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Marine Coastal Development
Submission date: August 2018
Supervisor: Nicole Aberle-Malzahn, IBI
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Unn Vagnsdóttir Johannesen

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Abstract

Marine larvae need a live feed with a high amount of PUFA especially EPA and DHA to have a healthy development. Copepods are the preferred choice of live feed since they are there natural food source and have the right biochemical composition for a healthy larval development. The main question was to examine the possibility to increase the EPA and DHA content in *A. tonsa* through a mono-diet of *R.* baltica and *R. salina* cultivated on four different nitrogen concentrations 0, 33, 66, and 100% $NaNO_3$. The experiment was divided in two the microalgae and the copepods. In the microalgae experiment was carried out by measuring the growth rate, carrying capacity, and biochemical composition (C:N molar ratio, TL, and FA) of *R. baltica* and *S. salina* reared on 0, 33, 66, and 100% $NaNO_3$ in the growth medium. The $NaNO_3$ treatment that had the highest DHA, EPA, and C:N ratio of each species was chosen to a feeding experiment of *A. tonsa*. *R. baltica* and *R. salina* cultivated in 66% $NaNO_3$ had statistically higher (ANOVA, $p < 0.05$) amounts of DHA and EPA in the stationary phase then the other treatments within each species. *R. baltica* and *R. salina* cultivated in 66% $NaNO_3$ showed Furthermore, *R. baltica* 100% $NaNO_3$ was also included in the feeding experiment. In the feeding experiment the three chosen treatments were fed as mono diets to *A. tonsa* where development, survival, C:N molar ratio, and the FA profile was studied for dietary effect at development stage NIV and CI (day 3 and 6). There appeared to be some slight variation in the DHA and EPA in the NIV stage however in the CI there was no difference in the FA % distribution in CI between the different treatments. The amounts of FA were overall slightly higher in the *A. tonsa* fed *R. baltica* 66% $NaNO_3$ and had the highest survival 25.5% after 6 days. Therefore, from the data collected in this study feeding *A. tonsa R. baltica* 66% $NaNO_3$ appears to be the sensible choice. Not can be definitively concluded since the were no statistical differences between any of the treatments in feeding experiment of *A. tonsa*. Moreover, the study showed *R. baltica* is superior to *R. salina* under the same cultivation conditions.

Key words: *Rhodomonas baltica*; *Rhodomonas marina*; *Rhodomonas salina*; *Acartia tonsa*; Fatty acids; EPA; DHA; ARA; C:N Stoichiometry

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1 Introduction

By the year 2050 the world population will reach 9.7 billion people as well as the consumption of fish per capita has steadily increased to 19.7 in 2013 and is estimated to increase the upcoming years. Therefore, it is important to consider how it would be possible to increase the world production of fish. The world capture fisheries and aquaculture production has been growing since the 1950's. The capture fisheries plateaued in the mid 1980's leaving the aquaculture the sole reason for the increase in the fish production to this day. The overall world aquaculture production increased from 55.7 to 73.8 million tons from 2009 to 2014 a growth of approximately 32.5% whereas, the world capture fisheries fluctuated around 90 million tons during this period. The world aquaculture has been divided into two sub groups to get a clearer overview of the production. The inland production involves the fresh water species while the marine production involves the species cultivated in saline waters. The inland production was 47.1 million tons in 2014 corresponding to 63.8% of the world aquaculture production in the same timeframe, furthermore the production increased from 34.3 to 47.1 million tons (37.3%) from 2009 to 2014. The marine production had a growth from 21.4 to 26.7 million tons (24,8%) from 2009 – 2014 moreover it only adds up to 36.2% of the overall aquaculture production in 2014. Although the overall aquaculture production has a rapid increase the sub groups clearly show the cultivation of marine species has encountered some obstacles throughout the cultivation compared to the cultivation of the inland species (FAO, 2016). One of the major obstacles in cultivation of pelagic marine larvae e.g. *Gadus morhua* is that most species have pelagic egg that results in small undeveloped larvae with low stress tolerance at hatching and have a small lipid sack with poorer nutrition which means their exogenous feeding starts early on compared with species that lay demersal eggs e.g. *Salmo salar* (Gjedrem et al., 1993, Falk-Petersen et al., 1999, Kjesbu, 1989).

1.1 Development of marine larvae

There are many factors that play a role in the development from a healthy marine pelagic larva to a healthy adult. These factors can be classified in two sets: intrinsic, and extrinsic factors. Intrinsic factors involve species-specific development pattern and show little variation within a species maturing in optimal conditions. The physical, chemical, and biological environment the larvae grows in are considered the extrinsic factors, moreover food availability and

nutritional value also belong in this category. The success of the larval development is dependent on the interaction of extrinsic factors upon intrinsic factors (Forskningsrådet, 2009). The small size of the of the newly hatched marine larvae and their early stages of exogenous feeding to have a rapid development requires high food availability with a diverse size distribution. (Hamre *et* al., 2013). In quantity proteins are the most important nutrition for the larvae however they are not enough on their own. One of the most important nutritional factors for a healthy larvae development is fatty acids (Houlihan *et* al., 1995; Conceição, 1997; (Izquierdo *et* al., 2000; Kanazawa, 1997). Fatty acids are known as micronutrients and are indispensable for the development pattern of the larvae however the larvae cannot synthesize fatty acids therefore they must be a part of their diet. The diet for pelagic marine larvae consists of live organisms i.e. various species and development stages of different zooplankton (Sargent *et* al., 1999; Sargent *et* al, 2002; Toivonen *et* al, 2001; Tocher, 2003). Furthermore, the marine pelagic larvae have a very low stress tolerance (Kjørsvik *et* al., 2007). One of the reasons marine pelagic larvae are difficult to cultivate is they are dependent on live feed rather than dry feed as used in cultivating fresh water species like salmon (*Salmo salar*). The dry feed particles are too large for the marine pelagic larvae to consume resulting in malnourishment.

1.1.1 Fatty acids (FA)

Poly-unsaturated fatty acids (PUFA)'s are essential for the development of marine larvae. The fatty acids 22:6 n-3 docosahexaenoic acid (DHA) and 20:5 n-3 eicosapentaenoic acid (EPA), and 20:4 n-6 (ARA) arachidonic acid belongs to the PUFA's are the primary once need for the marine fish larvae (Sargent *et* al., 1999a,b; Montero *et* al., 2004). DHA is precursor for the development of the neural tissue like the eyes and brain. Moreover, DHA is usually bound to phospholipids where it alters the cell wall by increasing the ion permeability amongst other things (Morgan *et* al., 2002). EPA bound to phospholipids maintain the cell wall and structure. Furthermore, EPA oxidizes to eicosanoids which are signaling molecules. The ARA must be consumed since marine fish cannot synthesis it (Bell and Sargent, 2003; Moraisa *et* al., 2001). The ARA competes with EPA to make eicosanoids, furthermore when ARA is bound to phospholipids its also has the function to maintain the cell wall and structure. The eicosanoids are signal powerful local hormone pathways for physiological and environmental stress ((Bell and Sargant, 2003; Stanley and Howard, 1998).

1.2 Live feed

There are several different live feed sources for marine larvae the most common are *Artemia*, rotifers, and copepods. Copepods are the natural food source for marine larvae therefore their nutritional profile is desired for the feed for the marine larvae in cultivation (van der Meeren *et* al., 2008). *Artemia* and rotifers have been used excessively as live feed because they are easy to cultivate and have to ability to change the nutritional value 2-24h prior to use with enrichment mediums (Olsen *et* al., 1993). However, their nutritional profile value is worse than that of copepods (McEvoy *et* al., 1998). Usually when cultivating copepods, they are limited to one or a mixture of up to three species of microalgae (McKinnon *et* al, 2003). For that reason, the biochemical composition of the microalgae is crucial for the quality of the copepods. The fatty acid profiles and carbon to nitrogen ratio amongst other biochemical compositions in the microalgae are dependent on the nutrient availability in the growth medium (Støttrup and Jensen, 1990; Lacoste *et* al., 2001). One of the motivations of using *Artemia* and rotifers over copepods is the high cost of cultivating copepods. The *Artemia* cysts quality varies thereby increasing the interest of copepod cultivation (Øie *et* al, 2011). "You are what you eat"!!!

1.2.1 Copepods

Copepods are an abundant group of zooplankton in the ocean and are a vital part of the diet for marine species (Humes, 1994). Copepods is a class of Crustaceans which contains 10 orders the three most abundant are the harpacticoid, cyclopoid, and calanoid (Dussart and Defaye, 2001). The order Calanoid contains the most studied species *Acartia* sp., *Centropages* sp., *Eurytemora* sp., *Parvocalanus* sp., and *Calanus finmarchicus* (Shields *et* al., 2005; Støttrup, 2005; McKinnon *et* al., 2003). Copepods are the natural food source for most species in marine aquaculture and have the right nutritional profile for marine larvae, hence the high interest. Copepods have high values of phospholipids, HUFA's (DHA, EPA, ARA), ascorbic acid (vitamin C), carotenoids, retinal/retinol, and micronutrients (van der Meeren *et* al., 2008; Hamre *et* al., 2008). The natural biochemical composition of copepods improves the pigment development, lowers the porportion of malformations, and improves the survival of marine larvae (Kanazawa, 1997; Fernández-Palacios *et* al., 2011). Copepods have six naupliar (NI-NVI) and five copepodite (CI-CV) stages ending the life cycle as an adult. The size of Copepods increases between each of the 12 development stages. The life cycle of Calanoid copepod is shown in Figure 1.1 (Algae Research and Supply). The generation time of copepods

can vary from days to years depending on species, temperature, salinity, feed quality and quantity. *C. finmarchicus* has a generation time of one year whereas as *A. tonsa* has a generation time of 12-14 days depending the temperature *C. finmarchicus* is the natural food for many of the newly introduced marine species, having pelagic larvae, in aquaculture. The short generation time of *A. tonsa* is desirable over *C. finmarchicus* due to a short life cycle. (Leandro *et* al., 2006; Heath et al., 2004). The cultivation of Copepods from egg to Adult is classified as intensive cultivation. The Copepods are grown to the desired size as feed for the fish larvae or they are cultivated in the purpose of producing eggs. Under the intensive cultivation of copepods there are several factors that play a vital role in a successful production. High oxygen concentration in the water and a light supply of 12^{+} h increases the egg the hatching. The oxygen supply is also important during the cultivation of copepods to keep the microalgae in suspension. The oxygen supply should minimize the occurrence of small bubbles since the copepods can get caught in them and die. After hatching the nauplii nauplii can tolerate a high density. As the nauplii develop into copepodites are more sensitive to the concentration of the individuals mL^{-1}. During egg production the females prefer a density of 1-10 individual mL^{-1}. Higher densities can reduce the egg production or cause mortality (Støttrup, 2003 & 2006; Lee et al., 2005). Furthermore, to optimize the production of Copepods all the environmental factors must be within the tolerance levels of the chosen species. The fecundity of Copepods is dependent on their consumption of EPA and DHA. A deficiency in n-3 fatty acids result in a more sensitive larvae with high mortality rate (Izquierdo 1996).

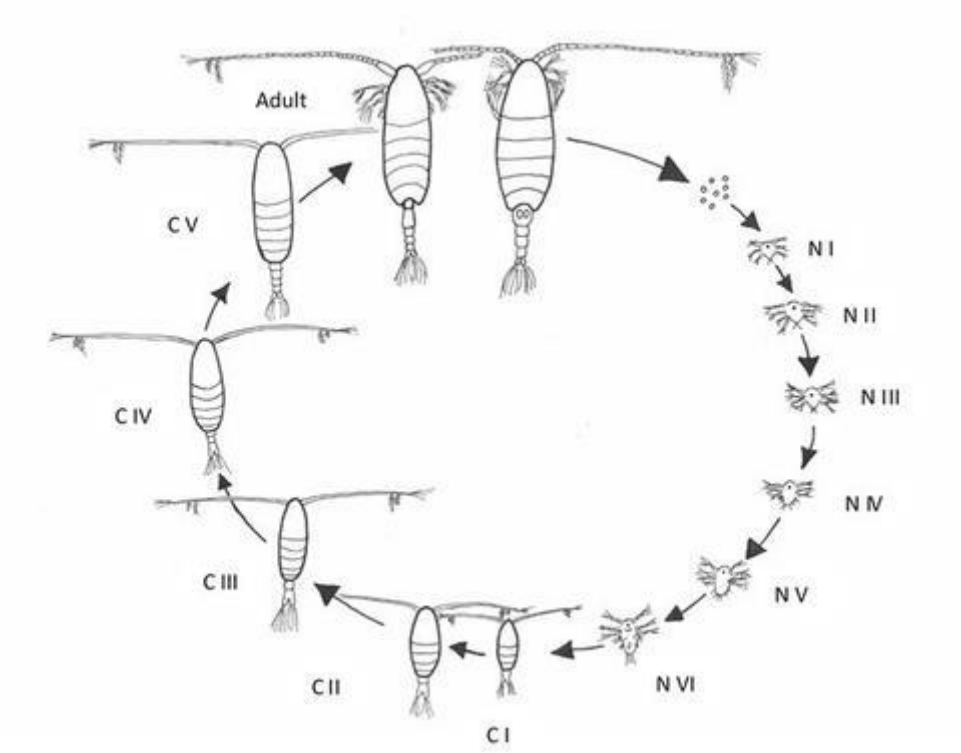


Figure 1.1. Shows the life cycle of a copepod from egg to adult. The six nauplii (NI-NVI) stages are shown and the six copepodite stages (CI-CVI). Furthermore, the figure shows in stage CVI the adults are differentiated between male and female (Algae Research and Supply).

1.2.2 *Acartia tonsa*

Acartia tonsa is a neritic Copepod species belonging to the order Calanoida of the subphylum crustacea that lives in brackish waters and tolerate high variations in temperature and salinity. They are distributed all around the world and feed on phytoplankton, ciliates, and rotifers (Kurashova and Abdullaeva,1984). They have a 12-step life cycle which is divided into six nauplii stages (NI-NVI) and six copepodite stages (C1-Adult). The nauplii metamorphosis into copepodites from nauplii stage NVI to C1 stage and becomes adult at stage CVI. Their life cycle takes approximately 12 days from hatching to adult under the conditions of 20ºC and a salinity of 30 with a sufficient amount of food. Furthermore, they are easy to cultivate and develop one stage a day at 20ºC making it simple to determine the copepod stage (Leandro *et* el., 2006). Their rapid development and ability to store fatty acids especially DHA/EPA makes them highly qualified as feed for marine larvae in cultivation (van der Meeren et al., 2008). Some of the marine larvae are very small when they start their first feeding and the stage NIV is used as start feed. Others marine larvae are larger and start with CI, CIV or Adult. Augustin and M. Boersma showed in 2006 *A. tonsa* fed on nitrogen deplete *Rhodomonas* sp. had an

increase in egg production. The *Rhodomonas* sp. had a C:N ratio of 4.5 in replete and 9.05 in the deplete treatment.

1.3 Microalgae

Microalgae are the unicellular photosynthetic organisms of the sea. They create energy by transferring sunlight with photosynthesis through different pigments in the cell Microalgae use the energy from the photosynthesis to produce carbohydrates, lipids, enzymes etc. from the available nutrients to maintain the cell and for preparing for cell division (Tomaselli *et* al., 2004). The nutrients required for a microalgae cell division is classified in to two groups: macro and micronutrients. The macronutrients primarily consist of nitrogen and phosphors however when growing diatoms silicate is included. The micronutrients consist of trace metals and vitamins. However, they are all essential for not limiting the growth. Furthermore, the temperature, light, and CO_2 are external factors which also can restrain the microalgae growth (Øie, 2011). Microalgae follow a five-phase growth pattern when cultivated. The lag or induction phase is when the addition of algae along with the growth medium where it takes some time for the algae to react to the excess amount of the growth factors. The exponential phase is when the algae have registered the growth factor/s are in excess and take advantage with exponential growth. The transition growth phase is when some of the growth factors are scarce thereby limiting the microalgae growth. The stationary phase is when the growth/death has reached an equilibrium of the microalgae. The death or crash phase is when the lack of a certain growth factor inhibits further growth and the cells thereby die (Figure 1.2) (Andersen, 2005).

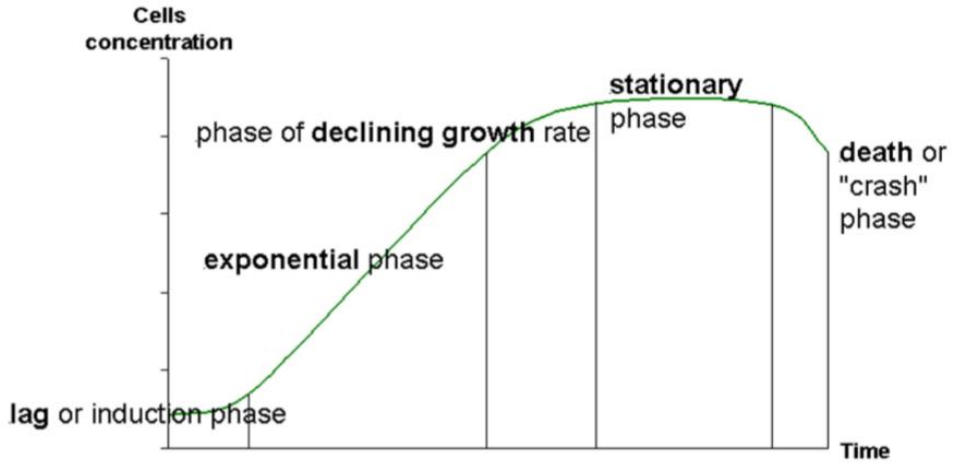


Figure 1.2. The graph illustrates the pattern microalgae grow when cultivated in the lab.

1.3.1 Nutrients

The factors that limit growth for the most part are: Light, and the macronutrients: nitrogen and phosphorus. Under nutrient limitation microalgae restrict cell division and increase their internal energy storage consisting of different lipids and carbohydrates. However, when cultivating microalgae in batch cultures nitrogen, phosphate, or light becomes the limiting factor and limits the growth. Therefore, the density of the culture and at which growth stage the microalgae are harvested effects their biochemical composition. The amounts of each nutrient for adequate growth is species dependent and can therefore be hard to generalize. Hence when cultivating algae all nutrients are in excess. In the exponential phase the microalgae have an excess of nutrients but in the stationary phase nutrient limitation or lack of light due to the high density of the microalgae limits the growth (Øie, 2011). Furthermore, the internal stoichiometry of carbon to nitrogen will change as the conditions in the growth medium change (Augustin and Boersma, 2006) The internal stoichiometry for the microalgae is as well dependent on which enrichment medium is used to create the growth medium. Some of the best know enrichment mediums are Conwy, f/2, K, and L1. The enrichment mediums were all designed for some specific microalgae yet can be used quite universal (Walne, 1970; Guillard and Ryther, 1962; Keller *et* al., 1987; Guillard, 1975). Moreover, the FA profile for the microalgae changes depending if it is in nutrient deplete or replete state. The FA accumulate in the microalgae in deplete state. Hence when choosing the microalgae for an experiment it is important to have in mind what is the desired FA profile in the end (Malzahn *et* al, 2007). For first feeding marine larvae and high amounts of the FA's DHA and EPA are needed for reducing abnormalities during development and for survival. Thus, the microalgae used for feeding the copepods that are feed to the marine larvae should contain high amounts of DHA and EPA (van der Meeren, 2008). Rhodomonas is a genus belonging to the phylum Cryptohyta that contains high amounts of DHA and EPA. "You are what you eat!"

1.3.3 *Rhodomonas*

Rhodomonas is a genus belonging to the phylum Cryptophyta. The characteristics are they contain plastids/chloroplasts (their foremost function is photosynthesis) and are flat with two asymmetrical flagellas placed in the anterior indention of the cell (Olenina, 2006). Another noteworthy characteristic is their high content of DHA and EPA which makes them interesting as a feed source for the copepods that are used as feed for first feeding marine larvae (Malzahn,

2007; Huerlimann, 2010). *Rhodomonas* is a genus with 28 different species and within each species there are several strands (Guiry and Guiry, 2018). The most know species are *R. baltica*, *R. lens*, *R. salina* they are also widely used in research as feed for copepods (Støttrup, 1986; Vu *et* al., 2016; Seisax, 2009). The specific use of the different species and strains is also country dependent *Rhodomonas baltica* 5/91 is used at SINTEF and CFeed (Norway) whereas *Rhodomonas salina* K-1487 is used in Denmark. (Kortner, 2011; Vu *et* al, 2016). The two species are very similar and are grown under the same conditions. They grow best with a temperature between 18-25ºC and a light irradiance of 100 µmol · m^{-2} · s^{-1}. The pH value in the culture should be around 8 to have a healthy growth to high or too low pH will inhibit the growth (Guevara, 2016). *Rhodomonas* are found in brackish waters therefore a salinity of around 28-30 is their preference (Jepsen, 2018). Furthermore, when *Rhodomonas* get nitrogen limited they are able relies the nitrogen bound to the red pigment (phycoerythrin) turning them green/yellow from red color (Thoison, 2017). Moreover, when the nitrogen deplete *Rhodomonas* get more nitrogen in the growth media they change back to the red color.

1.4 Scope of the study

The focus of the study was to determine if there was a possibility to improve the quality *A. tonsa* as live feed fed a mono-diet of different *Rhodomonas*. The *Rhodomonas* species were cultivated on four different nitrogen concentrations 0, 33, 66 and 100% $NaNO_3$. Three *Rhodomonas* species were chosen R. *baltica*, R. *marina*, and R. *salina* for the experiment. *R. baltica*, R. *marina*, and R. *salina* were analyzed for their growth, C:N molar ratio, and fatty acid profile for each of the four $NaNO_3$ treatments. The superior treatments from each of the microalgae species were chosen and fed as mono-diet to *A. tonsa*. The FA profile, C:N, survival, and development of *A. tonsa* was measured.

2 Aim of study

The aim of the study was to establish the development and survival rate of *Acartia tonsa* fed on mono-diets of one of three species of *Rhodomonas baltica, marina,* and *salina* cultured in four different nitrogen concentrations. The objectives of the study are

- to examine how the different nitrogen concentrations, affect the growth phases and carrying capacity of *R. baltica, R. marina,* and *R. salina*.

- to determine the effect nitrogen has on the stoichiometric ratio C:N and fatty acid composition of the *Rhodomonas* species.

- to evaluate which *Rhodomonas* species and respective nitrogen treatment constitutes the best feed for *A. Tonsa* according to development rate, survival, the C:N stoichiometry, and fatty acid profile with emphasis on DHA and EPA.

Hypothesis

1. There is no difference in the fatty acid profile for the four different treatments in the exponential phase; however, there should be a change in the fatty acid profile when nitrogen starts being limiting.

2. The changes in *Rhodomonas* stoichiometry and biochemistry represent changes in quality as food for herbivores although in the exponential phase in nitrogen replete conditions there will be no difference between the treatments.

3. The carrying capacity of *Rhodomonas* will vary between the different treatments.

4. The survival and development rate of *A. tonsa* is dependent on their diet.

5. The FA profile of *A. tonsa* is dependent on the fatty acid profile of their diet.

6. The C:N stoichiometry in *A. Tonsa* is dependent on their diet

3 Materials and methods

3.1 Microalgae experiment

3.1.1 Stock culture

The stock cultures *R. baltica* (Niva 5/91) and *R. marina* K-0435 were purchased from NIVA CCA. *R. salina* (NIVA 15/12) was received from Katharina Balding from Trondheim Biological Station. *R. baltica* and *salina* were maintained with autoclaved sand-filtered seawater enriched with full Conwy medium (1.5 mL/L). *R. marina* showed to be more difficult to grow and did not grow with the TL medium either.

3.1.2 Set up

Each set up was done for one species at a time. 16 bottles (four per treatment) were set up with 1.5L of growth medium (1mL enrichment media per liter of seawater) containing around 100,000 cells each for *R. baltica* and 75,000 *R. salina* and aerated. The light regime was 24h light furthermore pH and temperature were measured each day. Biomass samples were taken every day for determining the different growth phases with a growth curve, furthermore to define the growth rate and carrying capacity for each of the treatments. Fatty acid and particulate phosphate, nitrogen, and carbon samples were taken during the early logarithmic phase, late logarithmic phase, and during the stationary phase.

3.1.3 Preparations

Figure 3.1 shows a schematic of this setup. 16 1.5L soda bottles with a flat bottom were acid washed with 0.4% HCl and thoroughly rinsed with distilled water before use. There was continues lighting during the experiment and all bottles had a distance from the light to give a photon flux of 100 µmole m^{-2} s^{-1}. The seawater was sand filtered before chlorination with 0,25mL Sodium hypochlorite pr. 1L for 24 hours thereafter dechlorinated with 0,03g Sodium thiosulfate pr. 0,25mL Sodium hypochlorite with heavy aeration. One liter of Conwy medium without $NaNO_3$ was prepared (Walne, 1970). *R. marina* had problems growing when cultivated with Conwy enrichment therefor they were switched to TL medium used by SCCAP and NIVA CCA (Larsen et al., 1994). The nitrogen concentration in the TL medium and Conway medium is equal however the phosphate concentration is 5 times lower in the TL medium. The preparation of the different treatments for the TL medium are summed up in table 3.2. The

preparation of the four different nitrogen concentrations 0, 33, 66, and 100g $NaNO_3$ in the enrichment mediums are given in Table 3.1. Sufficient aeration was added to the cultures to keep the algae in suspension. The air was enriched with CO_2 (3200ppm). The aeration system was set up by six air-outlets where each one was connected with three 3x5mm silicone tubes with capillaries (0.8mm 1x3mm silicon tube with a 1.5 cm 0.5x1mm tube) to give a stable airflow. The light intensity was measured with the Quantumflux meter MQ-500: Full-Spectrum Quantum Meter from Apogee Instruments. Once a day the temperature was measured with a Digi-Sense® DUEL J·T·E·K™ THERMOCOUPLE THERMOMETER from Cole and Parmer and the pH was measured with a pH 3210 meter from WTW. All filters used during the experiment were precombusted (450ºC for 2 hours) 25mm GF/F Whatman® Glass microfiber filters.

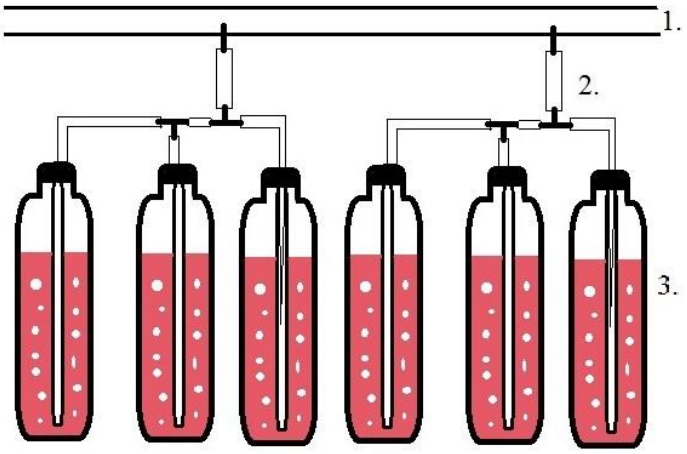


Figure 3.1. A schematic drawing of the algae set up. 1: The main air flow. 2: Two air outlets from the main airflow that is connected with the algae cultures. 3: Bottles of Rhodomonas cultures during the experiment.

Table 3.1. Overview of the nitrogen concentration of the Conwy enrichment medium for the four different treatments**.**

m(NaNO3)	Volume	Concentration of $NaNO_3$
g	L	g/L
0	0.1	0.00
3.3009	0.1	33.0
6.6018	0.1	66.0
25.0087	0.25	100.0

Table 3.2. Overview of the nitrogen concentration of the TL enrichment medium for the four different treatments.

m($NaNO_3$)	Volume	Concentration of $NaNO_3$
g	L	g/L
0	0.1	0.00
3.30	0.1	33.0
6.60	0.1	66.0
10.00	0.1	100.0

3.1.4 Biomass

Between 10,000 and 600,000 Rhodomonas cells from each culture were harvested and diluted up to 20 mL in a coulter counter beaker. The beaker was placed in the Multisizer™ 3 Coulter Counter and measured with the program multisizer 3 where 1000µl were measured three times under a current of -800 with a capillary of 100µl and the electrolyte was set to seawater. Equation (1) was used to calculate the density in the cultures.

$$Density_{Culture} = d * Density_{beaker} \qquad (1)$$

$Density_{Culture}$ is the density (cells/mL) in the culture and $Density_{beaker}$ are cells/mL given by the Coulter Counter while *d* is the dilution factor.

3.1.5 Particulate phosphorus

Usually you start such a paragraph like: Particulate phosphorus was measured following XXX as orthophosphate (I guess). Approximately 4,000,000 Rhodomonas cells were filtered on a 25mm GF/F Whatman filter and stored in scintillation vials at – 20ºC until analysis. The filters were thawed, 0.1 mL 4M H_2SO_4 are added to each scintillation vials and mixed. Thereafter the samples were oxidized with 2mL $K_2S_2O_8$ (50g L^{-1}). The samples were then autoclaved at 120ºC for 30 min and allowed to cool to room temperature. Before the samples were analyzed they were refiltered with a 0.45µm filter syringe. The samples were then stored in a – 20ºC freezer until the autoanalyzer was ready. To calculate the amount on the filter equation (2) was used.

$$PP = \frac{\left(P_{sample} - P_{blank}\right) \cdot 0.0121}{filtered\ volume} \qquad (2)$$

PP = µg P /L 0.0121 = coefficient filtered volume = L

P_{sample} = µg P/L P_{blank} = µg P/L

To calculate the molar concentration µM P thw PP was divided with the molar mass of phosphorus (30.9737 g/mol) and divide the number with 1L to get µmol/L (µM).

3.1.6 CHN analysis

Roughly four million cells were filtered on a 25mm GF/F Whatman filter and stored at -20 ºC until further preparation in 6-well plate to insure the filters would not have a crease line. I guess a sentence on the objective of the following would be good to help understand why you have done such an effort. An iron plate, a puncher and tweezers were washed in 96% ethanol and left on foil to evaporate. The work station was covered with foil before the equipment's was transferred to the station. The filters were placed on top of the iron plate and the part of the filter covered in algae was punched out with the hole puncher and the excess filter with no algae was descarded. The algae covered filter was packed in to a 5x9mm tin capsules for solids from Säntis analytical and formed as a tiny ball. All the samples were stored in a 96-well plate in a -20 ºC freezer until the samples were analyzed. The night before the samples would be analyzed they would be placed in a heating cabinet at 60 ºC to dry the samples before placed in CHN analyzer Elementar vario EL cube for combustion.

The CHN analyzer gives out the amount of particulate carbon and nitrogen as µg/filter. The amount was then transformed to mg/filter.

To calculate the concentration of particulate carbon or particulate nitrogen equation (3) was used.

$$mg/_L = \frac{\frac{mg}{Filter}}{filtered\ volume\ (L)} \qquad (3)$$

To calculate the molar concentration for particulate carbon or particulate nitrogen the result from formula (3) where divided with their molar weight 12.0107g/mol and 14.0067g/mol, respectively. Giving the molar concentration the unit µM C or N.

3.1.7 Fatty acids

Between 500mL to 50 mL of each culture was harvested depending on the density of the culture in a 50mL tube and centrifuged. The supernatant was removed, and nitrogen gas was added to the tube then stored at -80ºC until freeze dried. Around 1mg DW freeze dried sample of *Rhodomonas* was transferred to a 20mL glass vial used for the analysis. before starting the fatty

acid analyses the samples had to be digested with protease (Why?). 0.1 mL protease solution (10 mg *Streptomyces griseus* protease from Sigma Aldrich dissolved per mL 0.1 M Tris HCl) and 0.7 mL 0.1 M Tris HCl was added to each sample. Thereafter it was left to be incubate for 1h in room temperature (Jakobsen et al., 2008). Modifications were made on the Bligh and Dyer (1959) method for the extraction of lipids and the Metcalfe et al. (1966) method which used for the fatty acids methyl-esterification. A more detailed descriptive of the modifications are given below. Internal standard concentration (C23:0, NU-CKEK PREP, Inc.) was adjusted to 21µg per sample, which is approximately 14% of the expected lipid content of the samples. s dependent on the total lipid concentration of the sample.

The total lipid concentration of *R. baltica* is dependent on the growth conditions, therefor it was assumed it was around 15% furthermore the concentration of the internal standard should be 14% of the total lipid concentration. Therefor with a sample size of 1mg the total lipids contribute to an amount of 0.15mg corresponding to 15%.

Total lipid analysis

The tubes were put on ice after protease incubation. 2mL methanol and 1mL chloroform with internal standard were added to the tube and homogenized with IKA® $T10_{basic}$ – ULTRA-TURRAX® for one minute. 1mL of chloroform was transferred to the vial and homogenized for 20 seconds. 1mL of dH_2O from Elga PURE lab flex was added and homogenized for 20 seconds followed by 10 minutes of centrifuging at 4000rpm (Universal 32,Hettich). The tubes were kept on ice at all times until they got centrifuged. The 2 mL of chloroform in this method gave slightly more than 1.5mL of lipid extraction in chloroform. 0.5 mL of the lipid phase (lower phase) was transferred to a pre-weighed glass vial and the chloroform was evaporated with the Nitrogen evaporator OA-HEAT™ MODEL 5085 N-EVAP™ III. The vials were placed in a desiccator overnight before the final weighing. The amount of total lipid was multiplied by 4 given that 0.5mL of 2mL chloroform was transferred to the total lipid vial. Furthermore, the lipid concentrations were corrected by retracting the amount of internal standard (21µg) added to the sample to give the final result (µg/mg). The remaining 1 mL of the lipid containing phase was transferred to a new 20mL glass vial and the chloroform was evaporated. The method is summarized in Figure 3.2.

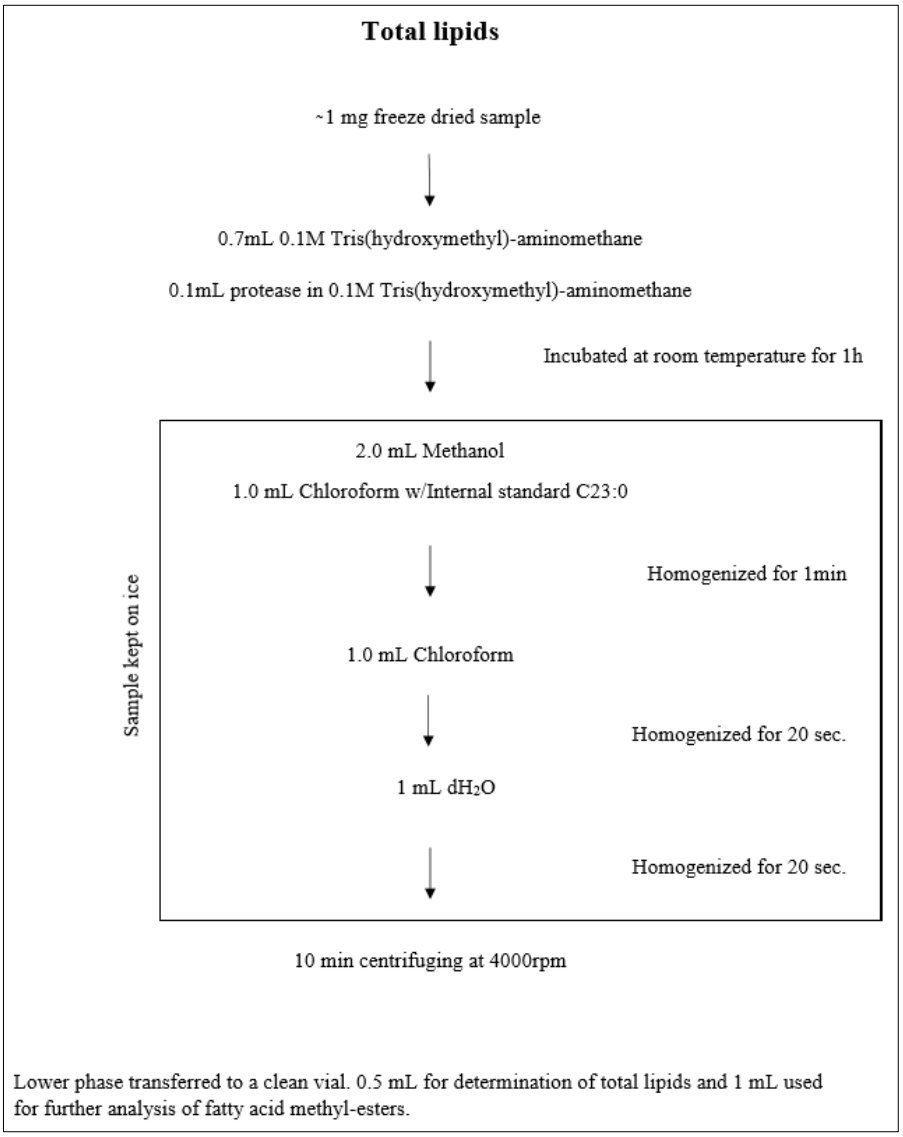


Figure 3.2. The modified method for the extraction of lipids.

Fatty acid methyl esterification (FAME)

The FA in the tube from the last step of the total lipids extraction was esterified by adding 1mL 0.5N NaOH-methanol to the tube, to dissolve the lipids, vortexed and heated for 15 minutes at 100ºC. 2mL of BF_3-methanol were added to the mixture, vortexed and heated for 5 minutes at 100ºC. 1mL isooctane was added to the mixture, vortexed and heated at 100ºC for 1 minute. The vials were cooled on ice between the heating and adding of a new chemical. 3.0mL saturated NaCl in dH_2O and 0.5mL isooctane was added and vortex then centrifuged for 3 minutes at 4000rpm. 0.5mL isooctane was added, vortexed and centrifuged for 3 minutes at 4000rpm the last step was repeated twice. The last was transferring the lipid phase (upper phase) to a small GC vial and analyzed with the gas chromatography Agilent Technologies 7890B GC system. The method is reviewed in Figure 3.3.

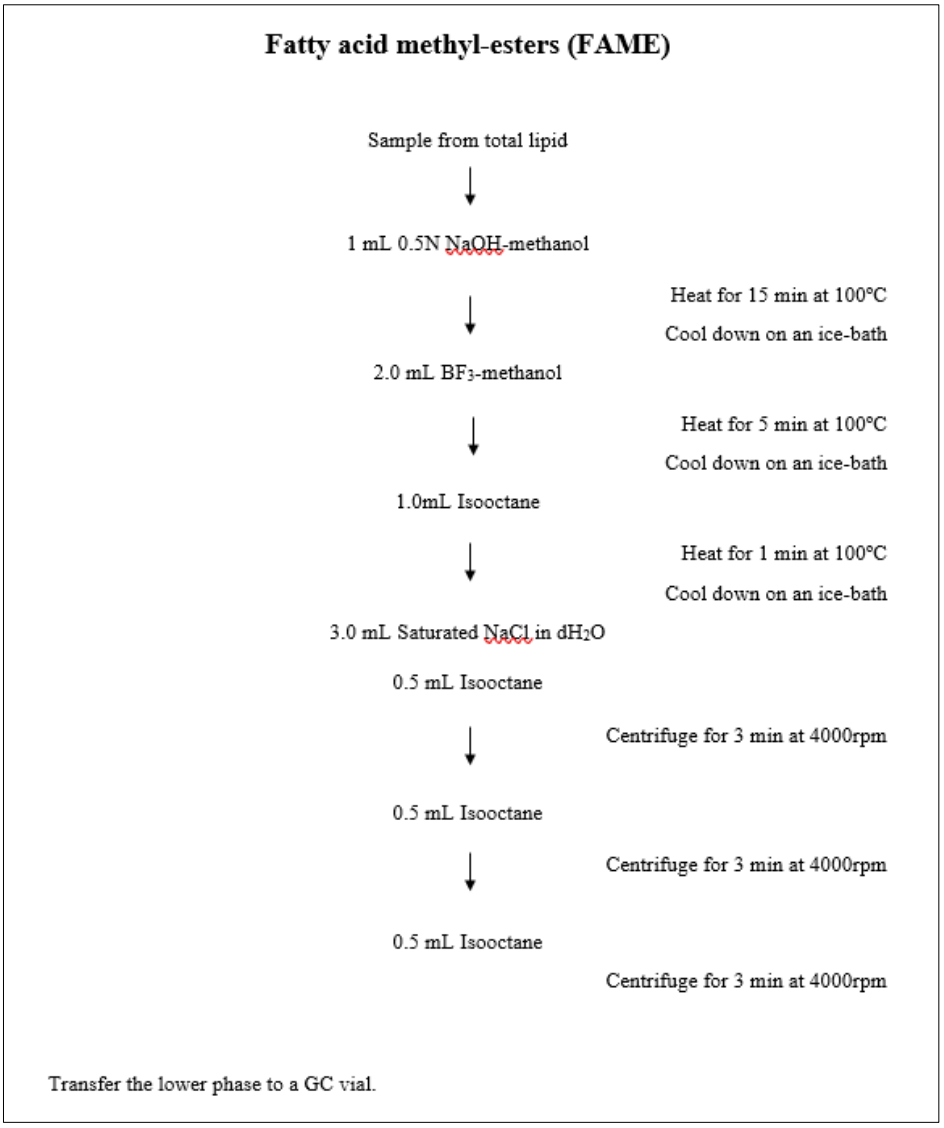


Figure 3.3. The modified method of fatty acid methyl esterification (FAME).

Split injections with helium as the carrying gas was used by the Gas Chromatography AgilentTechnology 7890B with a flame ionization detector (FID). The program Agilent 7890B GC - Acquisition calculates the concentration ng fatty acids μl^{-1} of the different known peaks from the internal standard in the chromatogram.The number given by the machine must be corrected with the exact weight of the samples and the dilution factor. 1 mL of 2 mL of chloroform was evaporated which and that leads to half of the total lipids sample is analyzed with the FAME. In the FAME method 2.5 mL of isooctane was added to the samples and only 1 µL is analyzed with the GC. This gives a dilution factor of 1250. The calculations for the different fatty acids were calculated with equation 4.

$$FA\left({}^{ng}/_{mL}\right) = \frac{{}^{ng}/_{mL}\,(GC) * 1250}{Sample\ DW} \qquad (4)$$

3.2 Copepod experiment

3.2.1 Set up

To test the effect of different algal qualities on growth and survival of copepods, three types of nutrient manipulated algae were used as food for the copepod *A. tonsa* in triplicates. Nine 5.9L polypropylene buckets from Emsafe, Emballator Lagan plast, were used for cultivating the copepods. A hole was drilled at the 5L marking in each bucket for a sieve (64µm) to create an overflow system. To enable to control temperature, the experimental containers were placed in waterbaths. Two water baths with drains were made with Euro container ED 86/22 2S from Auer packaging by drilling a hole in the bottom of the containers and connecting them with a drain to remove the overflow water from the copepod buckets. To keep the temperature steady at 20°C throughout the experiment a One Eco Therm 50W aquarium heater from Newa and a Platinum RTD sensor from Auber were connected to SYL-2372 PID temperature controller from Auber. Furthermore, water circulation was maintained with a water pump which was placed in the corner. A schematic drawing is illustrated in Figure 4.3. To minimize any external effect on the algae biochemical composition in the feeding tank artificial seawater was used without nitrogen and phosphorus. The artificial seawater was made with distilled water and aquavital sea salt (Aquarium Münster) and was used for the copepod tanks and the feeding tanks.The artificial seawater was made in portions of 40L with a salinity of ~ 30 in 50L containers from Bewi Norplasta made of polypropylene with heavy aeration 24h before use to get the salts to dissolve. 30L containers from RD plastics were used as feeding tanks and were connected to the copepod buckets with a peristaltic pump from Watson Marlow, 205S. To keep the algae in suspension throughout the experiment a steady low aeration was added to the buckets by tubes that were connected to a compressor from Hiblow, HP40.

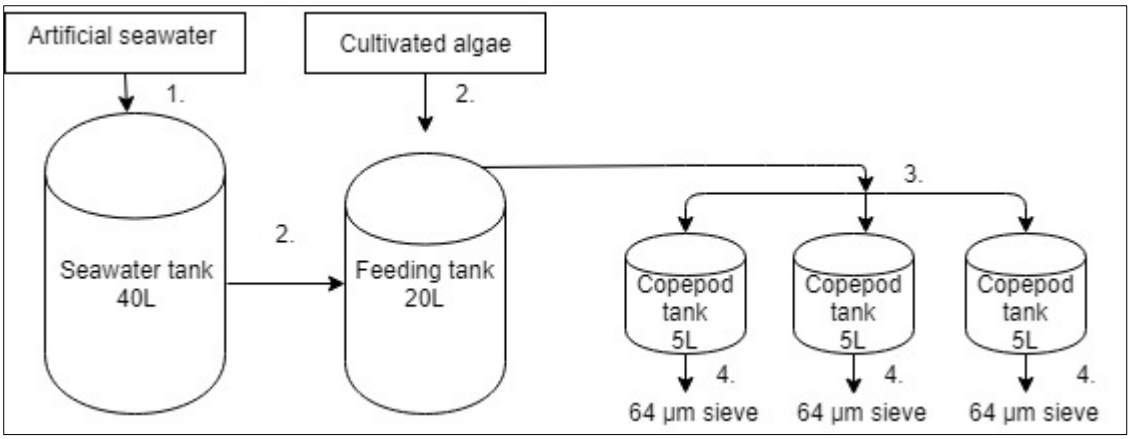


Figure 4.3. A schematic drawing of the copepod system. 1. The storing tank for artificial seawater. 2. The feeding tank where the cultivated algae and artificial seawater are mixed. 3. The feed mixture was pumped from the feeding tank and distributed evenly in to the three copepod tanks (15L/day). 4. The overflow water was filtered through 64 µm sieve to keep the copepod in the thanks.

3.2.2 Feeding

The selected microalgae treatments with the highest amount of DHA/EPA, total lipids, and total amount of fatty acids were chosen as mono-diets for *A. tonsa* which showed to be the stationary phase for *R. baltica* 66%, *R. salina* 66%, and *R. baltica* 100%. The algae were grown under the same conditions as in the algae experiment. The bottles were exchanged to 1L cell culture bottles due to the shape of the bottle the aeration had to be high and caused water to evaporate. Distilled water was added each day to the 1L mark of the bottles to keep the salinity constant throughout the experiment. Each treatment had three replicas resulting in 9 buckets with a volume of 5L. Five liters of artificial seawater with an algae concentration of 30,000 cells/mL was added to each bucket. The water exchange was with an overflow system with a peristaltic pump (5L/day). During the experiment as the copepod developed, feed concentrations were increased as shown in table 4.3. Each day a new algae mixture was made for the 20L feeding tank. Initial density of *A. tonsa* was around 20 nauplii/mL.

Table 4.3. Displays the concentration and amount of the feeding tanks prepared each day during the experiment.

Time	Algae concentration	Artificial seawater
Day	algae/mL	L
1	30,000	20
2	30,000	20
3	30,000	20
4	40,000	20
5	40,000	20
6	60,000	20

3.2.3 Hatching of Acartia tonsa

Acartia tonsa eggs were supplied by C-Feed AS (Norway). The eggs were put up for hatching in a bucket placed in the water bath with high aeration and an aquarium heater with a sensor to keep the temperature at 20ºC. 23h after the eggs were put up for hatching the nauplii hatched so far were discarded. Only the nauplii that hatched between hour 24-32 were collected and distributed between the buckets at a density of ind. mL^{-1}.

3.2.4 Development and survival

The staging of *A. tonsa* was done by taking pictures (Zeiss, Axiocam ERc 5s, attached to a microscope Nikon, Eclipse E200). The length of *A. tonsa* was measured with the program Zeiss which is a software for the camera. Approximately a 100 individuals were measured to get a good representation of the population at day 0, 4, and 7. The individuals were staged according to Alver et al. (2011) and (Leandro et al., 2006). The stages were given a number between 1-12 where 1-6 are the nauplii stages 1-6 and 7-12 represents the copepodite stages C1 stage to adult. Survival was estimated at day 3 and day 6 (experiment terminated) by taking a couple of mL of the culture and counting the individuals within it.

3.2.5 Particulate phosphate, carbon, and nitrogen

Individuals were collected on precombusted (450ºC for 2 hours) 25mm Whatman® GF-F filters and counted under the microscope. One sample for particulate phosphate analysis and one filter for C/N analysis were prepared. The analytical methods are more closely described in 4.1.5 and 4.1.6.

3.2.6 Fatty acids

Approximately 5mg of the *A. tonsa* culture was harvested (amount depending on size). The methods used are more closely described in 4.1.7. There was one minor change in the beginning of the total fatty acids protocol. No enzyme treatment was need and 0.8mL with ultra-distilled water with no incubation time. The fatty acid methyl esterification was extracted and calculated following the methods described in 3.1.7.

3.3 Statistics

The statistical analyses, graphs and tables were made in Microsoft® Office Excel and Word 2016 for Windows (Microsoft Corporation, USA). ANOVA was used to check for variance of the mean within treatments and between species.

4. Results

4.1 Microalgae

4.1.1 Growth

Figure 4.1 and Figure 4.2 show the growth curve for *R. baltica* and *R. salina*, respectively. *R. baltica* and *R. salina* both showed a there is a significant difference (ANOVA, $p < 0.05$) within each species where the response variable was biomass (cell number/mL) and predicting variable was time (days). Furthermore, there is a significant difference (ANOVA, $p < 0.05$) between *R. baltica* and *R. salina* for the equal treatments with error bars presenting the standard deviation. *R. baltica* and *R. salina* cultivated with 66% and 100% $NaNO_3$ in the growth medium have a normal growth curve. The microalgae grown on 33% $NaNO_3$ had a slight growth however the growth plateaued below 700.000 cells. *R. salina* did not tolerate the treatment with 0% $NaNO_3$ which resulted in the microalgae dying though the *R. baltica* did not die they just maintained the same density throughout the experiment. The growth rate for *R. baltica* and *R. salina* are shown in Figure 4.3 and 4.4, respectively. The response variable was growth rate and predicting variable was time (days). The *R. baltica* growth rates fluctuate during the experiment while the *R. salina* growth rate rises in the start of the experiment and decreases steadily until it reaches the stationary phase where it goes down to approximately 0. Furthermore, the *R. baltica* the growth rates and carrying capacity have a higher standard deviation then *R. salina* which indicate *R. baltica* have a higher natural difference within the cells or they are more sensitive to small changes in the environment. A summary of maximum growth rate and carrying capacity for each treatment and species is shown in table 4.1 and it also shows when each treatment reaches the carrying capacity and the maximum growth rate. *R. salina* reaches the maximum growth rate before *R. baltica* however *R. baltica* has a higher carrying capacity 3,370,650 cells/mL vs *R. salina* 2,547,950 cells/mL under the same growth conditions.

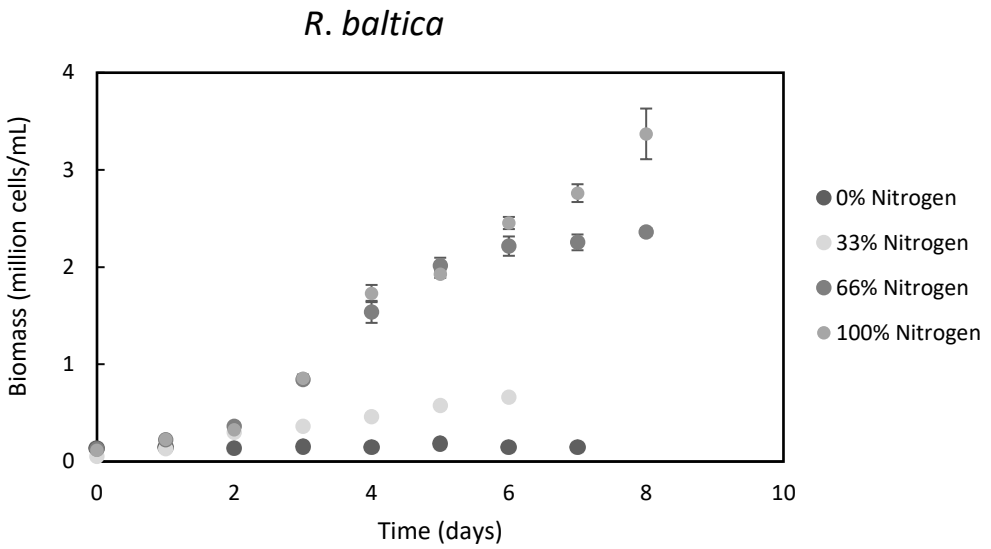


Figure 4.1. Growth curve of Rhodomonas baltica grown on different $NaNO_3$ concentration. Biomass (cell number/mL) as a function of time (days). Error bars show the standard error.

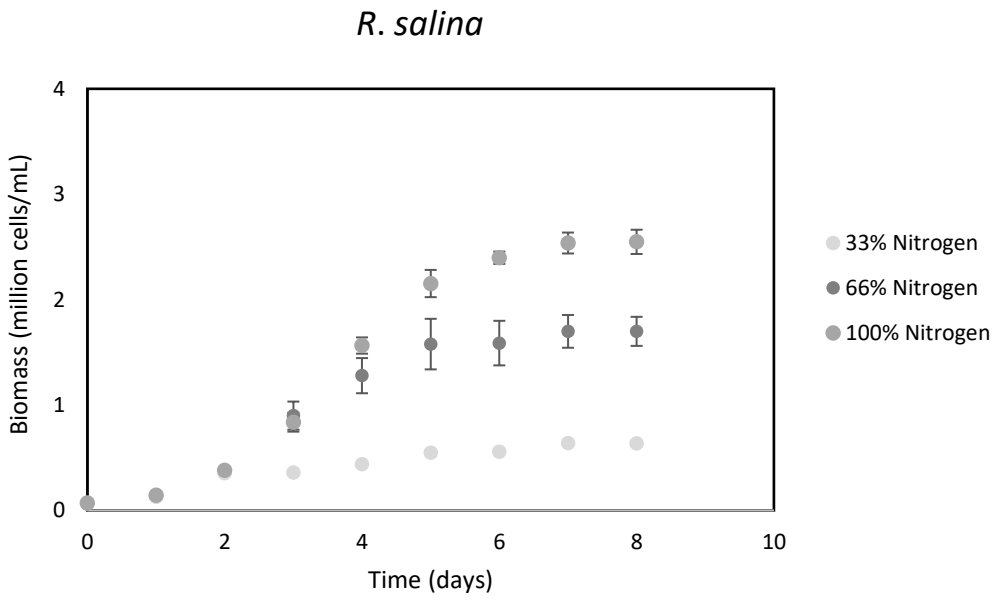
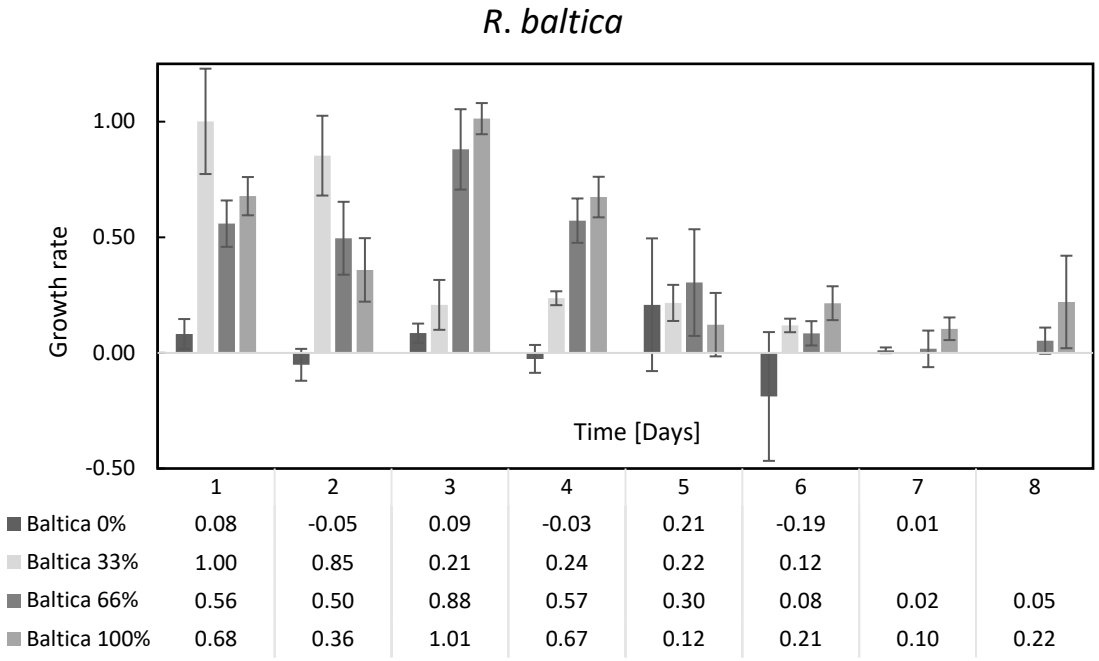
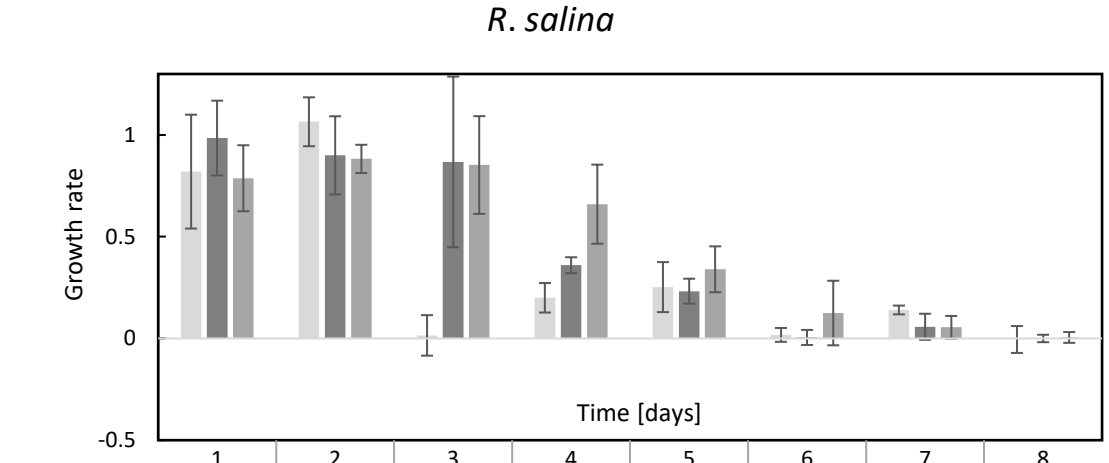


Figure 4.2. Growth curve of Rhodomonas salina grown on different $NaNO_3$ concentration. Biomass (cell number/mL) as a function of time (days). Error bars show the standard error.



	1	2	3	4	5	6	7	8
Baltica 0%	0.08	-0.05	0.09	-0.03	0.21	-0.19	0.01	
Baltica 33%	1.00	0.85	0.21	0.24	0.22	0.12		
Baltica 66%	0.56	0.50	0.88	0.57	0.30	0.08	0.02	0.05
Baltica 100%	0.68	0.36	1.01	0.67	0.12	0.21	0.10	0.22

Figure 4.3. The bar graph displays the relative growth rate for *R. baltica* throughout the experiment. The error bars represents the standard deviation.



	1	2	3	4	5	6	7	8
Salina 33%	0.82	1.065	0.015	0.2	0.2525	0.0175	0.14	-0.005
Salina 66%	0.985	0.9	0.8675	0.36	0.2325	0.005	0.0575	0
Salina 100%	0.7875	0.8825	0.8525	0.66	0.34	0.125	0.055	0.005

Figure 4.4. The bar graph displays the relative growth rate for *R. baltica* throughout the experiment. The error bars represents the standard deviation.

Table 4.1. The carrying capacity and maximum growth rate (Max GR) are summarized in the table for each species and treatment with the standard deviation. Furthermore, the numbers in brackets show what day the carrying capacity and maximum growth rate is reached.

Species	Treatment (% $NaNO_3$)	Carrying capacity	Max GR
R. baltica	0	179925 ± 51305 (5)	0.21 ± 0.29 (5)
R. baltica	33	663300 ± 55041 (6)	1.00 ± 0.23 (1)
R. baltica	66	2363150 ± 78994 (8)	0.88 ±0.17 (3)
R. baltica	100	3370650 ± 521066 (8)	1.01 ± 0.07 (3)
R. salina	33	637220 ± 12148 (7)	1.07 ± 0.12 (2)
R. salina	66	1698700 ± 275891 (8)	0.99 ± 0.18 (1)
R. salina	100	2547950 ± 230207 (8)	0.88 ± 0.07 (2)

4.1.2 Molar ratio: carbon and nitrogen

Table 4.2 summarizes the results taken from the main data sheet presented in A1 for the particulate carbon (C) and nitrogen (N) in the microalgae. Figure 4.5 shows the C and N molar ratio for each measuring point for each $NaNO_3$ concentration of *R. baltica* and figure 4.6 illustrates the data for *R. salina*. The error bars show the standard deviation. The significant difference was determent with ANOVA, $p < 0.05$.

Within treatments

In *R. baltica* there was a significant difference between the treatments grown *R. baltica* 0 % $NaNO_3$ stationary phase and exponential phase $p \approx 0.03$ and late exponential phase $p < 0.00$. Within *R. baltica* grown on 33% $NaNO_3$ there was a significant difference between the molar ratio in the exponential phase and late exponential as well as the stationary phase $p \approx 0.03$ and $p \approx 0.02$, respectively. For the *R. baltica* 66% $NaNO_3$ treatment the significant difference was $p \approx 0.01$ between the exponential phase and late exponential phase along with the stationary phase. The 100% $NaNO_3$ treatment for *R. baltica* there is a significant difference between the stationary phase and the exponential phase $p < 0.00$ and between the stationary phase and the late exponential phase $p < 0.00$. In *R. salina* 33% $NaNO_3$ treatment the exponential phase is statistically different from the late exponential phase and the stationary phase $p < 0.00$ for both. In the *R. salina* 66% $NaNO_3$ treatment there was a significant difference between the exponential phase and the stationary phase $p < 0.00$. In the *R. salina* 100% $NaNO_3$ treatment

there was a significant difference between the stationary phase and the exponential plus the late exponential phase $p < 0.00$ and $p \approx 0.01$, respectively.

Between treatments

The only significant difference between treatments and phases in *R. salina* was between the late exponential phase of the treatments 33% and 100% $NaNO_3$ $p \approx 0.01$. There were significant differences between the 0% $NaNO_3$ for *R. baltica* in the exponential phase and 33% $NaNO_3$ as well as 100% $NaNO_3$ $p \approx 0.01$ and $p \approx 0.05$ (rounded up), respectively. In the late exponential phase for *R. baltica* there was a significant difference between 100% $NaNO_3$ and 0% $p \approx 0.01$ furthermore there was a significant difference between the 100% $NaNO_3$ and the 66% $NaNO_3$ treatments $p \approx 0.01$. In the stationary phase there was a significant difference between the 0% $NaNO_3$ treatment and all the other treatments p values given from 33% to 100% $p \approx 0.02$; $p < 0.00$; $p < 0.00$. There were only two significant differences between treatments and between both in the stationary phase of the 66% and 100% $NaNO_3$ treatments $p \approx 0.01$ and $p < 0.00$.

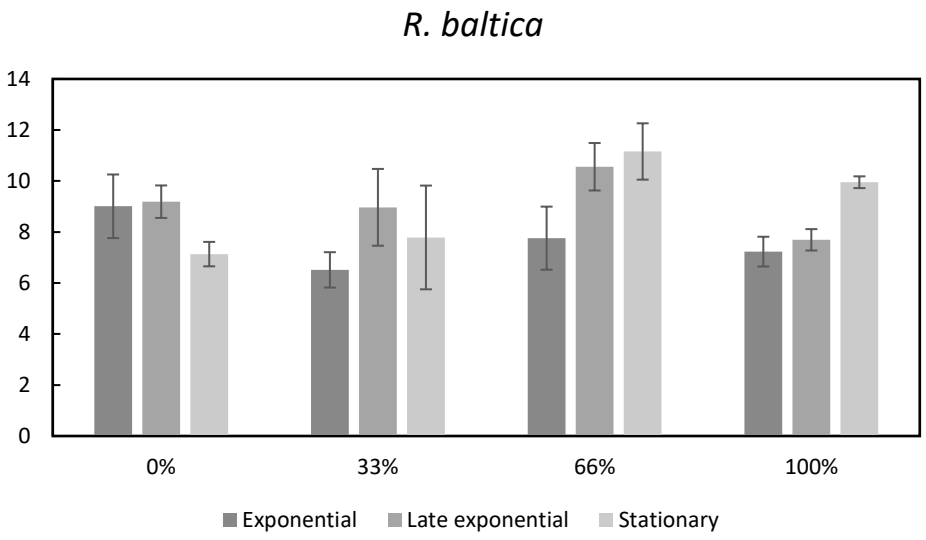


Figure 4.5. illustrates the molar ratio for the three-measuring points exponential, late exponential, and stationary phase for the four different treatments 0%, 33%, 66%, and 100% $NaNO_3$ for *R. baltica*. The error bars show the standard deviation. The values can be seen in table 4.2.

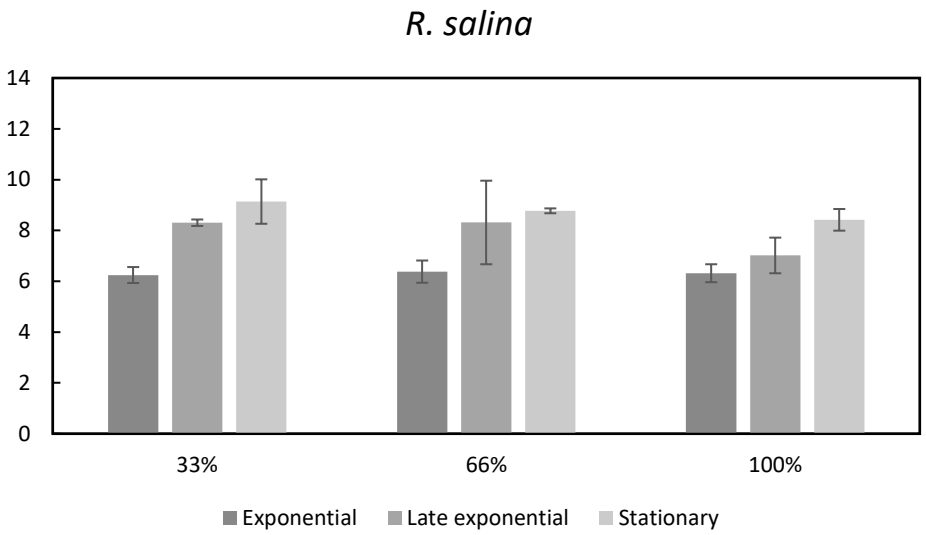


Figure 4.6. The bar graph illustrates the molar ratio for the three-measuring points exponential, late exponential, and stationary phase for the three different treatments 33%, 66%, and 100% $NaNO_3$ for *R. salina*. The error bars show the standard deviation. The numbers are shown in table A3.

4.1.3 Fatty acids

Due to the sizeable fatty acid data the data used in this paper was reduced to total lipids (TL), other lipids, fatty acids (FA), saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), EPA (Eicosapentaenoic *acid*), DHA (Docosahexaenoic acid), ARA (Arachidonic acid), other n-3, and other n-6. The data for the graphs 4.7, 4.8, and 4.9 are taken from A1 (Appendix A). The data was examined for significant difference with ANOVA, $p < 0.05$.

The total lipid distribution of *R. baltica* and *R. salina* for the four different treatments $NaNO_3$ are shown in Figure 4.7. The measuring points exponential, late exponential, and stationary phase are illustrated in separate frames where the seven treatments are presented. The total lipid distribution was divided in to four groups ∑SFA, ∑MUFA, ∑PUFA, and other lipids and measured in µg/mg dry weight (DW). Figure 4.8 shows the fatty acid distribution where the ∑SFA and ∑MUFA moreover it shows the division of EPA, DHA, ARA, other 3-n, and other n-6 within the ∑PUFA for *R. baltica* and *R. salina* for the four different treatments $NaNO_3$. Furthermore, the graph is divided in to three sub-graphs exponential, late exponential, and stationary phase and are shown µg/mg DW. In Figure 4.9 the fatty acid groups from Figure 4.8 are illustrated as a percentage of the TL for *R. baltica* and *R. salina* for the four different treatments $NaNO_3$. Additionally, the graph is broken up in to three parts exponential, late exponential, and stationary phase. Moreover, the remaining part of the TL are classified as other lipids.

Total lipids (TL)

R. baltica 0% $NaNO_3$ in the exponential phase declined during the late exponential phase ($p \approx 0.04$). In stationary phase for *R. baltica* 66% $NaNO_3$ was statistically higher then both the exponential phase ($p < 0.00$) and late exponential phase ($p \approx 0.01$). The *R. baltica* 33 % and 100% $NaNO_3$ had no statistically difference across the sampling points. There is a statistically difference ($p \approx 0.02$) between the exponential phase for *R. baltica* 0% $NaNO_3$ and *R. baltica* 33% $NaNO_3$ that latter being the higher amount. During the late exponential phase for *R. baltica* 33% $NaNO_3$ are significantly higher than *R. baltica* 66% and 100% $NaNO_3$ ($p \approx 0.03$ and $p \approx 0.05$, respectively). There was no statistical difference between the different treatments for *R. baltica* during the

stationary phase. The *R. salina* 33 % $NaNO_3$ had a statistical growth from the exponential to the late exponential phase ($p \approx 0.03$) followed be a drastic decline from the exponential phase to the stationary phase ($p < 0.00$). Furthermore, there was also a statistically difference between the exponential phase and the stationary phase ($p < 0.00$). For *R. salina* 66 % $NaNO_3$ had a steady growth from the exponential phase to the stationary phase and showed a statistically difference between the m ($p < 0.00$). In *R. salina* 100 % $NaNO_3$ had a decline from the exponential phase to the late exponential phase. A statistically different increase occurred between the late exponential phase and the stationary phase ($p \approx 0.04$). There was a significant difference ($p < 0.00$) in the exponential phase between *R. salina* 33 % $NaNO_3$ and *R. salina* 66 % $NaNO_3$ the latter being lower. In the late exponential phase, *R. salina* 33 % $NaNO_3$ was statistically higher then *R. salina* 66 % $NaNO_3$ and *R. salina* 100 % $NaNO_3$ ($p < 0.00$ and $p \approx 0.02$, respectively). The stationary phase shows *R. salina* 33 % $NaNO_3$ dropping drastically making *R. salina* 66 % $NaNO_3$ and *R. salina* 100 % $NaNO_3$ statistically higher ($p < 0.00$ and $p < 0.00$, respectively). *R. baltica* 33 % $NaNO_3$ was statistically higher then R. *salina* 33 % $NaNO_3$ during both the late exponential ($p \approx 0.02$) and the stationary phase ($p \approx 0.01$). During the stationary phase *R. baltica* 66 % $NaNO_3$ was statistically larger than the one for *R. salina* 66 % $NaNO_3$. There showed no statistically difference between *R. baltica* 100 % $NaNO_3$ and *R. salina* 100 % $NaNO_3$.

Fatty acid (FA)

In *R. baltica* 0% $NaNO_3$ have a statically increase from the exponential to the late exponential phase ($p \approx 0.03$) and decrease again in the stationary phase. In *R. baltica* 33% $NaNO_3$ had a steady increase over time however are statistically higher in the stationary phase compared to the exponential ($p \approx 0.01$) and late exponential phase ($p < 0.00$). In *R. baltica* 66% $NaNO_3$ follows the same pattern as *R. baltica* 33% $NaNO_3$ with the stationary phase being statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p < 0.00$). In *R. baltica* 100% $NaNO_3$ there was also a steady increase over time however not statistically different. In the exponential phase of *R. baltica* 0% $NaNO_3$ was statistically lower than the once from *R. baltica* 33% $NaNO_3$ ($p < 0.00$) and *R. baltica* 66% $NaNO_3$ ($p \approx 0.01$). FA was lowest in in *R. baltica* 0% $NaNO_3$ and higher in *R. baltica* 33% $NaNO_3$ there was a slight increase in the amount in *R. baltica* 66% $NaNO_3$ however it was lower than the *R. baltica* 33% $NaNO_3$ and *R. baltica* 66% $NaNO_3$ in *R. baltica* 100% $NaNO_3$

than *R. baltica* 0% $NaNO_3$. In the late exponential phase *R. baltica* had no statistical difference across the treatments. From the highest to the lowest 0% $NaNO_3$, 100% $NaNO_3$, 66% $NaNO_3$, and 33% $NaNO_3$. In the stationary phase *R. baltica* 0% $NaNO_3$ was statistically lower then *R. baltica* 33% $NaNO_3$ ($p < 0.00$) and *R. baltica* 66% $NaNO_3$ ($p < 0.00$). Furthermore, when comparing with *R. baltica* 33% $NaNO_3$ ($p \approx 0.2$) and *R. baltica* 66% $NaNO_3$ ($p \approx 0.1$) with *R. baltica* 100% $NaNO_3$ they were statistically higher. R. *salina* 33 % $NaNO_3$ had a statistically increase from the exponential to late exponential phase ($p < 0.00$) and a statistically decline from the late exponential to the stationary phase ($p < 0.00$). Furthermore, the late exponential phase was statistically higher than the stationary phase ($p \approx 0.02$). *R. salina* 66 % $NaNO_3$ had a steady increase over time. A statistical difference was between the exponential and stationary phase ($p < 0.00$). *R. salina* 100 % $NaNO_3$ decreased from the exponential to the late exponential phase and had an increase higher than the exponential phase from the late exponential to the stationary phase. The FA were highest in stationary phase and had a statistical difference between both the exponential ($p < 0.00$) and late exponential phase ($p \approx 0.01$). In the exponential phase for *R. salina* there was no statistically difference across the treatments *R. salina* 100 % $NaNO_3$ was slightly lower than that of *R. salina* 33 % $NaNO_3$ and *R. salina* 66 % $NaNO_3$ which were similar. The *R. salina* 33 % $NaNO_3$ in the late exponential phase was statistically higher than *R. salina* 66 % $NaNO_3$ ($p < 0.00$) and *R. salina* 100 % $NaNO_3$ ($p < 0.00$) being the lower one. The stationary phase for *R. salina* showed the *R. salina* 33 % $NaNO_3$ to be statistically lower than *R. salina* 66 % $NaNO_3$ ($p < 0.00$) and *R. salina* 100 % $NaNO_3$ ($p < 0.00$) being the higher one. Between *R. baltica* 33 % $NaNO_3$ and *R. salina* 33 % $NaNO_3$ *R. baltica* was statistically higher during the exponential ($p \approx 0.02$) and stationary ($p < 0.00$) phase. In the treatment of 66 % $NaNO_3$ *R. baltica* was statistically higher than *R. salina* during the stationary phase ($p \approx 0.01$). Comparing *R. baltica* and *R. salina* in the 100 % $NaNO_3$ treatment there was a statistical difference in the late exponential phase ($p \approx 0.01$) R. baltica being the higher amount.

Saturated fatty acids (SFA)

R. baltica 0 % $NaNO_3$ treatment had a statistical increase of SFA from the exponential to the late exponential phase ($p < 0.05$) and decreased in the stationary phase. The *R. baltica* 33 % $NaNO_3$ had a continues growth of SFA over time. *R. baltica* 33 % $NaNO_3$ in the stationary phase was

statistically higher compared with the exponential ($p < 0.00$) and late exponential phase ($p \approx 0.02$). *R. baltica* 66 % $NaNO_3$ also had a continues growth of SFA throughout the growth phases. The stationary phase for *R. baltica* 66 % $NaNO_3$ was statistically higher than the exponential ($p \approx 0.01$) and late exponential phase ($p \approx 0.02$). *R. baltica* 100 % $NaNO_3$ had an increase, although not statistically, in the SFA for the exponential to the late exponential phase moreover the SFA was the same in the late exponential and the stationary phase. In the exponential phase across the different treatments of *R. baltica* the only statistically difference was the treatments 33% $NaNO_3$ and 66 % $NaNO_3$ being statistically higher then 0% $NaNO_3$. *R. baltica* 33% $NaNO_3$ had the highest amount of SFA in the exponential phase of *R. baltica*. In the late exponential phase there was no statistical difference across the different treatments of *R. baltica* however was highest in the 33% $NaNO_3$. In the stationary phase for *R. baltica* the 0% treatment was statistically lower than 33% $NaNO_3$ ($p < 0.00$) and 66 % $NaNO_3$ ($p < 0.05$). Furthermore, the *R. baltica* 33% $NaNO_3$ was statistically higher than *R. baltica* 100% $NaNO_3$.In *R. salina* 33 % $NaNO_3$ the late exponential phase has the highest amount of SFA followed by the exponential then the stationary phase. The late exponential phase statistically higher than the exponential ($p < 0.00$) then the stationary phase ($p \approx 0.01$). In *R. salina* 66 % $NaNO_3$ had a slight decrease in the growth from the exponential to the late exponential phase however had an increase large increase in SFA in stationary phase. *R. salina* 100 % $NaNO_3$ had a decrease from the exponential phase to the late exponential phase and an increase above both the exponential and late exponential phase in the stationary phase. In the exponential phase for *R. salina* there was no statistical difference. In the late exponential phase for *R. salina* the treatment 33 % $NaNO_3$ was statistically higher than 66 % $NaNO_3$ ($p < 0.00$) and 100 % $NaNO_3$ ($p < 0.00$). In the stationary phase for *R. salina* the SFA were statistically lower than 66 % $NaNO_3$ ($p \approx 0.01$) and 100 % $NaNO_3$ ($p \approx 0.01$). Although, the amount of SFA in *R. baltica* 33 % $NaNO_3$ was higher than *R. salina* 33 % $NaNO_3$ in all the phases there was only a statistical difference in the exponential ($p \approx 0.02$) and stationary phase ($p < 0.00$). The SFA was higher in *R. baltica* 33 % $NaNO_3$ than *R. salina* 33 % $NaNO_3$ in all the phases however not statistically. *R. baltica* 100 % $NaNO_3$ was higher than *R. salina* 100 % $NaNO_3$ in the exponential, late exponential, and the stationary but not statistically.

Mono-unsaturated fatty acid (MUFA)

The treatment *R. baltica* 0% $NaNO_3$ had an increase in MUFA's from the exponential phase to the late exponential phase and a decline from the late exponential phase to the stationary phase. There was a statistical difference between the exponential phase and the late exponential phase ($p \approx 0.02$). *R. baltica* 33% $NaNO_3$ treatment had and steady increase of MUFA's over time. The stationary phase was statistically higher than the exponential ($p < 0$) and late exponential ($p \approx 0.01$) phase. *R. baltica* 66% $NaNO_3$ showed a steady growth throughout the experiment. The exponential ($p < 0.00$) and late exponential ($p < 0.0.0$) phases were statistically lower than the stationary phase. The amounts of MUFA's in *R. baltica* 66% $NaNO_3$ increased throughout the experiment. There was a statistical difference between the exponential and the stationary phase ($p \approx 0.04$). Between the exponential phase in the *R. baltica* the treatment of 0 % $NaNO_3$ was statistical lower between the 33% $NaNO_3$ and 66% $NaNO_3$ treatments. In the late exponential phase for *R. baltica* there was no statistical difference between the treatments. In the stationary phase for *R. baltica* the 0% $NaNO_3$ was statistically lower than the 33% ($p \approx 0.03$) and 66% $NaNO_3$ ($p \approx 0.01$) treatments. Furthermore, the 66% $NaNO_3$ was statistically higher than the 33% ($p < 0.05$) and 66% ($p \approx 0.01$) $NaNO_3$ treatments. In *R. salina* 33 % $NaNO_3$ the MUFA's increased form the exponential to the late exponential phase and declined below the amount in exponential in the stationary phase. The late exponential phase is statistically higher than both the exponential phase ($p \approx 0.01$) and the stationary phase ($p < 0.00$). *R. salina* 66 % $NaNO_3$ MUFA's steadily increased over time resulting in the stationary phase being statistically higher then both the exponential ($p < 0.00$) and late exponential phase ($p < 0.00$). In *R. salina* 100 % $NaNO_3$ the MUFA's increased throughout the experiment. The exponential ($p < 0.00$) and late exponential phase ($p < 0.00$) were statistically lower than the stationary phase. There was no difference in the amount of MUFA's in the exponential phase across the treatments for *R. salina*. In the late exponential phase for *R. salina* the treatment 33% $NaNO_3$ was statistically higher than the treatments 66% $NaNO_3$ ($p \approx 0.04$) and 100% $NaNO_3$ ($p \approx 0.01$). Furthermore, the treatment 33% $NaNO_3$ was statistically lower than treatments 66% $NaNO_3$ ($p < 0.00$) and 100% $NaNO_3$ ($p < 0.00$) in the stationary phase. Comparing *R. baltica* 33 % $NaNO_3$ and *R. salina* 33 % $NaNO_3$ showed *R. baltica* to be higher in all three phases. R. baltica was statistically higher in the exponential ($p < 0.00$) and the stationary ($p < 0.00$) phase. When comparing *R. baltica* 66 % $NaNO_3$ and *R. salina* 66 % $NaNO_3$ *R. baltica* was statistically higher in all the phases: exponential ($p \approx 0.01$), late exponential ($p \approx 0.04$), and

stationary ($p < 0.00$). *R. baltica* 100 % $NaNO_3$ showed to be higher in *R. salina* 100 % $NaNO_3$ in all phases however only statistically higher in the late exponential phase ($p < 0.00$).

Poly-unsaturated fatty acids (PUFA)

The PUFA's in treatment *R. baltica* 0 % $NaNO_3$ had an increase from the exponential phase to the late exponential then a slight decrease from the late exponential to the stationary phase however not any statistical differences between the different growth phases. The *R. baltica* 33 % $NaNO_3$ had a growth in the PUFA's over time. The stationary phase was statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p \approx 0.01$). The *R. baltica* 66 % $NaNO_3$ had a continues growth throughout the experiment. The exponential and late exponential phase were statistically lower than the stationary phase $p < 0.00$ and $p < 0.00$ respectively. *R. baltica* 100 % $NaNO_3$ had a continual growth in the PUFA's during the experiment although no statistical difference. In the exponential phase for all the different treatments of *R. baltica* the treatment 0% $NaNO_3$ was statistically lower than the 33% ($p \approx 0.01$) and 66% ($p \approx 0.04$) $NaNO_3$ treatments. There were no statistical differences between the late exponential phase for the different treatments of *R. baltica*. In the stationary phase of of *R. baltica* the treatment 0% $NaNO_3$ was statistically lower than the 33% ($p \approx 0.01$) and 66% ($p < 0.00$) $NaNO_3$ treatments. Furthermore, the 66% $NaNO_3$ treatment was statistical higher than both 33% ($p < 0.00$) and 100% ($p \approx 0.01$) $NaNO_3$. The *R. salina* 33 % $NaNO_3$ had a slight growth from the exponential to the late exponential phase but had a great decrease in the stationary phase of PUFA's resulting in the stationary phase being statistically lower then both the exponential ($p \approx 0.01$) and late exponential phase ($p < 0.00$). Furthermore, the late exponential phase was statistically higher than the exponential phase ($p < 0.00$). *R. salina* 66 % $NaNO_3$ had a continues growth over time with PUFA's. The stationary phase was statistically higher than the exponential ($p < 0.00$) and late exponential phase (0.00). The PUFA's in *R. salina* 100 % $NaNO_3$ declined from the exponential to the stationary phase and increased in the stationary phase. The stationary phase statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p < 0.00$). In *R. salina* there was no difference in PUFA's across the different treatments. The 33% $NaNO_3$ treatment was statistically higher in the late exponential phase in *R. salina* than the 66% ($p < 0.00$) and 100% (0.00) $NaNO_3$. In the *R. salina* stationary phase across treatments the 100% $NaNO_3$ was statistically higher than 33 % ($p < 0.00$)

and 66% (0.00) $NaNO_3$. *R. baltica* had higher amount of PUFA's than *R. salina* in all the treatments except in the late exponential phase of the 33 % $NaNO_3$ treatment. *R. baltica* was statistically higher in the stationary phase of the 33 % $NaNO_3$ ($p < 0.00$) and 66 % $NaNO_3$ ($p < 0.00$) treatments. In the 100% $NaNO_3$ treatment *R. baltica* was statistically higher than *R. salina* in the late exponential phase ($p \approx 0.01$).

Arachidonic acid (ARA)

In the *R. baltica* 0% $NaNO_3$ treatment the ARA grew steadily over time. The stationary phase was statistically higher than the exponential ($p \approx 0.02$) and late exponential phase ($p < 0.00$). The ARA in *R. baltica* 33% $NaNO_3$ treatment declined statistically from the late exponential to the stationary phase ($p < 0.00$). In the *R. baltica* 66% $NaNO_3$ treatment the ARA had an increasing growth throughout the experiment. The stationary phase was statistically higher than the exponential ($p \approx 0.01$) and late exponential phase ($p < 0.00$). The *R. baltica* 100% $NaNO_3$ treatment had an increase in the ARA over time. The exponential phase was statistically lower than the late exponential ($p \approx 0.04$) and the stationary phase ($p < 0.00$). In the exponential phase across all four *R. baltica* treatments there was no significant difference. The late exponential phase 0% $NaNO_3$ was statistically lower than 33% $NaNO_3$ treatment. Furthermore, the 33% $NaNO_3$ showed to be statistically lower than 66% $NaNO_3$ ($p < 0.05$) and 100% $NaNO_3$ ($p \approx 0.01$) treatments. In the stationary phase for the *R. baltica* treatments the 0% $NaNO_3$ treatment was statistically lower than 33% $NaNO_3$ treatment ($p \approx 0.02$). Moreover, the treatment 66% $NaNO_3$ was statistically higher than the treatments 0% $NaNO_3$ ($p \approx 0.02$) and 66% $NaNO_3$ ($p < 0.00$). At last the 33% $NaNO_3$ treatment was lower than the 100% $NaNO_3$. In the *R. salina* 33% $NaNO_3$ treatment there was a slight increase in the ARA from the exponential to the late exponential phase. From the late exponential to the stationary phase there was decline in the ARA below that of the exponential phase. The ARA in the stationary phase was statistically lower than the exponential ($p \approx 0.01$) and late exponential phase ($p < 0.00$). The *R. salina* 66% $NaNO_3$ treatment had a slight decline from the exponential phase to the late exponential phase. From the late exponential phase to the stationary phase there was an increase in the amount of ARA. There were no statistical differences. In the *R. salina* 100% $NaNO_3$ treatment there was a small decline in the ARA from the exponential to the late exponential phase. From the late exponential to the stationary phase the amount ARA

had tripled resulting in the stationary phase being statistically higher than the exponential ($p \approx 0.01$) and late exponential phase ($p \approx 0.02$). There was no statistical difference between the exponential phase in the *R. salina* treatments. In the late exponential phase, the 33% $NaNO_3$ treatment was statistically higher than the 100% $NaNO_3$ treatment ($p \approx 0.03$). Between the stationary phases the treatment 33% $NaNO_3$ was statistically lower than the treatments 66% $NaNO_3$ ($p \approx 0.01$) and 100% $NaNO_3$ ($p < 0.00$). Comparing *R. baltica* and *R. salina* the treatment 33% $NaNO_3$ *R. salina* had a lower amount of ARA's in the exponential and the stationary phase but had a higher amount in the late exponential phase. There was a statistical difference between *R. baltica* and *R. salina* 33% $NaNO_3$ in the late exponential phase ($p \approx 0.04$) and the stationary phase ($p < 0.00$). Between the *R. baltica* and *R. salina* in treatment 66% $NaNO_3$ *R. baltica* was lower than *R. salina* in the exponential phase but higher in the late exponential phase and statistically higher stationary phase ($p < 0.00$). Studying the differences between the *R. baltica* and *R. salina* in treatment 100% $NaNO_3$ *R. baltica* showed to be higher in all three phases although only significantly different in the late exponential phase ($p \approx 0.04$).

Eicosapentaenoic *acid* (EPA)

The treatment *R. baltica* 0% $NaNO_3$ had growth in EPA from the exponential phase to the late exponential phase thereafter it declined below the amount in the exponential phase in the stationary phase. The amount of EPA in *R. baltica* 33% $NaNO_3$ had a decrease in the exponential phase to the late exponential phase. From the late exponential phase to the stationary phase the EPA increased slightly above amount in the exponential phase. *R. baltica* 0% and 33% $NaNO_3$ showed no statistical difference in EPA between the phases. The EPA in *R. baltica* 66% $NaNO_3$ had a continues growth over time. The stationary phase was statistical higher than the exponential ($p < 0.00$) and the late exponential phase ($p < 0.00$). Furthermore, the exponential phase was statistically lower than the late exponential phase ($p < 0.00$). The EPA in *R. baltica* 100% $NaNO_3$ increased from the exponential phase to the late exponential phase and declined in the stationary phase. There were no statistical differences. In the exponential phase for all the *R. baltica* treatments there was a statistical difference in the EPA between the 33% and 66% $NaNO_3$ treatments ($p \approx 0.03$). In the late exponential phase for *R. baltica* there was one statistical difference between the treatments which was between the 33% and 66% $NaNO_3$ treatments ($p < 0.05$). The

amount of EPA in the stationary phase was statistically lower between the 0% $NaNO_3$ and 33% $NaNO_3$ ($p \approx 0.01$) treatments also between the 66% $NaNO_3$ treatment ($p \approx 0.01$). The 66% $NaNO_3$ treatment was statistically higher than 33% ($p < 0.00$) and 100% $NaNO_3$ ($p \approx 0.01$) treatments. The treatment *R. salina* 33% $NaNO_3$ had growth in EPA from the exponential phase to the late exponential phase and a drastic decline in the stationary phase below the exponential phase. There was a statistical difference between all the phase ($p < 0.00$). The *R. salina* 66% $NaNO_3$ treatment had a steady growth of EPA throughout the experiment. The stationary phase was statistically higher than the exponential phase ($p < 0.00$) and the late exponential phase ($p \approx 0.10$). The EPA for the *R. salina* 100% $NaNO_3$ treatment declined from the exponential to the late exponential phase and grew in the stationary phase above the amount in the exponential phase. The stationary phase was statistically higher than the exponential phase ($p < 0.00$) and the late exponential phase ($p \approx 0.02$). In the stationary phase the 33% $NaNO_3$ treatment was statistically lower than the 33% $NaNO_3$ ($p < 0.00$) and 100% $NaNO_3$ ($p <0.00$) treatments. Comparing the treatments across species the *R. salina* was lower in the exponential phase and the stationary phase ($p < 0.00$) than *R. baltica* in the 33% $NaNO_3$ treatment and higher in the late exponential phase ($p < 0.00$). *R. baltica* was higher than *R. salina* in the exponential, late exponential ($p \approx 0.02$), and stationary phase for the 66% $NaNO_3$ treatment. *R. baltica* was lower than *R. salina* in the exponential phase but higher in the late exponential and stationary phase however not statistically.

Docosahexaenoic acid (DHA)

The DHA in the treatment *R. baltica* 0% $NaNO_3$ increased from the exponential to the late exponential phase and declined in the stationary phase. There were no statistical differences between the phases. In *R. baltica* 33% $NaNO_3$ the DHA declined from the exponential phase to the late exponential phase and increased in the stationary phase. There was a statistical difference between all the treatments ($p < 0.00$). Treatment *R. baltica* 66% $NaNO_3$ had a steady increase of DHA over time. The stationary phase statistically higher than the exponential phase ($p < 0.00$). In the *R. baltica* 100% $NaNO_3$ treatment the DHA content increases from the exponential phase to the late exponential phase and decreases in the stationary phase below the amount in the exponential phase. The late exponential phase was statistically higher than the stationary phase ($p \approx 0.01$). In the exponential phase between the *R. baltica* treatments 33% $NaNO_3$ showed to be

statistically higher than 0% $NaNO_3$ ($p \approx 0.04$) and 66% $NaNO_3$ ($p \approx 0.03$). The late exponential phase of *R. baltica* for 100% $NaNO_3$ was statistically higher than the 33% $NaNO_3$ treatment ($p < 0.00$). The stationary phases for *R. baltica* had many statistical differences. 33% $NaNO_3$ was statistically higher than 0% ($p \approx 0.01$) and 33% $NaNO_3$ ($p < 0.00$). The 66% $NaNO_3$ was statistically higher than 0% ($p < 0.00$), 33% ($p \approx 0.03$) and 100% ($p < 0.00$) $NaNO_3$. In the *R. salina* 33% $NaNO_3$ the DHA increased from the exponential to the late exponential phase and had a great decline in the stationary phase. The stationary phase was statistically lower the exponential ($p < 0.00$) and late exponential phase ($p < 0.00$). The DHA in *R. salina* 66% $NaNO_3$ had a steady growth throughout the experiment. The stationary phase was statistically higher than the exponential ($p \approx 0.01$) and late exponential phase ($p \approx 0.03$). *R. salina* 100% $NaNO_3$ had a decrease in the DHA from the exponential to the late exponential phase and an increase to the stationary phase. The stationary phase was statistically higher than the exponential ($p < 0.00$) and the late exponential phase ($p \approx 0.04$). There were no statistical differences within the exponential and late exponential phases of DHA in *R. salina*. In the stationary phase the 33% $NaNO_3$ treatment was statistically lower than the treatments 66% ($p < 0.00$) and 100% $NaNO_3$ ($p < 0.00$) for *R. salina*. Comparing the 33% $NaNO_3$ treatments between the species shows that *R. baltica* had a higher content of DHA in the exponential phase and stationary phase ($p < 0.00$) than *R. salina*. Furthermore, *R. salina* had a higher content of DHA in the late exponential phase ($p < 0.00$). The DHA content of *R. baltica* and *R. salina* in the 66% $NaNO_3$ treatment showed to be similar in all three phases. In the 100% $NaNO_3$ treatment R. baltica was higher than R. salina in the exponential and late exponential phase but lower in the stationary phase ($p < 0.00$).

Other n-3 fatty acids (n-3)

The n-3 in *R. baltica* 0% $NaNO_3$ increased from the exponential to the late exponential phase and declined in the stationary phase however there were no statistical differences between the phases. The amount of n-3 in in *R. baltica* 33% $NaNO_3$ were similar in the exponential and late exponential phase but growth in the stationary phase. The stationary phase was statistically higher in the stationary phase in contrast to the exponential phase ($p < 0.00$). *R. baltica* 66% $NaNO_3$ had a persistent growth throughout the experiment. The stationary phase was statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p \approx 0.02$). The n-3 in *R. baltica* 100% $NaNO_3$

increased from the exponential phase to the late exponential phase and stagnated between the late exponential and the stationary phase. There were no statistical differences between the phases. In the exponential phase between all the different treatments of *R. baltica* the treatment 33% $NaNO_3$ was statistically higher than 0% $NaNO_3$ ($p \approx 0.02$). In the late exponential phase between the treatments of *R. baltica* there was no statistical difference. In the stationary phase for *R. baltica* the 66% $NaNO_3$ treatment was higher than the 0% ($p < 0.00$) and 100% treatments ($p \approx 0.01$), furthermore the 33% $NaNO_3$ treatment was statistically higher than the 100% $NaNO_3$. *R. salina* 33% $NaNO_3$ had an increase from the exponential to the late exponential phase and a rapid decline in the stationary phase in n-3. The late exponential phase was higher than the exponential ($p < 0.00$) and stationary phase ($p < 0.00$). Moreover, the exponential phase was statistically higher than the stationary phase ($p < 0.00$). *R. salina* 66% $NaNO_3$ had a steady growth over time with n-3. The stationary phase was statistically higher then the exponential phase ($p < 0.00$) and the late exponential phase ($p \approx 0.02$). In *R. salina* 100% $NaNO_3$ there was a decline in the n-3 from the exponential phase to the late exponential phase and an increase to the stationary phase. The stationary phase was statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p \approx 0.02$). Comparing the content of 3-n in *R. baltica* and *R. salina* between the 33% $NaNO_3$ treatment shows R. baltica has a higher content in the exponential phase and the stationary phase ($p < 0.00$). In the late exponential phase *R. salina* has a slightly higher content. The content of 3-n in the 66% $NaNO_3$ was higher in *R. baltica* than *R. salina* in all phases. 3-n content of the 100% $NaNO_3$ was higher in *R. baltica* than *R. salina* in the exponential and late exponential phase ($p \approx 0.04$). In the stationary phase *R. salina* was statistically higher than *R. baltica* ($p \approx 0.04$).

Other n-6 fatty acids (n-6)

The 0% $NaNO_3$ treatment for R. baltica had a great increase of n-6 from the exponential to the late exponential and a great decrease to the stationary phase. The was a statistical difference between the exponential and late exponential ($p \approx 0.02$) the latter being the higher. The was a steady growth throughout the experiment of n-6 in the 33, 66, and 100% $NaNO_3$ treatments of *R. baltica*. In 33% $NaNO_3$ there was the exponential phase was statistically lower than the late exponential ($p < 0.05$) and stationary phase ($p < 0.00$), furthermore there was a statistical difference between the late exponential and stationary phase ($p < 0.00$). In 66% $NaNO_3$ the stationary phase was statistically

higher than the exponential ($p \approx 0.02$) and late exponential phase ($p < 0.00$). In the 100% $NaNO_3$ the exponential phase was statistically lower than the late exponential ($p \approx 0.02$) and stationary phase ($p < 0.00$). The exponential phases in all the *R. baltica* only showed one statistical difference and it was between the 0% and 33% $NaNO_3$. In the late exponential phase there was no statistical difference between the treatments for *R. baltica*. The stationary phase for R. baltica showed to be statistically lower in the 0% $NaNO_3$ than 33% ($p \approx 0.02$) and 66% $NaNO_3$ ($p < 0.00$) treatment. Furthermore, n-6 showed to have higher in the stationary phase for 66% than 100% $NaNO_3$ treatment ($p \approx 0.001$). In the treatment 33% $NaNO_3$ in *R. salina* 6-n had an increase from the exponential to the late exponential phase and a decrease in the stationary phase. The late exponential phase was statistically higher than the exponential ($p < 0.00$) and the stationary phase ($p < 0.00$). The 66% $NaNO_3$ treatment for *R. salina* had a continues growth of n-6 over time. The stationary phase was statistically higher than the exponential ($p < 0.00$) and late exponential phase ($p < 0.00$). n-6 in the 100% $NaNO_3$ treatment had a decrease from the exponential to the late exponential phase and a great increase in the stationary phase. The stationary phase was statistically higher in than the exponential ($p < 0.00$) and the late exponential phase ($p < 0.00$). There showed to be no statistical difference between the exponential phases for *R. salina*. In the late exponential phase for *R. salina* the 33% $NaNO_3$ showed to be statistically higher than the 33% ($p < 0.00$) and 100% $NaNO_3$ treatments ($p < 0.00$). In the stationary phase the amount of n-3 declined drastically in the 33% $NaNO_3$ making it statistically lower than the 33% ($p < 0.00$) and 100% $NaNO_3$ treatments ($p < 0.00$). Comparing the treatments between the species *R. baltica* and *R. salina* showed that R. salina was higher in the exponential and late exponential phase ($p \approx 0.02$) however lower in the stationary phase ($p < 0.00$) in the treatment 33% $NaNO_3$. In the 66% treatment *R. baltica* is higher than *R. salina* in all phase but there was a statistical difference in the stationary phase ($p < 0.00$). *R. baltica* was higher than *R. salina* in all the phases in the 100% $NaNO_3$ treatment although only statistically higher in the late exponential phase ($p < 0.00$).

Percentage distribution

The % of other lipids is approximately the same over the all the different treatments in the exponential phase although the 66% $NaNO_3$ is a bit lower. The other lipids in the late exponential phase have decrease except the 66% $NaNO_3$ *R. baltica* and 100% $NaNO_3$ *R. salina* furthermore

the 33% *R. baltica* stays the same. In the stationary phase there is a decline in other lipids in % of TL in all except 0 and 100% $NaNO_3$ *R. baltica* which has a slight increase. Observing all three phases in the % lipid distribution the 66% $NaNO_3$ *R. baltica* and the 66% $NaNO_3$ *R. salina* had the lowest % of other lipids. Everything reaming after the Other lipids is grouped as FA and therefor the highest % of FA were observed in 66% $NaNO_3$ *R. baltica* and the 66% $NaNO_3$ *R. salina*. The % of SFA's and MUFA's grew steadily from the exponential to the stationary phase for all treatments. The PUFA's are a result of the ARA, EPA, DHA, Other-n3, and Other-6n fatty acids % distribution of the TL. The PUFA's had an increase over time for most treatments however had a decrease in the 0 and 66% $NaNO_3$ *R. baltica*. The highest % of PUFA's for both *R. baltica* and *R. salina* was in the 66% $NaNO_3$ treatment. n-3 had different % distribution patterns between species and their phases. The n-3 in 33 % $NaNO_3$ *R. baltica* and 33 and 66% $NaNO_3$ *R. salina* increased from the exponential to stationary phase. The 100% $NaNO_3$ *R. baltica* had a decrease in 3-n over time. The 0% $NaNO_3$ *R. baltica* increased from the exponential phase to the late exponential then had a slight decrease of n-3. *R. baltica* 66 % $NaNO_3$ and 100% $NaNO_3$ *R. salina* had a decrease in the 3-n from the exponential to the late exponential phase and an increase to the stationary phase. The highest % of n-3 for *R baltica* was in the 33% $NaNO_3$ treatment and for *R. salina* it was the 66% $NaNO_3$ treatment. The n-6 % distribution in the TL grew throughout the experiment in all treatments except the *R. baltica* 0% $NaNO_3$ where it increased from the exponential to the late exponential and declined in in the stationary phase. The highest % distribution for n-6 was in the 66% $NaNO_3$ treatments for both *R. baltica* and *R. salina*. The ARA was in general low in all treatments but was highest in the 66% $NaNO_3$ in both *R. baltica* and *R. salina*. The EPA and DHA % distribution of TL in the different treatments varied in different patterns. The *R. baltica* 0% $NaNO_3$ has a decline in the % DHA and EPA. The *R. baltica* 33% $NaNO_3$ had a decline in the % of EPA and DHA from the exponential to the late exponential phase then an increase in the stationary phase. *R. baltica* 66% $NaNO_3$ had a stable % distribution of EPA and DHA throughout the experiment. In *R. baltica* 100% $NaNO_3$ the % distribution of EPA and DHA were highest in the late exponential phase. In R. salina 33% $NaNO_3$ the % of DHA declines over time and the EPA increases from the exponential to the late exponential then decreases in the stationary phase. The % distribution of EPA and DHA in *R. salina* 66% $NaNO_3$ increases from the exponential to the stationary phase. The % distribution of DHA in *R. salina* 100% $NaNO_3$ had a continues growth over the phases and the EPA had a slight decline from the exponential to the late

exponential phase but an increase in the stationary phase. The highest EPA % distribution was in the stationary phase of *R. salina* 66% $NaNO_3$ and in the late exponential phase of *R. baltica* 100% $NaNO_3$. The highest DHA % distribution was in the exponential phase in *R. salina* 33% $NaNO_3$ and in *R. baltica* the exponential phase 0% $NaNO_3$ treatment. The next largest % distribution in *R. baltica* was the late exponential phase in the 66% $NaNO_3$ treatment. *R. salina* has in general had higher content of DHA and EPA % than *R. baltica* but contains lower amount of TL.

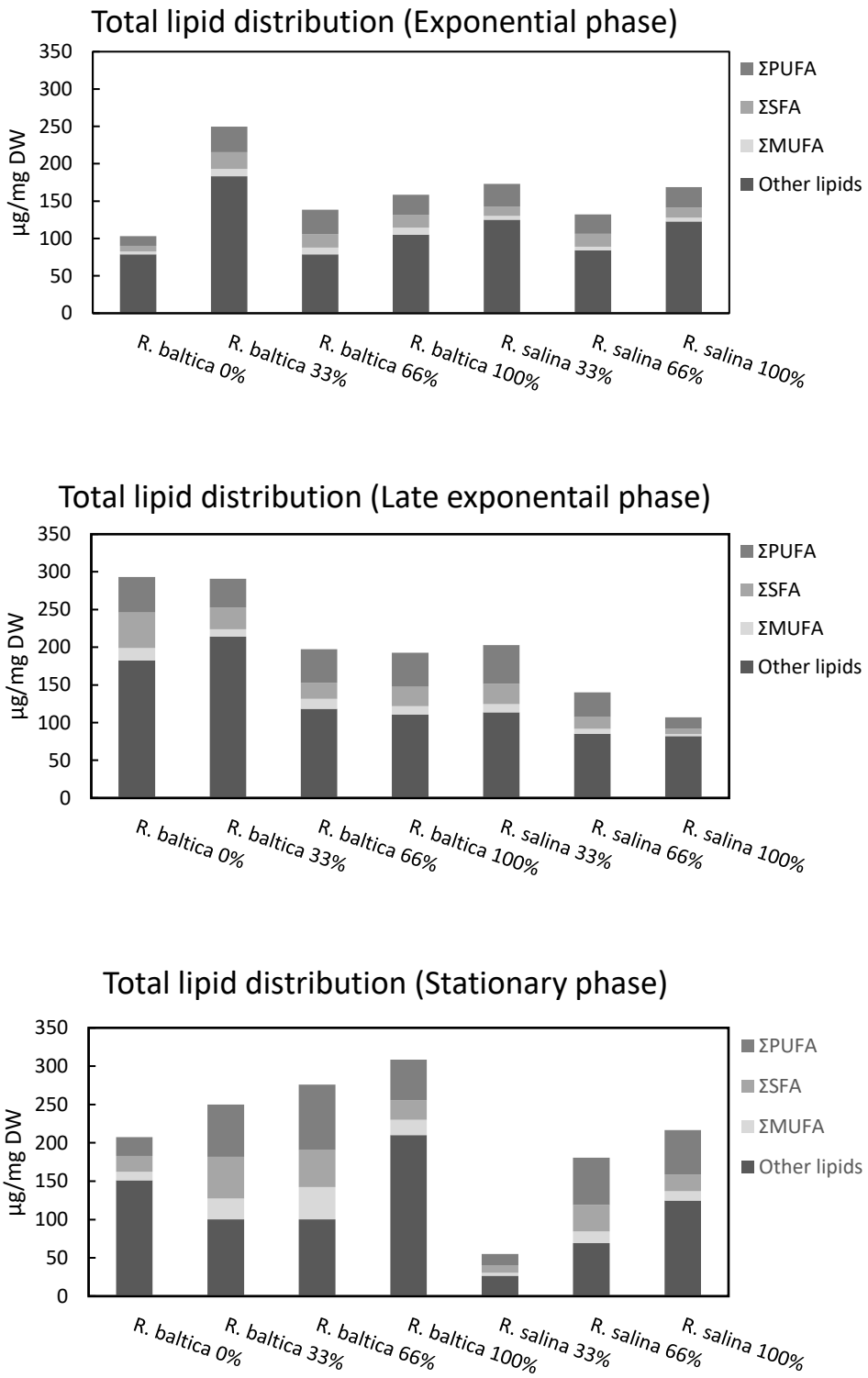


Figure 4.7. shows the distribution of total lipids divided in to four classes Other lipids, ∑SFA, ∑MUFA, ∑PUFA and measured in µg/mg dry weight. Furthermore, it shows it for the three-measuring points exponential, late exponential, and stationary phase for all $NaNO_3$ treatments for *R. baltica* and *R. salina*.

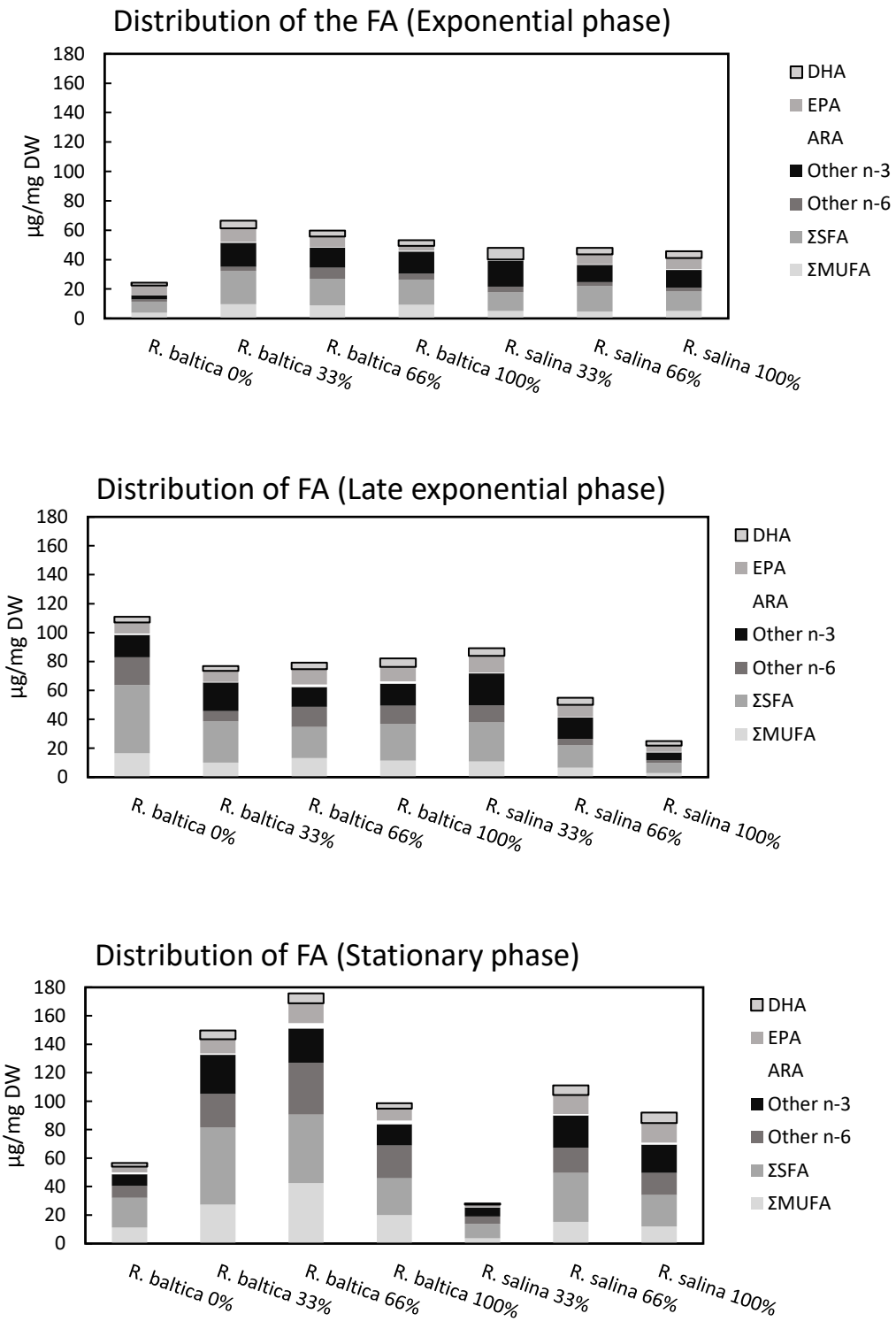
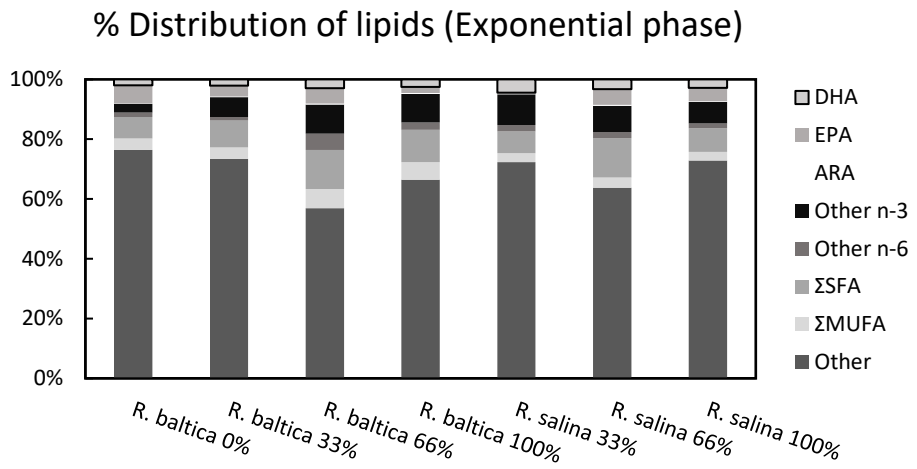
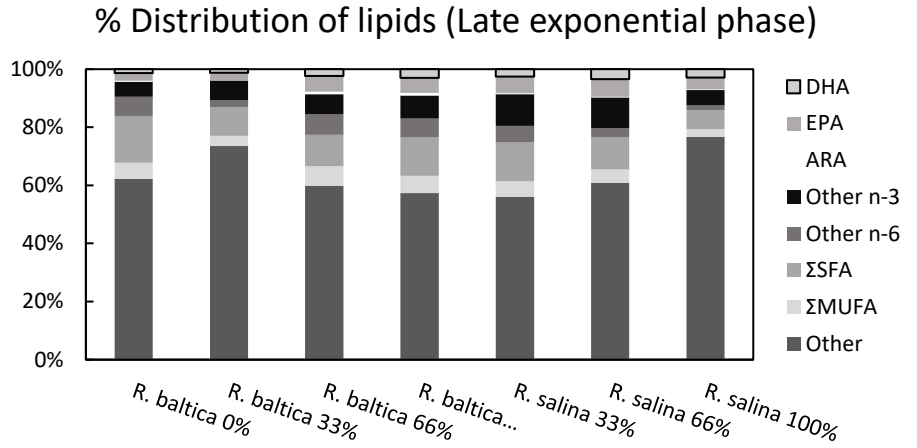


Figure 4.8. shows the fatty acid distribution of all $NaNO_3$ treatments for *R. baltica* and *R. salina* for the three different measuring stages exponential, late exponential, and stationary phase. The data graph takes examines the ∑PUFA closer and is divided in to EPA, DHA, ARA, Other 3-n, and Other 6-n as well as both ∑SFA and ∑MUFA and is measured in µg/mg DW.





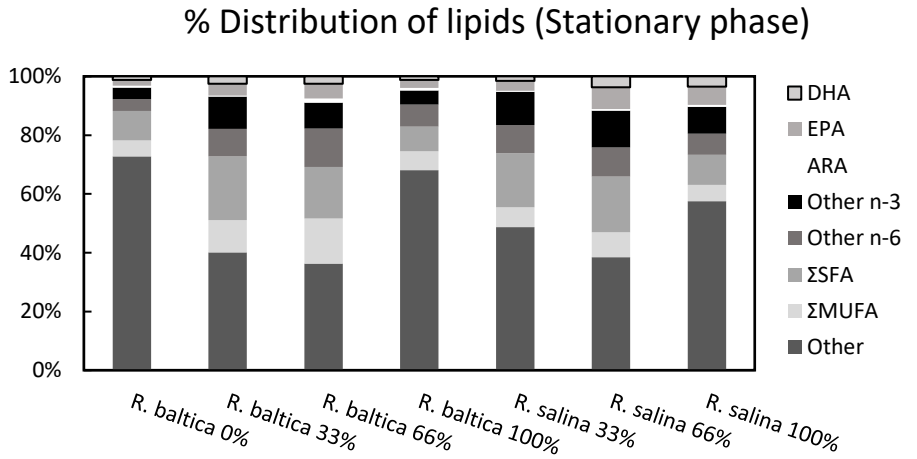


Figure 4.9. shows the % distribution of graph 4.8 in relation to the TL from graph 3.7 for all $NaNO_3$ treatments for *R. baltica* and *R. salina* for the three different measuring stages exponential, late exponential, and stationary phase. Furthermore, it includes the lasting lipids which all grouped together to Other.

4.2 Copepods

Acartia tonsa (A. tonsa) was the chosen species for the feeding experiment of the three most qualified algae from the microalga experiment in consideration of density, particulate carbon and nitrogen molar ratio, and lipid content. The analyzes of previous mentioned factors resulted in *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ were chosen as mono-diets for *A. tonsa* feeding experiment. Approximately 100 individuals of A. tonsa were staged in each replicate for each data point. In the chemical analysis of lipids, particulate carbon/nitrogen, and particulate phosphorus one sample was taken out of each replicate which resulted in a sample size of three. All the analyzes were made with ANOVA with $p < 0.05$.

4.2.1 Growth

Staging of *A. tonsa* was done on day 0, 3, and 6. The staging on day 0 was to correct for the development difference between the species and in the developmental rate (DR). Figure 4.10 shows the development of *A. tonsa* over time of fed on three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$. Each treatment has three replicates and each data point corresponds to the average. The error bars show the standard deviation for each point. The data for figure 4.10 was taken from table 4.3. As the figure shows there was only a slight difference in the development of *A. tonsa* between the different treatments although not statistically. The development rate between the three mono-diets were also very similar (table 4.3). *R. baltica* 100% $NaNO_3$ had the highest DR from day 0-3 additionally *R. salina* 66% $NaNO_3$ had the highest DR from day 0-6. *R. baltica* 66% $NaNO_3$ was statistical lower than *R. baltica* 100% $NaNO_3$ in the DR from day 0-3 ($p \approx 0.042$).

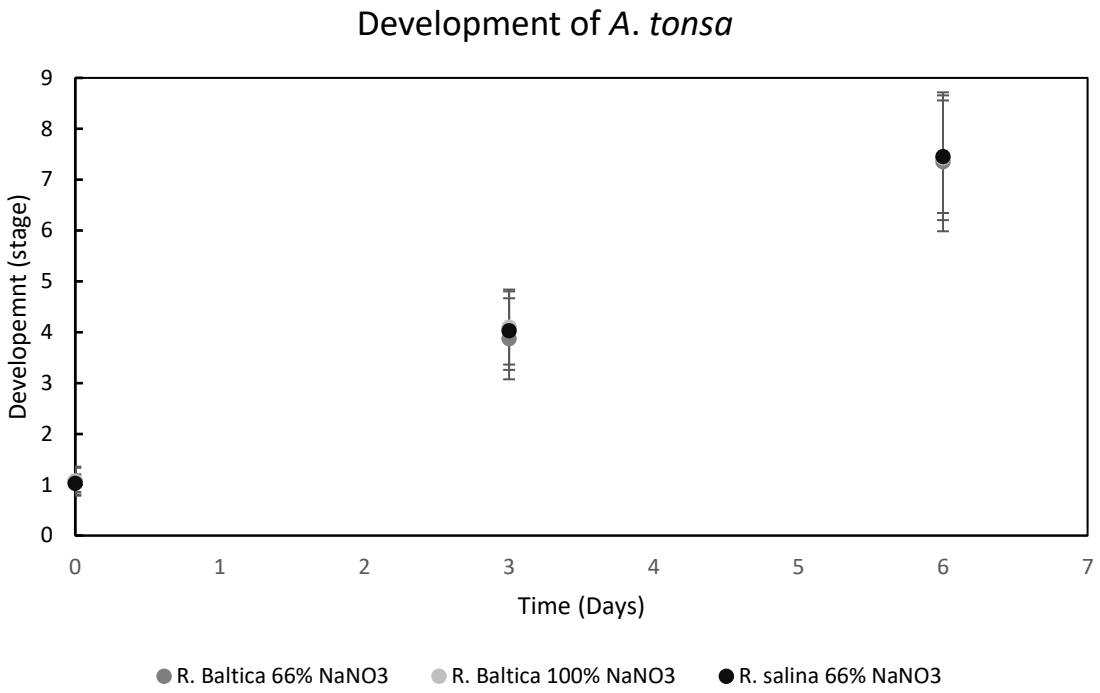


Figure 4.10. The figure pictures the development stage of A. tonsa fed three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$. Each treatment had three replicates and each point symbolizes the average. The error bars shows the standard deviation.

Table 4.3. The table shows the development and DR of *A. tonsa* fed on three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ as a function of time from the microalgae experiment. The sample size is three and the ± is the standard deviation.

	A. Tonsa					
	***R. baltica* 66% $NaNO_3$**		***R. baltica* 100% $NaNO_3$**		***R. salina* 66% $NaNO_3$**	
Day	Stage	DR	Stage	DR	Stage	DR
0	1.07 ± 0.26		1.07 ± 0.29		1.03 ± 0.17	
3	3.87 ± 0.80	0.93 ± 0.04	4.10 ± 0.74	1.01 ± 0.03	4.03 ± 0.77	1.00 ± 0.06
6	7.35 ± 1.37	1.05 ± 0.08	7.43 ± 1.23	1.06 ± 0.04	7.45 ± 1.11	1.07 ± 0.18

4.2.2 Survival

The survival of *A. tonsa* in the experiment was very low. Graph 4.11 shows the mortality of time in %. The data for graph 4.11 is extracted from table A3 (appendix C), moreover the table shows the amount of nauplii with standard deviation for each data point. *A. tonsa* fed *R. baltica* 100% $NaNO_3$ had the lowest survival and *R. baltica* 66% $NaNO_3$ the highest however there is no statistical difference. Due to an aeration problem during the experiment it is hard to differentiate what is the cause the aeration or the diet, more about this in the discussion.

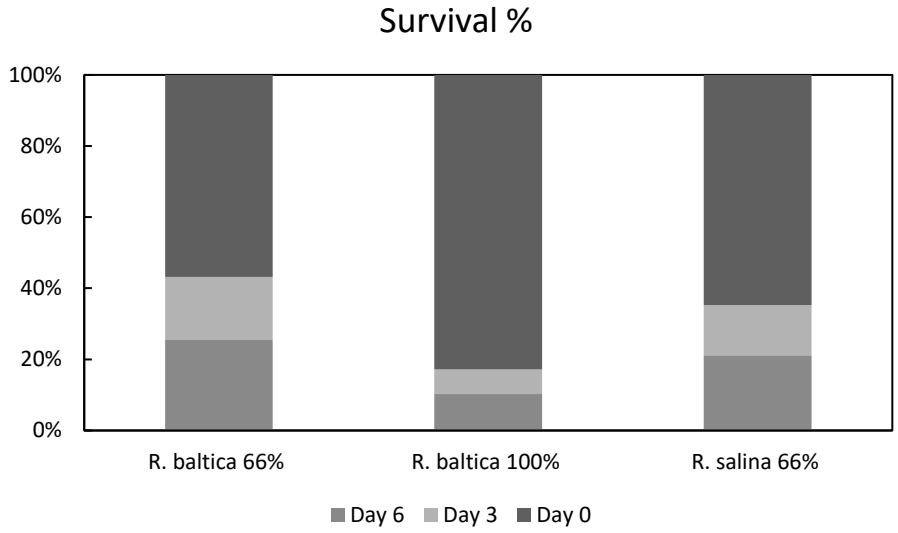


Figure 3.11. The bar graph shows the survival in % over time of A. tonsa fed three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$.

4.2.2 Molar ratio: carbon and nitrogen

The molar ratio for stages NIV and CI of *A. tonsa* fed three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ are shown in figure 4.12. The error bars represent the standard deviation. The data used in figure 4.12 is taken from appendix D In stage NIV for *A. tonsa* the C:N molar ratio is the highest (3.24) and lowest in the *R. salina* 66% $NaNO_3$ (2.15). In the stage CI the diet with *R. baltica* 100% $NaNO_3$ gave the highest C:N molar ratio (3.46), however the treatment with the lowest C:N ratio was *R. baltica* 66% $NaNO_3$ (2.50). The C/N molar ratio in *A. tonsa* increased in all treatments from the NIV to CI stage. Although, there was variation and a slight in crease there was no statistical difference within the treatments.

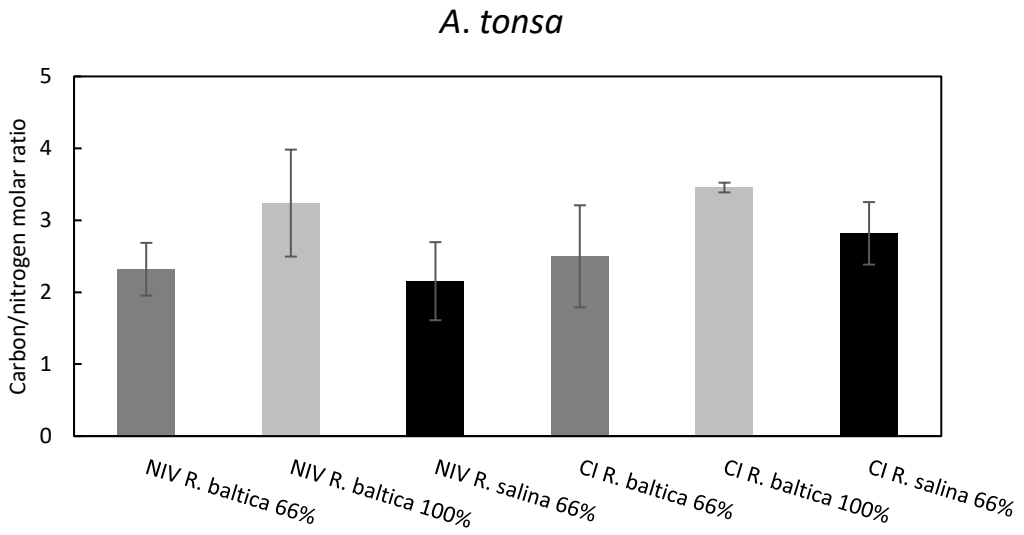


Figure 4.12. The bar graph shows the carbon/nitrogen ratio at development stage NIV and CI for *A. tonsa* fed three different mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$. The error bars signify the standard errors.

4.2.3 Lipid content of *A. tonsa*

Total lipid distribution of A. tonsa

The total lipid (TL) distribution of *A. tonsa* was divided in to four groups other lipids, ∑SFA's (saturated fatty acids), ∑MUFA's (mono-unsaturated fatty acids), and ∑PUFA's (poly-unsaturated fatty acids) as shown in Figure 4.13. The data for Figure 3.13 is based on the data in table A5 (appendix E) the table also contains the standard deviation for each data point except in some data points where there was only one value available. The ∑ of ∑SFA's, ∑MUFA's, and ∑PUFA's corresponds to the FA results from the Gas-Chromatography. In the *A. tonsa* stage CI the FA was highest in the in the *A. tonsa* fed *R. baltica* 66% $NaNO_3$ (33.52 µg/mg DW) and lowest in the *A. tonsa* fed *R. salina* 66% $NaNO_3$ (29.97 µg/mg DW) the *A. tonsa* fed *R. baltica* 100% $NaNO_3$ was in the middle (31.18 µg/mg DW). In *A. tonsa* stage NIV *R. baltica* 100% $NaNO_3$ had the highest amount of FA (28.99 µg/mg DW) and *A. tonsa* fed *R. salina* 66% $NaNO_3$ was still had the lowest amount (15.67 µg/mg DW). *A. tonsa* NIV fed *R. baltica* 66% $NaNO_3$ contained the amount of 26.30 µg/mg DW. ∑SFA's were higher in the CI stage than NIV. In the NIV stage *R. baltica* 100%

$NaNO_3$ had the highest amount (7.45 µg/mg DW) and in the CI stage *R. baltica* 66% $NaNO_3$ had the highest amount (9.41 µg/mg DW). ∑MUFA's were higher in the in NIV than CI stage except in the *A. tonsa* fed *R. salina* 66% $NaNO_3$. The ∑MUFA's were highest in NIV in *A. tonsa* fed *R. baltica* 66% $NaNO_3$ (2.43 µg/mg DW) and lowest when fed *R. salina* 66% $NaNO_3$ (µg/mg DW). In the CI stage the highest amount of ∑MUFA's were in the *R. baltica* 66% $NaNO_3$ (2.03 µg/mg DW) and lowest in *R. baltica* 100% $NaNO_3$ (0.99 µg/mg DW). The lowest amount of ∑PUFA's in both stages NIV and CI were the lowest in *A. tonsa* fed *R. salina* 66% $NaNO_3$ 10.33 and 19.40 µg/mg DW, respectively. ∑PUFA's in stage NIV were highest in *R. baltica* 100% $NaNO_3$ (20.27 µg/mg DW) and stage CI was highest in *R. baltica* 66% $NaNO_3$ (21.92 µg/mg DW). There were no statistical differences within each stage $p > 0.05$.

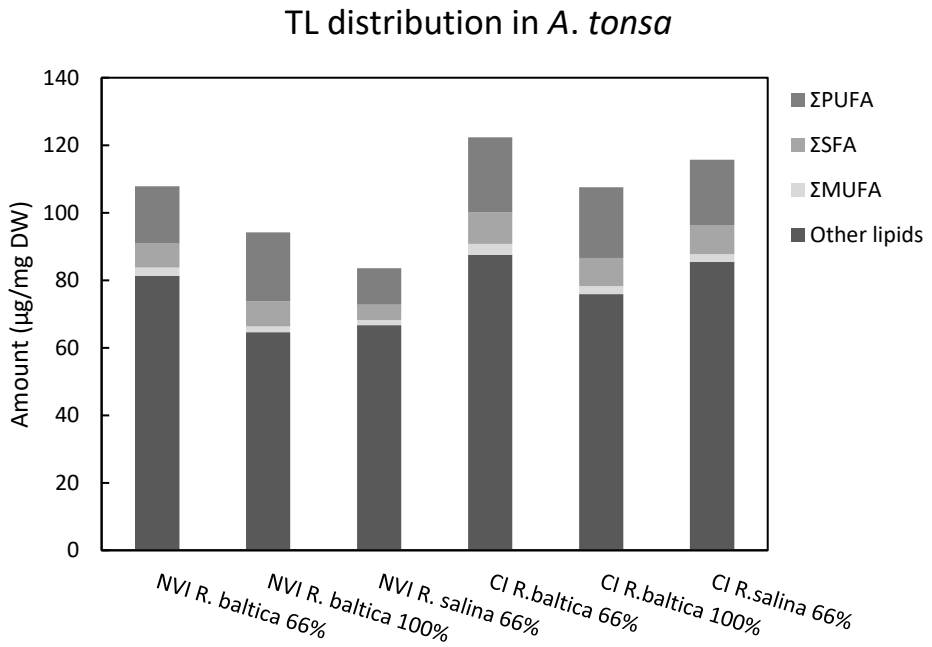


Figure 4.13. The bar graph shows the amount of total lipids in *A. tonsa* which is divided in to other lipids and FA. The FA divided in three main groups: ∑SFA's, ∑MUFA's, and ∑PUFA's. The amount of each group is given in µg/mg DW.

Distribution of FA in A. tonsa

The distribution of FA is separated into seven groups. ∑SFA', ∑MUFA's, and ∑PUFA's which is divided into five groups: Other n-3 (sum of the omega-3 fatty acids without EPA and DHA), Other n-6 (sum of the omega-6 fatty acids without ARA), EPA (Eicosapentaenoic *acid*), DHA (Docosahexaenoic acid), and ARA (Arachidonic acid). The ∑SFA's and ∑MUFA's are discussed in the sub chapter TL distribution of *A. tonsa*. *A. tonsa* at stage NIV and CI fed *R. baltica* 66% $NaNO_3$ had the highest amount of Other n-3 2.43 µg/mg DW and 2.19 µg/mg DW, respectively. *A. tonsa* fed *R. salina* 66% $NaNO_3$ had lowest amount in NIV (0.99 µg/mg DW) and CI (1.95 µg/mg DW). The amount Other n-6 in stage NIV was highest in *A. tonsa* fed *R. baltica* 100% $NaNO_3$ (5.53 µg/mg DW) and lowest in *A. tonsa* fed *R. salina* 66% $NaNO_3$. In CI *A. tonsa* fed *R. baltica* 66% $NaNO_3$ (5.18 µg/mg DW) had the highest amount of Other n-6 whilst *A. tonsa* fed *R. salina* 66% $NaNO_3$ (4.67 µg/mg DW) had the lowest amount. The highest amount of ARA in *A. tonsa* stage NIV was *A. tonsa* fed *R. baltica* 66% $NaNO_3$ (0.77 µg/mg DW) and the lowest was fed on *R. salina* 66% $NaNO_3$ (non). For the CI stage the highest amount of ARA was found in *A. tonsa* fed *R. salina* 66% $NaNO_3$ (1.57 µg/mg DW) and the lowest amount found in *A. tonsa* fed *R. baltica* 66% $NaNO_3$ (0.90 µg/mg DW). In EPA and DHA the highest and lowest amounts in stage NV1 are related to the mono-diet for *A. tonsa R. baltica* 66% $NaNO_3$ 3.88 and 5.71 µg/mg DW for the higher and *R. salina* 66% $NaNO_3$ for the lower 2.80 and 4.40 µg/mg DW. In the CI stage for *A. tonsa the R. baltica* 100% $NaNO_3$ mono-diet had the highest amount of EPA (5.24 µg/mg DW) and for DHA (8.69 µg/mg DW) was *R. baltica* 66% $NaNO_3$ fed *A. tonsa*. The lowest amounts of EPA and DHA were found in *R. salina* 66% $NaNO_3$ (4.62 µg/mg DW) and *R. baltica* 100% $NaNO_3$ (8.02 µg/mg DW), respectively. There were no statistical differences within each stage $p > 0.05$.

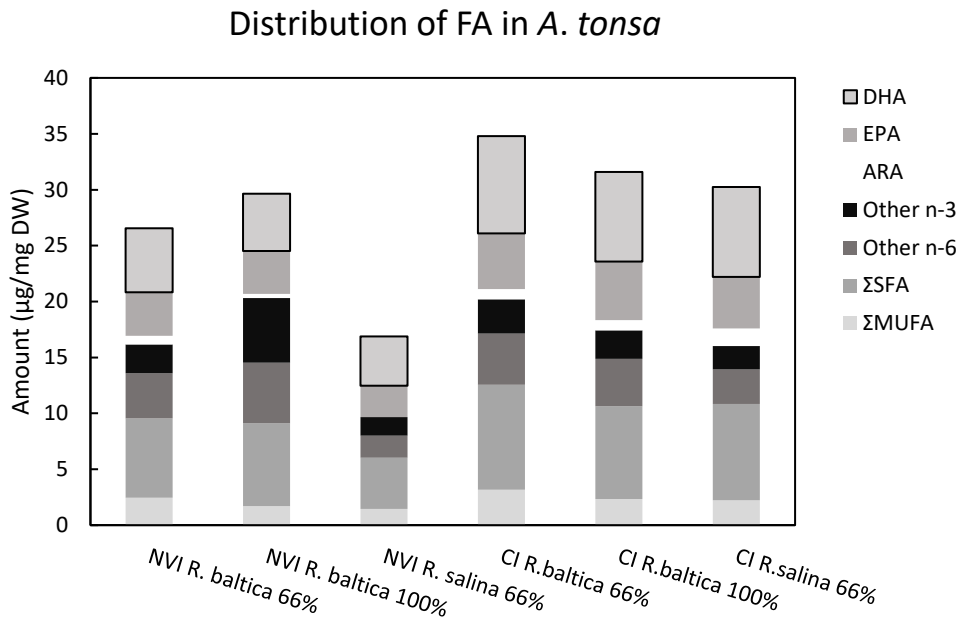


Figure 4.14. The bar graph shows the distribution of the seven groups FA in two development stages NIV and CI for *A. tonsa* fed on three mono-diets *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$. The FA groups are: ∑SFA's, ∑MUFA's, ∑PUFA's is divided into five groups: Other n-3 (sum of the omega-3 fatty acids), Other n-6 (sum of the omega-6 fatty acids), EPA (Eicosapentaenoic *acid*), DHA (Docosahexaenoic acid), and ARA (Arachidonic acid). The amount is given µg/mg DW.

% distribution of lipids in A. tonsa

The data for the % lipid distribution in relation to the TL in the NIV and CI stage for *A. tonsa* used in figure 4.15 can be found in table A6(appendix E). The % distribution of lipids in the NIV stage of *A. tonsa* fed *R. salina* 66% $NaNO_3$ had the lowest % in all groups: FA (20.19%), ∑SFA (5.48%), ∑MUFA (1.72%), ∑PUFA (12.99%), Other n-3 (2.01%), Other n6 (2.36%), ARA (0%), EPA (3.35%), and DHA (5.26%) *A. tonsa* fed *R. baltica* 100% $NaNO_3$ contained the highest % of FA (31.45%), ∑SFA (7.86%), ∑PUFA (21.77%), Other n-3 (6.14%), Other n-6 (5.74%), EPA (4.07%), and DHA (5.43%). *A. tonsa* fed *R. baltica* 66% $NaNO_3$ contained highest % of ∑MUFA (2.28%), ARA (0.72%). The *A. tonsa* fed *R. salina* 66% $NaNO_3$ also had the lowest % in all the different groups except in ARA the lowest amounts were as followed FA (26.12%), ∑SFA (7.44%), ∑MUFA (1.92%), ∑PUFA (16.76%), Other n-3 (1.81%), Other n-6 (2.67%), EPA (3.99%), and DHA (6.94%). ARA was the highest (1.36%). The *A. tonsa* fed *R. baltica* 66%

$NaNO_3$ had the highest % of Other n-3 (2.50%) and MUFA's (2.58%). The *A. tonsa* fed *R. baltica* 100% $NaNO_3$ had the highest % of FA (29.38%), SFA's (7.72%), PUFA's (19.49%), Other n-6 (3.93%), EPA (4.87%), and DHA (7.46%). The % of FA of the TL increased in Acartia tonsa from NIV to CI stage when fed *R. baltica* 66% $NaNO_3$, and *R. salina* 66% $NaNO_3$. However, *A. tonsa* fed on had a higher % of FA in both cases.

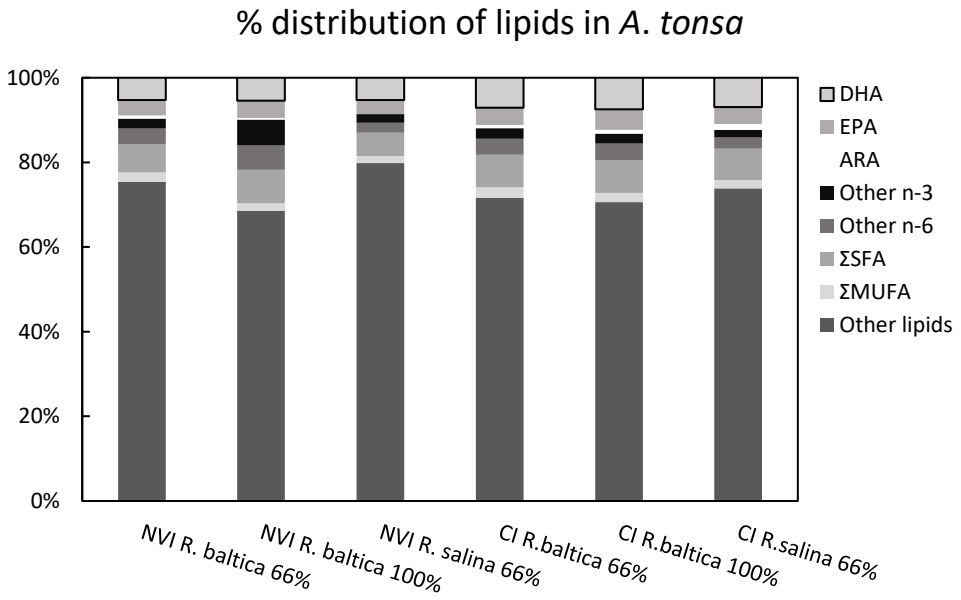


Figure 4.15. The % distribution of the FA in the TL for *A. tonsa* fed three different mono-diets of *R. baltica* 100% $NaNO_3$, *R. baltica* 66% $NaNO_3$, and *R. salina* 66% $NaNO_3$. The TL was divided into other lipids and FA (was divided into seven different groups: ∑MUFA's, ∑SFA's, Other n-3, Other n-6, ARA, EPA and DHA.

5. Discussion

The main objectives in the study was to establish the development and survival of A. tonsa fed mono-diets of R. baltica, R. salina, and R. marina cultured on four different nitrogen concentration 0, 33, 66, and 100 % $NaNO_3$. The discussion is divided up in to two parts the microalgae and copepods.

5.1 Microalgae

The objective of the microalgae experiment was to examine what effect the four different treatments 0, 33, 66, and 100 % $NaNO_3$ had on *R. baltica* and *R. salina* growth phases and carrying capacity. Furthermore, to determine the effect the nitrogen treatments had on the stoichiometric ratio C:N and the fatty acid profile. The three hypotheses were as followed:1. There is no difference in the fatty acid profile for the four different $NaNO_3$ treatments in the exponential phase; however, there should be a change in the fatty acid profile when nitrogen starts being limiting. 2. The stoichiometry between carbon and nitrogen is the same in the exponential phase although in nitrogen limited environment there is a variation between the treatments. 3. The carrying capacity of *Rhodomonas* will vary between the different treatments. Something along the lines that the changes in *Rhodomonas* stoichiometry and biochemistry represent changes in quality as food for herbivores.

5.1.1 Growth

R. marina showed to be more difficult to cultivate than *R. baltica* and *R. salina*. *R. marina* was first cultivated with Conwy where the cells died. *R. marina* was then cultivated with the same enrichment medium TL30 used in the NIVA CCA algae shop (Larsen et al, 1994). The reason for the failing to cultivate *R. marina* is unknown. The growth of *R. baltica* and *R. salina* was inhibited with nitrogen limitation Figure 4.1 and Figure 4.2. The more nitrogen was in the treatment higher the density of the culture (Augustin and Boersma, 2006). There was a statistical difference within both species between all the treatments. Moreover, *R. baltica* had a statistically higher cell density than *R. salina* in each treatment. *R. baltica* grown on 0% $NaNO_3$ had slight variation in the cell density over time due to the low cell density the measuring errors were larger for this treatment.

The growth rate for *R. baltica* grown on 0% $NaNO_3$ are therefore not exact. *R. salina* grown on 0% $NaNO_3$ could manage to stay alive to wait for favorable conditions. *R. baltica* reached the max growth rate at day 1 for the 33% $NaNO_3$ treatment. At day the 66 and 100% $NaNO_3$ treatments reached max growth rate in *R. baltica* (Figure 4.3). *R. salina* reached max growth rate in day 2 for the 33 and 100% $NaNO_3$. *R. salina* cultivated in 66% $NaNO_3$ reached the max growth rate at day 1 (Figure 4.4). To summarize the growth data for *R. baltica* and *R. salina* lower the concentration of $NaNO_3$ lower cell density. *R. salina* generally reaches the max GR earlier than *R. baltica*. *R. salina* appears to be more sensitive to a 0-nitrogen environment since they died in the 0% $NaNO_3$ treatment. Whereas *R. baltica* stayed stable waiting for favorable conditions, however since the measuring errors are too large in the 0% $NaNO_3$ the data is not considered under the deliberation of the hypothesis.

5.1.2 C:N ratio

R. baltica and *R. salina* show a trend where the C:N molar ratio increases as the nitrogen becomes limited for all treatments except in *R. baltica* 33% $NaNO_3$ where the C:N molar ratio has a slight decrease in the stationary phase (Figure 4.5 and Figure 4.6). In *R. baltica* and *R. salina* the exponential phase had to lowest C:N molar ratio compared to the late exponential phase and the stationary phase. This confirms the hypothesis that in the exponential phase the C:N molar ratio are comparable since the conditions are similar for each treatments. In the stationary phase for the treatments 66 and 100% $NaNO_3$ *R. baltica* was a significantly higher than *R. salina* $p \approx 0.01$ and $p < 0.00$, respectively. The C:N molar ratio was highest in the stationary phase for *R. baltica* 66% $NaNO_3$ treatment (11.16) and for *R. salina* the highest C:N molar ratio was in the stationary phase for the 33% $NaNO_3$ treatment (9.14).

5.1.3 Fatty acids

In *R. baltica* 33, 66, and 100% $NaNO_3$ had an increase in TL, FA, SFA's, MUFA's, and PUFA's as the nitrogen became limiting. Furthermore, within the PUFA's all the different groups (EPA, ARA, $\sum$ Other n-3, and $\sum$ Other n-6) had a steady increase as nitrogen became limiting. The only one who had a lower amount of any PUFA's in the stationary phase was the 100% $NaNO_3$ compared to the others was DHA (Figure 4.7, 4.8, and 4.9).

R. salina each phase had its own pattern for the lipid distribution. The exponential phase 33% $NaNO_3$ of the treatment had the highest amounts of TL, FA, SFA's, MUFA's, PUFA's. The 66% $NaNO_3$ had the lowest amounts of the lipid classes. The trend followed in the PUFA's distribution of EPA, DHA, ARA, ∑ Other n-3, and ∑ Other n-6. The late exponential phase the TL, FA, SFA's, MUFA's, PUFA's decline in between the treatments the highest being the 33% $NaNO_3$ and the lowest being the 100% $NaNO_3$ treatment. The same trends are followed in the PUFA's groups. In the stationary phase the TL are highest in the 100% $NaNO_3$ and lowest in the 33% $NaNO_3$ treatment. However, the 66% $NaNO_3$ treatment is higher in all the other FA groups. *R. baltica* has the higher values in most of the lipids and FA's except in DHA, %PUFA, % ∑ Other-n3, and % EPA where *R. salina* has the higher values (Figure 4.7, 4.8, and 4.9).

5.1.4 *Evaluation of treatments*

An overview of the lowest and highest values for each analysis for *R. baltica* and *R. salina* are given in table 5.1. The treatment names have been abbreviated *R. baltica* is B and *R. salina* is S. Each $NaNO_3$ treatment is abbreviated to only the number of the % concentration. The phases are abbreviated to E, LE, and S for exponential, late exponential, and stationary phase. Furthermore, the table also shows which of the species has the highest value per analysis. From the overview in table 5.1 its clear that *R. baltica* and *R. salina* cultivated in 66% $NaNO_3$ in the stationary phase has the best biochemical profile relative to C:N and FA. Moreover, Table 5.1 shows *R. baltica* in most analysis has a higher value then *R. salina*.

Table 5.1. An overview of the lowest and highest values for each analysis for *R. baltica* and *R. salina* are given in the table. The treatment names have been abbreviated *R. baltica* is B and *R. salina* is S. Each $NaNO_3$ treatment is abbreviated to only the number of the % concentration. The phases are abbreviated to E, LE, and S for exponential, late exponential, and stationary phase. Furthermore, the table also shows which of the species has the highest value per analysis.

	R. baltica		*R. salina*		*R. baltica* vs *R. salina*
Analysis	-	+	-	+	
Density	B33	B100	S33	S100	*R. baltica*
C:N	B33 E	B66 S	S33 E	S33 S	R. baltica
TL	B66 E	B100 S	S33 S	S100 S	R. baltica
FA	B 100 E	B66 S	S100 LE	S66 S	R. baltica
ΣSFA	B100 E	B33 S	S100 LE	S66 S	R. baltica
ΣMUFA	B66 E	B66 S	S100 LE	S66 S	R. baltica
ΣPUFA	B100 E	B66 S	B100 LE	S66 S	R. baltica
Σ Other n-3	B100 E	B66 S	B33 S	S66 S	R. baltica
Σ Other n-6	B33 E	B66 S	S100 LE	S66 S	R. baltica
ARA	B66 LE	B66 S	S33 S	S100 S	R. baltica
EPA	B100 E	B66 S	S33 E	S100 S	R. baltica
DHA	B66 LE	B66 S	S33 S	S33 E	R. salina
% FA	B33 LE	B66 S	S100 LE	S66 S	*R. baltica*
% ΣMUFA	B33 LE	B66 S	S100 LE	S66 S	*R. baltica*
% ΣSFA	B100 S	B33 S	S100 LE	S66 S	*R. baltica*
% ΣPUFA	B33 EL	B66 S	S100 LE	S66 S	*R. salina*
% Σ Other n-3	B100 S	B33 S	S100 LE	S66 S	*R. salina*
% Σ Other n-6	B33 E	B66 S	100 E	S66 S	*R. baltica*
% ARA	B33 E	B66 S	S100 E	S100 S	*R. baltica*
% EPA	B100 E	B66 LE	S33 E	S66 S	*R. salina*
% DHA	B33 LE	B100 LE	S33 S	S33 E	*R. baltica*

5.2 Copepod experiment

The best evaluated treatments from the microalgae experiment were chosen from the evaluation in 5.3 as a mono-diet for *A. tonsa* i.e. *R. baltica* 66% $NaNO_3$ and *R. baltica* 66% $NaNO_3$. Furthermore, *R. baltica* treatment 100% $NaNO_3$ was chosen since it is was is used currently in the industry. The objective of the copepod experiment was to evaluate which chosen mono-diet of *Rhodomonas* treatment constitutes the best feed for *A. tonsa* according to development rate, survival, the C:N stoichiometry, and fatty acid profile with emphasis on DHA and EPA. The three hypotheses were was followed 1. The survival and development rate of *A. tonsa* is dependent on

their diet. 2. The FA profile of *A. tonsa* is dependent on the fatty acid profile of their diet. 3. The C:N stoichiometry in *A. tonsa* is dependent on their diet

5.2.1 Development and survival

The development for the three different mono-diets for *A. tonsa R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ showed to have no statistical difference in the staging (Figure 4.10 and table 4.3). *A. tonsa* had a development of approximately a stage a day at 20 ºC coinciding with Leandro et al, 2006. The development rate for *A. tonsa* fed *R. baltica* 100% $NaNO_3$ was statistically higher than for *A. tonsa* fed *R. baltica* 66% $NaNO_3$ from day 0-3. The survival of the three different treatments of *A. tonsa* showed variation. The *A. tonsa* fed *R. baltica* 66% $NaNO_3$ had the highest % survival when the experiment was terminated 24.4 %. The *A. tonsa* fed *R. baltica* 100% $NaNO_3$ had the lowest % survival 10.3%. *R. salina* 66% $NaNO_3$ fed *A. tonsa* had relatively high % survival 21.2%. During the experiment the copepod thanks had slight aeration to keep the microalgae in suspension. Unfortunately, *A. tonsa* got caught inside the aeration bubbles and were transported on top of the overflow mesh. However, all the aeration pipes had capillaries in, hence all the copepod thanks got the same amount of bubbles. Although the % survival will not be accurate the % survival from Figure 4. 11 will give an indication of which diet in this study contributes to a higher survival of *A. tonsa*. Even though it is not possible to compare the survival of the *A. tonsa* treatments to literature it is possible to set them in perspective. Ismar et. al, 2008 and Galvao, 2004, discovered *A. tonsa* to have a survival of around 80% when fed *Rhodomonas* sp. and *R. baltica* which is extraordinarily higher survival then this study. In this study only 10-25% A. tonsa survived after six days.

5.2.2 Molar ratio carbon to nitrogen

The C:N molar ratio increased from the NIV stage to CI stage for *A. tonsa* in all the diets. The C:N ratio was highest in the *A. tonsa* fed *R. baltica* 100% $NaNO_3$ although not statistically (Figure 4.12). There was no apparent pattern comparing the C:N molar ratio of the algae with the C:N molar ratio of *A. tonsa* fed the corresponding algae.

5.2.3 Fatty acids

The FA and TL content increased from the NIV to CI stage for *A. tonsa* (Figure 4.13). The amounts in the fatty acid groups varied in CI in *A. tonsa* for all three treatments (Figure 4.14). However, by viewing the % distribution of lipids for all three treatments *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ in CI showed to have the same % distribution of Other lipids and the FA groups (Figure 4.15). The TL are slightly higher in NIV stage of *A. tonsa* fed *R. baltica* 66% $NaNO_3$ than *A. tonsa* fed *R. baltica* 100% $NaNO_3$ yet the *A. tonsa* fed *R. baltica* 100% $NaNO_3$ had a higher amount of FA than *A. tonsa* fed *R. baltica* 66% $NaNO_3$. *A. tonsa* fed *R. salina* 66% $NaNO_3$ had the lowest amounts of both TL and FA's. There is no relation between the TL and FA's profile of the algae the copepods in neither NIV or CI stage.

5.2.4 Evaluation of treatments

An overview of the lowest and highest values for each analysis for the feeding of *R. baltica* 66 and 100% $NaNO_3$ and *R. salina* 66% $NaNO_3$ to *A. tonsa* are given in Table 5.2. Furthermore, Table 5.2 shows the both development stages: NIV and CI. The treatment names have been abbreviated *R. baltica* is B and *R. salina* is S. Each $NaNO_3$ treatment is abbreviated to only the number of the % concentration. Table 5.2 confirms that although the 100% $NaNO_3$ *R. baltica* has the overall higher % distribution of in NIV stage of *A. tonsa* but it has the poorest survival. The *A. tonsa* fed *R.* baltica 66% $NaNO_3$ had the best survival but the worst DR in the NIV stage. In the CI stage the *A. tonsa* fed *R.* baltica 66% $NaNO_3$ had the highest amounts of FA and TL moreover it has the best survival yet the poorest DR. The *A. tonsa* fed *R.* baltica 100% $NaNO_3$ had the highest overall FA% of the TL but had the poorest survival. The *R. salina* 66% $NaNO_3$ fed *A. tonsa* was had the poorest overall results in both stages. None of the analysis showed any significant difference therefore it is not possible to conclude that any of the mono-diets is superior.

Table 5.2. An overview of the lowest and highest values for each analysis for the feeding of *R. baltica* 66 and 100% $NaNO_3$ and *R. salina* 66% $NaNO_3$ to *A. tonsa*. Furthermore, it shows the both development stages: NIV and CI. The treatment names have been abbreviated *R. baltica* is B and *R. salina* is S. Each $NaNO_3$ treatment is abbreviated to only the number of the % concentration.

	A. tonsa			
	NIV		CI	
Analysis	-	+	-	+
DR	B66	B100	B66	S66
Survival	B100	B66	B100	B66
C:N	S66	B100	S66	B100
TL	S66	B66	B100	B66
FA	B66	B100	S66	B66
ΣMUFA	S66	B66	B100	B66
ΣSFA	S66	B100	B100	B66
ΣPUFA	S66	B100	S66	B66
Σn-3	S66	B100	S66	B66
Σn-6	S66	B100	S66	B66
ARA	S66	B100	B100	S66
EPA	S66	B66	B66	B100
DHA	S66	B66	S66	B100
% FA	S66	B100	S66	B100
% ΣMUFA	S66	B66	S66	B66
% ΣSFA	S66	B100	S66	B100
% ΣPUFA	S66	B100	S66	B100
% Σn-3	S66	B100	S66	B66
% Σn-6	S66	B100	S66	B100
% ARA	S66	B66	B100	S66
% EPA	S66	B100	S66	B100
% DHA	S66	B100	S66	B100

6. Conclusion

The main aim of the study was to establish the development and survival rate of *A. tonsa* fed on mono-diets of one of three species of *Rhodomonas baltica*, *marina*, and *salina* cultured in 0%, 33%, 66%, and 100% $NaNO_3$. *R. marina* was more difficult to cultivate than *R. baltica* and *R. salina* and was there for excluded hence *R. baltica* 100% $NaNO_3$ was used in the feeding experiment in its place. The *A. tonsa* fed *R. baltica* 66% $NaNO_3$ had the highest survival but the poorest development rate. *R.* salina 66% $NaNO_3$ fed *A. tonsa* had the highest development rate from day 0-6 and had a survival slightly lower than *A. tonsa* fed *R. baltica* 66% $NaNO_3$. *A. tonsa* fed *R. baltica* 100% $NaNO_3$ had the worst survival though it had the highest DR from day 0-3. Although, there were no statistical differences the *A. tonsa* fed *R. baltica* 66% $NaNO_3$ showed to have the overall best biochemical composition.

The microalgae experiment showed nitrogen limited the growth and reduced the carrying capacity which was expected in *R. baltica* and *R. salina*. The more nitrogen limited the algae the higher the C:N molar ratio. The hypothesis about the stoichiometry between carbon and nitrogen was the same in the exponential phase although in nitrogen limited environment there would be a variation between the treatments was true. In the exponential phase only the 0% nitrogen was statistically different but was not taken in to account during the evaluation of the different treatments due to measuring inaccuracy. There was a statistically difference between the carrying capacity within each species the highest concentration of $NaNO_3$ resulted in a higher carrying capacity. Furthermore, *R. baltica* had a higher carrying capacity than *R. salina* in each treatment. There was no difference in the fatty acid profile for the four different treatments in the exponential phase however the EPA and DHA for the *R. baltica* grown on 33 and 66% $NaNO_3$ was different. In addition to the fatty acid profile changed when the algae became nitrogen limited.

Out of all four $NaNO_3$ treatments for each species the 66% showed to be best suited for the *A. tonsa* feeding experiment however, in the end they showed no statistical difference in FA's, TL, C:N, and development. Only difference was that *A. tonsa* fed *R. baltica* 66% $NaNO_3$ had a higher survival. There was no evidence supporting that the FA profile and C:N molar ratio in *A. tonsa* was dependent on the diet. As a mono-diet for *A. tonsa* the only evident difference between *R. baltica* 66% $NaNO_3$, *R. baltica* 100% $NaNO_3$, and *R. salina* 66% $NaNO_3$ was *R. baltica* 66% $NaNO_3$ higher survival.

7. Future study

The study showed under nitrogen limited conditions *R. baltica* had a C:N molar ratio of 11 cultivated in 66% $NaNO_3$ by cultivating with different % of $NaNO_3$ it might be possible to get it higher. The chemical composition is also dependent of the phosphorus concentration in the culture medium therefore it would be of interest to use the same set up as in this study to examine the biochemical and development changes.

Examine *R. marina* closer and learn how to cultivate it and examine the biochemical composition.

Density analyses of *R. salina* and *R. baltica* showed when stresses in a certain way both species made much smaller cells. There it would be interesting to study if *R. baltica* and *R. salina* have sexual reproduction.

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Appendix

Appendix A

TL and fatty acid composition of *R. baltica* and *R. salina*

Table A1. The table shows the FA and TL results for the seven different treatments and the three different measuring points exponential, late exponential, and the stationary phase in the microalgae experiment: *R. baltica* cultivated on 0, 33, 66, and 100% $NaNO_3$ and R. salina cultivated on 33, 66, and 100% $NaNO_3$.

	R. baltica 0% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.47 ± 0.43	0.49 ± 0.38	3.27 ± 0.49
C14:0	3.30 ± 1.64	10.63 ± 0.26	8.09 ± 4.53
C14:1n5	0.02 ± 0.01	0.01 ± 0.01	0.06 ± 0.03
C15:0	-	0.18 ± 0.15	-
C16:0	3.14 ± 3.45	22.27 ± 13.06	9.58 ± 6.95
C16:1n7	0.20 ± 0.26	1.35 ± 0.81	0.94 ± 0.81
C17:0	-	0.40 ± 0.39	-
C18:0	0.38 ± 0.22	13.33 ± 16.54	1.67 ± 0.15
C18:1n9	0.69 ± 0.59	9.74 ± 5.68	3.97 ± 3.15
C18:1n7	1.11 ± 1.37	4.47 ± 2.53	2.89 ± 2.19
C18:2n6	1.40 ± 0.91	18.93 ± 10.93	8.79 ± 7.60
C18:3n3	4.14 ± 6.21	15.02 ± 8.33	6.74 ± 5.15
C20:0	0.04	0.17 ± 0.23	0.02
C20:1n9	-	0.02	-
C20:2n6	0.21 ± 0.21	0.06 ± 0.02	0.11 ± 0.03
C20:3n3	0.65 ± 0.55	0.35 ± 0.36	1.41 ± 0.51
C20:4n6	0.11 ± 0.11	1.18 ± 0.70	1.46 ± 0.09
C20:5n3	4.85 ± 5.07	7.70 ± 4.52	3.93 ± 3.03
C21:0	-	-	-
C22:0	0.14 ± 0.12	0.14 ± 0.07	0.24 ± 0.14
C22:1n9	0.07 ± 0.08	0.07 ± 0.04	0.18 ± 0.06
C22:6n3	1.64 ± 2.75	3.93 ± 2.31	2.59 ± 1.97
C24:0	0.01	-	-
C24:1	2.57 ± 1.58	1.73 ± 1.04	3.21 ± 1.10
TL	103.12 ± 61.95	293.35 ± 131.84	207.53 ± 106.97
ΣFA	24.36 ± 17.82	110.96 ± 62.89	56.51 ± 37.29
ΣSFA	7.41 ± 4.83	47.32 ± 31.74	20.74 ± 37.29
ΣMUFA	4.00 ± 2.04	16.47 ± 8.27	11.23 ± 7.06
ΣPUFA	12.95 ± 12.74	47.17 ± 26.08	24.54 ± 18.76
Σn-3	11.28 ± 12.13	27.00 ± 14.77	14.67 ± 10.36
Σn-6	1.67 ± 0.93	20.17 ± 11.63	9.87 ± 8.41

Table A1 continues.

	R. baltica 33% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.86 ± 0.36	0.76 ± 0.92	10.61 ± 0.96
C14:0	9.12 ± 2.31	10.08 ± 5.95	18.96 ± 1.34
C14:1n5	0.02 ± 0.02	0.03 ± 0.01	0.03 ± 0.03
C15:0	-	0.07	0.04 ± 0.03
C16:0	11.43 ± 1.87	15.81 ± 7.40	32.34 ± 2.38
C16:1n7	0.92 ± 0.27	1.42 ± 9.01	5.34 ± 0.33
C17:0	-	0.06	-
C18:0	0.88 ± 0.43	1.57 ± 1.19	2.30 ± 0.51
C18:1n9	2.31 ± 0.71	6.64 ± 3.01	15.19 ± 1.14
C18:1n7	3.93 ± 0.59	3.46 ± 9.01	4.64 ± 0.35
C18:2n6	2.92 ± 0.62	6.94 ± 9.01	23.46 ± 1.73
C18:3n3	15 ± 1.39	19.16 ± 3.01	26.79 ± 1.63
C20:0	0.06 ± 0.03	0.07 ± 0.04	0.1
C20:1n9	-	0.03	0.05 ± 0.05
C20:2n6	0.03 ± 0.01	0.14 ± 0.01	0.10 ± 0.11
C20:3n3	0.73 ± 0.14	0.73 ± 7.01	0.64 ± 1.14
C20:4n6	0.48 ± 0.39	0.28 ± 4.01	0.94 ± 0.08
C20:5n3	9.22 ± 0.60	7.59 ± 2.01	9.99 ± 0.78
C21:0	-	0.05	-
C22:0	0.15 ± 0.02	0.17 ± 0.15	-
C22:1n9	0.08 ± 0.02	0.09 ± 0.01	0.06 ± 0.12
C22:6n3	5.18 ± 0.39	3.48 ± 1.01	6.23 ± 0.24
C24:0	-	-	-
C24:1	2.47 ± 0.23	3.52 ± 2.58	8.37
TL	249.82 ± 74.15	290.85 ± 53.92	249.95 ± 89.23
ΣFA	66.43 ± 7.76	76.90 ± 37.65	149.78 ± 12.63
ΣSFA	22.41 ± 4.68	28.52 ± 15.19	54.24 ± 4.75
ΣMUFA	9.72 ± 1.58	10.10 ± 8.08	27.41 ± 5.20
ΣPUFA	34.30 ± 1.76	38.29 ± 14.45	68.13 ± 2.89
Σn-3	30.87 ± 1.43	30.96 ± 11.49	43.65 ± 2.01
Σn-6	3.43 ± 0.50	7.33 ± 3.06	24.48 ± 1.88

Table A1 continues.

	R. baltica 66% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.63 ± 0.19	0.67 ± 0.41	14.50 ± 0.59
C14:0	8.54 ± 2.12	10.67 ± 3.99	28.16 ± 6.76
C14:1n5	0.02 ± 0.01	0.03 ± 0.01	0.06 ± 0.02
C15:0	-	-	0.25 ± 0.02
C16:0	7.39 ± 5.22	9.31 ± 3.50	17.99 ± 7.77
C16:1n7	0.55 ± 0.21	1.37 ± 0.59	4.48 ± 0.82
C17:0	-	-	-
C18:0	1.05 ± 0.62	0.91 ± 0.27	0.96 ± 0.32
C18:1n9	3.10 ± 2.29	5.87 ± 1.60	26.65 ± 11.48
C18:1n7	3.37 ± 0.97	4.85 ± 2.68	9.44 ± 2.28
C18:2n6	7.56 ± 5.99	14.20 ± 7.92	36.24 ± 1.38
C18:3n3	13.01 ± 2.67	13.19 ± 6.77	23.50 ± 3.16
C20:0	0.04 ± 0.02	0.02 ± 0.00	-
C20:1n9	-	0.01	0.15
C20:2n6	0.07 ± 0.03	0.02 ± 0.00	0.04 ± 0.01
C20:3n3	0.58 ± 0.36	0.43 ± 0.32	0.63 ± 0.40
C20:4n6	0.44 ± 0.35	1.57 ± 1.03	3.84 ± 0.86
C20:5n3	7.07 ± 1.34	10.57 ± 0.75	13.81 ± 0.99
C21:0	1.03	1.03	-
C22:0	0.09 ± 0.05	0.12 ± 0.07	0.19 ± 0.05
C22:1n9	0.05 ± 0.04	0.05 ± 0.04	0.10 ± 0.03
C22:6n3	4.07 ± 0.70	4.59 ± 2.85	7.05 ± 0.54
C24:0	-	-	-
C24:1	2.48 ± 0.63	1.47 ± 0.50	2.27 ± 0.81
TL	138.63 ± 20.69	197.48 ± 40.33	275.84 ± 24.33
ΣFA	59.72 ± 11.80	79.27 ± 30.32	175.78 ± 28.60
ΣSFA	17.99 ± 4.08	21.45 ± 7.44	48.24 ± 15.02
ΣMUFA	8.94 ± 1.74	13.26 ± 4.92	42.45 ± 10.72
ΣPUFA	32.79 ± 7.98	44.56 ± 18.25	85.09 ± 3.93
Σn-3	24.74 ± 4.59	28.78 ± 9.82	44.99 ± 4.22
Σn-6	8.05 ± 6.14	15.78 ± 8.95	40.10 ± 2.00

Table A1 continues.

	R. baltica 100% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.97 ± 0.63	0.51 ± 0.43	9.06 ± 0.55
C14:0	6.48 ± 4.94	11.64 ± 2.87	16.09 ± 6.29
C14:1n5	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
C15:0	-	-	0.16
C16:0	8.62 ± 7.57	11.21 ± 3.72	7.86 ± 3.33
C16:1n7	0.69 ± 0.65	1.41 ± 0.45	2.68 ± 0.86
C17:0	-	-	-
C18:0	0.84 ± 0.50	2.17 ± 3.16	0.93 ± 0.84
C18:1n9	1.69 ± 1.51	3.90 ± 1.42	7.33 ± 2.93
C18:1n7	2.88 ± 2.52	5.08 ± 1.38	7.76 ± 2.25
C18:2n6	4.01 ± 2.78	12.54 ± 5.05	22.94 ± 7.23
C18:3n3	11.23 ± 11.02	14.76 ± 2.05	13.99 ± 4.57
C20:0	0.02 ± 0.02	0.05 ± 0.04	0.03
C20:1n9	-	-	-
C20:2n6	0.10 ± 0.05	0.03 ± 0.03	0.08 ± 0.10
C20:3n3	0.66 ± 0.64	0.36 ± 0.34	0.84 ± 0.75
C20:4n6	0.47 ± 0.33	1.55 ± 0.77	2.68 ± 0.79
C20:5n3	6.44 ± 6.73	9.96 ± 1.17	8.40 ± 2.59
C21:0	-	-	-
C22:0	0.11 ± 0.08	0.11 ± 0.07	0.21 ± 0.08
C22:1n9	0.10 ± 0.06	0.04 ± 0.04	0.12 ± 0.05
C22:6n3	3.92 ± 3.65	5.98 ± 0.68	3.70 ± 1.13
C24:0	-	-	-
C24:1	4.03 ± 0.71	1.95 ± 0.84	2.84 ± 2.10
TL	158.48 ± 106.04	192.66 ± 41.68	308.39 ± 76.40
ΣFA	53.23 ± 41.60	82.14 ± 21.08	98.48 ± 31.70
ΣSFA	17.04 ± 13.27	25.55 ± 8.50	25.87 ± 10.95
ΣMUFA	9.40 ± 5.03	11.42 ± 3.68	20.00 ± 6.72
ΣPUFA	26.79 ± 23.45	45.18 ± 9.17	52.62 ± 15.41
Σn-3	22.26 ± 21.14	31.07 ± 3.51	26.93 ± 7.68
Σn-6	4.53 ± 3.12	14.11 ± 5.82	25.69 ± 7.90

Table A1 continues.

	R. salina 33% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.07 ± 0.01	0.09 ± 0.09	1.33 ± 0.08
C14:0	4.63 ± 0.04	7.84 ± 0.42	2.64 ± 1.39
C14:1n5	-	-	-
C15:0	-	-	-
C16:0	7.54 ± 1.44	17.46 ±	5.12 ± 2.42
C16:1n7	0.54 ± 0.04	1.10 ± 0.08	0.39 ± 0.27
C17:0	-	-	-
C18:0	0.39 ± 0.02	1.63 ± 1.09	0.51 ± 0.15
C18:1n9	1.47 ± 0.73	5.58 ± 2.05	1.76 ± 0.95
C18:1n7	3.14 ± 0.51	4.17 ± 0.06	0.82 ± 0.47
C18:2n6	3.72 ± 1.04	11.62 ± 0.77	4.31 ± 1.96
C18:3n3	13.21 ± 1.51	22.05 ± 0.81	4.56 ± 2.43
C20:0	0.04 ± 0.02	0.04 ± 0.03	0.00 ± 0.00
C20:1n9	-	-	-
C20:2n6	0.03 ± 0.00	0.04 ± 0.03	0.00
C20:3n3	0.06 ± 0.04	0.14 ± 0.10	0.02 ± 0.00
C20:4n6	0.45 ± 0.11	0.48 ± 0.02	0.14 ± 0.10
C20:5n3	5.83 ± 4.09	11.62 ± 0.28	1.88 ± 1.16
C21:0	-	-	-
C22:0	0.01 ± 0.00	0.01	-
C22:1n9	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
C22:6n3	4.93 ± 1.28	5.28 ± 0.16	0.83 ± 0.55
C24:0	-	-	-
C24:1	-	-	-
TL	173.16 ± 13.07	202.77 ± 12.39	41.24 ± 30.55
ΣFA	47.97 ± 4.68	89.15 ± 5.90	23.11 ± 11.87
ΣSFA	12.65 ± 1.47	27.06 ± 3.25	8.40 ± 4.05
ΣMUFA	5.15 ± 0.26	10.86 ± 2.60	2.97 ± 1.68
ΣPUFA	30.17 ± 3.47	51.23 ± 0.49	11.74 ± 6.18
Σn-3	25.97 ± 4.38	39.09 ± 0.89	7.28 ± 4.15
Σn-6	4.19 ± 0.92	12.14 ± 0.82	4.46 ± 2.06

Table A1 continues.

	R. salina 66% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.09 ± 0.14	0.25 ± 0.30	6.20 ± 0.44
C14:0	4.42 ± 1.04	6.02 ± 1.52	12.08 ± 4.41
C14:1n5	-	-	-
C15:0	-	-	0.22 ± 0.14
C16:0	9.60 ± 5.65	8.49 ± 1.50	18.24 ± 8.02
C16:1n7	0.52 ± 0.21	0.81 ± 0.25	1.87 ± 0.81
C17:0	0.15 ± 0.15	-	0.20 ± 0.20
C18:0	3.16 ± 5.41	0.79 ± 0.46	2.66 ± 2.53
C18:1n9	1.19 ± 0.47	2.30 ± 1.58	9.14 ± 1.62
C18:1n7	2.87 ± 0.46	3.32 ± 0.42	4.27 ± 0.78
C18:2n6	2.72 ± 0.39	4.42 ± 2.47	17.73 ± 2.01
C18:3n3	11.58 ± 1.63	14.67 ± 3.26	22.61 ± 4.94
C20:0	0.05 ± 0.05	0.02 ± 0.02	0.01 ± 0.01
C20:1n9	-	-	
C20:2n6	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00
C20:3n3	0.09 ± 0.02	0.06 ± 0.02	0.05 ± 0.04
C20:4n6	0.43 ± 0.02	0.41 ± 0.17	1.06 ± 0.53
C20:5n3	6.85 ± 1.16	8.38 ± 1.20	13.30 ± 2.21
C21:0	-	-	-
C22:0	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.01
C22:1n9	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C22:6n3	4.36 ± 0.53	4.85 ± 0.80	6.69 ± 1.00
C24:0	-	-	-
C24:1	-	-	-
TL	132.32 ± 5.68	140.04 ± 40.20	180.44 ± 29.70
ΣFA	48.01 ± 16.41	54.81 ± 12.13	111.06 ± 21.37
ΣSFA	17.38 ± 12.24	15.58 ± 2.91	34.30 ± 13.66
ΣMUFA	4.59 ± 1.08	6.44 ± 2.15	15.29 ± 2.50
ΣPUFA	26.04 ± 3.57	32.80 ± 7.35	61.47 ± 9.56
Σn-3	22.88 ± 3.24	27.95 ± 5.10	42.65 ± 7.69
Σn-6	3.16 ± 0.40	4.84 ± 2.58	18.82 ± 2.07

Table A1 continues.

	R. salina 100% $NaNO_3$		
	Exponential	Late exponential	Stationary
	µg/mg DW		
C12:0	0.11 ± 0.13	0.18 ± 0.03	4.48 ± 1.61
C14:0	4.40 ± 0.53	2.74 ± 2.89	9.68 ± 3.54
C14:1n5	-	-	-
C15:0	-	-	0.01
C16:0	7.69 ± 1.66	3.65 ± 3.76	10.41 ± 0.76
C16:1n7	0.59 ± 0.18	0.59 ± 0.62	1.24 ± 0.19
C17:0	-	-	-
C18:0	1.15 ± 1.31	0.51 ± 0.16	1.13 ± 0.32
C18:1n9	1.31 ± 0.84	0.94 ± 0.71	5.74 ± 0.88
C18:1n7	3.09 ± 0.30	1.61 ± 1.57	5.14 ± 0.65
C18:2n6	2.55 ± 0.28	1.83 ± 1.56	15.57 ± 3.85
C18:3n3	12.10 ± 0.95	6.40 ± 6.67	19.82 ± 2.65
C20:0	0.05 ± 0.03	0.01 ± 0.02	0.01 ± 0.01
C20:1n9	0.08 ± 0.08	-	0.08 ± 0.08
C20:2n6	0.01	0.02	0.03 ± 0.01
C20:3n3	0.08 ± 0.02	0.05 ± 0.05	0.05 ± 0.01
C20:4n6	0.42 ± 0.05	0.26 ± 0.15	1.38 ± 0.47
C20:5n3	7.53 ± 0.48	4.33 ± 4.55	13.59 ± 1.02
C21:0	-	-	-
C22:0	-	0	0.01
C22:1n9	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C22:6n3	4.77 ± 0.40	3.11 ± 2.47	7.50 ± 0.54
C24:0	-	-	-
C24:1	-	-	-
TL	168.57 ± 46.93	107.07 ± 59.58	216.84 ± 56.49
ΣFA	45.80 ± 6.01	24.87 ± 24.87	92.05 ± 7.16
ΣSFA	13.35 ± 3.51	7.00 ± 6.79	22.00 ± 5.44
ΣMUFA	5.01 ± 1.29	2.76 ± 2.86	12.12 ± 1.65
ΣPUFA	27.45 ± 2.05	15.10 ± 15.24	57.93 ± 4.01
Σn-3	24.48 ± 1.75	13.08 ± 13.80	40.96 ± 4.20
Σn-6	2.97 ± 0.32	2.02 ± 1.73	16.98 ± 4.33

Appendix B
Carbon / Nitrogen ration of *R. baltica* and *R. salina*

Table A2. The carbon and nitrogen content of the *R. baltica* and *R. salina* cultivated in the four different treatments 0%, 33%, 66%, and 100% $NaNO_3$ in the growth medium. The molar ratio is showed with the standard deviation.

Species	Treatment	Sampling	Molar ratio
	% NaNo3	**Phase**	**C:N**
R. baltica	0	Exponential	9.01 ± 1.25
		Late exponential	9.19 ± 0.64
		Stationary	7.13 ± 0.48
	33	Exponential	6.52 ± 0.69
		Late exponential	8.97 ± 1.51
		Stationary	7.79 ± 2.03
	66	Exponential	7.76 ± 1.24
		Late exponential	10.56 ± 0.93
		Stationary	11.16 ± 1.10
	100	Exponential	7.23 ± 0.58
		Late exponential	7.69 ± 0.42
		Stationary	9.95 ± 0.23
R. salina	33	Exponential	6.25 ± 0.31
		Late exponential	8.31 ± 0.13
		Stationary	9.14 ± 0.87
	66	Exponential	6.40 ± 0.44
		Late exponential	8.31 ± 1.64
		Stationary	8.77 ± 0.10
	100	Exponential	6.32 ± 0.35
		Late exponential	7.02 ± 0.70
		Stationary	8.42 ± 0.43

Appendix C
Survival of Acartia tonsa

Table A3. The table shows the amount of *A. tonsa* nauplii there were in the beginning of experiment for each of the three treatments *R. baltica* and *R. salina* grown on 66% or 100% $NaNO_3$. Furthermore, it shows the survival in % over time.

	A. Tonsa					
	***R. baltica* 66% $NaNO_3$**		***R. baltica* 100% $NaNO_3$**		***R. salina* 66% $NaNO_3$**	
Day	Density #	%	Density #	%	Density #	%
0	22 ± 2.29	100	22 ± 2.75	100	20 ± 4.54	100
3	10 ± 2.75	43.18	4 ± 2.93	17.16	7 ± 2.65	35.29
6	6 ± 1.65	25.45	2 ± 0.35	10.30	4 ± 1.59	21.18

Appendix D
Carbon and nitrogen data for *A. tonsa*

Table A4. Carbon (C) and nitrogen (N) content of *A. tonsa* fed mono-diets of three different feeds *R. baltica* and *R. salina* grown on 66% or 100% $NaNO_3$ in the Conwy medium. The C and N content was measured at development stage NIV and CI in each treatment there were three replicates. Furthermore, the mol for C and N were calculated to find the molar ratio. The data is represented as the average of the three replicates ± standard deviation.

A. tonsa fed	Stage	Carbon		Nitrogen		Ratio
		µg/filter	mol	µg/filter	mol	molar
R. baltica 66% $NaNO_3$	NIV (4)	14.05 ± 5.03	1.17 ± 0.42	6.91 ± 1.51	0.49 ± 0.11	2.32 ± 0.37
	CI (7)	19.57 ± 15.16	1.63 ± 1.26	8.38 ± 4.05	0.60 ± 0.29	2.50 ± 0.71
R. baltica 100% $NaNO_3$	NIV (4)	34.53 ± 24.79	2.88 ± 2.06	11.58 ± 5.70	0.83 ± 0.41	3.24 ± 0.74
	CI (7)	36.66 ± 2.47	3.05 ± 0.21	12.37 ± 0.78	0.88 ± 0.06	3.46 ± 0.07
R. salina 66% $NaNO_3$	NIV (4)	12.75 ± 5.34	1.06 ± 0.44	6.71 ± 1.14	0.48 ± 0.08	2.15 ± 0.54
	CI (7)	22.34 ± 7.23	1.86 ± 0.60	9.09 ± 1.55	0.65 ± 0.11	2.82 ± 0.43

Appendix E
Fatty acid data for Acartia tonsa

Table A5. Fatty acid data for *A. tonsa* cultivated on both nitrogen deplete and replete algae. *A. tonsa* were fed on *R. baltica* 66% $NaNO_3$ and *R. baltica* 100% $NaNO_3$ *R. salina* 66% $NaNO_3$. The data shows the fatty acid composition for development stages NIV and C1 for *A. tonsa*. The fatty acids values are an average of three samples and are given up in µg/mg DW ± SD.

	R. baltica 66% NaNO		R. baltica 100% NaNO		*R. salina* 66% NaNO	
	NIV	CI	NIV	CI	NIV	CI
	µg/mg DW ± SD		µg/mg DW ± SD		µg/mg DW ± SD	
C14:0	1.77 ± 0.50	1.76 ± 0.48	1.80 ± 0.84	1.55 ± 0.41	0.99 ± 0.61	1.33 ± 0.25
C16:0	4.10 ± 1.06	5.67 ± 1.26	4.30 ± 2.13	5.07 ± 1.25	2.61 ± 1.66	4.96 ± 0.42
C16:1n7	-	0.50 ± 0.17	-	0.44	-	0.40
C17:0	0.38 ± 0.10	0.57 ± 0.17	0.60 ± 0.41	0.52 ± 0.16	0.28 ± 0.20	0.55 ± 0.04
C18:0	0.80 ± 0.14	1.41 ± 0.47	0.71 ± 0.39	1.16 ± 0.29	0.70 ± 0.67	1.77 ± 0.94
C18:1n9	1.13 ± 0.29	1.46 ± 0.36	0.69 ± 0.32	0.92 ± 0.28	0.80 ± 0.64	1.10 ± 0.49
C18:1n7	0.74 ± 0.18	0.95 ± 0.32	0.62 ± 0.31	0.81 ± 0.21	0.55 ± 0.45	0.54 ± 0.04
C18:2n6	3.38 ± 1.08	3.64 ± 1.65	3.02 ± 1.31	3.44 ± 0.94	1.81 ± 1.05	2.42 ± 1.08
C18:3n3	2.23 ± 0.78	1.91 ± 0.69	2.48 ± 1.01	1.98 ± 1.42	1.42 ± 1.11	1.48 ± 0.40
C18:3n6	0.40 ± 0.10	0.94 ± 0.81	2.38 ± 1.60	0.61 ± 0.31	0.16 ± 0.12	0.67 ± 0.35
C20:0	0.05	-	-	-	-	-
C20:1n9	0.08 ± 0.03	0.07 ± 0.04	0.03 ± 0.01	0.05 ± 0.00	0.03 ± 0.04	0.06 ± 0.01
C20:2n6	0.25	-	-	0.18	-	-
C20:3n3	0.03	-	-	-	-	-
C20:4n6	0.77 ± 0.20	0.90 ± 0.32	0.37	0.92 ± 0.24	-	1.57 ± 1.40
C20:5n3	3.88 ± 1.18	4.99 ± 1.23	3.84 ± 1.51	5.24 ± 1.59	2.80 ± 1.84	4.62 ± 1.34
C22:0	0.01	-	-	-	-	-
C22:1n9	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
C22:5n3	0.32 ± 0.06	1.15 ± 1.00	3.31 ± 2.39	0.58 ± 0.46	0.26 ± 0.17	0.61 ± 0.47
C22:6n3	5.71 ± 1.56	8.69 ± 2.69	5.12 ± 1.98	8.02 ± 2.40	4.40 ± 2.96	8.03 ± 2.46
C24:1	0.05 ± 0.01	0.16 ± 0.11	0.35 ± 0.25	0.09 ± 0.02	0.04 ± 0.02	0.11 ± 0.03
TL	107.81 ± 18.04	122.38 ± 38.53	94.23 ± 40.41	107.52 ± 55.08	83.62 ± 53.34	115.74 ± 10.08
ΣFA	26.54 ± 7.24	34.79.52 ± 9.36	29.63 ± 12.73	31.59 ± 7.59	16.88 ± 10.93	30.23 ± 2.29
ΣSFA	7.10 ± 1.75	9.41 ± 2.14	7.41 ± 3.15	8.31 ± 2.09	4.58 ± 3.00	8.61 ± 1.20
ΣMUFA	2.45 ± 0.63	3.16 ± 1.83	1.71 ± 0.82	2.33 ± 0.71	1.44 ± 1.15	2.22 ± 0.46
ΣPUFA	16.98 ± 4.87	22.22 ± 6.48	20.52 ± 9.56	20.96 ± 4.88	10.86 ± 6.95	19.40 ± 3.42
Σn-3	2.58 ± 0.81	3.06 ± 1.67	5.79 ± 3.39	2.55 ± 0.23	1.68 ± 1.16	2.09 ± 0.43
Σn-6	4.04 ± 1.13	4.58 ± 2.43	5.40 ± 2.91	4.23 ± 0.79	1.97 1.15	3.09 ± 0.96

Table A6. Fatty acid data as % of TL for *A. tonsa* fed on *R. baltica* 66% $NaNO_3$ and *R. baltica* 100% $NaNO_3$ *R. salina* 66% $NaNO_3$. The table shows the % distribution of ARA, EPA, DHA, ∑SFA's, ∑MUFA's, Other n-3, Other n-6, and Other lipids of the TL for development stages NIV and C1 for *A. tonsa*. The FA values are an average of three samples and are given up in %/TL

	R. baltica 66% NaNO		*R. baltica* 100% NaNO		*R. salina* 66% NaNO	
	NIV	CI	NIV	CI	NIV	CI
ARA	0.72	0.39		0.74	0.85	1.36
EPA	3.60	4.07	3.35	4.08	4.87	3.99
DHA	5.30	5.43	5.26	7.10	7.46	6.94
ΣSFA	6.59	7.86	5.48	7.69	7.72	7.44
ΣMUFA	2.28	1.81	1.72	2.58	2.16	1.92
Other n-3	2.39	6.14	2.01	2.50	2.38	1.81
Other n-6	3.75	5.74	2.36	3.74	3.93	2.67
Other lipids	75.38	68.55	79.81	71.57	70.62	73.88