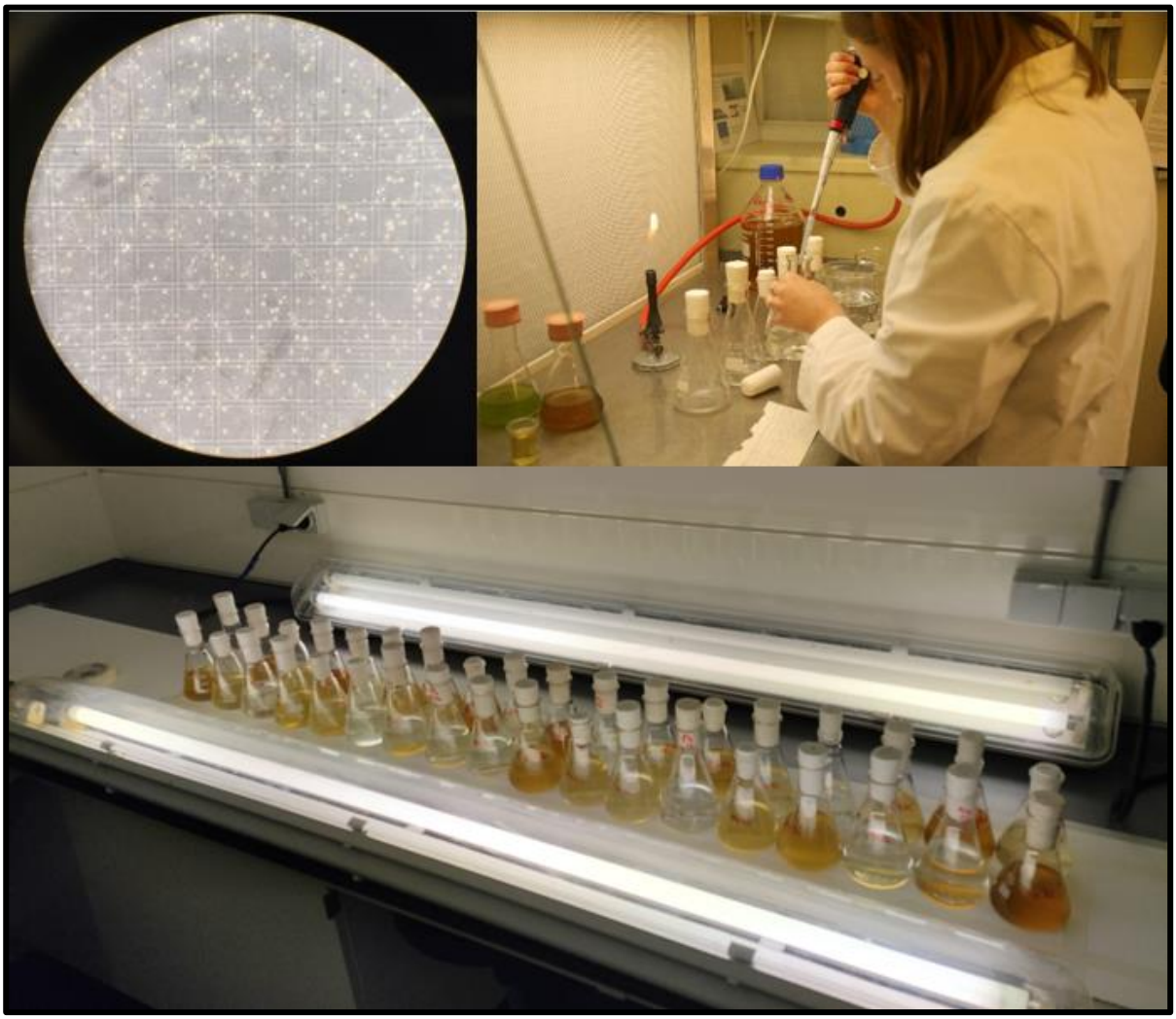
Harbour porpoise (*Phocoena phocoena*) faeces effects on phytoplankton growth in the North Sea and Eastern Scheldt



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Summary

CO_2 emissions are rising and form the predominant driver of climate change. The ocean acts as a carbon sink with phytoplankton acting as a primary producer. Whales enhance primary production through nutrient-rich faeces that add limited nutrients such as nitrogen, phosphor and iron to the surface waters. Studies have been focussing on greater whales and a knowledge gap exists on whether much smaller cetaceans provide these same services. The harbour porpoise (*Phocoena phocoena*) is one of the smallest cetaceans in the world and the most common whale species in the North Sea and its estuaries, but its contribution to the biological pump is unknown in these productive waters. A preliminary study by Rugvin Foundation by Van Burken in 2022 gave the first insight into the effects of harbour porpoise faeces on phytoplankton growth in the Eastern Scheldt. This study was continued but aimed at investigating these effects in the North Sea. A set of experiments were performed to monitor the growth (cell density and biomass) of the algae species *Phaeodactylum tricornutum, Nannochloropsis, Skeletonema costatum, and Phaeocystis globosa* over time in North Sea water when added to different concentrations of harbour porpoise faeces. Data from the preliminary study was included to analyse differences between these locations. Results show that the growth of the phytoplankton in most cases was enhanced by the harbour porpoise faeces. However, no clear relationship was found between the different faeces concentrations and the amount of algal growth in most cases. The phosphor and iron in the faeces had respectively a 559 and 191 times higher concentration than measured in seawater, providing many nutrients for algal growth that are otherwise limited in seawater. Nutrient concentration of the Eastern Scheldt was found to be higher than for the North Sea, but growth was found to be stronger when North Sea water was used. To determine the contribution of harbour porpoise faeces to primary production more factors like population densities, distribution, diet and environmental factors need to be studied. Also, more research on whale faecal nutrient composition should be executed to compare smaller cetaceans with great whales. Multiple adjustments need to be made to the methods to accurately compare results between studies. These suggestions would provide a stronger base to make statements on how the harbour porpoise can contribute to primary production in Dutch waters, contribute to nutrient cycling and how the impact of small cetaceans differs from great whales in less productive oceans.

Contents

1. Introduction

CO_2 emissions are rising and form the predominant driver of climate change. Emissions from fossil fuels, industry and land use change have been exponentially rising since the 1950s reaching a global emission of 37 billion tonnes per year in 2021 (Ritchie et al., 2020). The ocean acts as a carbon sink and has absorbed an estimated 25% of all anthropogenic CO_2 emissions from the atmosphere from the 1960s to the 2010s (Gruber et al., 2023). The biological carbon pump of the ocean takes CO_2 out of the atmosphere (Boyd, 2015; Sanders et al., 2014) because phytoplankton acts as a primary producer, by fixing organic and inorganic carbon in the euphotic zone and transferring it to the ocean interior and sediments (Chisholm, 1995; Hülse et al., 2017). Half of the primary production on earth is estimated to be contributed to phytoplankton fixing about 50 Gt of carbon per year (Carr et al., 2006; Field et al., 1998; Kulk et al., 2020). Rising atmospheric CO_2 levels are expected to change the marine environment (Basu & Mackey, 2018). Increasing sea surface temperatures cause enhanced stratification and fewer mixing events which are expected to lead to nutrient limitation in the euphoric layer (Li et al., 2012; Riebesell et al., 2007). This could limit the ability of phytoplankton to fix atmospheric carbon (Basu & Mackey, 2018).

Recent studies show that marine vertebrates positively influence the capacity of marine ecosystems to fix or sequester carbon (Atwood et al., 2018; Lutz & Martin, 2014; Meynecke et al., 2023; Roman et al., 2014; Roman & McCarthy, 2010). Whales enhance primary production through carbon sequestering, horizontal transfer and vertical mixing of nutrients plus recycling and sequestering of carbon (Lavery et al., 2010; Roman et al., 2014; Roman & McCarthy, 2010; Smith et al., 2013). Firstly, the foraging behaviour of whales, which entails diving and surfacing, enhances the upward transport of nutrients to surface waters from nutrient-rich deep waters also called the “whale pump” (Dewar et al., 2006; Roman et al., 2014). Secondly, the whale conveyor belt, meaning the migration of whales between feeding areas and calving grounds, transports nutrients from carcasses, urea, and placenta from high-productivity to low-productivity areas (Roman et al., 2014). Moreover, carcasses from whales that sink to the ocean floor (whale falls) act as a carbon sink, sequestering approximately 1600 kg of carbon into the deep sea (e.g. one 40 tonnes gray whale (*Eschrichtius robustus*))(Pershing et al., 2010; Smith, 2007). Lastly, multiple studies show that the nutrient-rich faeces of whales play an important role in marine nutrient cycling (Lavery et al., 2014; Nicol et al., 2010; Smith et al., 2013). In marine systems, nitrogen is often a limiting nutrient for phytoplankton primary production (Rabalais, 2002). Nitrogen is added to surface waters, where phytoplankton grows, because of floating faecal plumes and urine when whales defecate, feed and rest near the surface (Pennisi, 2015; Roman & McCarthy, 2010). The plumes also contain a high iron and phosphor concentration which are also often limited micronutrients in the ocean (Freitas et al., 2023; Nicol et al., 2010; Smetacek et al., 2012).

Research focusing on the effects of whale faeces on phytoplankton growth showed that the faeces of the pygmy blue whale (*Balaenoptera musculus brevicauda)* increased biomass of multiple phytoplankton species, with increasing faecal concentrations (Smith et al., 2013) and that in the small management areas around Svalbard, faeces from an estimated 15000 minke whales (*Balaenoptera acutorostrata)*, due to the release of phosphorus, could stimulate the primary production of around 400 tonnes of carbon per day (Freitas et al., 2023).

All in all, the fact that whales can play an important role as ecosystem engineers and contribute to increased phytoplankton primary production and a healthy food web is clear. However, studies have been focussing mostly on greater whales (baleen whales and the sperm whale) and a knowledge gap exists on whether much smaller cetaceans provide these same services.

The harbour porpoise (*Phocoena phocoena*) is one of the smallest cetaceans in the world and the most common whale species in the North Sea (Zanderink & Osinga, 2020). Whether this smaller, but abundant, cetacean with different behavioural and physiological characteristics contributes similarly to the biological pump is unknown. Compared to the open ocean, the North Sea and its estuaries are considered to be more productive (Capuzzo et al., 2017; Watson et al., 2015). The North Sea deals with relatively high anthropogenic input of nitrogen and phosphor from rivers (Jickells, 1998; Lancelot et al., 2014), however, riverine nutrient loads have decreased due to stricter policy on the use of fertilizers (Burson et al., 2016).

The question remains to what extent the harbour porpoise can contribute to phytoplankton growth and primary production in the North Sea strengthening the base of the food web, and possibly mitigating climate change. A preliminary study by Rugvin Foundation in 2022 gave the first insight into the effects of harbour porpoise faeces on phytoplankton growth in the Eastern Scheldt. Due to expected differences in productivity between the North Sea and the Eastern Scheldt, and in general the limited area of the Eastern Scheldt, this study continued the research but aimed at investigating the effects of harbour porpoise faeces on the growth of phytoplankton in the North Sea and looked at differences in impact between the two locations. In addition, this study focussed on the differences in water quality (N, P, Fe) between the North Sea (nearshore and offshore) and Eastern Scheldt (East and West), and the differences in faeces' nutrient composition between greater whales and small cetaceans. A set of experiments were performed to monitor the growth of multiple algae species in North Sea water after the addition of harbour porpoise faeces in different concentrations. Data from the preliminary study performed by Van Burken in 2022, was included to analyse differences between these locations. Due to higher overall nutrient concentrations in coastal waters, it is expected that the algae species will grow less with the North Sea water than the water from the Eastern Scheldt. Also, it is expected that there is a positive relationship between faeces concentration and algal growth, for some but not all species, based on preliminary results by Van Burken.

2. Methods

2.1 Location

This study was conducted in a laboratory at the Zeeschelp Foundation in Kamperland, Zeeland. Zeeschelp Foundation is an aquaculture farm specialising in algae, seaweed, bivalve, flatfish and cetacean culture.

2.2 Water sample

Water samples from the North Sea were collected twice between the end of April and the beginning of May approximately 100 km offshore and 6 meters deep by a Stena Line ferry (route Hoek van Holland, NL – Harwich, UK). The water was removed from any algae, bacteria, larvae etc. without changing the nutrient composition by filtering the water with 1.2µm Whatman glass microfiber filters and a vacuum filtration flask. Data on the nutrient composition of surface water from the North Sea and Eastern Scheldt were retrieved by Rijkswaterstaat due to difficulties and time constraints in finding an available external party for nutrient analysis.

2.3 Harbour porpoise faeces

2.3.1 Sample collection

Faeces samples were collected during necropsies of stranded harbour porpoises along the Dutch North Sea coast by the faculty of Veterinary Medicine at Utrecht University. Faecal samples were collected and frozen. During the first series of experiments previously stored samples at the Zeeschelp Foundation were used. For the second series of experiments new samples were obtained from Utrecht University.

2.3.2 Sample preparations

Samples of nine individuals (first batch) and six individuals (second batch) were mixed excluding samples of calves due to expected different nutrient compositions in comparison to adults. The filtered seawater and faeces samples were mixed according to the method used by Van Burken where 936 mL of faeces solution was made with 5.24 gr of faeces (wet weight) and filtered seawater. The solution was then placed on a mixing table for a minimum of 18 hours to prevent clotting when filtering the solution again with the 1.2µm Whatman glass microfiber filters and vacuum filtration flask. The second batch of samples contained faeces contaminated with the Brucella bacteria (zoonosis). Therefore, more safety measures were followed including working in a fume hood and wearing a medical mask, lab coat, and gloves to prevent infection.

No faeces was left for nutrient analysis, however, a few faecal samples were analysed for nutrient contents during the previous study by van Burken (2020). This data was used to get an estimation of the faecal nutrient contents of the harbour porpoise. The faeces solution was also analysed for nutrient content because of differences in colour between the faeces solution made with

the first batch of samples and the second batch. The pH, ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and iron (Fe) concentrations were determined with simplistic water quality tests which gave an indication of the nutrient concentrations rather than an accurate value. A literature study was performed to study the nutrient composition of the faeces of greater whales.

2.4 Marine phytoplankton

2.4.1 Species

The marine phytoplankton species chosen for this study are *Phaeodactylum tricornutum, Phaeocystis globose, Nannochloropsis oceanica, and Skeletonema costatum*. All species are common in Dutch waters (Australian Government Office of the Gene Technology Regulator, 2019; Karlson et al., 2021; Martino et al., 2007; Peeters & Peperzak, 1990) and have been successfully cultured at the Zeeschelp Foundation. *Phaeocystis* blooms, on the other hand, can have harmful effects on the environment (Karlson et al., 2021) and this species was added to test whether harbour porpoise faeces could also stimulate the growth of harmful species.

2.4.2 Culture

Start-up cultures of the algae species were available at Zeeschelp Foundation and were inoculated in 250 mL erlenmeyers with filtered seawater. The culture was left for a minimum of seven days in a stable environment with fluorescent lights at a temperature of ±20-22°C to grow to a high density. Before the start of the experiment, the cell density of algae was checked by counting the cells under the microscope (for detailed methods see Chapter 2.5.3). The target density was $15*10^6$ cells/mL since this starting density was maintained by Van Burken (2022). In case the starting density was too low, samples were centrifuged and water was removed until the sample had reached a more favourable density.

2.5 Effects of porpoise faeces on phytoplankton growth

2.5.1 Treatments and set-up

Sterilized erlenmeyers (250 mL) were filled with a 200 mL solution of a mix of filtered water from the North Sea, one of the four faecal solutions and one of the four algae species (Table 1), according to proportions used by Van Burken (2022). The absolute concentrations were doubled for every treatment since more solution was needed since this study also included the parameter biomass. In addition, a positive control and negative control were included in the experiment. Instead of the faeces solution, the growth medium L1 was used for the positive control. The medium is used at Zeeschelp Foundation to optimally grow algae and serves as a health check of the algae. No faeces solution was added to the negative control.

Table 1 The quantities of faeces solution, seawater, algae and growth medium per treatment.

	Control (+)	Control (-)	Faeces concentration 1	Faeces concentration 2	Faeces concentration 3	Faeces concentration 4
Faeces solution (mL)			36	64	112	200
Seawater (mL)	197.4	198	162	134	86	0
Algae (mL)	2	2	2	2	2	2
Grow medium L1 (mL)	0.6					
Total	*200*	*200*	*200*	*200*	*200*	*200*

All combinations were performed in triplets. In total 72 combinations, with 36 erlenmeyers at a time (so two rounds) were placed in a line between fluorescent tubes for two weeks (Figure 1). The lights were turned on all day to enhance algal growth. The room's ambient temperature was set at 20-21°C. A randomised block design was used to compensate for the erlenmeyers at the ends of the set-up potentially having less light (Appendix I: Randomised block design). To prevent clogging of algae, the solution was carefully stirred every day by moving the erlenmeyers in a circular motion.

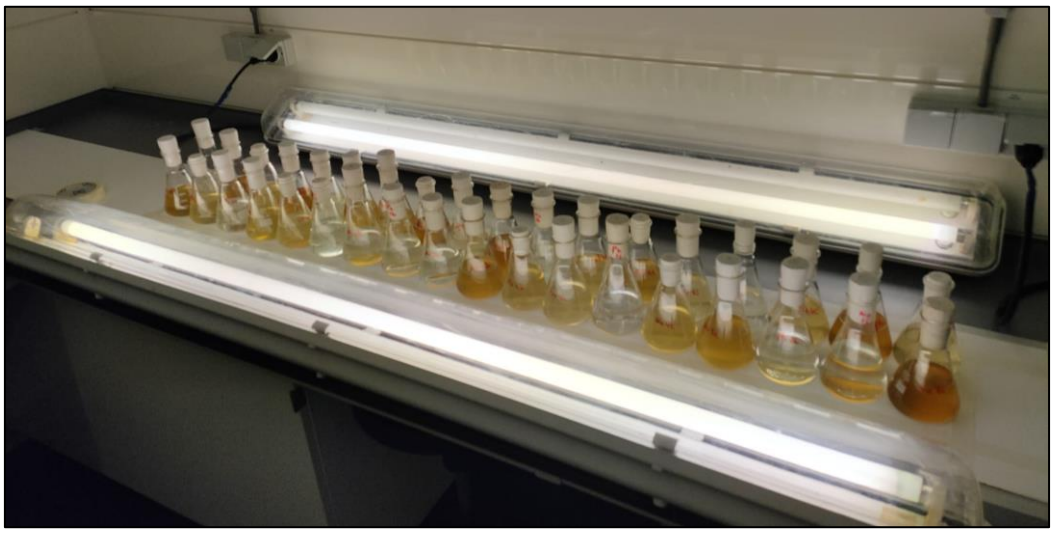

Figure 1 Experimental set-up with 36 erlenmeyers with seawater, algae and faeces between two white light tubes.

2.5.2 Measurements

Cell density

The cell density of the algae was determined per erlenmeyer every 2 or 3 days for two weeks. An mL sample was taken with a pipet by stirring the erlenmeyer before sampling and by briefly heating the neck of the erlenmeyer with a burner before and after sampling. The stopper was also briefly heated before closing the erlenmeyer. Using the burner served to minimize bacteria entering the erlenmeyers. The samples were fixated with one drop of Lugol Iodine solution.

The algae cell density was determined with a Bürker-Türk counting chamber, microscope and mechanical counter. The cells are counted per square of the counting chamber and the cell density (cells/mL) was calculated as $\frac{nr\ cells}{(nr \times lentgh \times width\ squares) * (1 * 10^{-4})}$

Dry weight

In comparison to Van Burken (2022), a second parameter was added to the experiments. The dry weight of the algae was estimated every 3 or 4 days per erlenmeyer. A 30 mL sample was taken (again using the burner to prevent bacteria entering the flask) from every erlenmeyer and 1.2 µm filter papers and a vacuum filtration flask were used to filter the algae from the solution. The dry weight (g/mL) could be calculated by weighing the filter paper before filtration and after filtration after the sample was dried in a dry oven for 24 hours. Filters were kept in a desiccator to cool before weighing. Since every treatment started with the same concentration and volume of algae, only one measurement to estimate the biomass per algae species was done at t0. A separate 2 mL of algae was diluted with 198 mL of seawater. A 30 mL sample was taken from this solution for the measurement.

2.5.3 Data analysis

Data on the cell density and biomass over time for all treatments was put in Excel and data from the Eastern Scheldt trial (Van Burken 2022) was added. Due to differences in starting density of the algae, making comparison between data for both locations and between species difficult, an adjusted cell density was calculated for every erlenmeyer as t_x-t_0, with a cell density of 0 cells/mL at t_0 for all erlenmeyers. Also, the growth rate (cells/day and g/day) for every measurement was calculated as (t_x-t_{x-1})/t.

In R studio (V 4.1.1), the maximum cell density (cells/mL), maximum biomass (g/mL), the total number of cells (nr cells), total biomass (g) and the maximum growth rate (cells/day and g/day) per erlenmeyer were calculated. Shapiro-Wilk tests were performed on all parameters to check normality. Log- and square root transformations were tried on parameters with a non-normal distribution. The total biomass, log-transformed maximum biomass and growth rate (g/day) showed a normal distribution. Polynomial trendlines of the cell density and biomass over time were plotted to visualize growth and boxplots were created per treatment per location for every algae species to visualize the distribution of data. Significant differences between the treatments, locations, species and were tested by generating General Linear Models. The family per model was based on the distribution and nature of the data. Assumptions attributed to the families were tested. The total cell count, the maximum cell density and the maximum growth (cells/day) are count data and were not normally distributed. The data was over dispersed and a negative binomial was concluded as the best fit, although not all assumptions attributed to this family were met. Data on the total biomass, the log-transformed maximum biomass and growth rate (g/day) were normally distributed and therefore a Gaussian family was chosen for these GLMs. The factors “treatment”, “location” and “species”, were added to the model to predict the effects of the abovementioned parameters.

3. Results

Algal growth was studied in relation to different concentrations of porpoise faeces in a solution of filtered water from the North Sea. Data was successfully recorded for *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Phaeocystis globosa*. However, the culture of *Skeletonema costatum* was taken over twice by a second unidentified algae species, suppressing the *Skeletonema* culture, which quickly collapsed. Therefore, no data for this species during the North Sea water trial is presented. In addition, the faeces solution made with the first batch of samples turned out darker than the second solution and the solution used by Van Burken. Therefore, it is highly likely that the first solution used for the trial with *Phaeodactylum* and *Nannochloropsis* contained a higher concentration of nutrients. Data of all four species from the Eastern Scheldt trial performed by Van Burken (2022) was added for analysis. The parameters sum cell count/biomass and maximum growth are discussed in this chapter. The results for the maximum cell count/biomass can be found in Appendix V: Supplementary results.

3.1 Cell density

Algal growth in terms of cell density over time is shown in Figure 2 for the four algae species for both locations. The North Sea trial was conducted until t14 and data for the Eastern Scheldt trial was available until t10 or t12. For all trials, the positive control showed normal growth meaning the trials were conducted with a successful culture of algae. An exception, however, is the positive control for the *Phaeocystis* trial (North Sea water) which was taken over by *Skeletonema* cells, causing the *Phaeocystis* to crash.

From Figure 2 can be stated that cell density did not directly increase after t0, but after a couple of days and sometimes cell density even dropped after t0 before growth started. Some growth curves have reached a maximum after which growth collapsed while other the cell density of other species is still increasing at the last measuring day.

Nannochloropsis: For both North Sea and Eastern Scheldt trials the treatments showed a higher density of cells than the negative control. However, the higher concentrations of faeces do not show an increased cell density.

Phaeodactylum: For the Eastern Scheldt trial the treatments with a higher faeces concentration showed an overall higher cell density. This cannot be seen for the North Sea trial.

Phaeocystis: For the North Sea trial a clear pattern is visible between increased faeces concentration and an increased cell density. However, a different pattern can be seen for the Eastern Scheldt trial since all concentrations of faeces resulted in lower cell density than the negative control.

Skeletonema: For the Eastern Scheldt trial growth for concentration 1-3 was higher than for the negative control. However, the highest concentration of faeces was lower than the negative control.

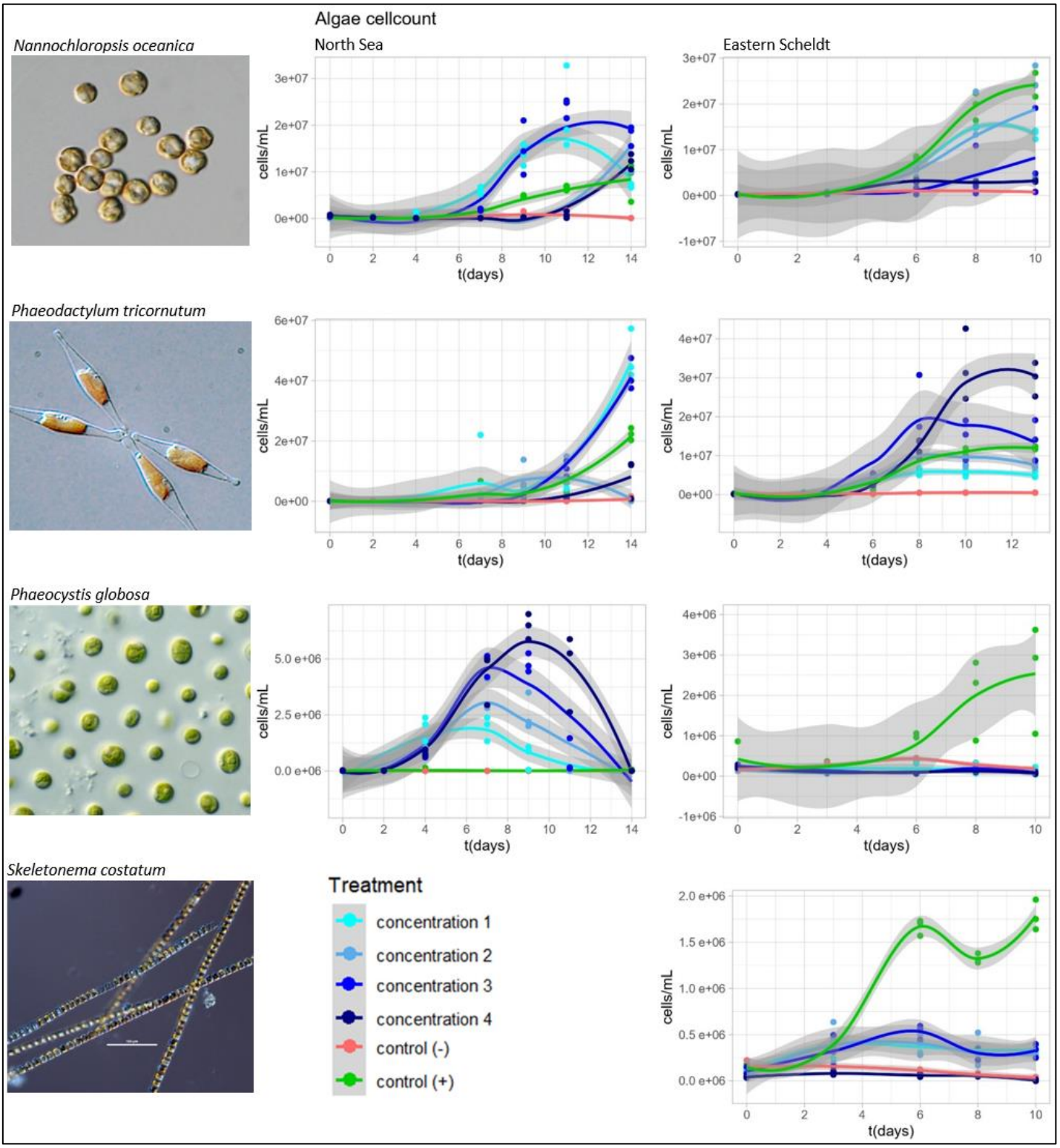

Figure 2 Growth curves of the cell density (cells/mL) for the four algae species when exposed to different concentrations of harbour porpoise faeces for both the North Sea and Eastern Scheldt trial. The graphs show polynomial growth lines fitting cell density data (cells/mL) with a confidence interval of 80% over a period of maximum 14 days.

3.1.1 Total cell density

The distribution of the data on the total cells counted for every treatment for both locations is shown in **Error! Reference source not found.**3 &4Table 2. For the whole dataset, significant differences between the total counted cells were found between the species and locations. The model* shows that all treatments have a significantly higher total cell count than the negative control (Table 2). No differences in algal growth, in terms of total cell count, were found between the treatments. However, an increase in total cells counted is visible for *Phaeodactylum* with increasing faecal concentrations during the Eastern Scheldt trial (Figure 2). The total cell count is significantly higher for the trial performed with water from the North Sea water, in comparison to water from the Eastern Scheldt. In addition, the total cell count for *Nannochloropsis* and *Phaeodactylum* species is significantly higher than for *Phaeocystis* and *Skeletonema* (Table 2 & Appendix IV: Output GLM, Table 8). No differences between *Nannochloropsis-Phaeodactylum* and *Phaeocystis-Skeletonema* were found.

Table 2 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for the total cell count with a null deviance and residual deviance of respectively 104 and 94 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept)	13.394	0.274	48.892	< 2e-16 ***
Treatment concentration 1	1.788	0.294	6.077	1.23e-09 ***
Treatment concentration 2	1.629	0.294	5.535	3.11e-08 ***
Treatment concentration 3	1.927	0.294	6.547	5.87e-11 ***
Treatment concentration 4	1.771	0.294	6.017	1.78e-09 ***
Location-North Sea	0.487	0.201	2.421	0.0155 *
Species- Nannochloropsis oceanica	1.700	0.246	6.905	5.02e-12 ***
Species- Phaeodactylum tricornutum	2.0097	0.246	8.163	3.27e-16 ***
Species-Skeletonema costatum	-0.5116	0.318	-1.610	0.108

**The intercept should be interpreted as the reference to which the total cell count of treatments, locations and species are compared. In this case, the intercept represents the negative control, the location Eastern Scheldt and the species Phaeocystis globosa. A positive estimate shows a higher total cell count in comparison to the reference (intercept).*

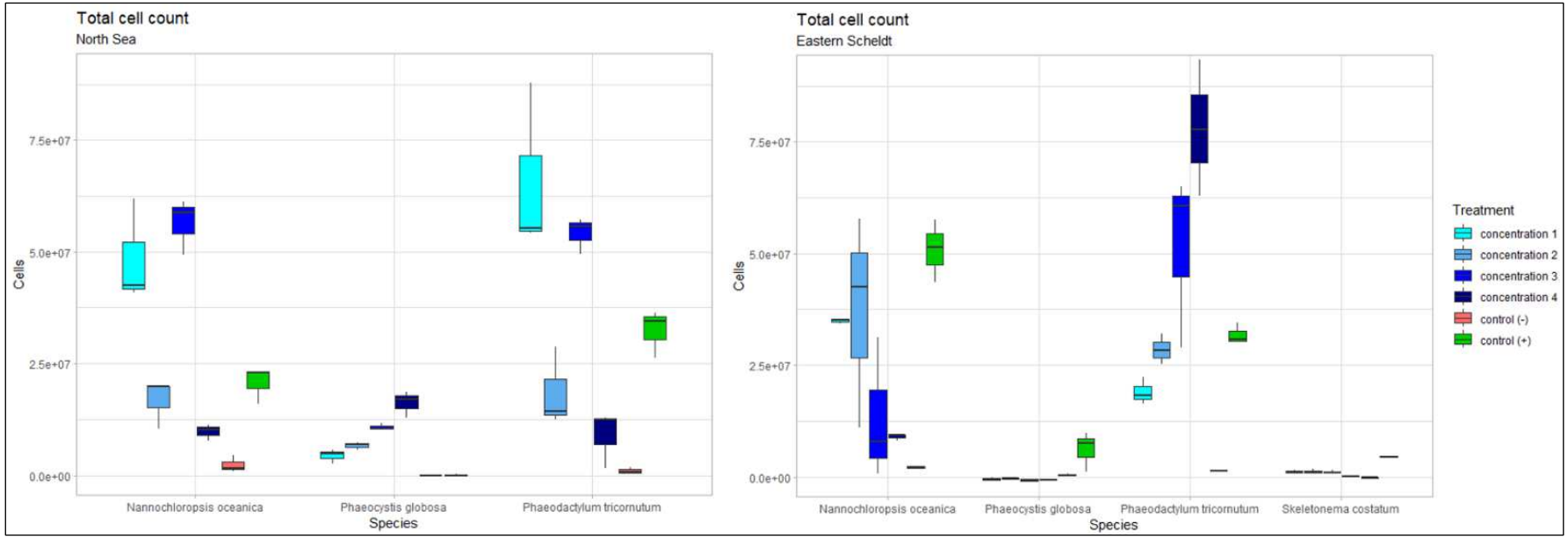

Figure 3 Boxplots summarising the distribution of the total cell count for the four algae species (Nannochloropsis, Phaeocystis, Phaeodactylum and Skeletonema) for both the North Sea trial and Eastern Scheldt trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

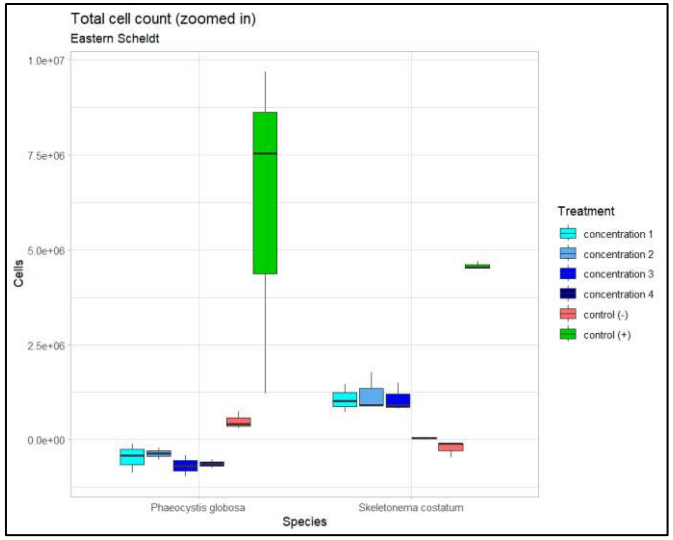

Figure 4 Boxplot summarising the distribution of the total cell count zoomed in on Phaeocystis and Skeletonema during the Eastern Scheldt trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

3.1.3 Maximum growth rate

The distribution of the data on maximum growth rate (cells/day) for every treatment for both locations is shown in Figure 5-6. For the whole dataset, significant differences in the maximum growth rate were found between the species and locations. The model shows that all treatments have a significantly higher maximum growth rate than the negative control (Table 3) but no differences between treatments were found. In addition, the maximum growth rate is significantly higher for the trial performed with water from the North Sea water, in comparison to water from the Eastern Scheldt. In addition, the total cell count for *Nannochloropsis* and *Phaeodactylum* species is significantly higher than for *Phaeocystis* and *Skeletonema*. No differences were found between *Nannochloropsis-Phaeodactylum* (Appendix IV: Output GLM, Table 10).

Table 3 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for the maximum cell density with a null deviance and residual deviance of respectively 104 and 94 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept)	10.261	0.271	37.844	< 2e-16 ***
Treatment concentration 1	2.421	0.291	8.312	< 2e-16 ***
Treatment concentration 2	2.359	0.291	8.099	5.55e-16 ***
Treatment concentration 3	2.623	0.291	9.006	< 2e-16 ***
Treatment concentration 4	2.322	0.291	7.973	1.55e-15 ***
Location-North Sea	0.798	0.199	4.009	6.10e-05 ***
Species- Nannochloropsis oceanica	2.0646	0.244	8.473	< 2e-16 ***
Species- Phaeodactylum tricornutum	2.488	0.244	10.212	< 2e-16 ***
Species-Skeletonema costatum	-0.973	0.315	-3.093	0.00198 **

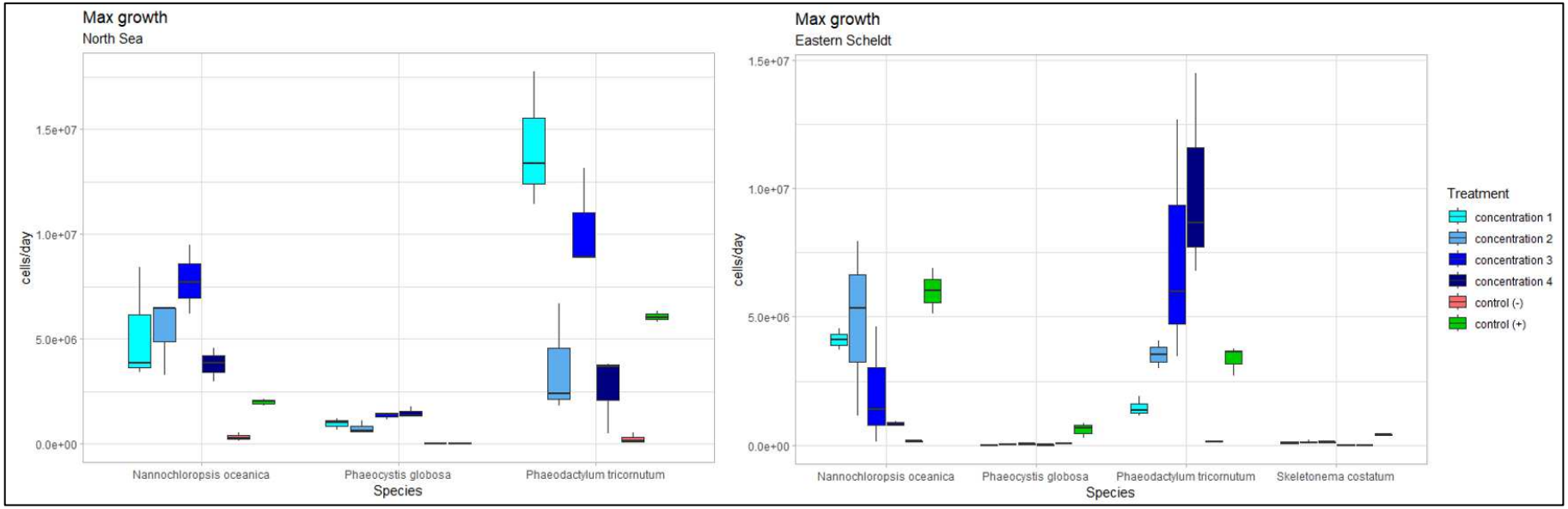

Figure 5 Boxplots summarising the distribution of maximum growth (cells/day) for the four algae species (Nannochloropsis, Phaeocystis, Phaeodactylum and Skeletonema) for both the North Sea trial and Eastern Scheldt trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations

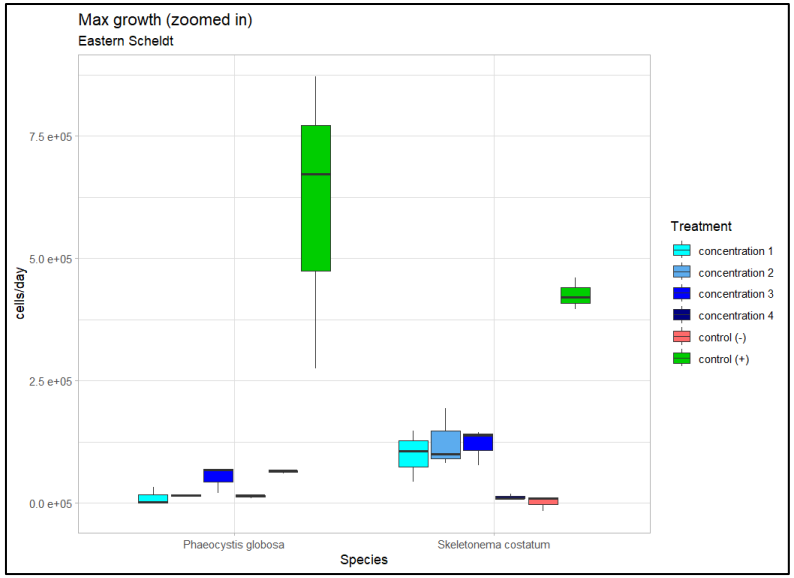

Figure 6 Boxplot summarising the distribution of the maximum growth zoomed in on Skeletonema and Phaeocystis during the Eastern Scheldt trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

3.2 Biomass

The growth of the algae in terms of increased dry weight over a period of 14 days is shown in Figure 7 for the three algae species for trials with water from the North Sea. The 80% confidence intervals are removed for clearer visualisation of the trendlines but can be viewed in Appendix II: Growth curves biomass including confidence interval, Figure 11. At t0 no algae solution of *Phaeocystis* was left to perform a dry weight analysis. Moreover, due to a mistake in the dilution of algae in seawater to measure biomass at t0 for *Nannochloropsis* and *Phaeodactylum,* both starting biomasses were converted manually to the right ratio. Therefore, data for t0 for both species is a calculated value rather than a measured value.

Both *Nannochloropsis* and *Phaeodactylum* show from t0 an increased biomass until t4, whereafter biomass drops until t7, after which the biomass again increases. Most treatments of *Nannochloropsis* seem to have collapsed at t14, whereas some treatments for *Phaeodactylum* show increased biomass in comparison to t11. Concentrations 1 and 3 show an overall higher growth curve than concentrations 2 and 4 for Nannochloropsis. *Phaeocystis* shows in general a higher growth curve for higher levels of faeces.

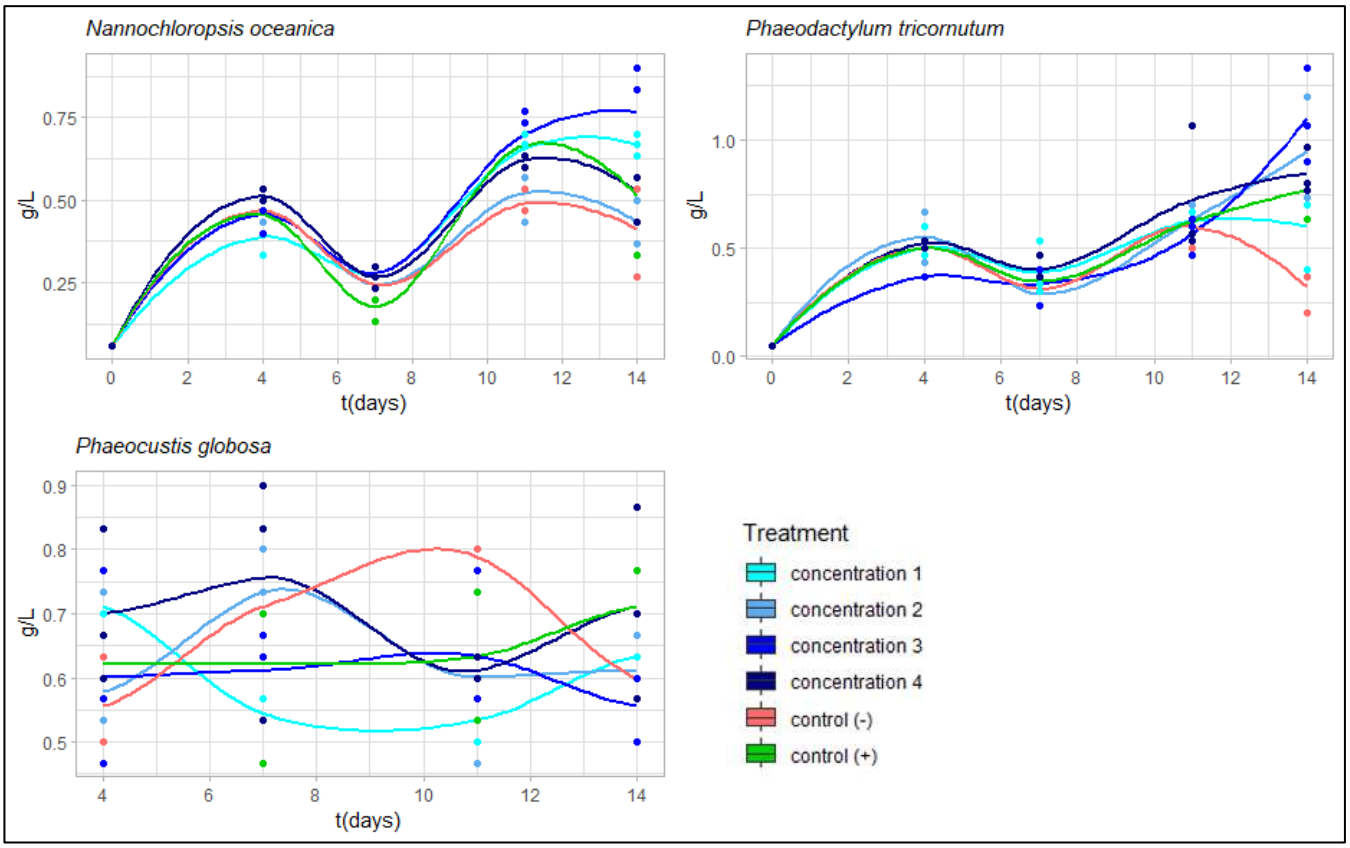

Figure 7 Growth curves for the biomass (g/L) of two beneficial algae (Nannochloropsis and Phaeodactylum) and one potentially harmful algae (Phaeocystis) when exposed to different concentrations of harbour porpoise faeces for the North Sea trial. The graphs show polynomial growth lines fitting biomass measurements over a period of maximum 14 days. The confidence interval of 80% is removed to have a clearer view of the trendlines.

3.2.1 Total biomass

The distribution of the total biomass (g) for every treatment for the North Sea trial is shown in Figure 8. For the whole dataset, significant differences between the total biomass were found between the species and treatments. The model shows that treatments 3 and 4 have significantly higher total biomass in comparison to the negative control (Table 4), but no differences between treatments were found. In addition, the total cell count for the *Nannochloropsis* and *Phaeodactylum* species is significantly higher than for *Phaeocystis.* No difference between *Nannochloropsis* and *Phaeodactylum* was found.

Table 4 Estimated regression parameters, standard errors, z-values and p-values for the Gaussian GLM for the total biomass with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept)	2.268	0.129	17.648	< 2e-16 ***
Treatment concentration 1	0.285	0.154	1.857	0.0711
Treatment concentration 2	0.252	0.154	1.641	0.109
Treatment concentration 3	0.393	0.154	2.559	0.0146 *
Treatment concentration 4	0.508	0.154	3.304	0.00208 **
Species- Nannochloropsis oceanica	-0.61780	0.119	-5.193	7.26e-06 ***
Species- Phaeodactylum tricornutum	-0.410	0.119	-3.446	0.00140 **

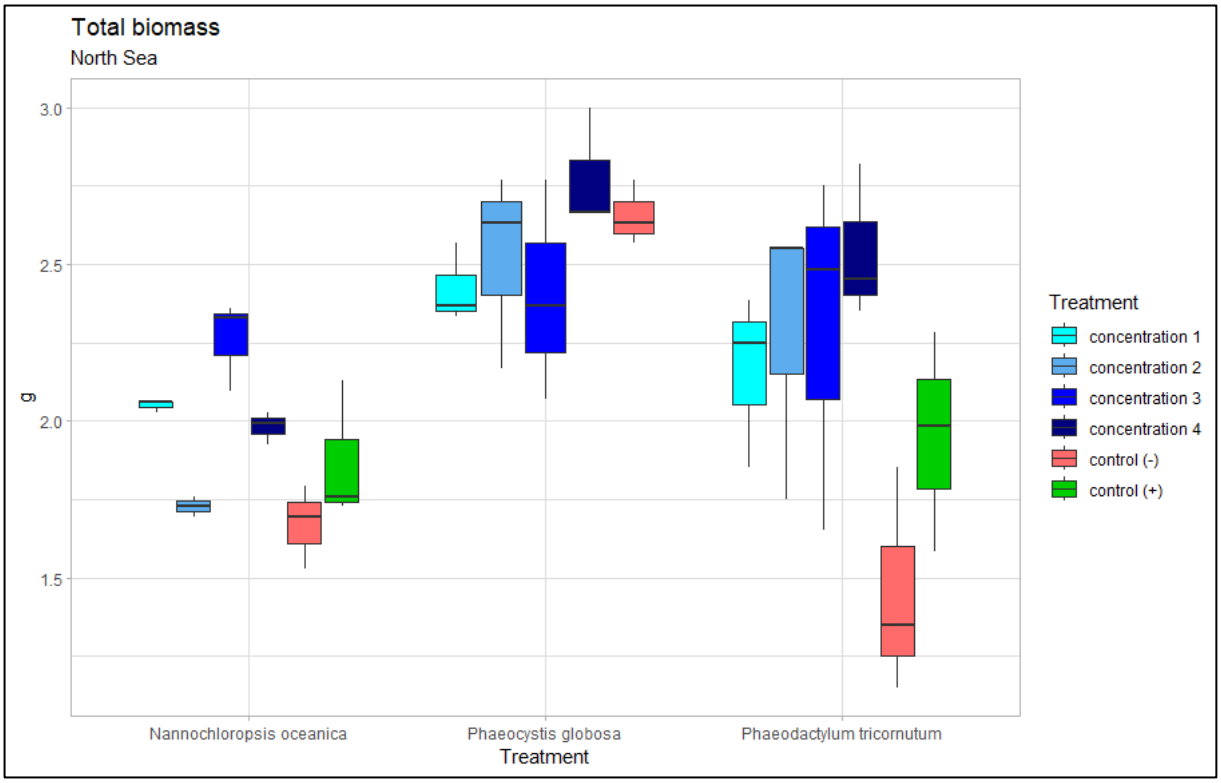

Figure 8 Boxplots summarising the distribution of the total biomass (g) for the three algae species (Nannochloropsis, Phaeocystis and Phaeodactylum) for both the North Sea trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

3.2.3 Maximum growth rate

The distribution of the maximum growth rate (g/day) for every treatment for the North Sea trial is shown in Figure 9. The model shows no significant differences between the maximum growth rate between the species and treatments (Table 5). However, the maximum growth rate for the species *Nannochloropsis* and *Phaeodactylum* are significantly higher than for *Phaeocystis* and *Phaeodactylum* significantly higher than *Nannochloropsis* (Table 5 & Appendix IV: Output GLMTable 12).

Table 5 Estimated regression parameters, standard errors, z-values and p-values for the Gaussian GLM for the maximum growth (g/day) with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept)	0.0402	0.0122	3.296	0.00213 **
Treatment concentration 1	-0.00267	0.0146	-0.183	0.856
Treatment concentration 2	0.0101	0.0146	0.694	0.4922
Treatment concentration 3	0.0206	0.0146	1.410	0.170
Treatment concentration 4	0.0220	0.0146	1.509	0.140
Species- Nannochloropsis oceanica	0.0560	0.0113	4.959	1.51e-05 ***
Species- Phaeodactylum tricornutum	0.0834	0.0113	7.385	7.45e-09 ***

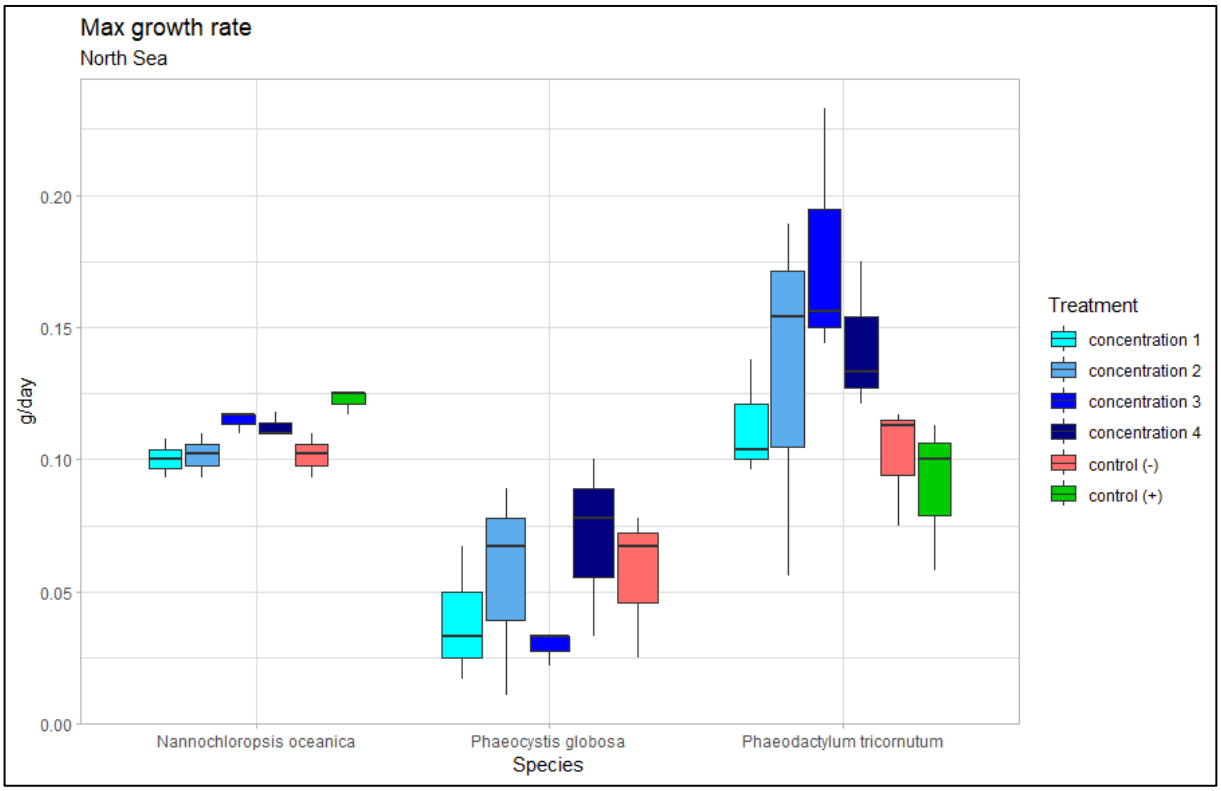

Figure 9 Boxplots summarising the distribution of the maximum growth rate (g/day) for the three algae species (Nannochloropsis, Phaeocystis and Phaeodactylum) for both the North Sea trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

3.3 Nutrient composition

3.3.1 Faeces

The faeces solution was analysed (with simplistic water quality tests) for pH, ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and iron (Fe). As previously mentioned, the first batches of samples resulted in a darker solution than the second batch and the previous study by Van Burken. The analysis confirmed a higher phosphate concentration (approximately 1.5x) in the first batch (Table 6). All other parameters were similar. Four faeces samples and seawater were analysed during the previous study by Van Burken (2022) by the NIOZ for nutrient composition in 2022 (Table 7). The value for the nitrogen (dry weight) concentration is calculated from the average total N (wt%), determined with a C: N analysis, and the sample mass. The concentration of phosphor was 559 times higher in the faeces samples than in the seawater sample and the iron was 191 times higher.

Table 6 Nutrient analysis for both batches of faeces solution.

	Batch 1	**Batch 2**
pH	7.8	7.8
NH_4^+ (mg/L)	Range 3.9-5.5	Range 3.9-5.5
NO_3^- (mg/L)	0	0
PO_4^{3-} (mg/L)	30.2	21.2
Fe (mg/L)	<0.02	<0.02

Table 7 Average N, P and Fe concentrations for dry weight and wet weight of four harbour porpoise faeces samples used by Van Burken.

	Faeces		**Seawater**	***Enrichment factor***
	Dry weight	*Wet weight*		
N	368 g kg^{-1}			
P	7.984 g kg^{-1}	0.0573 g/L	0.088 mg/L	559
Fe	0.100 g kg^{-1}	0.0007 g/L	0,0034 mg/L	191

3.3.2 Seawater

Water samples of surface water of the North Sea were retrieved by Stena Line 100 km from the coast (Appendix III: **Error! Reference source not found.**). For the Eastern Scheldt trial water was retrieved in the West Eastern Scheldt. Data on the nutrient composition of surface water from the North Sea and Eastern Scheldt were retrieved by Rijkswaterstaat. Data showed an overall higher concentration of P, N and Fe in the Eastern Scheldt than in the North Sea (Appendix III: Figure 13-15).

4. Discussion

Results show that the growth of the phytoplankton was enhanced by the harbour porpoise faeces. However, no clear relationship was found between faeces concentration and algal growth. The growth curve of *Phaeodactylum* during the Eastern Scheldt trial was the only example where the cell density increased with higher faeces concentrations. Moreover, multiple significant differences were found in growth between the species and the locations.

The increased algal growth can be explained by the enrichment factor of the harbour porpoise faeces. The phosphor and iron in the faeces had respectively a 559 and 191 times higher concentration than measured in seawater, providing many nutrients for algal growth that are otherwise limited in seawater. Preliminary studies show estimated the enrichment factor of phosphate for a harbour porpoise at 682 for harbour porpoises near Alaska (Pearson, 2021) and the enrichment factor of iron of sperm whales in the Southern Ocean at 10 million (Lavery et al., 2010; Pearson, 2021). Rather than the smaller size of the porpoise, the large difference in the enrichment factor of iron can be explained by the diet of the sperm whale, which includes iron-rich cephalopods (Lavery et al., 2010) and the overall low concentration of iron in the Southern Ocean (Toulza et al., 2012). Unfortunately, studies on the enrichment factor of nitrogen of harbour porpoise faeces are lacking. More faecal nutrient analyses of harbour porpoises and whales, in general, are needed to compare the impacts on primary production between different species in low and high-productivity areas.

Data from Rijkswaterstaat (n.d.) confirmed that the nutrient concentration of phosphor, nitrogen and iron were lower offshore in comparison to the coast, however, the total cell count, maximum cell density and maximum growth (cells/day) were significantly higher for the algae grown in North Sea water than water from the Eastern Scheldt. This suggests that algal growth benefits less from the porpoise faeces in waters with higher nutrient concentrations. However, a higher concentration of phosphates in the faeces solution used for 2 out of 3 species during the North Sea trial should also be considered. Another explanation could be an unbalanced N:P ratio. An unbalanced reduction of riverine input of phosphorus and nitrogen since the 1990s caused an offshore gradient from phosphorus to nitrogen limitation (Burson et al. 2016). This caused a P deficiency for phytoplankton in the ROFI (region of freshwater influence, reaching 30-50 km offshore) which was described for both *Phaeocystis* and *Skeletonema* (Burson et al., 2016; Simpson et al., 1993).

A reason that for most cases higher faeces concentration did not necessarily lead to increased algal growth can be explained by the fact that algae need to adapt when exposed to a new environment (Krishnan et al., 2015). This can be seen in the growth curves where in most cases the cell density stays the same or even decreases before the algae start exponentially growing. This “lag phase” is also described by Smith et al. (2013) where the growth response of phytoplankton, was initiated 2 to 7 days

after the addition of faecal nutrients of pygmy blue whales. Further hinder in growth for some treatments can be a sign that the algae has trouble adapting because the nutrient concentration, to which the algae is exposed is either too high or low to properly adapt to the new environment. From the cell density growth curves can be noted that for some treatments the algae cell density collapses because a point is reached where the nutrient supply left in the erlenmeyer is insufficient. This pattern is also noted by Smith et al. (2013), however, their study showed a clear interaction between treatment and time, where higher faecal concentrations led to earlier nutrient exhaustion. This pattern is not visible for this study, but the timing or occurrence of exhaustion does differ between the trials and, therefore, affects the outcomes of this study. Algae species starting with a higher starting density, and therefore possibly higher total cells counted and maximum cell density, were corrected for analysis by adjusting the starting cell density to 0. However, it should be considered that the time to the collapsing point is also dependent on the starting density. For future research, it would be advisable to monitor the start-up culture more closely to be able to steer growth by adding more nutrients when needed and only start the experiment when the standardized starting density is reached. Also, to compare data for every parameter more precisely, the length of the trial should be altered to the number of days to which all species have reached the collapsing point. Data up to this point should then be used for further analysis.

For many measurements, less growth of the species Phaeocystis and Skeletonema was observed in comparison to *Nannochloropsis* and *Phaeodactylum.* The hypothesis that the harbour porpoise faeces could hinder the growth of the potentially harmful *Phaeocystis* cannot be assumed since the reason behind the limited growth is more likely caused because *Nannochloropsis* and *Phaeodactylum* are known to be relatively easy species for culture. This is less known for Phaeocystis since it's generally less cultured. However, it should not be ignored that *Nannochloropsis* and *Phaeodactylum* were studied simultaneously during the first trial and the faeces solution with higher phosphate concentration was used for these species.

The parameter of biomass was added to the experiment to check whether cell density and biomass were correlated. When the growth curves for the cell density and biomass are compared, very different patterns are shown. However, when the boxplots for the parameters for the cell density and biomass measurements are compared, similar patterns can be seen for *Nannochloropsis* and *Phaeodactylum*, but not for *Phaeocystis.* This shows that a higher cell density is not that easily translated into higher biomass.

Overall, to determine whether harbour porpoise faeces can contribute the primary production more factors should be considered. Next to the nutrient concentration of the faeces, the population densities, distribution, diet and other environmental factors should be modelled for the studied cetacean species. Moreover, interactions and competition between the algae species in Dutch waters

should be studied to determine which species has a better advantage. The culture of *Skeletonema*, for instance, failed twice due to an infestation of another unidentified algae species. It's hypothesized that this species was already present in the *Skeletonema* start-up culture in such a low density that it was not visible when analysing the start density but bloomed due to the harbour porpoise faeces.

Results should be interpreted with care due to the many factors influencing algal growth and the methodological challenges related to performing pilot studies. For instance, the ratio between the wet weight of the faeces samples and seawater to make a faeces solution seemed insufficient. The first batch of faeces solution made during this study was thought to be more concentrated than the second batch plus the solution made by Van Burken due to the darker colour. A higher phosphate concentration was confirmed by the nutrient analysis. For further research, a fixed ratio of faeces, measured in dry weight, and seawater should be established. Furthermore, when comparing results from Van Burken to this study, inter-observer variability in counting the algae cells should be considered. Variation was decreased by communicating the counting "rules", however, to minimize differences a protocol should be written. Also, some algae species were more difficult to recognize and distinguish from non-algae cells than others, which took some practice. To prevent having less accurate data at the beginning of the experiment, it would be advisable for future trials to practise counting the cells with an algae expert. In addition, when taking seawater samples in the future it's important to consider seasonal changes in nutrient concentrations in the marine environment due to biannual algal blooms (Silva et al., 2021). The first study by Van Burken started in late summer (end of August-mid October) after the summer phytoplankton bloom when nutrients were depleted. This study was performed during the spring bloom (end of April- mid June) which could have resulted in relatively lower nutrient depletion of the seawater sample. For future studies, it is important to take samples within a fixed timeframe under similar weather conditions, preferably before an algal bloom so nutrients are not depleted to be able to compare data more accurately between studies. Also, during this study, it was perceived that some species were more free-floating in the water, while others clumped more to the bottom of the erlenmeyer. Therefore, it should be taken into consideration that for some species more shaking or stirring is needed before sampling to equalize the distribution of algae in the erlenmeyer. A solution would be to stir the erlenmeyers mechanically before sampling to ensure equal distribution of algae. Lastly, the total cell count and maximum cell density gave very similar results suggesting using one of both parameters should be sufficient for further research.

To conclude, harbour porpoise faeces did in most cases increase algal growth, but higher faecal concentration did not directly lead to more growth. To determine the contribution of harbour porpoise faeces to primary production more factors like population densities, distribution, diet and environmental factors need to be studied. Also, more research on whale faecal nutrient composition

should be executed to compare smaller cetaceans with great whales. Moreover, interactions and competition between algae species should be investigated to predict possible changes in the algae community due to porpoise faeces. Multiple adjustments need to be made to the methods to accurately compare results between studies. A protocol should, therefore, be written. These suggestions would provide a stronger base to make statements on how the harbour porpoise can contribute to nutrient cycling and primary production in Dutch waters, and how the impact of small cetaceans differs from great whales in less productive oceans.

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Appendices

Appendix I: Randomised block design

A randomised block design was applied during the study to compensate for erlenmeyers on the ends of the set-up possibly getting less light. In total 36 erlenmeyers with six different treatments (nr 1-6) in triplets (letter a, b, c) for two algae species at a time (*Nannochloropsis-Phaeodactylum tricornutum* and *Phaeocystis globosa-Skeletonema costatum*) were placed between fluorescent tubes for two weeks. The triplets were divided into three blocks shown in yellow, orange and blue (Figure 10). The erlenmeyers were switched from block after 3.5 days and after seven 7 days since the start of the experiment. The experiment lasted 10.5 days, making the time spent in each block equal. Erlenmeyers within the blocks were randomised at the start of the experiment and again when switched from block. Due to extending the experiment during execution by three days, the erlenmeyers spend one more day extra in each block the last 3 days.

Note: During the second round, the treatments with *Skeletonema* failed due to an infestation of *Phaeocystis.* The treatments were removed and replaced by dummy erlenmeyers filled with seawater to maintain the block design.

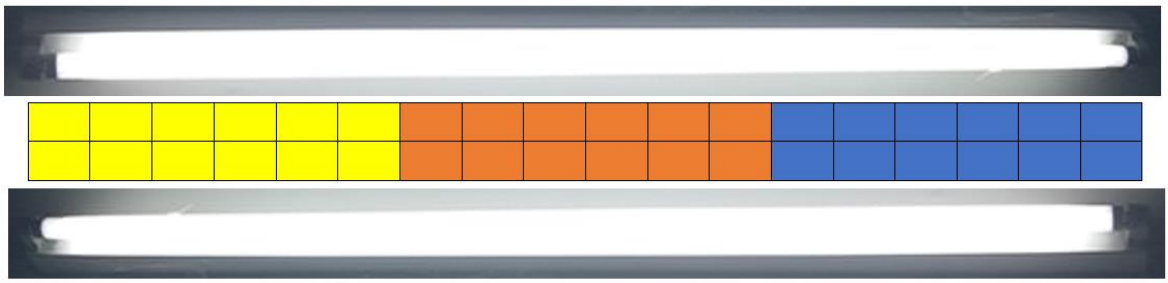


Figure 10 Set-up of the randomized block design. The block design is divided into 3 blocks (yellow, orange and blue). Each colour represents one out of three triplets. The three blocks are switched and the erlenmeyers within each block are randomized with each switch.

Appendix II: Growth curves biomass including confidence interval

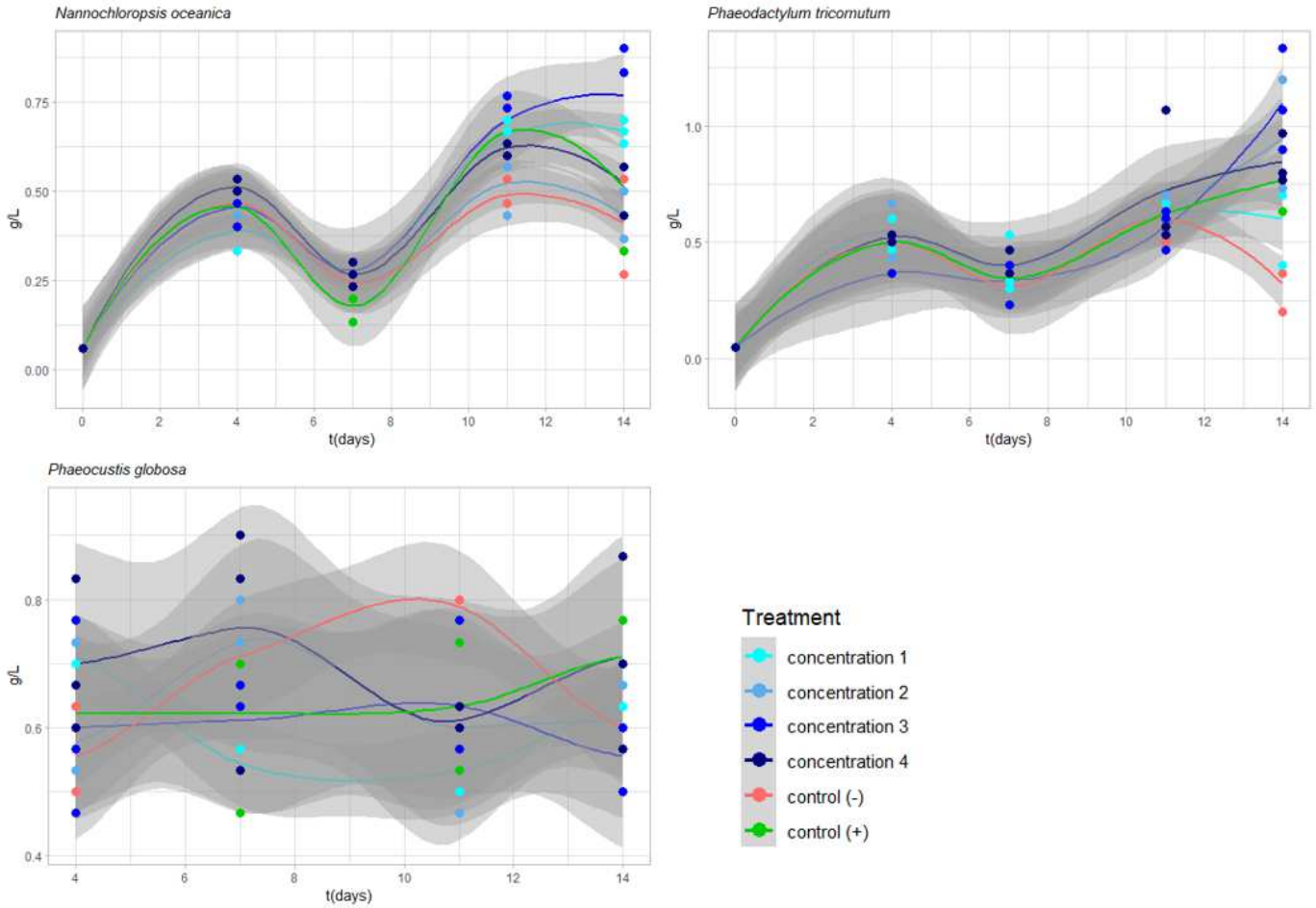
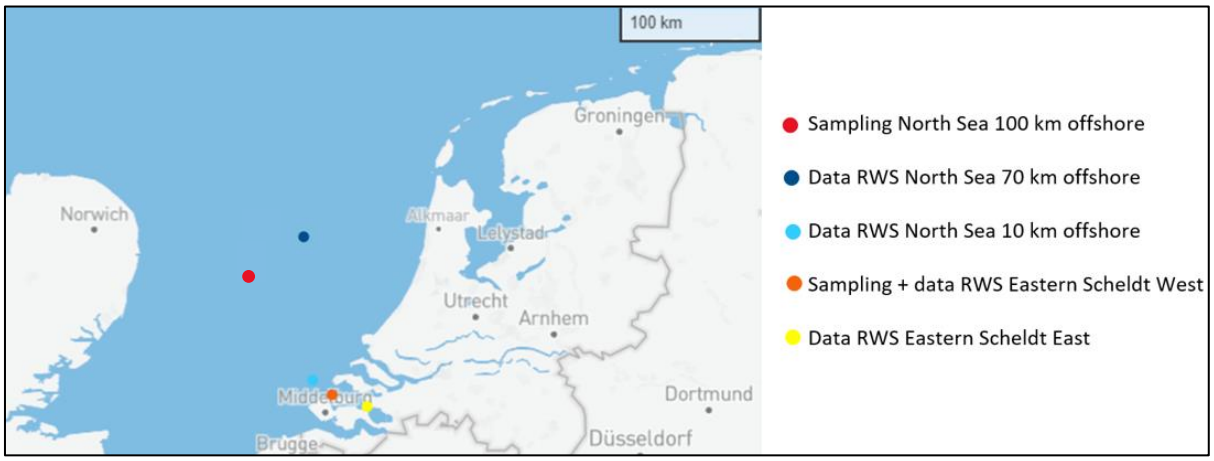


Figure 11 Growth curves for the biomass (g/L) for the four species (Phaeocystis, Phaeodactylum, Nannochloropsis and Skeletonema) when exposed to different concentrations of harbour porpoise faeces for the North Sea trial. The graphs show polynomial growth lines fitting biomass measurements with a confidence interval of 80% over a period of maximum 14 days.

Appendix III: Nutrient composition North Sea and Eastern Scheldt

Water samples of surface water of the North Sea were retrieved by Stena Line 100 km offshore (**Error! Reference source not found.**). For the Eastern Scheldt trial water was retrieved in the West Eastern Scheldt. Data on the nutrient composition of surface water from the North Sea and Eastern Scheldt were retrieved by Rijkswaterstaat (Rijkswaterstaat, n.d.). Data on the total phosphor (mg/L), total nitrogen (mg/L) and total iron (mg/L) concentration of surface water from the 1990s to the 2020s was available for the Eastern Scheldt (West and East) and North Sea (10 and 70 km from coast). Extreme outliers in the data were deleted. Some locations did not have data on all the abovementioned parameters.



Graphs made with data from Rijkswaterstaat (Rijkswaterstaat, n.d.), show a similar trend in iron concentration between the Eastern Scheldt (west) and the North Sea at 10 km from the coast (Figure 13). Most samples ranged between 0 and 1.5 mg/L. The total iron measured in the North Sea, further from the coast (70 km offshore) is lower with a range between 0 and 0.25 mg/L.

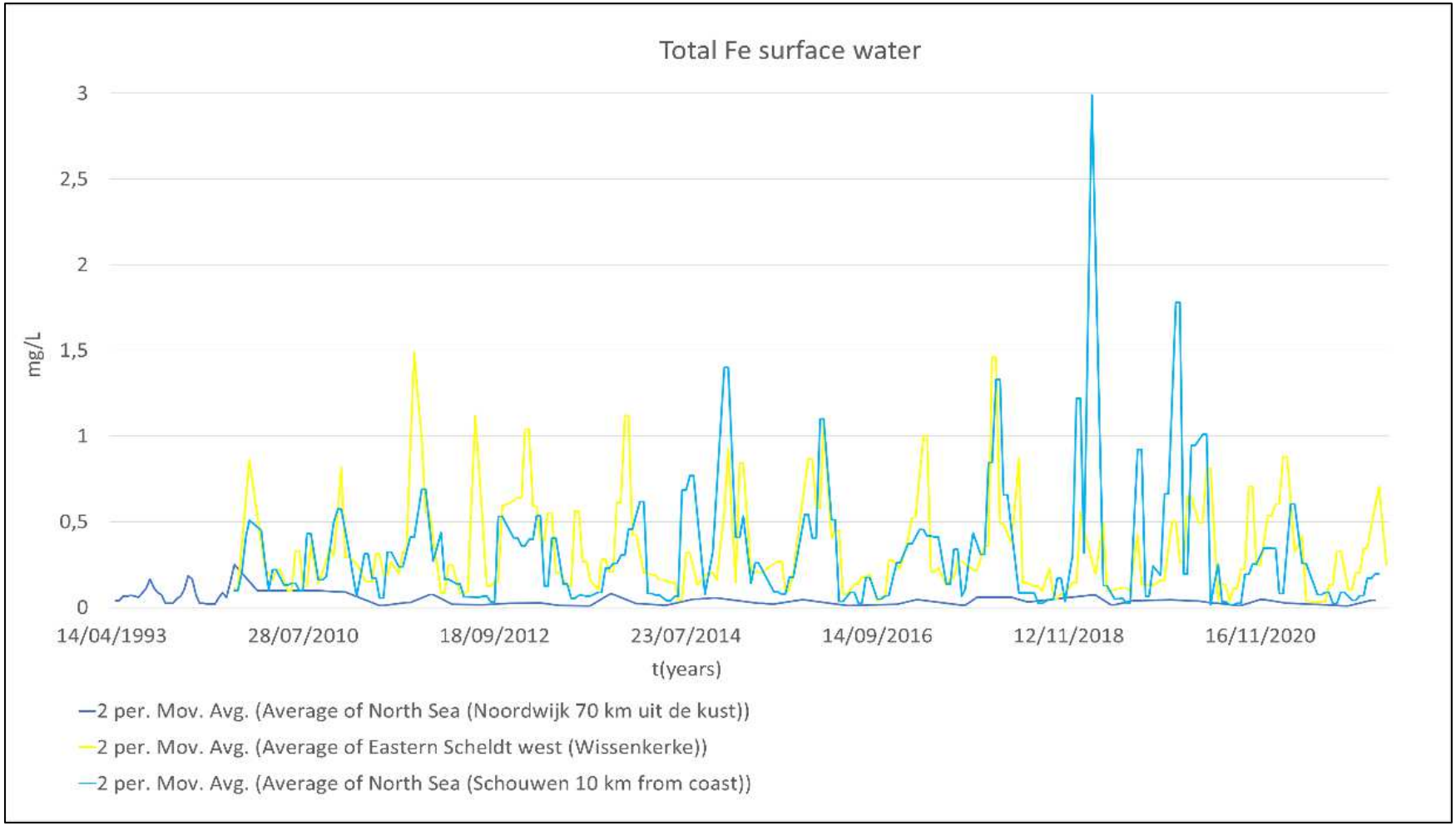


Figure 12 Average total Fe concentration (mg/L) per year for the surface water of the North Sea (70 +10 km from coast) and the Eastern Scheldt (West) since the 1990's.

Data on the total phosphor (mg/L) of surface water of the Eastern Scheldt (West and East) and North Sea (70 km from the coast), show a similar trend in concentration between both locations in the Eastern Scheldt (Figure 14Figure 14). Most samples ranged between 0.02 and 0.1 mg/L. The total phosphor measured in the North Sea shows a lower overall concentration, where most samples range between 0 and 0.04 mg/L.

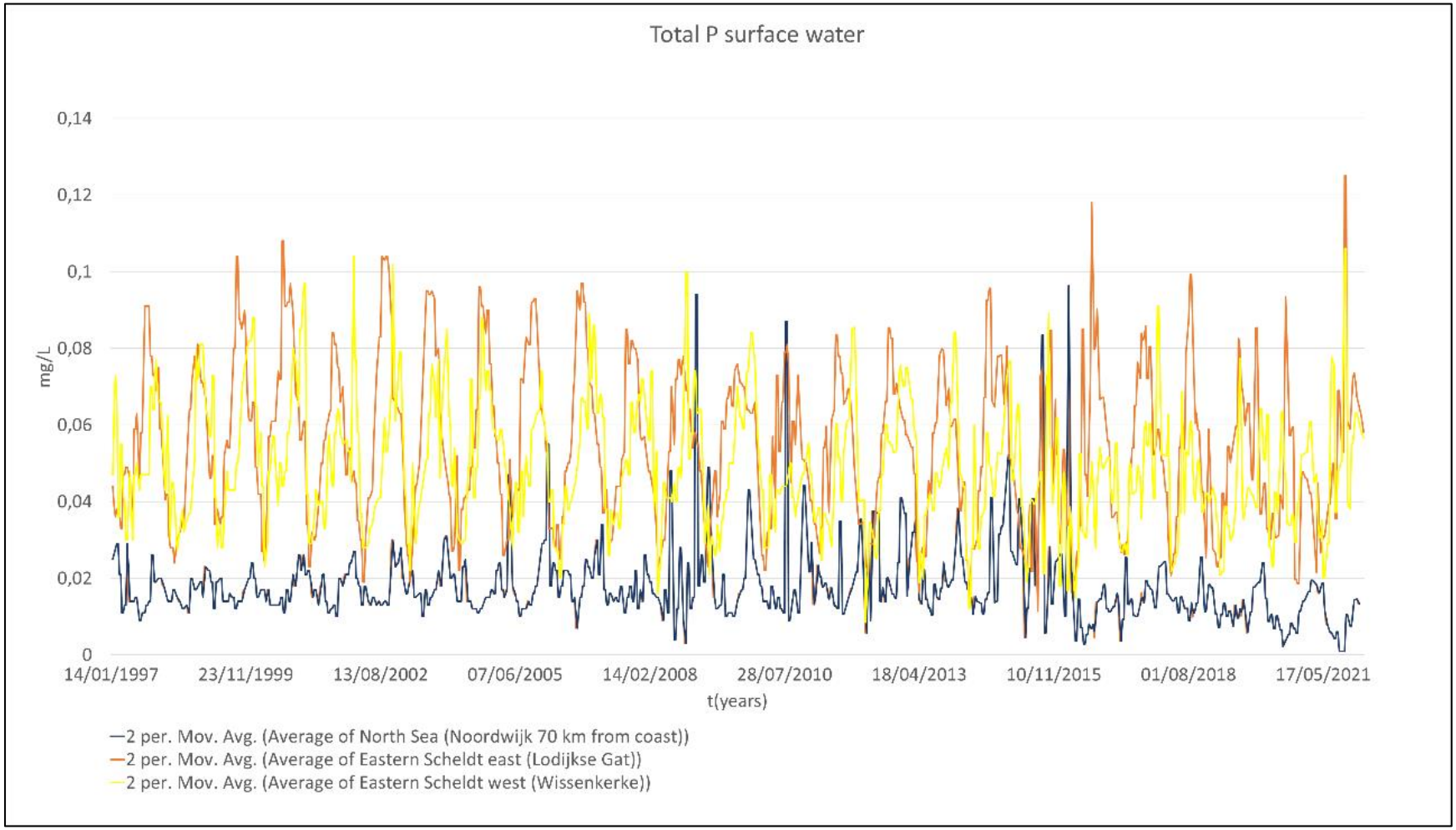


Figure 13 Average total P concentration (mg/L) per year for the surface water of the North Sea (70 km from coast) and the Eastern Scheldt (East + West) since the 1990's.

Data on the total nitrogen (mg/L) of surface water of the Eastern Scheldt (West and East) and North Sea (70 km from the coast), show a similar trend in concentration between both locations in the Eastern Scheldt (Figure 15). Most samples ranged between 0.2 and 1.1 mg/L. The total nitrogen measured in the North Sea shows a lower overall concentration, where most samples range between 0.1 and 0.3 mg/L.

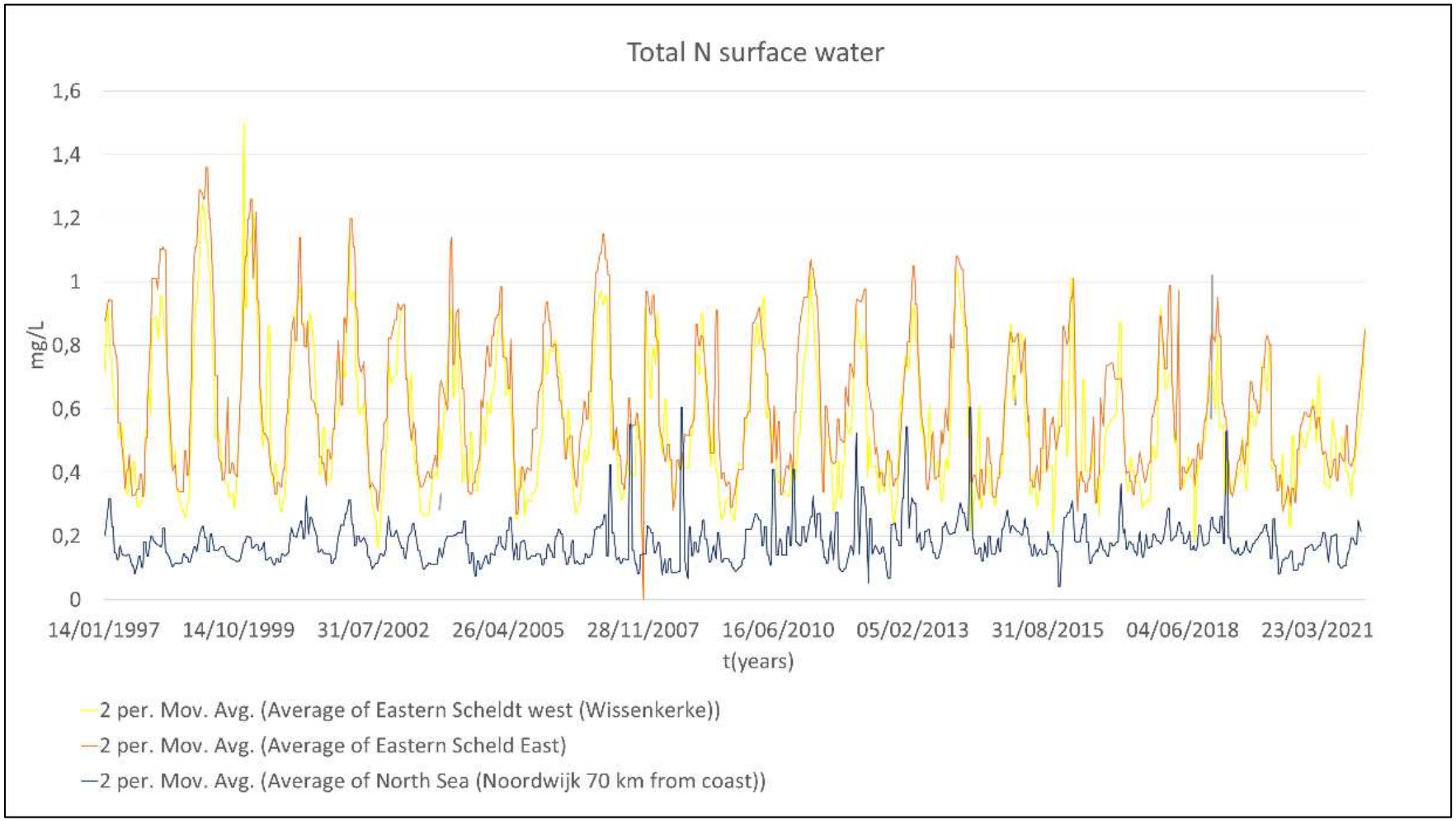


Figure 14 Average total N concentration (mg/L) per year for the surface water of the North Sea (70 +10 km from coast) and the Eastern Scheldt (West) since the 1990's.

Appendix IV: Output GLM

Total cell density

Table 8 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for the total cell count with a null deviance and residual deviance of respectively 104 and 94 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept)(Skeletonema-Eastern Scheldt)	2.882	0.309	41.740	< 2e-16 ***
Species- Nannochloropsis oceanica	2.2116	0.3178	6.958	3.45e-12 ***
Species- Phaeodactylum tricornutum	2.521	0.318	7.933	2.14e-15 ***

Maximum cell density

Table 9 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for maximum cell density with a null deviance and residual deviance of respectively 104 and 96 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept) (Skeletonema)	11.000	0.342	32.141	< 2e-16 ***
Species- Nannochloropsis oceanica	2.843	0.353	8.066	7.26e-16 ***
Species- Phaeocystis globosa	0.775	0.353	2.198	0.0280 *
Species- Phaeodactylum tricornutum	3.148	0.353	8.930	< 2e-16 ***

Maximum growth rate (cells/day)

Table 10 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for the maximum growth rate (cells/day) with a null deviance and residual deviance of respectively 104 and 94 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept) (Skeletonema-Eastern Scheldt)	9.288	0.306	30.406	< 2e-16 ***
Species- Nannochloropsis oceanica	3.0375	0.315	9.656	< 2e-16 ***
Species- Phaeodactylum tricornutum	3.461	0.315	11.003	< 2e-16 ***

Maximum biomass

Table 11 Estimated regression parameters, standard errors, t-values and p-values for the Gaussian GLM for the maximum biomass with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept) (Phaeodactylum-concentration 1)	-0.260	0.0712	-3.635	0.000820 ***
Treatment-Concentration 3	0.203	0.0855	2.372	0.0229 *
Species Nannochloropsis oceanica	-0.269	0.0662	-4.059	0.000237 ***

Maximum growth rate (g/day)

Table 12 Estimated regression parameters, standard errors, t-values and p-values for the Gaussian GLM for the maximum growth (g/day) with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept) (Phaeodactylum)	0.124	0.0122	10.133	2.36e-12 ***
Species-Phaeocystis globosa	- 0.0834	0.0113	-7.385	7.45e-09 ***
Species Nannochloropsis oceanica	-0.0274	0.0113	-2.426	0.0201 *

Appendix V: Supplementary results

Maximum cell count

The distribution of the data on the maximum cell density (cells/mL) for every treatment for both locations is shown in **Error! Reference source not found.**6-8. For the whole dataset, significant differences in maximum cell density were found between the species and locations. The model showed that all treatments have a significantly higher maximum cell density than the negative control (*Table 13*), but no differences between treatments were found. In addition, the maximum cell density is significantly higher for the trial performed with water from the North Sea water, in comparison to water from the Eastern Scheldt. Lastly, the total cell count for the species *Nannochloropsis*, *Phaeodactylum* are higher than for *Phaeocystis*. *Skeletonema* showed a significantly lower maximum cell density than the other three species (Appendix IV*: Output GLM*, Table 9). No difference was found between *Nannochloropsis* and *Phaeodactylum*.

Table 13 Estimated regression parameters, standard errors, z-values and p-values for the negative binomial GLM for the maximum cell density with a null deviance and residual deviance of respectively 104 and 96 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**z value**	**Pr (>|t|)**
(Intercept)	11.775	0.304	38.760	< 2e-16 ***
Treatment concentration 1	2.114	0.326	6.480	9.18e-11 ***
Treatment concentration 2	2.095	0.326	6.420	1.36e-10 ***
Treatment concentration 3	2.3278	0.326	7.134	9.76e-13 ***
Treatment concentration 4	2.129	0.326	6.525	6.78e-11 ***
Location-North Sea	0.832	0.223	3.731	0.000191 ***
Species- Nannochloropsis oceanica	2.0684	0.273	7.576	3.56e-14 ***
Species- Phaeodactylum tricornutum	2.373	0.273	8.692	< 2e-16 ***
Species-Skeletonema costatum	-0.775	0.353	-2.198	0.02797 *

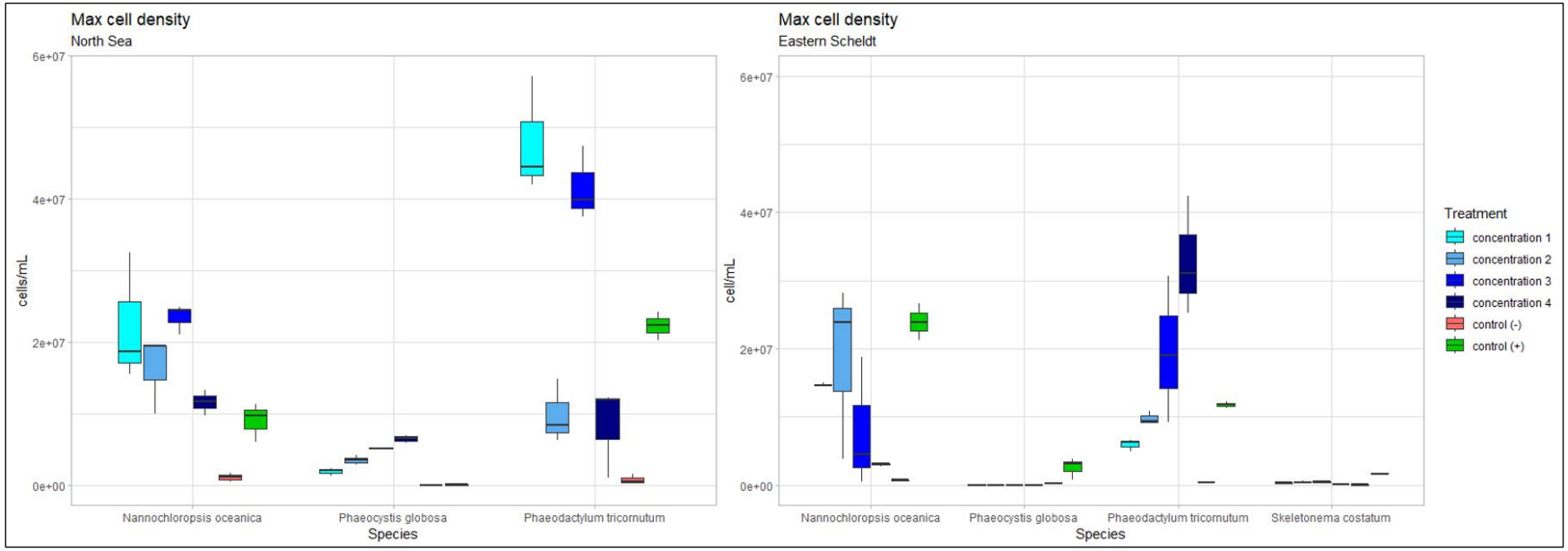


Figure 15 Boxplots summarising the distribution of maximum cell count (cells/mL) for the four algae species (Nannochloropsis, Phaeocystis, Phaeodactylum and Skeletonema) for both the North Sea trial (A) and Eastern Scheldt trial (B). The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

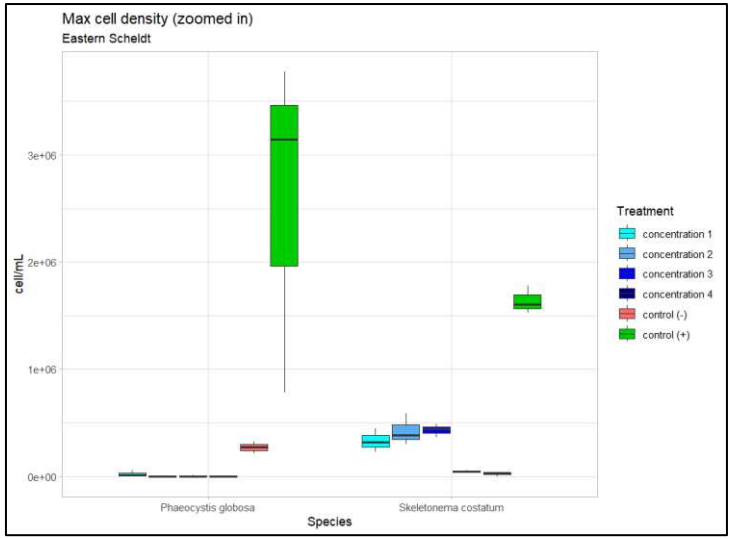


Figure 16 Boxplot summarising the distribution of the maximum cell density zoomed in on Skeletonema and Phaeocystis during the Eastern Scheldt trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.

Maximum biomass(log)

The distribution of the log-transformed maximum biomass (g) for every treatment for the North Sea trial is shown in Figure 134. For the whole dataset, significant differences between the maximum biomass (log) were found between the species and treatments. The model shows that treatments 3 and 4 have significantly higher total biomass in comparison to the negative control (

Table 14). Also, concentration 3 showed significantly higher maximum biomass than concentration 1 (Appendix IV: Output GLM, Table 11 Estimated regression parameters, standard errors, t-values and p-values for the Gaussian GLM for the maximum biomass with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.. In addition, the maximum biomass was significantly lower for the species *Nannochloropsis* in comparison to the other two species (Appendix IV: Output GLM, Table 11 Estimated regression parameters, standard errors, t-values and p-values for the Gaussian GLM for the maximum biomass with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom. No differences between *Skeletonema* and *Phaeodactylum were* found.

Table 14 Estimated regression parameters, standard errors, z-values and p-values for the Gaussian GLM for the log-transformed maximum biomass with a null deviance and residual deviance of respectively 44 and 38 degrees of freedom.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept)	-0.448	0.0716	-6.243	2.64e-07 ***
Treatment concentration 1	0.0975	0.0855	1.140	0.261
Treatment concentration 2	0.144	0.0855	1.678	0.101
Treatment concentration 3	0.300	0.0855	3.512	0.00117 **
Treatment concentration 4	0.2447	0.0855	2.861	0.00683 **
Species- Nannochloropsis oceanica	-0.180	0.0662	-2.713	0.00996 **
Species- Phaeodactylum tricornutum	0.0891	0.0662	1.346	0.186

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr (>|t|)**
(Intercept)	-0.448	0.0716	-6.243	2.64e-07 ***
Treatment concentration 1	0.0975	0.0855	1.140	0.261
Treatment concentration 2	0.144	0.0855	1.678	0.101
Treatment concentration 3	0.300	0.0855	3.512	0.00117 **
Treatment concentration 4	0.2447	0.0855	2.861	0.00683 **
Species- Nannochloropsis oceanica	-0.180	0.0662	-2.713	0.00996 **
Species- Phaeodactylum tricornutum	0.0891	0.0662	1.346	0.186

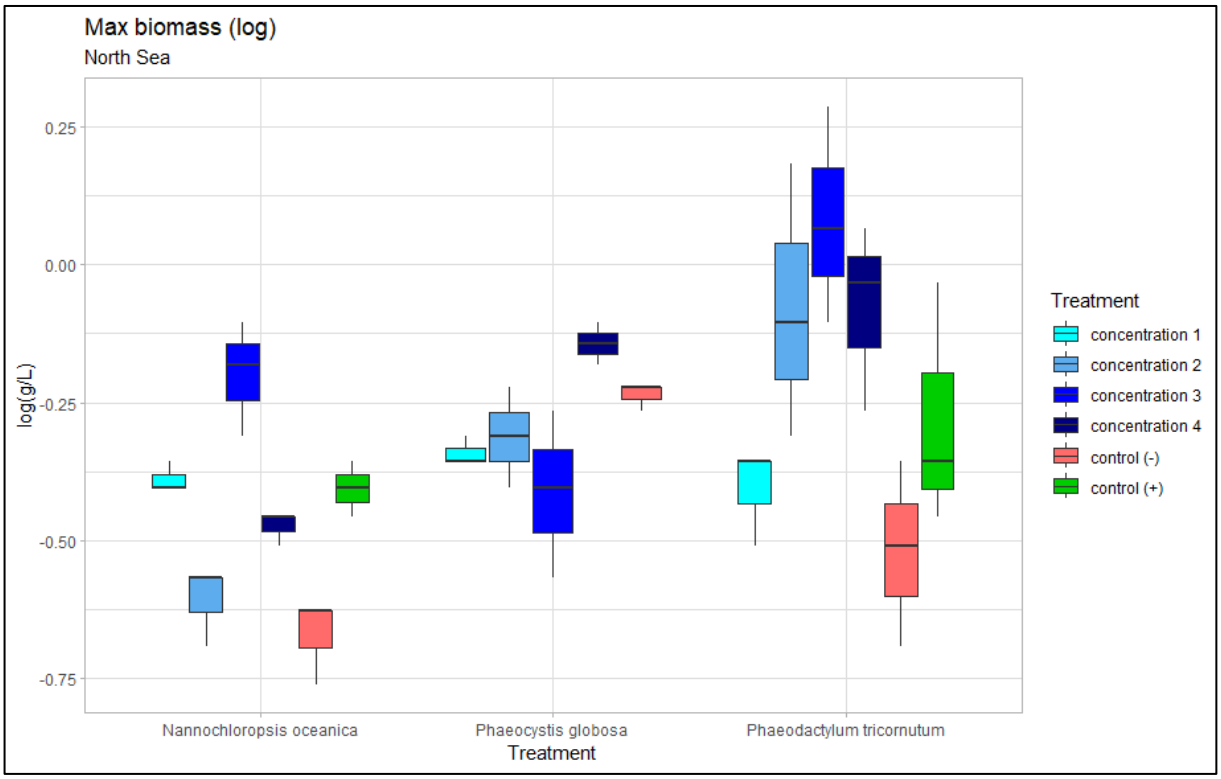


Figure 17 Boxplots summarising the distribution of the maximum biomass (g/L) for the three algae species (Nannochloropsis, Phaeocystis and Phaeodactylum) for both the North Sea trial. The negative control is shown in red, the positive control in green and the treatments a darker blue colour with increasing faecal concentrations.