drops below a set concentration and stops the pump when the desired level is reached again.

Monitored parameters where: nitrite and ammonium with Merckoquant® test strips (Merck) and chlorophyll a with an Algae Online Analyser (bbe Moldaenke). The water in the containers was refreshed twice a week. Clams that died during the experiment where recorded daily, removed and stored in a freezer at -20˚C.

Table 2. Algae concentration and number of clams per batch in the growth containers.

Container	µg chl-a/l	# Ind. batch 1 (28th June 2010)	# Ind. batch 2 (28th June 2010)	# Ind. batch 3 (28th June 2010)	# Ind. batch 4 (28th June 2010)	# Ind. batch 5 (15th July 2010)	Total #
1	0	29	4	9	3	19	64
2	2	29	4	9	3	20	65
3	5	28	4	9	3	20	64
4	20	30	4	9	3	20	66
5	40	30	4	9	3	20	66

For the filtration and oxygen consumption experiments new clams were used (Batch 6, size <80mm and batch 7; size >80mm, Table 3). They were acclimated for 1 week at each food concentration. The acclimated group of clams used for the concentration 2 µg chla/l - were also used for concentration 20 µg chla/l. Similarly the group of clams used for concentration 5 µg chla/l was also used for 40 µg chla/l.

Table 3. Number of clams used for filtration and oxygen consumption measurements at different dates and food concentrations.

	Filtration				Oxygen			
Food concentration	Size <80mm, batch 6		Size >80mm, batch 7		Size <80mm, batch 6		Size >80mm, batch 7	
µg chla/l	Number	Date	Number	Date	Number	Date	Number	Date
2	6	3 Nov 2010	6	2 Nov 2010	5	5 Nov 2010	9	5 Nov 2010
5	4	3 Nov 2010	6	2 Nov 2010	8	4 Nov 2010	10	4 Nov 2010
20	5	6 Oct 2010	5	8 Oct 2010	7	20 Oct 2010	9	20 Oct 2010
40	5	12 Oct 2010	6	5 Oct 2010	8	19 Oct 2010	10	19 Oct 2010

3.4 Filtration

To measure the filtration rate at the different food concentrations an experimental flow-through system was used (Fig. 1). The 600-liter containers with different food concentrations were used as a storage tank. Water was pumped with a peristaltic pump through small grazing chambers (volume 60 ml) containing 1 clam each and back into the storage container. The bigger clams, size >80 mm were put in grazing chambers with artificial sediment (1-2 mm caviar brown aquarium gravel), chamber volume with sand approximately 200 ml. The smaller clams often came out of the sediment and kept their siphons above the water. Therefore, they were measured in small grazing chambers without sediment, chamber volume 60 ml. One chamber did not contain a clam and was used as reference treatment. The flow was optimized such that there was a clear difference in concentration but not more than around 30% clearance, to prevent re-filtration effects. For the small clams the flow was 25 ml/min, for the big clams the flow was set to 50 ml/min.

From container 1 to 5, clams of 2 different size classes were measured. Some clams died during the experiments or were inactive. The results of these clams were not used. The number of clams measured per food concentration ranged from 10 to 12.

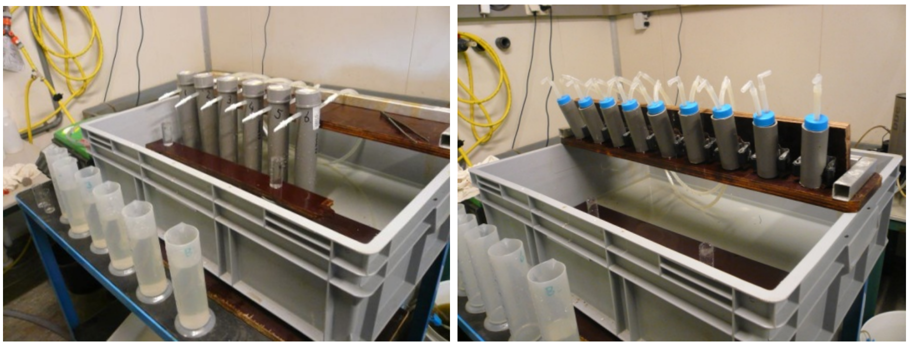


Figure 1. Filtration setup, left for the small clams, right for the big clams.

To determine filtration rates, we measured clearance rates. Clearance rate is the rate with 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, we directly determined filtration rates. We measured particle concentrations in the water flowing in and out of the containers with a Z2 particle counter (Beckman coulter). From the difference in particle concentration we calculated the clearance rate using the following equation:

$$CR=((C_{in} - C_{out})/C_{out}) * Q$$

where CR= clearance rate in l/h per individual, C_{in} = particle concentration of the outflow of the blanco, C_{out} = particle concentration of the outlow and Q = flow rate in l/h (Widdows, 1985).

To correct for particle settlement inside the containers (also resulting in decreasing concentrations) we measured the particle concentration in the water flowing out of the reference treatment and used this as a values for particle concentration in the water flowing into the containers.

3.5 Respiration

Clams (batch 6 in Table 2) were put in closed containers, filled with seawater of a known oxygen concentration (Fig. 2). Small clams were put in plastic 60-ml centrifuge tubes and left undisturbed for 10 to 20 minutes. The big clams where put in plastic 1-liter containers and left undisturbed for 1 to 2 hours. No sediment was added.

Figure 2. Clams incubating for oxygen measurement, left the small clams, right the big clams.

After the incubation time the containers where opened and the water was gently stirred by moving the measuring probe before oxygen measurement. This was repeated several times for each food concentration. Each run, two blanks were included. To keep the temperature constant the containers were left floating in a water bath during incubation. Dissolved oxygen was measured with a LDO probe to the nearest 0.01 mg/l (Hach Lange).

Ten clams of 2 different size classes from container 1 to 5 were measured. Some clams died during the experiments or were inactive. These were not used. The number measured per food concentration ranged from 14 to 18.

3.6 Statistical analysis

Differences in algal concentration between containers, and effects of clam origin or food concentration on filtration, respiration and growth and mortality rates were tested with ANOVA. The homogeneity of variances was tested with a Levene test. If 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. Linearity of the data was examined with residual plots. Since the assumptions for ANOVA were violated even after transformation of the data the non-parametric Kruskal-Wallis test or Mann-Whitney U test was used. As a consequence tests of significance of interactions was not possible. The significance level for all tests was $p<0.05$ and the analyses were made using PASW Statistics 17.0.

4 Results

4.1 Filtration

Filtration rate significantly increased with clam size to a maximum value of 1.8 l/h/individual at 1 g ADW (Fig. 3a and Table 4). There was also an effect of origin of clams (batch number) on filtration rate (Table 4). Filtration rate was significantly different between food levels, but there was no relation between food concentration and filtration rate (Fig. 3a and Table 4). Average clam size did not differ between containers (Annex 3). Relative filtration rate showed a significant decrease with clam size (Fig. 3b and Table 4). Average values per size class are presented in Table 5.

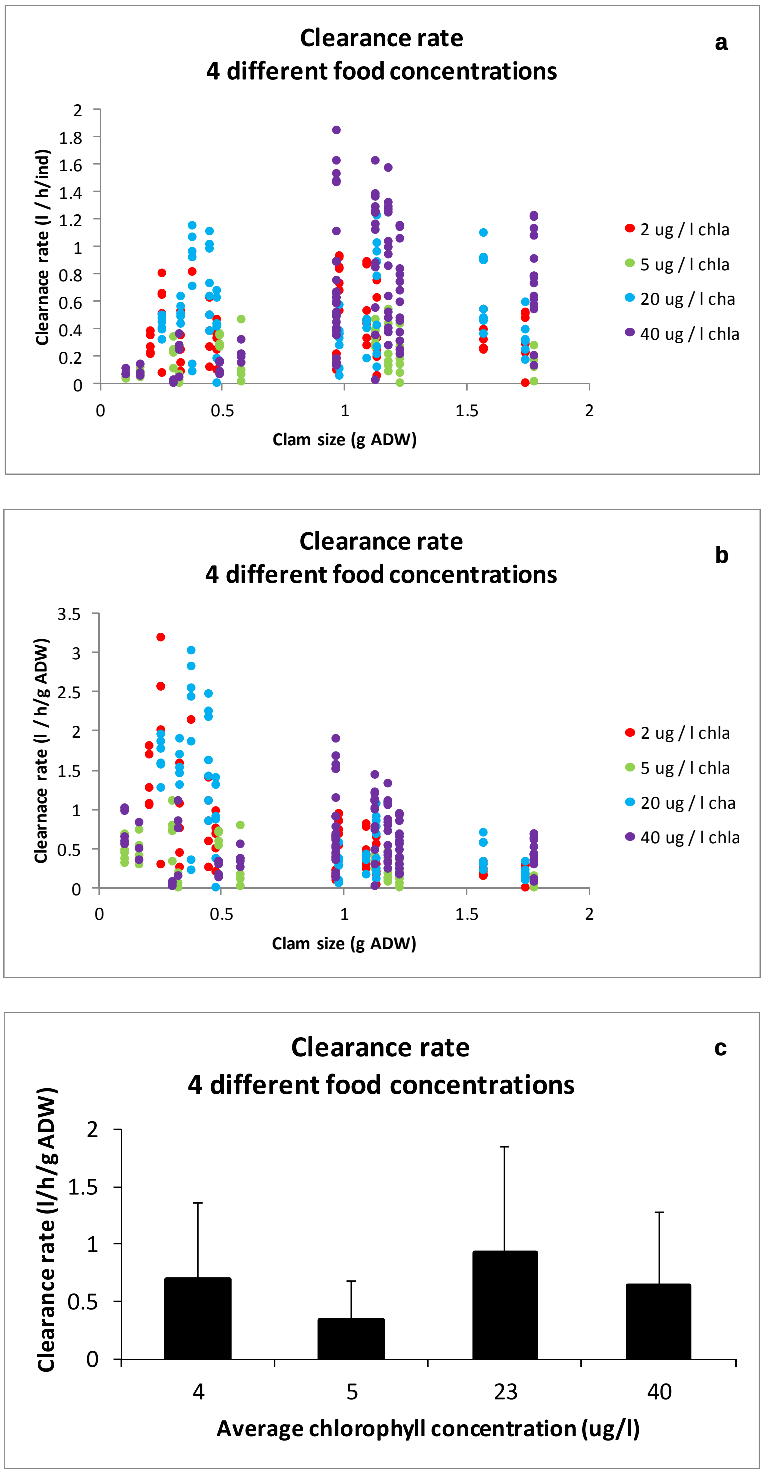


Figure 3. Relation between clearance rate and clam size or food concentration expressed per individual (a), or per g ADW (b) and average per food concentration (c).

Table 4. P-values of Kruskal-Wallis (K) or Mann-Whitney U (M) tests. For details see Annex 3. In bold the results that were significant.

Tested variable	Clam size	Origin of clams	Food level
Filtration rate per individual	**0.000** (K)	**0.000** (K)	**0.000** (K)
Filtration rate per g ADW	**0.000** (K)	**0.000** (K)	**0.000** (K)
Respiration rate per individual	**0.033** (K)	**0.000** (K)	**0.000** (K)
Respiration rate per g ADW	**0.000** (K)	**0.000** (K)	**0.000** (K)
Growth rate in WW	**0.000** (K)	**0.000** (K)	**0.000** (K)
Growth rate in mm	**0.030** (K)	**0.000** (K)	**0.008** (K)
Survival	0.153 (M)	**0.026** (K)	0.265 (K)

Table 5. Average filtration rate (CR) per food level and per size class.

Food concentration (µg chla/l)	Size class (mm)	Average CR (l/h /individual)	Stdev	Average CR (l/h/g ADW)	Stdev
2	<80	0.37	0.21	1.20	0.80
	>80	0.42	0.26	0.36	0.27
5	<80	0.15	0.13	0.49	0.29
	>80	0.24	0.14	0.20	0.13
20	<80	0.57	0.30	1.54	0.74
	>80	0.50	0.31	0.39	0.25
40	<80	0.13	0.09	0.47	0.31
	>80	0.80	0.42	0.69	0.40

4.2 Respiration

Respiration rate showed a significant increase with clam size (Fig. 4a and Table 4). In addition, there was an effect of origin of clams (batch number) and food level (container number) on respiration rate (table 4). Relative respiration rate showed a significant decrease with clam size (Fig. 4b and table 4). Average values per size class are presented in Table 6.

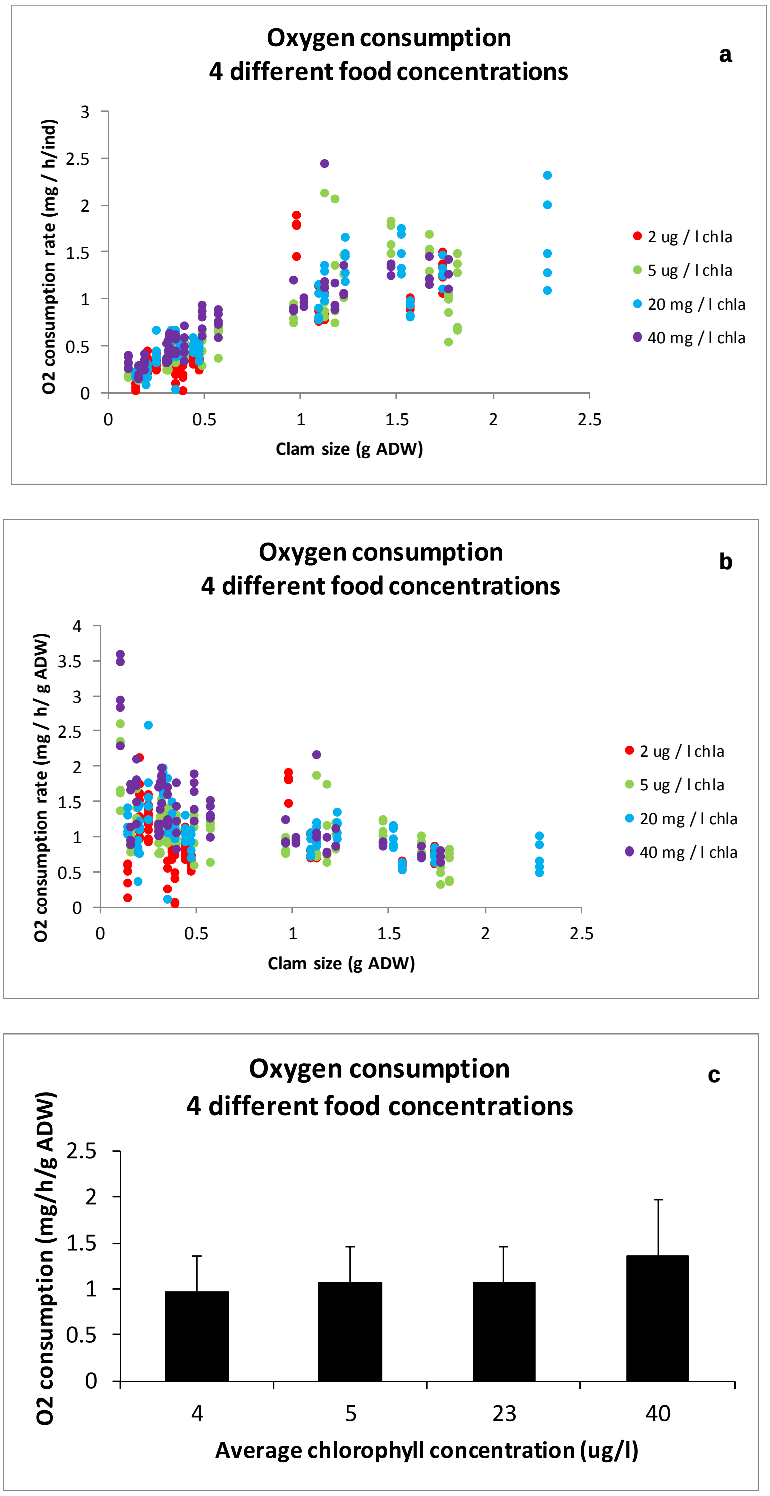


Figure 4. Relation between oxygen consumption and clam size or food concentration expressed per individual (a), or per g ADW (b) and average per food concentration (c).

Table 6. Average respiration rate (RR) per food level and per size class.

Food concentration (µg chla/l)	Size class (mm)	Average RR (l/h)	Stdev	Average RR (l/h/g ADW)	Stdev
2	<80	0.32	0.12	0.97	0.39
	>80	1.17	0.96	0.35	0.43
5	<80	0.36	1.18	0.16	0.38
	>80	1.19	0.88	0.39	0.32
20	<80	0.38	1.21	0.16	0.42
	>80	1.25	0.85	0.35	0.23
40	<80	0.49	1.57	0.20	0.60
	>80	1.18	0.93	0.32	0.29

4.3 Growth

We had problems keeping the algae concentration constant, possibly due to the large volume and rectangular shape of the containers. This resulted in large fluctuations in chlorophyll concentration (Table 7 and Annex 2). However, the concentrations in the containers differed significantly (Table 7 and Annex 3). Other environmental parameters are presented in (Annex 2). Ammonium was always 0 mg l-1, but in the containers with high food concentrations levels higher than 0.5 mg l nitrite were measured on 4 days and in container 4 and 9 days in container 5. Oxygen fluctuated between 7.5-9.6 mg/l and temperature between 17.3 and 20.1 0C. Salinity fluctuated between 25.6 and 36.4 ‰, but at the beginning of the experiment, the reliability of the meter was questionable. Differences between containers were not observed for oxygen, temperature and salinity.

Table 7. Average algae concentration in µg chlorophyll a per liter during the growth experiment.

Container	Average	stdev
1	0.00	0.00
2	4.10	4.29
3	4.96	4.09
4	23.21	14.86
5	40.31	28.81

Growth was expressed as increase in wet weight (Fig. 5a) and increase in shell length (Fig. 5b).

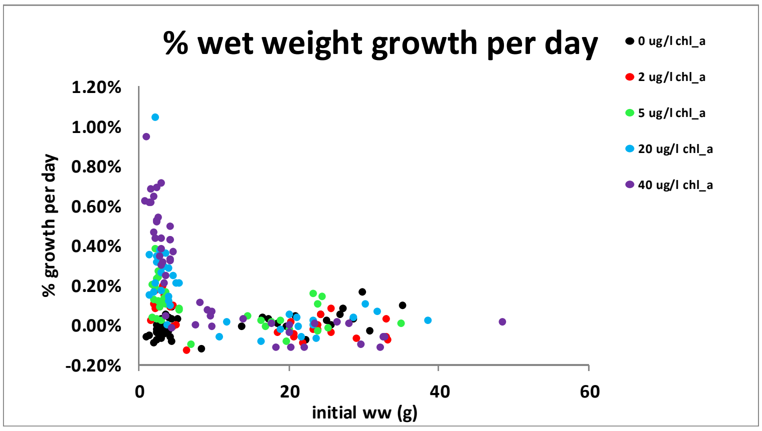
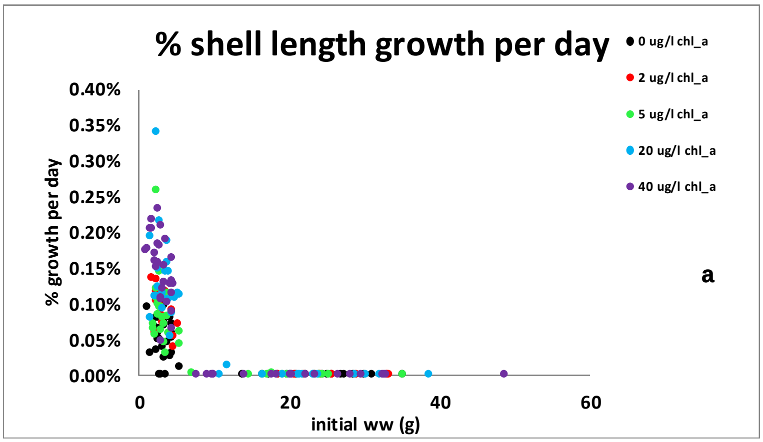
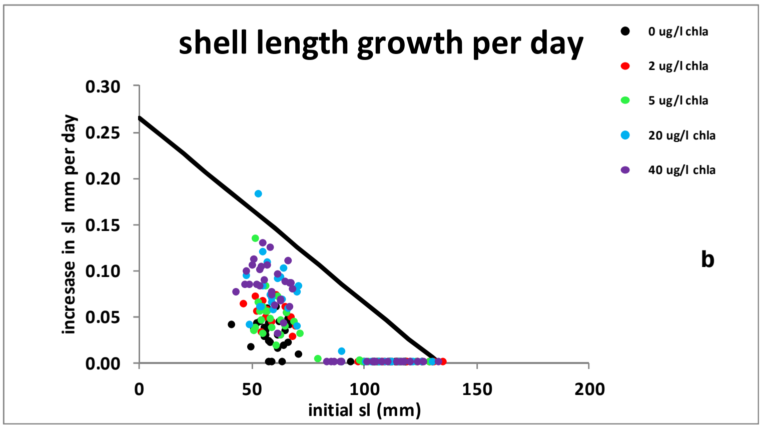


Figure 5a. Relation between growth rate and clam size or food concentration expressed in % increase in wet weight





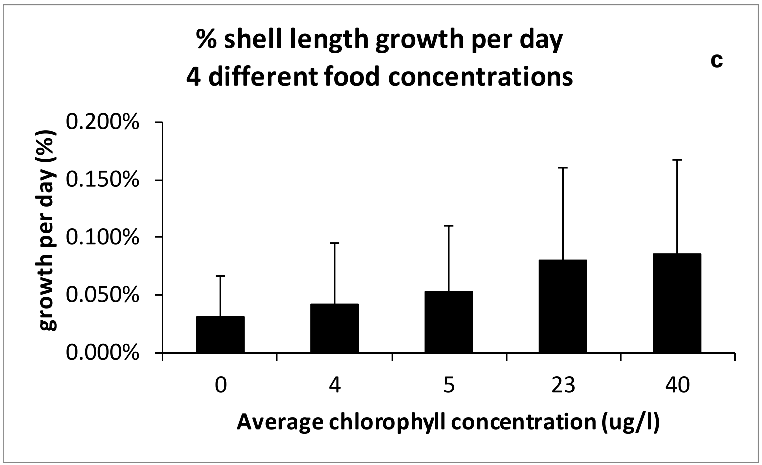
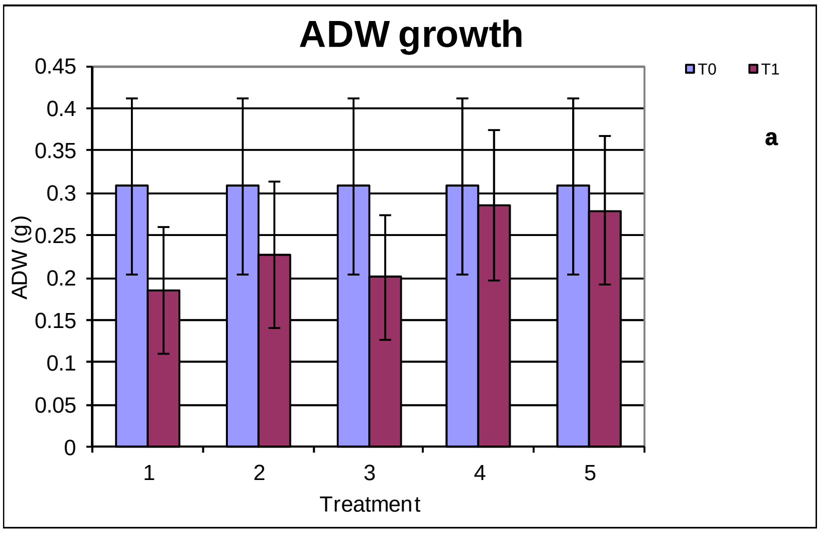


Figure 5b. Relation between growth rate and clam size or food concentration expressed in % increase in shell length (a), or mm increase in shell length (b) solid line indicates maximal growth expected according to the Von Bertalanffy equation $dL/dt = 0.002 * k(L_\infty - L_x)$ based on literature data from Cardoso et al (2011), and average per food concentration (c).

Clam size, origin of clams (batch number) and food level (container number) significantly affected growth rate (Fig. 5 and Table 4).

Clams smaller than 5 gram WW and 75 mm shell length showed more growth than larger clams (Fig. 5). All clams showed less growth than what was theoretically possible according to the Von Bertalanffy growth curve (Fig. 5). All clams had a lower ash-free dry weight than the initial sample (Fig. 6). This suggests suboptimal growing conditions. Indeed, in the small clams the difference was less in the high-food concentration treatments compared to the unfed and low-food concentrations (Fig. 6a). In the large clams this was not the case (Fig. 6b).



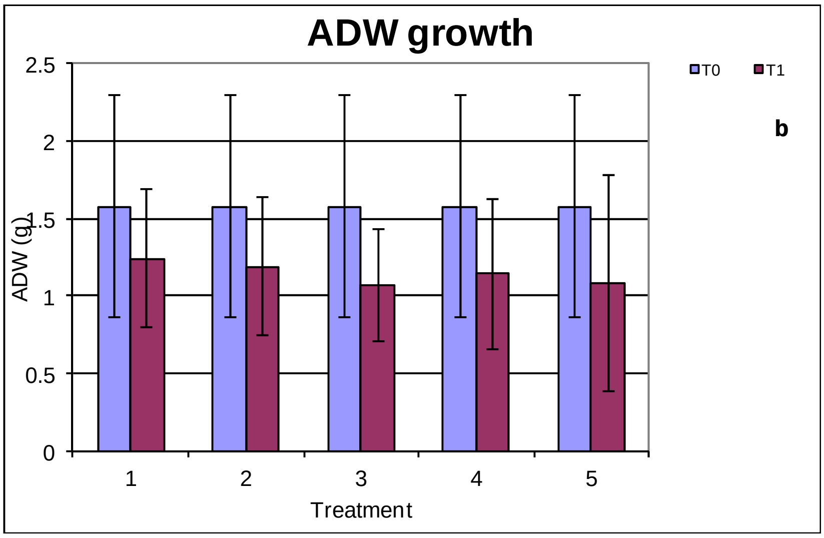


Figure 6. Ash-free dry weight (average in g with sd) of initial (T0) and final (T1) sample for clams smaller than 75 mm (a) and larger than 75 mm (b).

4.4 Survival

Survival was affected by clam origin (batch number) (Fig. 7). This effect was significant for percentage of survival (Table 4). Food concentration did not affect percentage of survival (Fig. 7 and Table 4). Clam size did not affect survival (Table 4).

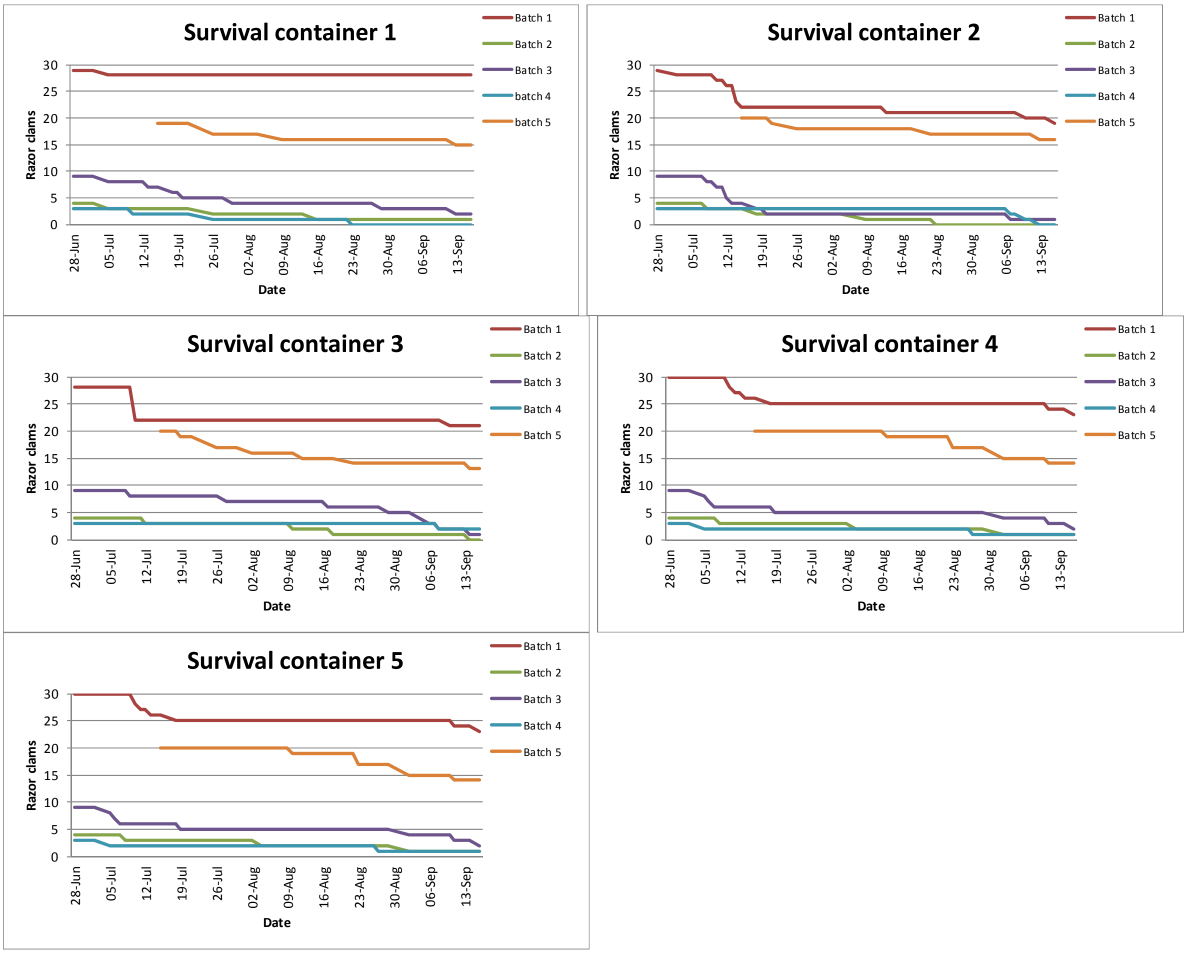


Figure 7. Survival of different clam batches (1-5) at five chlorophyl concentrations (container 1: 0 µg/l, container 2: 2 µg/l; container 3: 5 µg/l, container 4: 20 µg/l, container 5: 40 µg/l).

5 Discussion and conclusion

The laboratory experiments provide information on filtration, respiration, growth and survival rates of *Ensis directus* and how these rates are related to clam size and food concentration. To our knowledge, this is the first study to measure these parameters for *E. directus* in combination with food concentration. It can be concluded that filtration rates increase with clam size and food concentration. These positive relations have been observed for other bivalve species as well. Winter (1973) showed this for *Mytilus edulis*. 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*. Our results for *E. directus* show that respiration rates also increase with clam size and food concentration. Riisgard & Randlov (1981) measured the same for *M. edulis*. Growth rates were higher for small clams than for large clams and higher at high food levels compared to low food levels. However, large clams may not have performed optimally in our set-up. Food concentration did not affect survival.

Literature data on filtration, growth and survival of *E. directus* is limited and non-existent for respiration. Shumway et al (1985) measured an average filtration rate of 0.93 l/h/g DW. Troost & van Duren (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 present values of 0.1-3.9 l/h/g DW. Filtration rate measurements carried out in 2011 showed higher values and range from 0.3 to 14.4 l/h/ g DW (Kamermans et al., in prep). A possible explanation can be that the filtration measurements of 2010 were carried out with clams that were used in the growth experiment. The low growth rates during the growth experiment suggest that these clams were stressed. In 2011 the clams measured were collected only for the filtration measurements.

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 0.08% increase in shell length per day. Our growth rates for this size class were similar in the unfed treatment (up to 0.10 % per day), and higher in the fed treatments (up to 0.35% per day). Based on shell size and age determinations, Cardoso et al (2011) estimated the Von Bertalanffy growth curve. The observed growth rates of our experiment were much lower than the theoretical growth rates. The average survival rate recorded by Freudendahl et al (2010) was 57% in 9 weeks or 0.9% per day. Our daily survival rates depended on batch number and average rates were 1.0% for Batch 1, 0.2% for Batch 2, 0.4% for Batch 3, 0.5% for Batch 4, 1.2% for Batch 5. This indicates that for some batches survival was comparable to the field data of Freudendahl et al (2010).

A striking result is the low growth of individuals lager than 75 mm. For some reason these individuals showed no or low growth. Possibly the duration of the experiment (80 days) was not long enough to detect changes in the large clams. Another explanation may be that the provided algal diet contained only one species, which may not be sufficient to support growth in the clams. Most shellfish need the fatty acids EPA (present in diatoms) and DHA (present in flagellates) (Helm et al, 2004). The reduction in ash-free dry weight in all treatments is unexpected, because this was observed even in the small clams that did show shell growth. A possible explanation is that the clams first showed growth, which is visible in an increase in shell length, but then lost weight during the remaining part of the experiment.

Concluding, results on *Ensis directus* as a laboratory species have been achieved. The species seems to be very sensitive as shown by the mortality in the laboratory. However, similar mortality rates have been observed in the field. Currently, the empirical relations for filtration and respiration provided by this study are used in the DEB model together with filtration rates obtained in 2011 (Wijsman 2011). Given the decrease in ash-free dry weight it is not advisable to use the present growth results. In 2011 another growth experiment is carried out.

The 2011 study includes improvement of the laboratory conditions for growth experiments with *Ensis directus*. A more balanced diet of two algal species (a diatom and a flagellate) is provided and better flow conditions in the tanks are realised to ensure efficient distribution of the algae to all clams. This is achieved through the use of circular tanks and rotor blades to prevent sedimentation of food particles. In addition to this, the effect of different silt concentrations (50, 150 and 300 mg/l) and food concentrations (6 and 15 µg chla/l) on filtration rate and growth rate is tested in 2011.

6 Acknowledgements

We would like to thank Peter Pekaar, Joël Cuperus and Arnold Bakker and Karin Troost for collecting razor clams for the experiments, Ad van Gool, Angelo Hofman, Johan Jol, Noortje Ros, Rob Witbaard and Carola van Zweeden for practical assistance and Jaap van der Meer and Marcel Rozemeijer, Saa Kabuta, John de Ronde for comments on an earlier draft of the manuscript. The research was funded by the Monitoring Programme Sandmining of RWS and LaMER.

7 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: C115/11
Project Number: 4303100601

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

Approved: Dr. Karin Troost
Researcher

Signature:

Date: October 3, 2011

Approved: Dr. B.D. Dauwe
Head Department Delta, IMARES Yerseke

Signature:

Date: October 3, 2011

Annex 1. Protocol for collecting, transport and maintenance of *Ensis directus* in the laboratory

Pauline Kamermans, Rob Witbaard, Emiel Brummelhuis, Arnold Bakker and Joël Cuperus, December 2010

Collecting

Use a 10L bucket for each 25 Ensis and fill it for 80% with sediment collected at the Ensis location. Small animals (<7 cm) can be placed 5.5L buckets. Place large individuals (>13/14 cm) in deeper buckets. Collect the animals with a boxcore. Sieve the content of the core over a 5 mm sieve with water and place the animals directly in the buckets with sediment. Place the buckets in larger containers with running seawater. Add air when the flow rate of the water is low. Keep the temperature close to *in-situ* conditions.

Transport

Place each bucket in a plastic bag filled with oxygen. Transport the buckets in a car and make sure they are stable.

Maintenance

Place the buckets in a basin with running seawater. Place a net over each bucket to avoid jumping of the Ensis and predation by birds. Add algae once a day. Avoid temperatures above 25 ^{0}C by adjusting the renewal rate of the water. Remove all animals that appear on the sediment surface.

Experimental set-up

Leave the animals at least one week in the basis to avoid working with weak individuals. Give the animals enough space to move around. You can fit 200 individuals of 40 mm to 100 individuals of 140 mm in a 1m^2 container.

Annex 2. Water quality parameters measured during the growth experiment

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
8-Jul-10	1			0				
8-Jul-10	2			2				
8-Jul-10	3			5				
8-Jul-10	4			20				
8-Jul-10	5			40				
9-Jul-10	1	0.2				7.98	17.9	25.8
9-Jul-10	2	0.2				7.95	18.5	25.6
9-Jul-10	3	0.2		17		7.95	20.1	26.2
9-Jul-10	4	0.2				7.95	18.0	25.9
9-Jul-10	5	0.2				7.95	18.0	26.0
10-Jul-10	1	0.2	0		9.08	8.11	18.0	33.8
10-Jul-10	2	0.1	0		9.23	8.13	18.0	33.8
10-Jul-10	3	0.1	0		9.18	8.12	18.0	29.1
10-Jul-10	4	0.2	0		9.25	8.12	17.8	34.1
10-Jul-10	5	0.1	0		9.29	7.99	17.8	34.4
11-Jul-10	1	0.2	0		9.43	8.19	17.5	34.2
11-Jul-10	2	0.1	0		9.36	8.14	17.5	34.1
11-Jul-10	3	0.1	0		9.36	8.17	17.6	33.6
11-Jul-10	4	0.2	0		9.37	8.16	17.5	34.2
11-Jul-10	5	0.1	0		9.34	7.92	17.7	34.3
12-Jul-10	1	0.1	0		7.76	7.84	18.8	25.8
12-Jul-10	2	0.2	0		7.73	7.94	18.3	25.7
12-Jul-10	3	0.2	0		7.74	7.76	18.4	25.8
12-Jul-10	4	0.3	0		7.74	7.92	18.8	26.0
12-Jul-10	5	0.1	0		7.71	8.05	18.0	26.2
13-Jul-10	1	0.1			7.9	8.16	17.9	26.0
13-Jul-10	2	0.1			7.9	8.14	17.9	25.9
13-Jul-10	3	0.1			7.9	8.13	18.0	25.8
13-Jul-10	4	0.2			7.8	8.19	18.1	25.9
13-Jul-10	5	0.2			7.5	8.85	18.7	26.2
14-Jul-10	1	0.1			99.5%	8.16	18.2	34.0
14-Jul-10	2	0.1			99.2%	8.14	18.1	33.9
14-Jul-10	3	0.2			99.4%	7.20	17.9	34.0
14-Jul-10	4	0.3		15	99.5%	7.88	17.8	34.5
14-Jul-10	5	0.3		55	99.5%	8.10	18.1	34.9
15-Jul-10	1	0.1			99.3%	8.06	18.6	34.0
15-Jul-10	2	0.2			98.5%	8.05	18.4	33.9
15-Jul-10	3	0.1			98.7%	8.02	18.2	34.0
15-Jul-10	4	0.4			98.7%	8.02	18.1	34.4
15-Jul-10	5	0.4			98.4%	8.06	18.2	34.9
16-Jul-10	1	0.2	0		9.18	8.10	18.2	52.1 ms/cm
16-Jul-10	2	0.2	0		9.29	8.08	17.9	52.1 ms/cm

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
16-Jul-10	3	0.1	0		9.32	7.97	17.8	52.0 ms/cm
16-Jul-10	4	0.2	0		9.33	8.17	17.9	52.6 ms/cm
16-Jul-10	5	0.4	0		9.32	8.27	17.9	53.2 ms/cm
17-Jul-10	1	0.2	0		9.39	8.12	18.0	52.1 ms/cm
17-Jul-10	2	0.1	0		9.26	8.05	18.0	52.1 ms/cm
17-Jul-10	3	0.1	0		9.23	8.05	18.0	52.0 ms/cm
17-Jul-10	4	0.1	0		9.37	8.26	18.1	52.4 ms/cm
17-Jul-10	5	0.4	0		9.27	8.31	18.2	52.8 ms/cm
18-Jul-10	1	0.3	0		9.49	8.09	17.7	52.0 ms/cm
18-Jul-10	2	0.2	0		9.5	8.08	17.8	45.5 ms/cm
18-Jul-10	3	0.2	0		9.51	8.11	17.6	51.6 ms/cm
18-Jul-10	4	0.3	0		9.59	8.40	17.6	52.5 ms/cm
18-Jul-10	5	0.6	0		9.61	8.44	17.7	53.1 ms/cm
19-Jul-10	1	0.4			7.9	8.05	18.6	51.5 ms/cm
19-Jul-10	2	0.3			7.9	8.02	18.3	52.4 ms/cm
19-Jul-10	3	0.5			7.8	8.16	18.2	51.8 ms/cm
19-Jul-10	4	1			7.9	7.98	18.3	52.5 ms/cm
19-Jul-10	5	1.8			8.1	8.08	18.1	52.8 ms/cm
20-Jul-10	1	0.2			9.32	8.10	18.2	34.0
20-Jul-10	2	0.4			9.23	8.02	18.0	34.3
20-Jul-10	3	0.4			9.18	7.91	18.0	34.2
20-Jul-10	4	0.6			9.24	7.96	18.1	34.4
20-Jul-10	5	1			9.27	8.00	18.2	34.7
21-Jul-10	1	0.2		0	9.28	8.07	18.5	34.0
21-Jul-10	2	0.4		10	9.22	7.96	18.2	34.2
21-Jul-10	3	0.4		8	9.07	7.70	18.3	34.2
21-Jul-10	4	0.6		21	9.17	7.84	18.3	34.4
21-Jul-10	5	1		42	9.24	7.75	18.3	34.7
22-Jul-10	1	0.2			9.23	8.18	18.4	33.9
22-Jul-10	2	0.4			9.27	7.93	18.1	34.2
22-Jul-10	3	0.4			9.19	7.60	18.0	34.3
22-Jul-10	4	0.6			9.20	7.69	18.0	34.4
22-Jul-10	5	0.6			9.29	7.67	18.0	34.7
23-Jul-10	1	0.1		0	8.2	8.21	18.1	
23-Jul-10	2	0.3		2.5	8.2	7.89	18.0	
23-Jul-10	3	0.3		6	8.1	7.86	18.1	
23-Jul-10	4	0.3		19	8.1	7.76	18.1	
23-Jul-10	5	0.5		38	8.1	7.79	18.1	
24-Jul-10	1	0.1			8.1	8.16	18.6	34.0
24-Jul-10	2	0.2			8.1	8.05	18.2	34.2
24-Jul-10	3	0.2			8.0	8.02	18.2	34.3
24-Jul-10	4	0.2			8.0	8.00	18.3	31.5
24-Jul-10	5	0.3			8.1	8.11	18.3	34.8
25-Jul-10	1	0.1			8.2	8.59	17.8	34.3
25-Jul-10	2	0.2			8.1	8.33	17.8	34.4
25-Jul-10	3	0.2			8.1	7.92	17.9	34.2

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
25-Jul-10	4	0.2			8.1	7.90	18.0	34.5
25-Jul-10	5	0.3			8.0	8.49	18.5	34.5
26-Jul-10	1	0.1			8.0	8.37	18.4	51.5 ms/cm
26-Jul-10	2	0.1			8.2	8.30	18.1	52.0 ms/cm
26-Jul-10	3	0.2			8.1	8.12	18.0	52.1 ms/cm
26-Jul-10	4	0.2		3	8.1	8.22	18.1	52.4 ms/cm
26-Jul-10	5	0.3		90	8.1	7.79	18.1	52.8 ms/cm
28-Jul-10	1	0.2			8.3	8.86	18.0	52.0 ms/cm
28-Jul-10	2	0.1			8.2	8.18	17.9	52.4 ms/cm
28-Jul-10	3	0.2			8.2	8.11	17.8	52.5 ms/cm
28-Jul-10	4	0.3			8.1	7.81	17.8	52.7 ms/cm
28-Jul-10	5	0.5			8.2	7.89	17.9	53.0 ms/cm
29-Jul-10	1	0.1	0		8.0	8.50	18.7	
29-Jul-10	2	0.1	0		8.2	8.10	17.8	
29-Jul-10	3	0.2	0		8.2	7.94	17.8	
29-Jul-10	4	0.2	0		8.1	8.06	17.9	
29-Jul-10	5	0.3	0		8.1	8.23	17.9	
30-Jul-10	1				8.3	8.28	17.7	34.3
30-Jul-10	2			107	7.9	8.28	19.6	34.4
30-Jul-10	3				8.1	8.34	18.1	34.4
30-Jul-10	4			28	8.2	7.90	17.8	34.5
30-Jul-10	5			39	8.3	8.17	17.7	34.9
31-Jul-10	1	0.1		0	8.3	8.56	18.2	34.6
31-Jul-10	2	0.1		1.38	8.2	8.39	17.9	31.6
31-Jul-10	3	0.2		8.48	7.8	8.15	17.7	34.7
31-Jul-10	4	0.2		25.6	8.1	7.63	17.8	34.7
31-Jul-10	5	0.4		31.4	8.2	7.67	17.5	35.0
1-Aug-10	1	0.1		0	8.4	8.46	17.8	34.3
1-Aug-10	2	0.1		5.2	8.3	8.14	17.8	34.2
1-Aug-10	3	0.2		5.68	8.2	7.93	17.6	32.6
1-Aug-10	4	0.2		19.27	8.1	8.34	17.7	34.8
1-Aug-10	5	0.4		20.63	8.2	8.47	17.6	35.1
2-Aug-10	1	0.1	0	0	8.4	8.09	17.8	34.3
2-Aug-10	2	0.1	0	1.33	8.4	7.96	17.5	34.2
2-Aug-10	3	0.2	0	9.15	8.3	7.74	17.5	34.7
2-Aug-10	4	0.2	0	21.22	8.3	7.70	17.5	34.8
2-Aug-10	5	0.5	0	15.2	8.4	7.87	17.5	35.1
3-Aug-10	1	0.1	0	0	8.4	8.12	17.7	34.3
3-Aug-10	2	0.1	0	16	8.4	8.04	17.5	34.3
3-Aug-10	3	0.2	0	10	8.3	7.92	17.5	34.8
3-Aug-10	4	0.2	0	10	8.4	8.00	17.5	34.9
3-Aug-10	5	0.4	0	38	8.4	8.09	17.5	35.3
4-Aug-10	1	0.1	0	0	8.1	8.10	18.5	34.4
4-Aug-10	2	0.1	0	5.2	8.2	8.07	18.1	34.4
4-Aug-10	3	0.2	0	4.9	8.2	7.99	18.1	34.8
4-Aug-10	4	0.2	0	19.8	8.2	7.95	18.1	35.8

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
4-Aug-10	5	0.4	0	45.4	8.2	7.89	18.2	35.0
5-Aug-10	1	0.1	0	0	8.3	8.09	17.6	34.5
5-Aug-10	2	0.1	0	3.3	8.2	8.05	17.7	34.5
5-Aug-10	3	0.2	0	2.66	8.2	7.94	17.8	34.7
5-Aug-10	4	0.2	0	28.55	8.0	8.08	18.1	34.6
5-Aug-10	5	0.2	0	42.82	8.0	8.09	18.2	34.5
6-Aug-10	1	0.1		0	7.9	8.22	17.8	34.2
6-Aug-10	2	0.1		2.74	8.3	8.06	17.7	34.3
6-Aug-10	3	0.2		4	8.3	7.94	17.8	34.8
6-Aug-10	4	0.2		21.52	8.3	7.75	17.6	34.9
6-Aug-10	5	0.3		46.05	8.3	8.24	17.6	35.0
7-Aug-10	1	0.1		0	8.2	8.42	17.4	34.6
7-Aug-10	2	0.1		1.37	8.4	8.44	17.3	34.7
7-Aug-10	3	0.2		7.06	8.4	8.19	17.3	34.9
7-Aug-10	4	0.2		12.25	8.4	8.29	17.4	35.0
7-Aug-10	5	0.2		45.79	8.3	8.51	17.5	35.1
8-Aug-10	1	0.1		0	8.4	8.06	17.6	34.7
8-Aug-10	2	0.1		0.65	8.4	7.92	17.5	34.7
8-Aug-10	3	0.2		5.16	8.4	7.85	17.5	34.9
8-Aug-10	4	0.2		7.25	8.4	7.99	17.6	34.9
8-Aug-10	5	0.3		33.87	8.3	8.09	17.6	35.1
9-Aug-10	1	0.1		0	8.0	8.35	18.5	34.0
9-Aug-10	2	0.2		0.96	8.2	8.29	18.2	34.4
9-Aug-10	3	0.2		6.5	8.2	7.81	18.1	34.8
9-Aug-10	4	0.3		19.07	8.3	7.85	18.2	34.8
9-Aug-10	5	0.5		36.2	8.3	8.06	18.1	35.0
10-Aug-10	1	0.1		0	8.4	8.14	17.5	34.7
10-Aug-10	2	0.2		2.54	8.3	8.04	17.6	34.7
10-Aug-10	3	0.2		13.4	8.3	7.95	17.6	35.0
10-Aug-10	4	0.2		33.07	8.3	7.89	17.8	34.9
10-Aug-10	5	0.3		47.99	8.0	7.78	18.1	34.6
11-Aug-10	1	0.1		0	8.4	8.48	17.5	34.8
11-Aug-10	2	0.1		0	8.4	8.39	17.5	34.8
11-Aug-10	3	0.1		3.94	8.3	8.27	17.7	35.1
11-Aug-10	4	0.2		20.75	8.2	8.33	18.0	34.9
11-Aug-10	5	0.4		37.9	7.9	8.34	18.7	34.7
12-Aug-10	1	0.1		0	8.5	8.53	17.3	34.9
12-Aug-10	2	0.1		0	8.4	8.56	17.4	34.8
12-Aug-10	3	0.1		1.82	8.5	8.4.3	17.5	35.1
12-Aug-10	4	0.2		33.09	8.3	8.47	17.6	35.3
12-Aug-10	5	0.4		37.21	8.3	8.44	17.6	35.4
13-Aug-10	1	0.1		0	8.5	8.25	17.3	34.9
13-Aug-10	2	0.15		1.5	8.5	8.19	17.4	34.8
13-Aug-10	3	0.1		8.9	8.4	7.83	17.5	35.1
13-Aug-10	4	0.3		74.5	8.3	7.84	17.6	35.3
13-Aug-10	5	0.6		15.9	8.2	7.92	17.6	35.2

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
14-Aug-10	1	0.1		0	9.39	8.30	17.4	34
14-Aug-10	2	0.1		0	9.30	8.32	17.5	34
14-Aug-10	3	0.1		0.93	9.24	8.24	17.7	34
14-Aug-10	4	0.2		75.25	9.14	7.86	17.8	34
14-Aug-10	5	0.4		46.37	8.76	8.02	18.0	34
15-Aug-10	1	0.1		0	9.39	8.27	17.4	34
15-Aug-10	2	0.1		0	9.32	7.40	17.5	31
15-Aug-10	3	0.1		0.25	9.31	7.83	17.5	30
15-Aug-10	4	0.3		47.85	9.18	6.60	17.8	31
15-Aug-10	5	0.5		25.44	9.19	7.58	17.7	32
16-Aug-10	1	0.1		0	9.26	8.16	17.7	35.0
16-Aug-10	2	0.1		0.1	9.22	8.55	17.7	34.9
16-Aug-10	3	0.1		0.31	9.15	7.01	17.7	35.2
16-Aug-10	4	0.2		32.37	9.16	8.18	18.0	35.2
16-Aug-10	5	0.3		18.96	8.26	8.89	18.6	35.1
17-Aug-10	1	0		0	8.70	7.99	17.8	35.0
17-Aug-10	2	0.1		5	8.70	7.78	17.9	35.0
17-Aug-10	3	0.1		12.9	8.50	7.68	18.1	35.3
17-Aug-10	4	0.3		30	8.35	7.67	18.1	35.4
17-Aug-10	5	0.4		52	8.15	7.82	18.2	35.0
18-Aug-10	1	0.1		0	8.1	8.65	17.9	34.5
18-Aug-10	2	0.1		6.33	8.2	8.51	17.9	35.0
18-Aug-10	3	0.1		2.93	8.2	8.31	17.9	35.3
18-Aug-10	4	0.3		43.6	8.1	7.79	17.9	35.6
18-Aug-10	5	0.4		17	8.2	7.74	17.8	35.5
19-Aug-10	1	0.1		0				
19-Aug-10	2	0.1		2.64	8.3	8.35	17.5	35.1
19-Aug-10	3	0.1		1.35	8.0	8.39	18.4	34.9
19-Aug-10	4	0.3		32.3	8.2	7.78	17.7	35.8
19-Aug-10	5	0.4		29.6	8.3	7.91	17.6	35.7
20-Aug-10	1	0.1		0	8.4	8.58	17.6	35.1
20-Aug-10	2	0.1		8.79	8.3	8.51	17.7	35.2
20-Aug-10	3	0.1		3.8	8.3	8.52	17.8	35.4
20-Aug-10	4	0.2		17.42	8.3	8.45	17.9	35.7
20-Aug-10	5	0.3		35	8.0	8.49	18.1	35.4
21-Aug-10	1	0.1		0	7.9	8.49	18.7	35.1
21-Aug-10	2	0.1		11.14	8.3	8.43	18.0	35.2
21-Aug-10	3	0.1		0.16	8.4	8.51	18.0	35.4
21-Aug-10	4	0.2		11.59	8.4	7.97	17.9	35.7
21-Aug-10	5	0.3		181.76	8.4	8.04	17.9	35.4
22-Aug-10	1	0.1		0	8.4	8.89	18.1	35.1
22-Aug-10	2	0.1		4.74	8.3	8.71	17.9	35.3
22-Aug-10	3	0.1		0.01	8.3	8.47	18.0	35.4
22-Aug-10	4	0.2		6.32	8.3	8.35	17.9	35.7
22-Aug-10	5	0.3		115.74	8.0	7.99	18.0	36.2
23-Aug-10	1	0.1		0	8.3	8.69	18.0	35.3

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
23-Aug-10	2	0.2		2.33	8.3	8.45	18.0	35.4
23-Aug-10	3	0.1		0.63	8.3	8.52	18.0	35.5
23-Aug-10	4	0.3		4.37	8.1	8.32	18.0	35.8
23-Aug-10	5	0.4		61.86	7.8	8.08	18.2	36.4
24-Aug-10	1	0		0	8.3	8.67	18.2	35.0
24-Aug-10	2	0.2		0.58	8.2	8.59	18.0	35.2
24-Aug-10	3	0.1		11.75	8.3	8.58	18.1	35.5
24-Aug-10	4	0.3		31.15	8.2	8.54	18.1	35.7
24-Aug-10	5	0.5		21.31	8.0	8.45	18.3	35.8
25-Aug-10	1	0		0	8.4	8.28	17.5	35.1
25-Aug-10	2	0.2		2.64	8.3	8.25	17.5	35.3
25-Aug-10	3	0.1		5.27	8.3	8.15	17.7	35.5
25-Aug-10	4	0.2		26.76	8.3	8.01	17.9	35.9
25-Aug-10	5	0.4		41.54	8.1	8.03	18.0	35.8
26-Aug-10	1	0.05		0	8.3	8.64	17.6	35.1
26-Aug-10	2	0.2		4.2	8.3	8.51	17.6	35.4
26-Aug-10	3	0.1		6.36	8.3	8.53	17.7	35.6
26-Aug-10	4	0.2		24.68	8.2	8.47	17.8	36.0
26-Aug-10	5	0.6		17	7.9	8.47	18.3	35.5
27-Aug-10	1	0.05		0	8.2	8.56	17.7	35.1
27-Aug-10	2	0.1		3.44	8.2	8.49	17.8	35.3
27-Aug-10	3	0.1		4.53	8.2	8.52	17.8	35.6
27-Aug-10	4	0.2		20.14	8.1	8.40	17.9	35.6
27-Aug-10	5	0.4		17	8.0	8.45	17.8	35.5
28-Aug-10	1	0		0	8.3	8.58	17.8	34.3
28-Aug-10	2	0.2		13.56	8.4	8.47	17.9	35.2
28-Aug-10	3	0.1		10.26	8.4	8.43	18.0	35.6
28-Aug-10	4	0.2		13.33	8.3	8.33	18.1	35.8
28-Aug-10	5	0.6		40	8.3	8.26	18.1	35.8
29-Aug-10	1	0		0	8.3	8.37	17.9	34.5
29-Aug-10	2	0.2		6.96	8.3	8.26	17.8	35.3
29-Aug-10	3	0.1		0	8.3	8.21	17.9	35.4
29-Aug-10	4	0.2		17.58	8.3	8.02	17.9	35.8
29-Aug-10	5	0.6		41.05	8.3	7.94	17.9	35.5
30-Aug-10	1	0		7.28	8.4	8.63	17.9	34.5
30-Aug-10	2	0.2		1.77	8.4	8.44	17.9	35.2
30-Aug-10	3	0.1		2.37	8.4	8.11	17.9	35.4
30-Aug-10	4	0.2		16	8.3	7.81	18.0	35.8
30-Aug-10	5	0.6		17.77	8.3	7.90	18.0	35.5
31-Aug-10	1	0.05		0	8.2	8.36	18.5	34.3
31-Aug-10	2	0.1		17.25	8.3	8.27	17.8	35.1
31-Aug-10	3	0.05		3.4	8.3	7.86	17.8	35.2
31-Aug-10	4	0.2		25.82	8.3	7.73	17.9	35.7
31-Aug-10	5	0.3		55.63	8.3	7.74	18.2	34.9
2-Sep-10	1	0.2			8.4	8.33	17.4	34.7
2-Sep-10	2	0.2			8.4	8.29	17.5	35.3

Date	container	NO2 (mg/l)	NH4 (mg/l)	chla (ug/l)	O2 (mg/l)	pH	Temp	Sal
2-Sep-10	3	0.1			8.3	8.03	17.7	35.3
2-Sep-10	4	0.2		7.4	8.3	8.29	17.9	35.7
2-Sep-10	5	0.3		14.86	8.3	8.29	17.9	35.1
3-Sep-10	2			0				
3-Sep-10	3			0				
6-Sep-10	1	0.1			8.4	8.36	17.5	
6-Sep-10	2	0.2			8.4	8.26	17.4	35.5
6-Sep-10	3	0.1			8.4	8.01	17.5	35.3
6-Sep-10	4	0.2		7	8.3	7.75	17.6	35.5
6-Sep-10	5	0.5		4.6	8.2	8.06		35.7
8-Sep-10	4			31.7				
8-Sep-10	5			39.5				
10-Sep-10	1	0.1			8.64		18.1	
10-Sep-10	2	0.3					18.0	
10-Sep-10	3	0.2			8.41		18.0	
10-Sep-10	4	0.5					18.0	
10-Sep-10	5	0.3					17.9	
10-Sep-10	1	0.05						
10-Sep-10	2	0.1			8.35			
10-Sep-10	3	0.1			8.55		17.9	
10-Sep-10	4	0.1						
10-Sep-10	5	0.2						
11-Sep-10	1	0.05			8.36	8.12	17.8	34.9
11-Sep-10	2	0.1			8.42	8.12	17.8	35.5
11-Sep-10	3	0.1			8.45	8.13	17.9	35.3
11-Sep-10	4	0.1			8.46	8.16	18.2	35.4
11-Sep-10	5	0.2			8.48	8.17	18.9	34.7
12-Sep-10	1	0.05			8.16	8.16	18.1	
12-Sep-10	2	0.1			8.44	8.20	17.9	
12-Sep-10	3	0.1			8.49	8.16	17.9	
12-Sep-10	4	0.1			8.52	8.21	18.0	
12-Sep-10	5	0.2			8.47	8.27	18.0	

Annex 3. Results of statistical tests

3-1 Filtration

Ranks

	adw	N	Mean Rank
Cr/ind	.000	160	130.79
	1.000	142	174.84
	Total	302	

Test Statistics[a,b]

	Cr
Chi-Square	19.144
Df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: adw

Ranks

	adw	N	Mean Rank
Cr/adw	.000	160	176.24
	1.000	142	123.62
	Total	302	

Test Statistics[a,b]

	cr
Chi-Square	27.321
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: adw

Ranks

	batch	N	Mean Rank
Cr/ind	6	110	110.92
	7	192	174.75
	Total	302	

Test Statistics[a,b]

	Cr
Chi-Square	37.367
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	batch	N	Mean Rank
Cr/adw	6	110	187.05
	7	192	131.14
	Total	302	

Test Statistics[a,b]

	Cr
Chi-Square	28.668
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	Container	N	Mean Rank
Cr/ind	2	67	140.19
	3	54	77.30
	4	67	173.36
	5	114	180.45
	Total	302	

Test Statistics[a,b]

	Cr
Chi-Square	56.840
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

Test Statistics[a,b]

	Cr
Chi-Square	56.840
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

Ranks

	Container	N	Mean Rank
Cr/adw	2	67	147.37
	3	54	97.81
	4	67	176.63
	5	114	164.59
	Total	302	

Test Statistics[a,b]

	Cr
Chi-Square	28.667
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

Ranks

	Container	N	Mean Rank
adw	2	67	141.25
	3	54	142.33
	4	67	143.54
	5	114	166.54
	Total	302	

Test Statistics[a,b]

	Adw
Chi-Square	5.480
df	3
Asymp. Sig.	.140

a. Kruskal Wallis Test

b. Grouping Variable: container

3-2 Respiration

Ranks

	Adw	N	Mean Rank
o2/ind	1.0000	106	54.58
	2.0000	5	86.00
	Total	111	

Test Statistics[a,b]

	o2opname
Chi-Square	4.549
df	1
Asymp. Sig.	.033

a. Kruskal Wallis Test

b. Grouping Variable: adw

Ranks

	Adw	N	Mean Rank
O2/adw	.0000	270	220.22
	1.0000	106	121.99
	2.0000	5	76.00
	Total	381	

Test Statistics[a,b]

	O2adw
Chi-Square	66.083
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: adw

Ranks

	batch	N	Mean Rank
o2/ind	6	262	132.97
	7	119	318.76
	Total	381	

Test Statistics[a,b]

	o2opname
Chi-Square	232.909
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	Batch	N	Mean Rank
O2/adw	6	262	216.24
	7	119	135.44
	Total	381	

Test Statistics[a,b]

	O2adw
Chi-Square	44.049
df	1
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	container	N	Mean Rank
o2/ind	2	112	154.09
	3	110	193.07
	4	85	215.40
	5	74	215.76
	Total	381	

Test Statistics[a,b]

	o2opname
Chi-Square	20.532
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

Ranks

	container	N	Mean Rank
O2/adw	2	112	161.38
	3	110	188.96
	4	85	187.16
	5	74	243.27
	Total	381	

Test Statistics[a,b]

	O2adw
Chi-Square	24.915
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

3-3 Food concentration

Ranks

	container	N	Mean Rank
chla	1.00	35	22.09
	2.00	35	65.60
	3.00	35	74.21
	4.00	35	129.61
	5.00	35	148.49
	Total	175	

Test Statistics[a,b]

	chla
Chi-Square	143.776
Df	4
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

3-4 Growth

Ranks

	iniww	N	Mean Rank
growthww	1.00	10	139.50
	2.00	40	125.73
	3.00	34	119.26
	4.00	22	119.09
	5.00	6	113.25
	6.00	1	1.00
	7.00	2	33.00
	8.00	2	66.00
	9.00	3	107.67
	10.00	2	35.50
	11.00	1	78.00
	13.00	1	57.00
	14.00	2	95.00
	16.00	3	64.33
	17.00	4	72.25
	18.00	3	37.00
	19.00	4	48.50
	20.00	8	48.69
	21.00	4	70.00
	22.00	3	9.33
	23.00	6	71.50
	24.00	7	88.57
	25.00	5	69.70
	26.00	1	76.00
	27.00	2	110.50
	28.00	3	85.50
	29.00	2	12.50
	30.00	2	134.50
	31.00	1	39.00
	32.00	3	46.67
	33.00	4	37.25
	35.00	2	98.00

	38.00	1	81.00
	48.00	1	79.00
	Total	195	

Test Statistics[a,b]

	growthww
Chi-Square	72.844
Df	33
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: iniww

Ranks

	inisl	N	Mean Rank
growthww	42.00	1	26.00
	43.00	1	188.00
	46.00	1	1.00
	47.00	1	193.00
	48.00	2	178.50
	49.00	2	164.00
	50.00	2	106.50
	51.00	4	131.00
	52.00	5	116.40
	53.00	3	164.33
	54.00	6	127.50
	55.00	10	136.70
	56.00	4	102.50
	57.00	8	110.44
	58.00	6	75.00
	59.00	10	131.35
	60.00	1	158.00
	61.00	5	101.70
	62.00	6	115.25
	63.00	5	153.40
	64.00	3	71.17

	65.00	7	124.21
	66.00	4	130.00
	67.00	6	99.67
	68.00	2	109.50
	69.00	2	130.50
	70.00	2	138.50
	71.00	3	125.33
	72.00	1	114.50
	79.00	1	6.00
	81.00	1	24.00
	83.00	1	58.00
	85.00	2	120.00
	87.00	1	108.00
	90.00	4	61.75
	94.00	1	56.00
	97.00	1	35.00
	98.00	1	76.00
	99.00	1	50.00
	100.00	1	86.00
	101.00	2	41.50
	102.00	1	83.00
	104.00	5	63.00
	105.00	3	35.33
	106.00	5	59.10
	107.00	2	60.50
	108.00	2	14.00
	109.00	2	63.00
	110.00	1	74.00
	111.00	5	64.20
	112.00	2	59.00
	113.00	2	26.50
	114.00	2	67.00
	115.00	5	87.30
	116.00	2	72.00
	117.00	2	38.00
	118.00	3	85.67

	119.00	3	57.33
	120.00	1	75.00
	121.00	2	115.00
	122.00	1	95.50
	123.00	1	47.00
	124.00	1	23.00
	126.00	4	62.75
	129.00	2	107.50
	130.00	2	101.00
	131.00	1	13.00
	133.00	1	78.00
	135.00	1	89.00
	Total	194	

Test Statistics[a,b]

	growthww
Chi-Square	91.617
Df	68
Asymp. Sig.	.030

a. Kruskal Wallis Test

b. Grouping Variable: inisl

Ranks

	batch	N	Mean Rank
growthww	1.00	107	121.87
	2.00	3	52.33
	3.00	11	60.18
	4.00	4	167.25
	5.00	70	65.45
	Total	195	

Test Statistics[a,b]

	growthww
Chi-Square	55.360
Df	4
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	batch	N	Mean Rank
growthsl	1.00	107	138.21
	2.00	3	44.00
	3.00	11	40.18
	4.00	4	145.75
	5.00	70	45.21
	Total	195	

Test Statistics[a,b]

	growthsl
Chi-Square	142.944
Df	4
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	container	N	Mean Rank
growthww	1.00	45	61.73
	2.00	30	78.68
	3.00	35	106.79
	4.00	36	117.50
	5.00	49	122.53
	Total	195	

Test Statistics[a,b]

	growthww
Chi-Square	36.502
Df	4
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: container

Ranks

	container	N	Mean Rank
growthsl	1.00	45	78.48
	2.00	30	85.75
	3.00	35	97.29
	4.00	36	111.86
	5.00	49	113.76
	Total	195	

Test Statistics[a,b]

	growthsl
Chi-Square	13.782
Df	4
Asymp. Sig.	.008

a. Kruskal Wallis Test

b. Grouping Variable: container

3-5 Survival

Ranks

	batch	N	Mean Rank
surv	1.00	5	19.50
	2.00	5	7.00
	3.00	5	9.20
	4.00	5	11.40
	5.00	5	17.90
	Total	25	

Test Statistics[a,b]

	Surv
Chi-Square	11.081
df	4
Asymp. Sig.	.026

a. Kruskal Wallis Test

b. Grouping Variable: batch

Ranks

	container	N	Mean Rank
surv	1.00	5	13.00
	2.00	5	9.30
	3.00	5	10.70
	4.00	5	12.90
	5.00	5	19.10
	Total	25	

Test Statistics[a,b]

	Surv
Chi-Square	5.222
df	4
Asymp. Sig.	.265

a. Kruskal Wallis Test

b. Grouping Variable: container