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Cultivation of *Acartia tonsa* with 3 different marine microalgae to optimize productivity and nutritional value

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Marine Coastal Development
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Abstract

The conventional method for first feeding of marine fish is to use enriched rotifers (*Brachionus* spp.) in the beginning and followed with enriched *Artemia*, before transfer to dry feed. Recent researches have shown that fish larvae fed on calanoid copepods have higher growth rate, survival rate, fewer deformities and stronger pigmentation than larvae fed on rotifers and *Artemia.* The reason for the difference is discussed to be due to better nutritional quality of the copepods, which met the larvae requirement, mainly the Highly Unsaturated Fatty Acids (HUFAs); DHA, EPA and ARA. However, it is generally considered to be difficult, labour intensive and expensive to have a large-scale production of copepods. It is therefore important to continue to develop and optimize the cultivation methods of copepods, making the production inexpensive and simple.

In this thesis, *Acartia tonsa* was cultivated with three different monoalgal diets with the microalgae species, *Rhodomonas baltica, Tisochrysis lutea* and *Conticribra weissflogii*, to optimize cultivation productivity and nutritional value of *A. tonsa*. Two separate studies were performed, first a nutritional study in a flow through system (FTS), and a second test study where *A. tonsa* was cultivated in a Recirculation Aquaculture System (RAS), integrated with a microalgae cultivation, a so-called copeponics system.

In conclusion, the study of the nutritional value of *A. tonsa* and microalgae species, showed that it was possible to manipulate the fatty acid composition of *A. tonsa* through the diet. The survival and growth rate of *A. tonsa* was highest when reared on *R. baltica* and *T. lutea*. The fatty acid composition of *A. tonsa* reared on *R. baltica* resulted in the most suitable HUFA ratio of DHA/EPA (~2) and EPA/ARA (~6) for marine fish larvae. The copeponics system functioned when using the microalgae *R. baltica* and the results gave strong indication that it was possible to reduce the water and nutrient consumption by ~90% and ~53%, respectively. However, the copeponics system should be further investigated for optimization.

Keywords: *Acartia tonsa*, Microalgae, Fatty acids, Cultivations methods, Copeponics

Abbreviations

ARA	Arachidonic Acid
CT	Copepod tank
DHA	Docosahexaenoic Acid
DIN	Dissolved Inorganic Nitrogen
DW	Dry Weight
EPA	Eicosapentaenoic Acid
FAME	Fatty Acid Methyl Esterification
FAs	Fatty Acids
FTS	Flow-Through System
GC	Gas Chromatography
HUFAs	Highly-Unsaturated Fatty Acids
IMTA	Integrated Multitrophic Aquaculture
MUFAs	Mono-Unsaturated Fatty Acids
PE	Polyethylene
PP	Polypropylene
PUFAs	Poly-Unsaturated Fatty Acids
RAS	Recirculation Aquaculture System
SD	Standard Deviation
SFAs	Saturated Fatty Acids
SGR	Specific Growth Rate
TAN	Total Ammonia Nitrogen
TL	Total Lipids

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1. Introduction

Aquaculture is believed to becoming more important in future production of protein for human consumption, and especially marine aquaculture has the highest potential for future growth (FAO, 2016). Much effort has been made to establish aquaculture of marine fish species. The juvenile production of marine fish species often requires the use of live feed for successful rearing, as the earliest stages of many marine fish species include a small larvae stage that cannot utilize conventional dry feed. These small larvae need small prey that fits into their small developing mouths and guts (Lee et al., 2008).

1.1 First feeding of marine fish larvae

The conventional method for first feeding of marine fish is to use enriched rotifers (*Brachionus* spp.) in the beginning and followed with enriched *Artemia*, before transfer to dry feed (Baskerville-Bridges and Kling, 2000). Rotifers and *Artemia* are not a part of the natural diet of marine fish larvae but are used due to their simplicity in cultivation. In the wild it is normal for marine fish larvae to regularly encounter calanoid copepod nauplii, in fact copepods can represent up to 80% of the mesozooplankton (Blaxter et al., 1998) and play a key role in the food web, especially for planktivorous fish and fish larvae (Fox et al., 1999, Möllmann et al., 2004).

Recent studies have shown that fish larvae reared on calanoid copepods have higher growth rate, survival rate, fewer deformities and stronger pigmentation than larvae reared on rotifers and *Artemia* (Holmefjord et al., 1993, Næss et al., 1995, Shields et al., 1999, Olsen et al., 2014, Øie et al., 2015, Evjemo et al., 2014). Figure 1, Figure 2 and Figure 3, show different species of marine fish larvae reared on rotifers and *Artemia*, compared with the use of the copepod *Acartia tonsa*. In all cases the larvae reared on copepods showed higher survival, growth rates and fewer deformities, as the figures indicate. The reason for the difference is believed to be due to better nutritional quality of the copepods, which met the larvae requirement. The important nutritional difference between the live preys are believed to be lipids and fatty acid compositions (Øie et al., 2015).

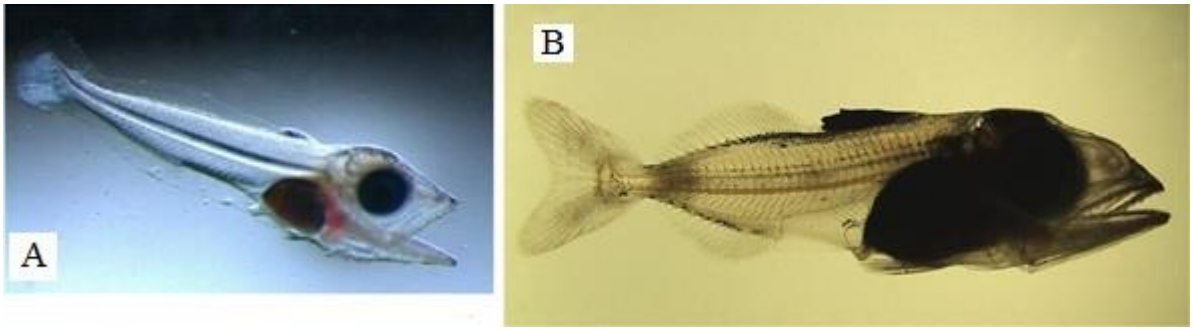

Figure 1 – Atlantic Bluefin Tuna (*Thunnus thynnus*), 15 days post hatch. A a larva reared on rotifers and *Artemia.* B a larva reared on the copepod *Acartia tonsa* and (Evjemo et al., 2014).

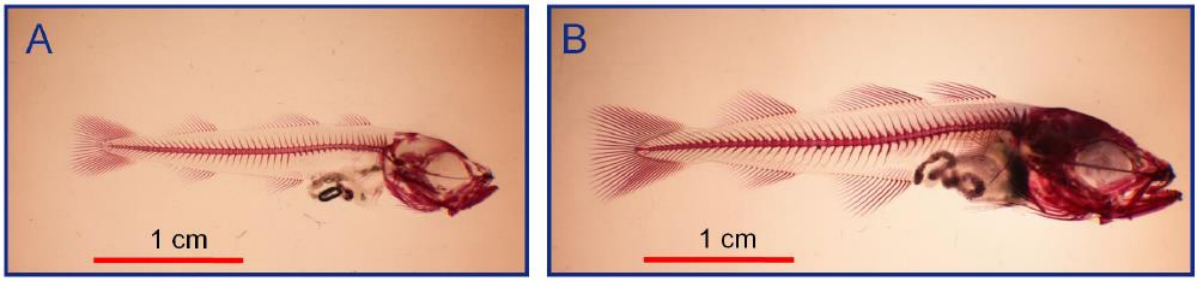

Figure 2 – Atlantic cod (*Gadus morhua*), 60 days post hatch. A a larva reared on rotifers and *Artemia.* B a larva reared on the copepod *Acartia tonsa* (Øie et al., 2015).

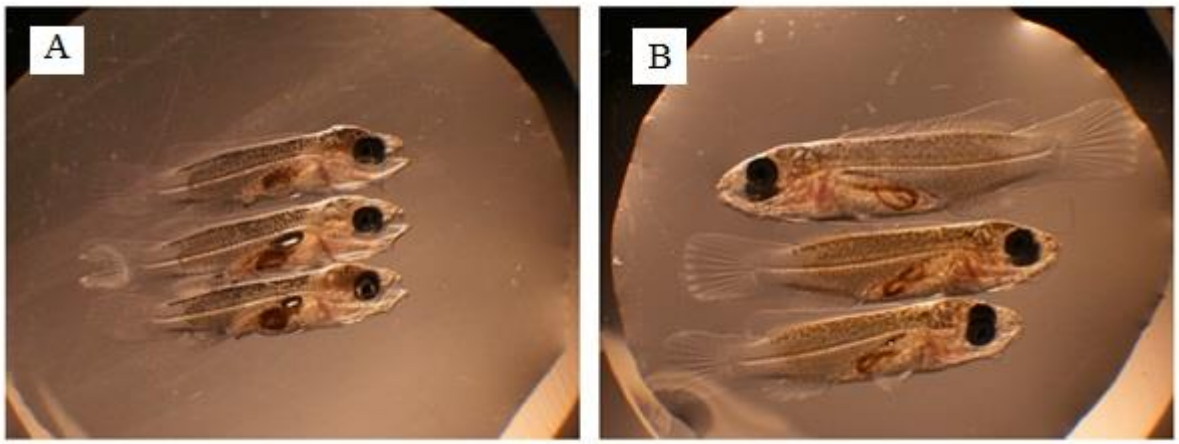

Figure 3 - Ballan wrasse (*Labrus bergylta*), 34 days post hatch. A. larvae reared on rotifers and *Artemia*. B larvae reared on the copepod *Acartia tonsa* (Øie et al., 2015).

1.2 Lipids

Lipids are divided into different classes depending on their molecular structure, including waxesters, sterols, fat soluble vitamins, monoglycerides, diglycerides, triglycerides and phospholipids (Fahy et al., 2009). Figure 4 shows the molecular structure of different lipids; a free fatty acid, triglyceride, and phospholipid.

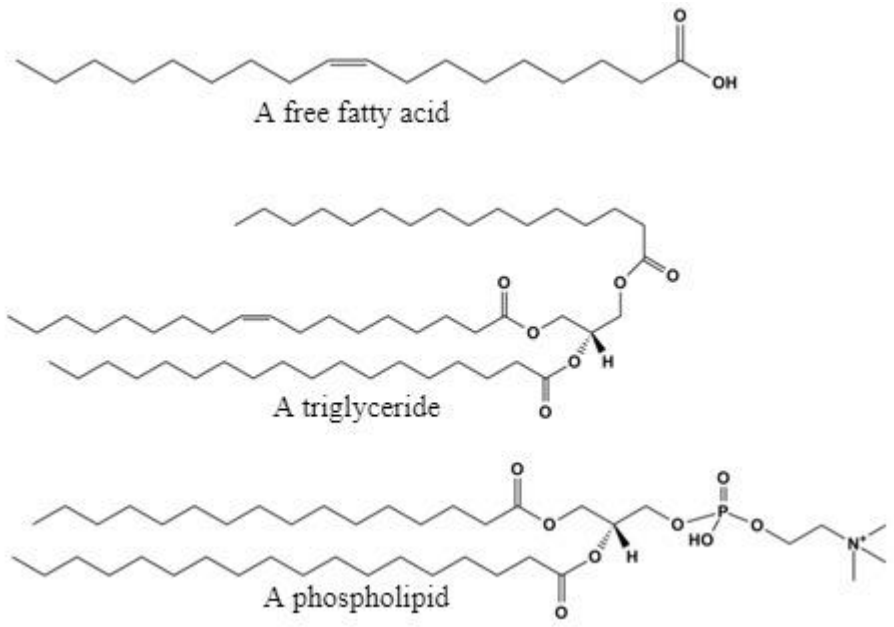

Figure 4 – The structures of common lipids, the lines represent connection between carbon molecules that are saturated by hydrogen molecules. The double lines represent a double bond. Based on information from (Akoh, 2017).

Fatty acids are usually derived from triglycerides or phospholipids. They have multiple roles within the cell where they play a part in signal-transduction pathways, cellular fuel sources, the composition of hormones and lipids, modification of proteins and energy storage in the form of triacylglycerols (Rustan and Drevon, 2005).

A fatty acid is a carboxylic acid with a carbon chain and the naming depends upon the structure and number of carbons. Natural occurring fatty acids normally have a chain of 4 to 28 carbons and in most cases the chain has an even number (McNaught, 1997). Every fatty acid is given numbers in form of X:Yn-Z, where the "X" represents the number of carbons in the chain, "Y" represents the number of double bonds in the carbon chain and "Z" represents the position of the first double bond in the chain from the methyl-end (Brett and Müller-Navarra, 1997).

Fatty acids are broken down into two main groups, saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). The SFA group does not have any double bonds and are metabolized mainly as a source of energy and used as lipid storage (Arts et al., 2001). The UFA group has double bonds and are further divided according to the number of double bonds. Fatty acids with one double bond are called monounsaturated fatty acids (MUFAs) and can be synthesized *de novo*

by almost by all organisms (Arts et al., 2001), and those with more than one double bond are called polyunsaturated fatty acids (PUFAs) (Vance and Vance, 1985). Figure 5 shows the structure of different SFAs and UFAs.

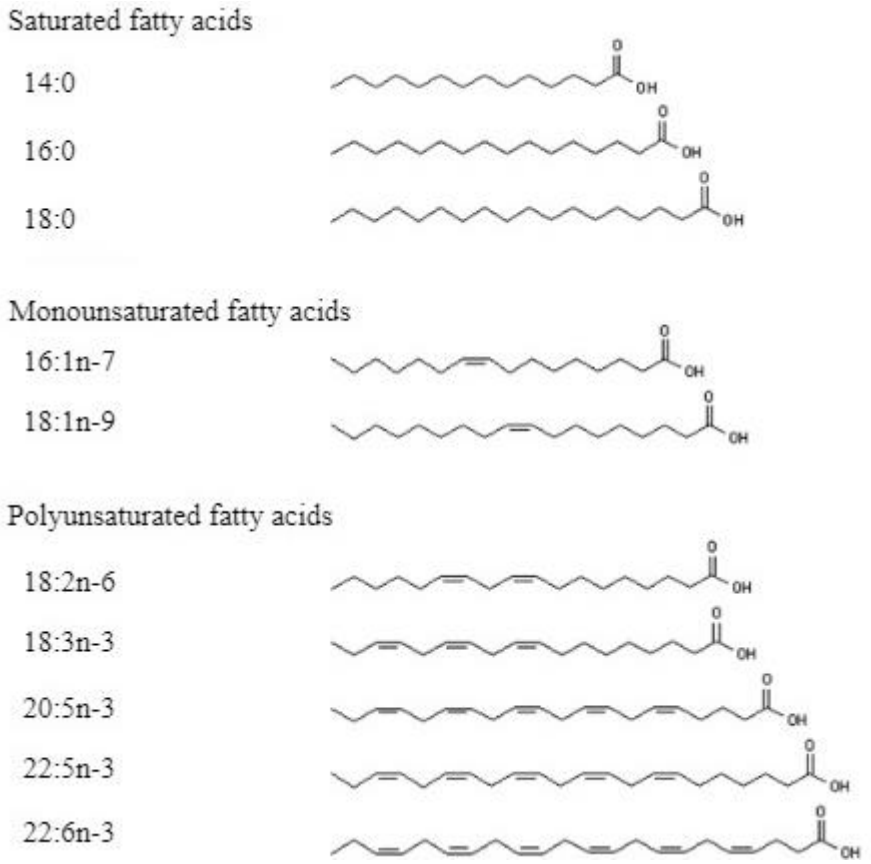

Figure 5 – The structures and numbers of common saturated, monosaturated and polyunsaturated fatty acids. The lines represent connection between carbon molecules that are saturated by hydrogen molecules. The double lines represent a double bond. Based on information from (Akoh, 2017).

Microalgae can synthesise *de novo* n-3 PUFAs and are the main producers of them (Sargent and Whittle, 1981), although some higher trophic organisms can convert linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) with elongation and desaturation to docosahexaenoic acid (DHA; 22:6 n-3), eicosapentaenoic acid (EPA; 20:5 n-3) and arachidonic acid (ARA; 20:4 n-6), however the conversion is usually inefficient (Brett and Müller-Navarra, 1997, Arts et al., 2001).

All vertebrates, such as fish, require three PUFAs in their diet for normal growth and development; DHA, EPA and ARA (Sargent et al., 1993, Sargent et al., 1997). Those fatty acids are commonly named highly unsaturated fatty acids (HUFAs) and play a role in maintaining the structural and functional integrity of cell membranes and a more specific role as a precursors for hormones known collectively as eicosanoids (Sargent et al., 1999).

1.3 Fatty acids and phospholipids in live feed

Copepods contain high levels of n-3 HUFAs, especially in their phospholipids when compared to rotifers and *Artemia* (Evjemo et al., 2003, van der Meeren et al., 2008) and marine fish larvae show better growth and development when fed with high levels of HUFAs (Olsen et al., 2014). However, not only are high levels of HUFAs important but also the ratio of DHA/EPA and EPA/ARA, which have been especially linked together in relation to early larval development (Reitan et al., 1994, Reitan et al., 1997, Bell et al., 2003).

Tocher and Sargent (1984) showed that the HUFA composition in many marine fish species was similar to each other and rich in content of DHA and EPA, and usually in DHA/EPA ratio of ≥ 2. The eggs of the marine fish contain a certain HUFA composition, and by using the same composition in live feed, a significantly higher growth rate has been found compared to when the HUFA composition is poorer than in the eggs. Therefore the eggs indicate the HUFA requirement of the fish larvae (Rodriguez et al., 1997).

Table 1 shows the HUFA composition in both cod and halibut eggs as well as in enriched and unenriched rotifers and *Artemia*. Naturally, rotifers and *Artemia* are very low in HUFAs and are therefore enriched with HUFAs before being used as live feed for fish larvae. Reaching a DHA/EPA ratio >2 can prove difficult, especially for *Artemia* as they are naturally high in lipid content before enrichment, especially of fatty acids with poor nutritional value (18:1n-9, 18:2n-6, 18:3n-3) (Bell et al., 2003). *Artemia* also has a rapid rate of retro conversion of EPA to DHA (Navarro et al., 1999), this makes *Artemia* less suitable as a first feed for marine fish larvae.

The copepod *Acartia tonsa* has high contents of HUFAs compared to enriched rotifers and *Artemia*, as well as a better ratio of DHA/EPA and EPA/ARA, and this makes the copepods far superior as live feed.

Table 1 – HUFA composition (% of total fatty acids) in total lipids of cod eggs and halibut eggs, enriched and unenriched rotifers and *Artemia* nauplii and *Acartia tonsa*. (TOO – Tuna orbital oil, MO – Marinol, a blend of sardine, anchovy and pilchard oils).

Sample	DHA 22:6 n-3	EPA 20:5 n-3	ARA 20:4 n-6	DHA/EPA	EPA/ARA	Source
Cod eggs	29.3%	14.8%	1.7%	2.0	8.7	(Klungsøyr et al., 1989)
Halibut eggs	27.9%	13.8%	1.6%	2.0	8.6	(Bruce, 1999)
Rotifer (unenriched)	0.1%	0.2%	Trace	0.5	-	(Rodriguez et al., 1997)
Rotifer (TOO)	12.7%	4.6%	1.1%	2.8	4.2	(Estevez et al., 1999)
Rotifer (MO)	6.5%	13.1%	0.8%	0.5	16.7	(Estevez et al., 1999)
Artemia (unenriched)	0.0%	5.3%	1.2%	0.0	4.1	(Estevez et al., 1999)
Artemia (TOO)	10.0%	7.6%	1.8%	1.3	4.2	(Estevez et al., 1999)
Artemia (MO)	3.0%	11.6%	1.2%	0.3	9.5	(Estevez et al., 1999)
Acartia tonsa	30.3%	6.8%	0.8%	4.5	9.2	(Støttrup et al., 1999)

Calanoid copepods are known to have high fractions of phospholipids (van der Meeren et al., 2008), 50% more than other live prey species (McEvoy et al., 1998). Dietary phospholipids have shown to be important for fish larvae (Cahu et al., 2009), as they increase their growth and development (Kanazawa, 1993, Geurden et al., 1995), and significantly increase their ingestion rate (Koven et al., 1998). However, need for dietary phospholipids reduces as the larvae grows and the reason for that might be related to the development of the gut and stomach of fish larvae, but it lacks fully functional digestive track and digestive enzymes until metamorphosis (Munilla-Moran and Stark, 1989, Bisbal and Bengtson, 1995). Since copepods contain higher contents of phospholipids, as percentage of total lipids, than in rotifers and *Artemia*, it makes them again more suitable as live feed for fish larvae (McEvoy et al., 1998).

Despite that copepods show higher nutritional values than rotifer and *Artemia*, they are only used by few commercial hatcheries as live feed. It has been a challenge to intensively cultivate copepods and wild harvests of copepods have unpredictable factors such as weather condition, handling and storage (Drillet et al., 2011). Wild caught copepods may also carry parasites, bacteria or viruses which could lead to disease problems and greatly affect the production of fish larvae (Marcogliese, 1995). However, at least 60 species of copepods have been raised to some extent (Blaxter et al., 1998) but one of them, *Acartia tonsa*, has shown highest cultivation potential, and is mass cultivated by the company Cfeed AS.

Figure 6 – *Acartia tonsa* in copepodid stages (Cfeed, 2018)

1.4 *Acartia tonsa*

Acartia tonsa is a pelagic calanoid copepod and is found worldwide. It is euryhaline and eurythermal, meaning it tolerates a wide range of salinity, from 1 to 38 ppt, and temperatures from 0°C to 30°C, making it to a very hardy species (Mauchline, 1998). *A. tonsa* is most commonly found near shore on depths from 0-20 m, although they have been reported down to

600 m depth. Because of its distribution, many fish species encounter and feed on *A. tonsa*. This makes the species to an ideal prey to cultivate as a live feed for marine fish larvae (Marchus and Wilcox, 2007).

A. tonsa, and copepods in general, have a complex life cycle made up from two phases, nauplii and copepodid. They have six nauplii stages (NI - NVI) and go through metamorphosis after the last nauplii stage. After metamorphosis they develop into copepodid and go through six copepodid stages (CI - CVI) where the last stage is adult (Seebens et al., 2009). Figure 7 shows the developmental stages from eggs to adult. The stages are an advantage when copepods are used as live feed as the appropriate size or stage can be chosen to fit the size of fish larvae.

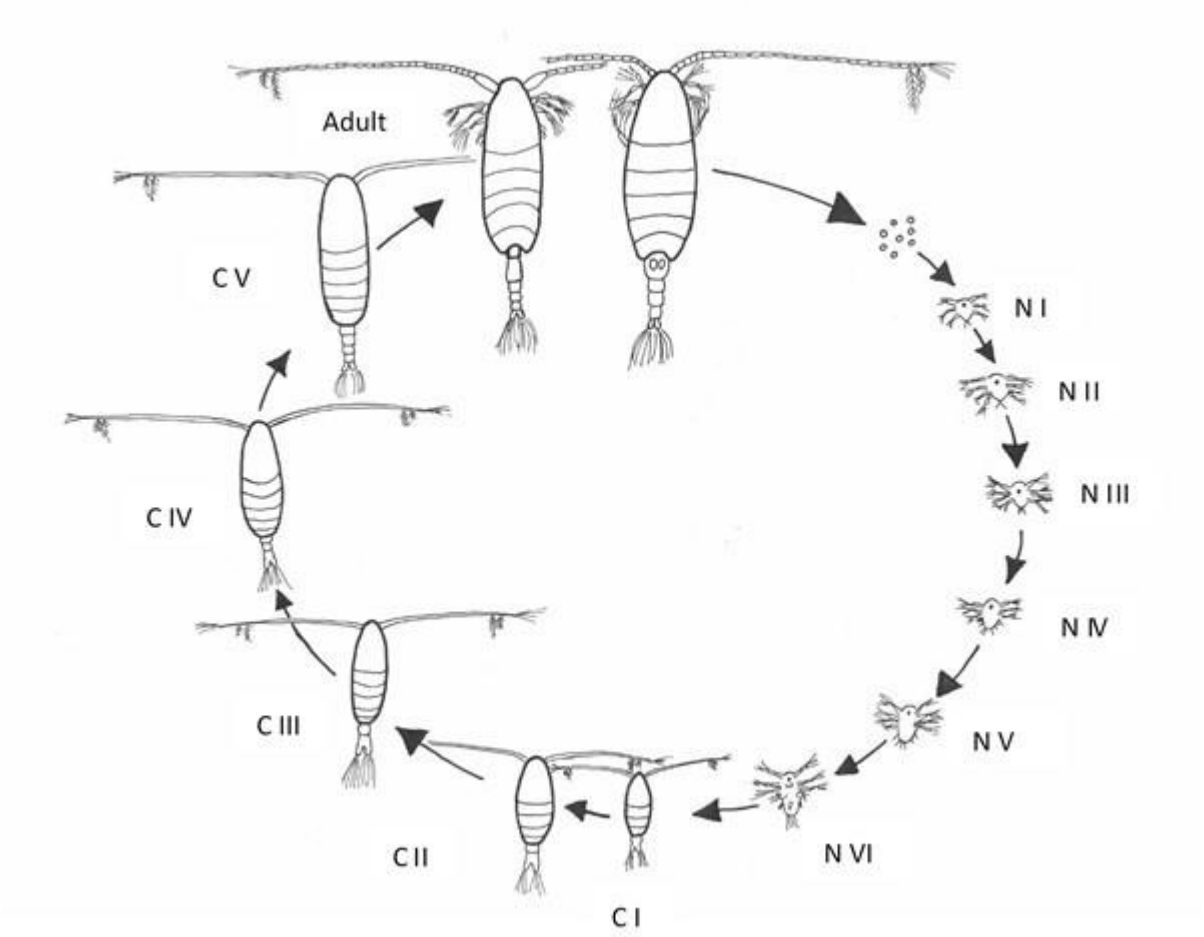

Figure 7 – The developmental stages of copepods from nauplii stages (NI -NVI), copepodid stages (CI - CVI) and to adult (Soki, 2015).

A. tonsa mainly feeds on microalgae but also on ciliates, rotifers, on their own eggs and nauplii (Mauchline, 1998). *A. tonsa* is usually cultivated on microalgae as they are simple to mass cultivate and usually high in lipids and PUFAs. A correlation has been made between some fatty acids in microalgae and the copepod feeding on them (Anderson and Pond, 2000, Brett et al., 2009) which indicates that the fatty acid profile of the copepod can be manipulated through diet. This can be important for delivering the required fatty acids in the right ratio to the fish

larvae, which may differ between species. It is therefore important to choose the right microalgae to feed to the copepods.

1.5 Microalgae

Microalgae are primary producers and they play a crucial nutritional role for marine copepods, and most marine invertebrates depend on them as a food source their whole life cycle. It is therefore no wonder that the use of microalgae in aquaculture has been around for a long time and over the last decades several hundred microalgae species have been tested as feed, but only around 20 species gained widespread use as feed (Guedes and Malcata, 2012).

The nutritional value of microalgae significantly varies between species but also depends upon the culture conditions (Brown et al., 1997, Enright et al., 1986). Marine microalgae are a major producer of PUFAs (Rasdi and Qin, 2016) and most microalgae produce high percentage of EPA (7-34%). The microalgae classes such as prymnesiophytes (e.g. *Tisochrysis lutea*) and cryptomonads (e.g. *Rhodomonas baltica*) are relatively high in DHA (0.2-11%) and eustigmatophytes (e.g. *Nannochloropsis*) and diatoms (e.g. *Conticribra weissflogii*) have the highest percentage of ARA (up to 4%) (Guedes and Malcata, 2012).

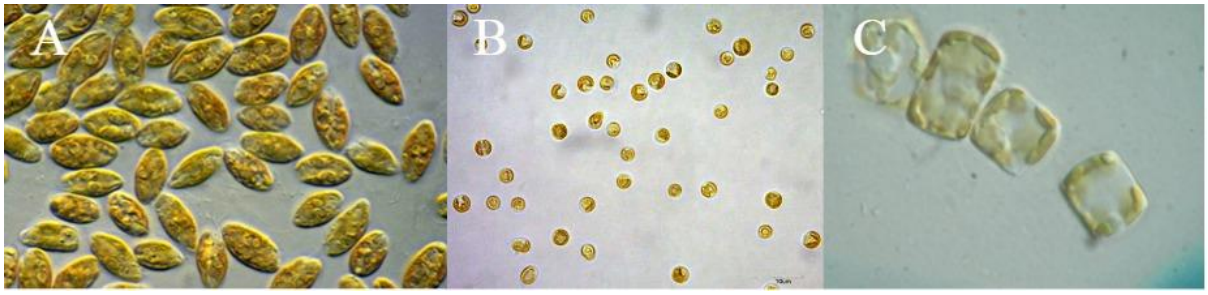

Figure 8 - Microscopic pictures of the microalgae *Rhodomonas baltica* (A), *Tisochrysis lutea* (B) and *Conticribra weissflogii* (C). (Lipout, 2015, PhycoKey, 2017, Nordic-Microalgae, 2018).

Støttrup et al. (1986), which continuously cultivated *Acartia tonsa*, used the cryptophyte *Rhodomonas baltica* with good success. Many other researchers have also used *R. baltica* as a feed for copepods as it's known to give good growth (Berggreen et al., 1988, Schipp et al., 1999, Bellas and Thor, 2007, Teixeira et al., 2010).

1.6 Cultivation of copepods

A successful intensive production of copepods needs a cost-efficient cultivation method. It is generally considered to be difficult, labour intensive and expensive to have a large scale production of copepods (Støttrup, 2000), however Cfeed AS mass produces the eggs of the copepod *Acartia tonsa*. This implies that cultivation methods and techniques have improved

over the years. However, it is important to develop new methods and strive to improve them to increase production, cost efficiency but also increase the sustainability.

In aquaculture the concept of integrated multitrophic aquaculture (IMTA) has gained more attention throughout the years as it may be a way to bring more sustainability to the industry. IMTA is the idea of cultivating species belonging to different trophic levels together and using one species waste as another one's food source. Figure 9 shows the concept with the copepod *A. tonsa* and the microalgae *R. baltica*. The waste from *A. tonsa* is partly taken up by *R. baltica* and *R. baltica* is then used as a feed for *A. tonsa*, creating a circulation of nutrients.

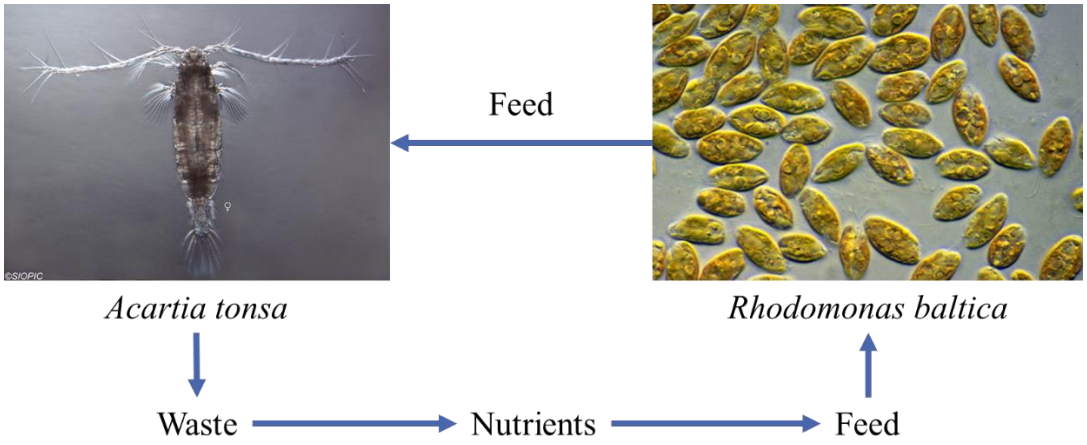

Figure 9 – Integrated multitrophic aquaculture (IMTA) with the copepod *A. tonsa* and the microalgae *R. baltica*. The waste from *A. tonsa* is used as a feed for *R. baltica* and *R. baltica* is then used as a feed for *A. tonsa*. Modified from (PhycoKey, 2017, SCRIPPS).

The goal of IMTA is to increase production and improve utilization of the feed, and by doing so increase environmental sustainability and social acceptance of aquaculture (Barrington et al., 2009). To introduce IMTA to the production of copepods would be a step forward in cultivation methods. However, the intensive production of copepods is done on land in flow-through systems where the waste water is lead to the ocean. To introduce the IMTA concept in copepod production, a recirculation aquaculture system (RAS) need to be used, and when the two systems are used together, the concept is given the name "Copeponics" and will henceforth be referred as that.

RAS are closed systems where the water is reused, and such systems use 90 to 99% less water than the conventional flow-through systems. By constantly reusing the water and only adding small volume of new water into the system it becomes easier to keep an optimal temperature and the heating requirements, and consequently the energy requirements, is lowered. However, by constantly reusing the water, the water quality steadily declines as the organism within it discreates metabolites and therefore the water needs to be treated to keep adequate water

quality. The general rule is, the lower the water exchange is, more water treatments are required (Ebeling and Timmons, 2012).

As the organism grows within the system, it will consume oxygen, give off carbon dioxide, nitrogen waste and faeces. The water is treated to counter those affects and bring balance to the water quality. The most common treatments done are oxygenation, aeration for carbon dioxide removal, particle removal, transformation of ammonia to nitrate through nitrification using a biofilter (Ebeling and Timmons, 2012).

1.7 Aims of study

The aim of this study was to characterize the nutritional value of *A. tonsa* by analysing the fatty acids after feeding with 3 selected monoalgal diets. The cultivation of the copepods with the use of those 3 microalgae species was then optimized by implementing the use of a so-called copeponics system. This study was designed with an aquaculture approach and aimed to provide the fish famers with information about how to optimize the nutritional quality of *A. tonsa* and describe cultivation of *A. tonsa* in a copeponics system to save water and nutrients.

The objectives were divided into:

- To optimize the nutritional value of the copepod *A. tonsa* by finding out how the fatty acids profiles of *R. baltica*, *T. lutea* and *C. weissflogii* are converted into *A. tonsa*.
- To optimize the cultivation method of the copepod *A. tonsa* with the use of the same microalgae species by using a copeponics and reducing the nutrients and water needed for the cultivation.

The hypothesis were divided into:

- "The fatty acid composition of *A. tonsa* can be manipulated through the diet."
- "Less nutrients and water have to be added when cultivating *A. tonsa* in a copeponics system compared to cultivation in a traditional flow through system."

2. Materials and methods

Two separate studies were performed using two different systems. A nutritional study of *A. tonsa* reared on three different monoalgal diets in flow through system (FTS) to characterize the nutritional value, and a test study of *A. tonsa* in a copeponics system with the same three different microalgae.

In this chapter the system schematics, system materials, the rearing of *A. tonsa* and cultivation of microalgae methods will be described.

2.1 Nutritional value of *A. tonsa*

Growth, survival, fatty acid profiles and carbon analysis were studied in a nutritional study of *A. tonsa* reared on three different microalgae diets, *R. baltica*, *T. lutea* and *C. weissflogii*.

2.1.1 System schematics and equipment

The system used in this study was an FTS where three parallels were run for each microalgae diet at the same time. Figure 10 below shows the schematic setup and data on volume and flow of all compartments in each system.

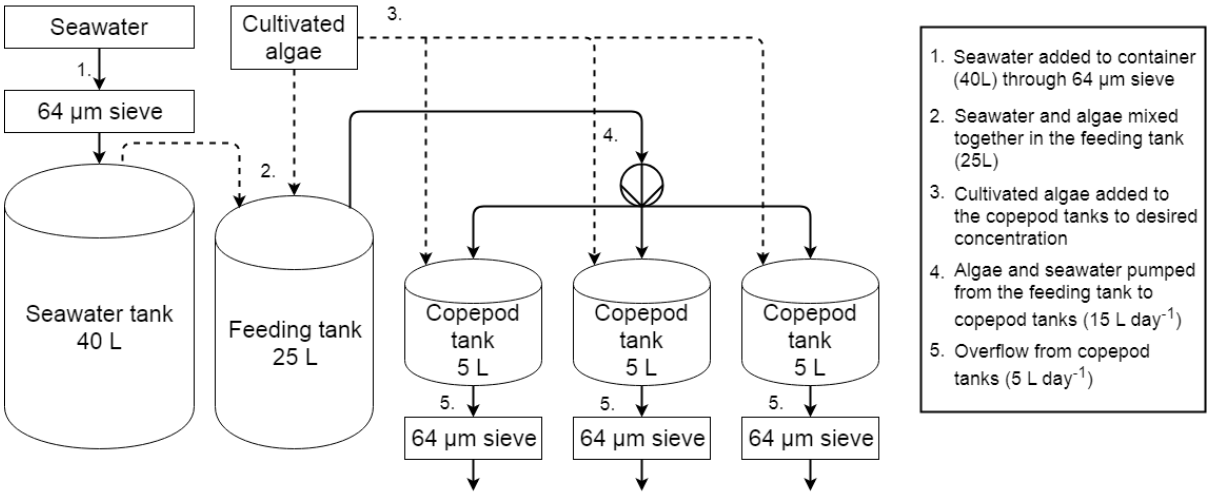

Figure 10 - Schematic drawing and flow scheme of the FTS.

Seawater was strained through a 64 µm sieve and collected into 40 L cylindrical tank with a flat bottom, the so-called seawater tank. The seawater had a temperature of 10°C and was heated to room temperature with heavy aeration. After 24 hours, roughly ~23 - 24.5 L of seawater was transferred to a 25 L cylindrical feeding tank with a flat bottom and ~0.5 – 1.5 L of cultivated algae were added (microalgae cultivation is described further in chapter 0). This

process was repeated every day of the study and the feeding tanks were emptied and cleaned every day to prevent growth of unwanted organisms.

The copepod tanks were 5 L buckets and had an overflow covered by a 64 µm sieve (further explained in Figure 13) which were rinsed regularly to avoid clogging. A peristaltic pump (205U, Watson Marlow, UK) pumped 5 L day^{-1} of algae solution to each copepod tank, supplying the copepods with constant feeding and a 100% water exchange per day. In total, 15 L of the algae solution were used of the total 25 L mixed, leaving 10 L unused. This was done in order to prevent the algae from settling on the bottom.

The copepod tanks, 3 replicates for each microalgae, were randomly distributed and kept in a temperature-controlled water baths at 20°C (Figure 11). The heaters in the water baths were aquarium heaters (Eco Therm 50W, Newa, Italy) controlled by a proportional-integral-derivative (PID) controllers (SYL-2372, Auber, USA) and temperature sensors (Platinum RTD, Auber, USA). Small pumps in the water baths circulated the water and prevented any forming of heat gradient in the water baths. By using this method, the temperature was kept stable throughout the study.

The overflow from the copepod tanks flowed into the water baths which had a drain to take the excess water. An air pump (Hiblow HP-40, Techno Takatsuki co ltd, Japan) was used to add aeration to the copepod tanks and a 24-hour light regime was used during the study.

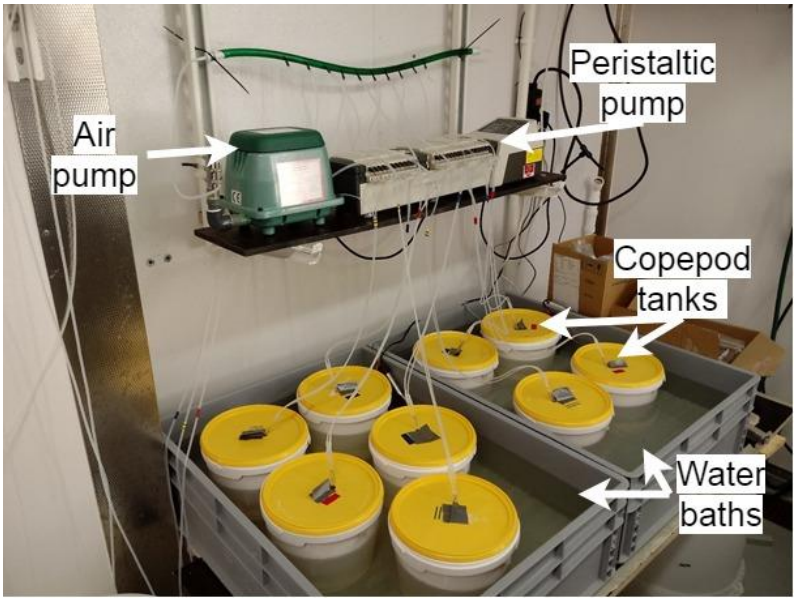

Figure 11 - The arrangement of the water baths, copepod tanks, peristaltic pump and air pump.

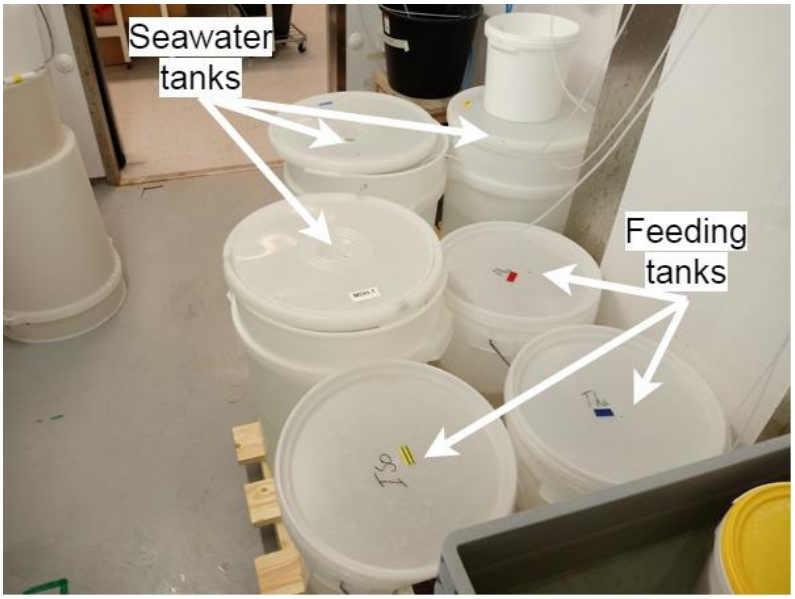

Figure 12 – The arrangement of feeding tanks and seawater tanks.

2.1.2 System materials

Table 2 shows the containers used in the system as well as type of plastic material and name of producer.

Table 2 – Containers used, type of plastic material and name of producer (PP – polypropylene).

Name	Type of plastic	Source
Copepod tank	PP	Emsafe 5,9 ltr, Emballator Lagan plast, Sweden
Water baths	PP	Euro container ED 86/22 2S, Auer packaging, UK
Feeding tanks	PP	25L container, RD plastics, Holland
Seawater tanks	PP	40L container, Bewi Norplasta, Norway

Silicone tubes (Silicone tubing, VWR, USA) in three different diameters 3x5, 2x4 and 1x3 (Inner diameter (mm) x outer diameter (mm)) were used for the study. The 3x5 tubes were used in 10 cm pieces for the peristaltic pump. The 2x4 tubes were led from each feeding tank to the peristaltic pump. The 1x3 tubes were used from the peristaltic pump and the air pump to the copepod tanks.

Two 1 ml pipettes (Serological pipette, Sigma, USA) were attached on the end of the silicone tubing which led to the copepod tanks, one from the peristaltic pump and the other from the air pump, to ensure the tubes would stay secure in the tank. They can be seen on Figure 13 (D) along with how the sieves used in the copepod tanks were constructed.

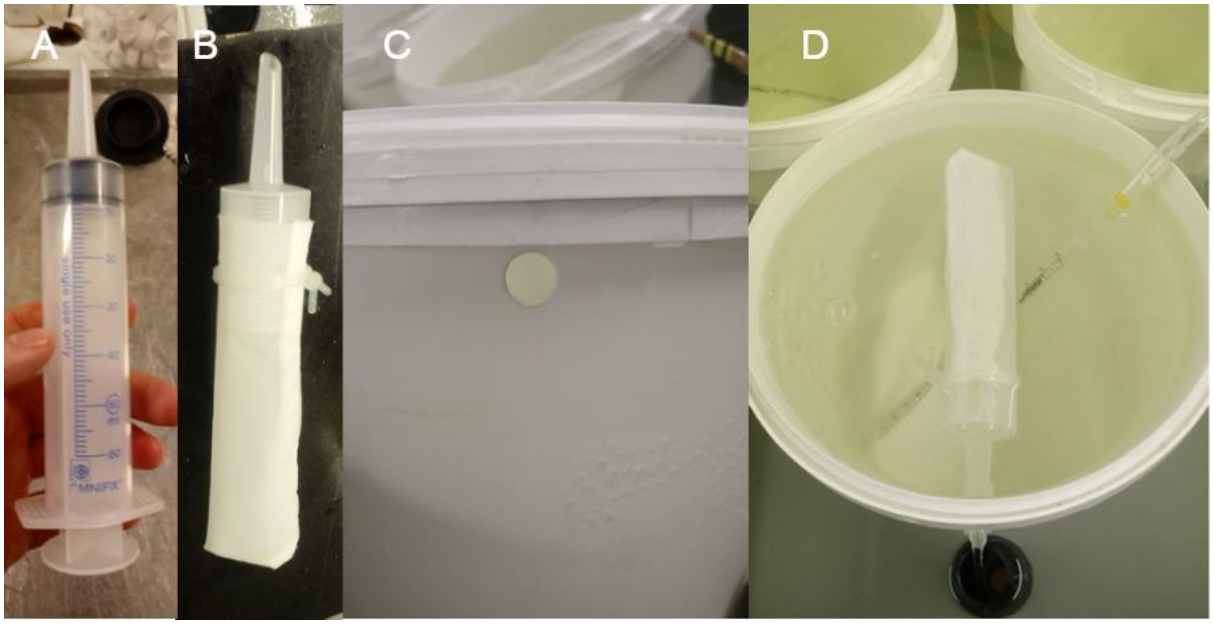

Figure 13 – The sieve in the overflow of the copepod tanks and how they were constructed. A. 50 ml syringe, B. The lower part of a syringe was cut off and the sieve attached to it. C. The hole in the copepod tank where the end of the syringe and a small piece of silicone tubing was fitted. D. The sieve attached to the copepod tank.

2.1.3 Rearing of *A. tonsa*

At day zero, 15 ml of eggs supplied from Cfeed AS were incubated in 15 L of seawater in cylindrical tank with a flat bottom (25 L container, RD plastics, Holland) with heavy aeration to prevent the eggs from settling on the bottom. The seawater was temperature controlled at 20°C with an aquarium heater and a temperature sensor (Figure 14) (the same as used in the water baths described in chapter 2.1.1 System schematics and equipment). After 36 hours the nauplii were moved to the copepod tanks at densities of ~50 individuals ml^{-1}.

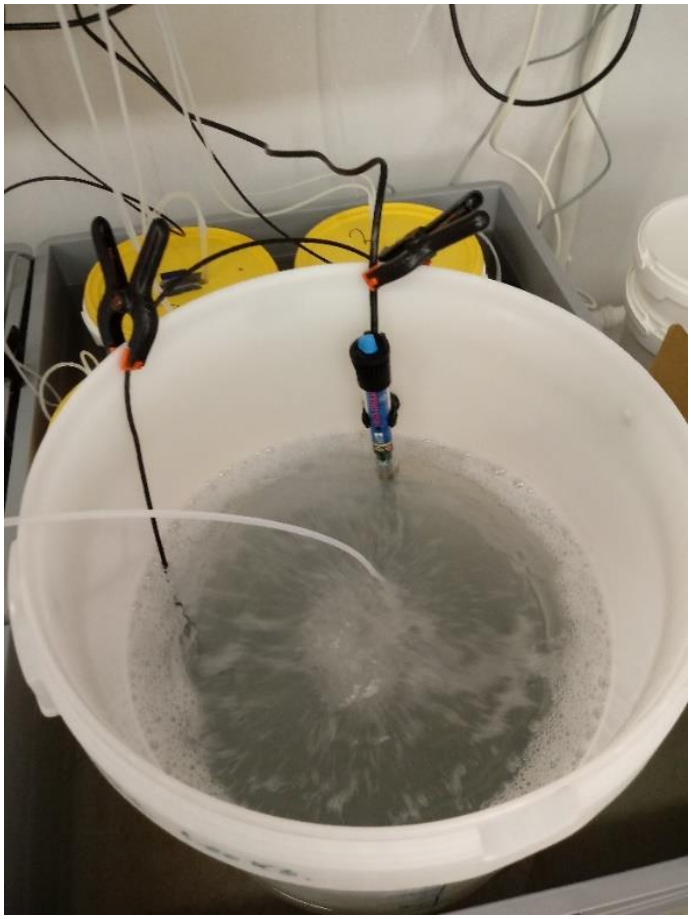

Figure 14 - Hatching tank for *A. tonsa* in a 25 L cylindrical tank with a flat bottom. The temperature was controlled at 20°C and heavy aeration was added to prevent the eggs from settling.

A. tonsa was reared on three different microalgae diets, *R. baltica, T. lutea* and *C. weissflogii*. The cultivation method of the microalgae and stock cultures is described in chapter 0. As three different microalgae species were used as feed for the study, they algae cells were equalized. A fixed volume $\mu m^3\ ml^{-1}$ of algae cells was used instead of using a fixed number of algae cells ml^{-1}. This was done because the cell volume can change from day to day, and therefore using a fixed content of cells ml^{-1} would result in feeding different volume each day. The volume of feed used was aimed to overfeed the copepods and was adjusted the first days of the study as needed. Table 3 shows the volume $\mu m^3\ ml^{-1}$ used for each day of the study as well as an estimate of the cell density for each species which the volume would correspond to.

Table 3 - Feeding regime during the study showed in volume µm³ ml⁻¹ and corresponding cell density (cells ml^{-1}) for each species.

		Density (cells ml⁻¹)		
Days	**Volume (µm³ ml⁻¹)**	***R. baltica***	***T. lutea***	***C. weissflogii***
2 - 3	10,000	50,000	200,000	10,000
4	20,000	100,000	400,000	20,000
5 - 14	40,000	200,000	800,000	40,000

As mentioned in chapter 2.1.1 System schematics and equipment, the feeding tanks were filled with ~23 - 24.5 L of seawater and ~0.5 - 1.5 L of cultivated algae, depending on the desired concentration. Every day of the study, samples were taken from the algae cultures and the cell density and volume of the algae was estimated using a Coulter counter (Multisizer 3 Coulter counter, Beckman Coulter, USA). The purpose of this was to estimate how much algae needed to be added to the feeding tanks. This was then repeated for the copepod tanks. Each copepod tank was supplied with 5 L day⁻¹ of algae solution from the feeding tanks at the desired volume shown in Table 3. To ensure enough feed in each copepod tank, cultivated algae was added manually every day to each the copepod tanks to reach the desired volume.

2.2 Copeponics

Nitrogen dynamics and growth of *A. tonsa* were studied in a copeponics system. The studies were performed with the microalgae species *R. baltica, T. lutea* and *C. weissflogii*.

2.2.1 System schematics and equipment

Three parallel systems were run at the same time. Figure 15 presents a schematic setup and data on volume and flow of all compartments in each system. During the study the system was optimized and the description here is of the resulting system. The systems had two circulations within them, one between the biofilter and the copepod tank and another between the copepod tank and the algae tank.

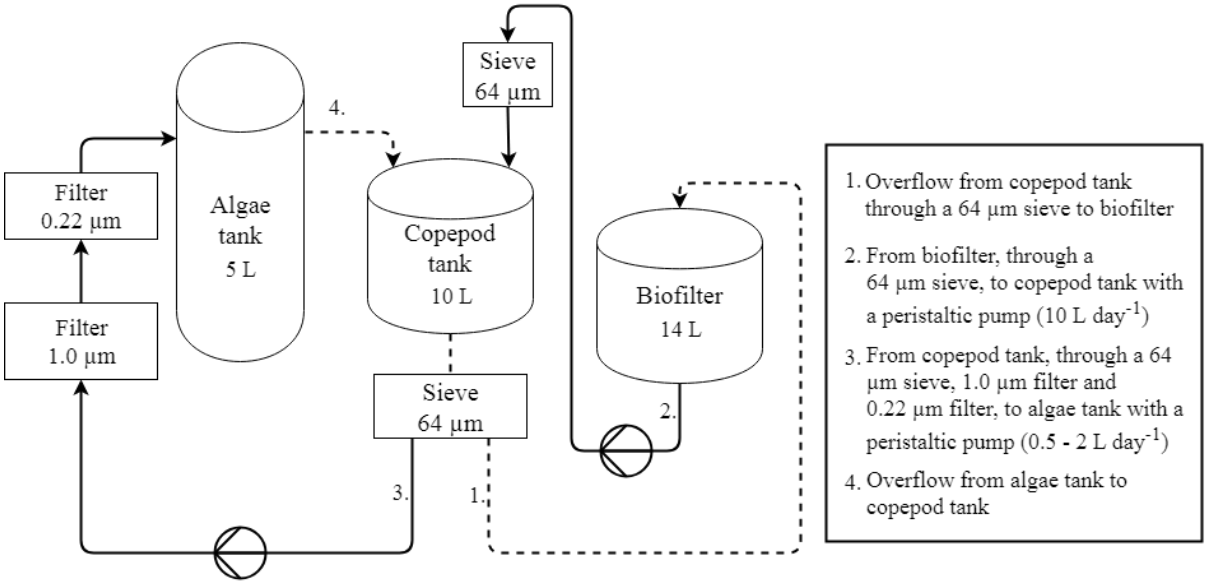

Figure 15 - Schematic drawing and flow scheme of the recirculation system with data on volume and flow of all tanks; copepod tank, biofilter and algae tank. The dotted line shows overflows and the solid line flows with pumps.

The outflow of the copepod tanks was covered with a 64 µm sieve which kept the copepods within in the tank. The copepod tanks had an overflow that lead to the biofilters for nitrification. The water was pumped from the biofilter, through a 64 µm sieve and back to the copepod tank with a peristaltic pump (520U, Watson Marlow, UK), 10 L day^{-1} for 100% water exchange in the copepod tank.

The other circulation was between the copepod tanks and the algae tanks. Water was pumped from the copepod tanks to the algae tanks with a peristaltic pump (520S, Watson Marlow, UK) but before going to the algae tank the water went through 1.0 µm capsule filter (Polycap HD 75, Whatman, USA) and 0.22 µm filter unit (Sterivex GP, Millipore, USA). This was done to prevent any debris and microorganisms from entering the algae tanks. The algae tanks had overflows that led back to the copepod tanks, keeping the water level stable in the algae tanks. The pumping rate to the algae tanks controlled the feeding rate for the copepods, as more water added to the algae tank would lead to more algae going through the overflow to the copepod tanks. The pumping rate was increased from 0.5 to 2.5 L day^{-1} during the 14-day period or until sufficient algae density was measured in the copepod tanks (> 60,000 cells ml^{-1}).

All tanks were aerated, the copepod tanks had gentle aeration with small air stones. The biofilter and the algae tanks got heavier aeration but CO_2 (2000 ppm) enriched air was added only to the algae tanks.

2.2.2 System materials

The copepod and the biofilter tanks were made of black 16 L polyethylene (PE) buckets (Murbøtte 362771, Biltema, Norway). The biofilter tanks had a flat bottom and the copepod

tanks had a cone shaped bottom which was made by heating the bottom of the bucket and moulding it into a cone shape.

All tanks were supported by aluminium frames and the frames for the copepod tanks and the biofilters were designed to keep them supported from all sides, as seen on Figure 16. There was a 9 cm height difference between the frames of the copepod tanks and the biofilter, elevating the copepod tank higher than the biofilters.

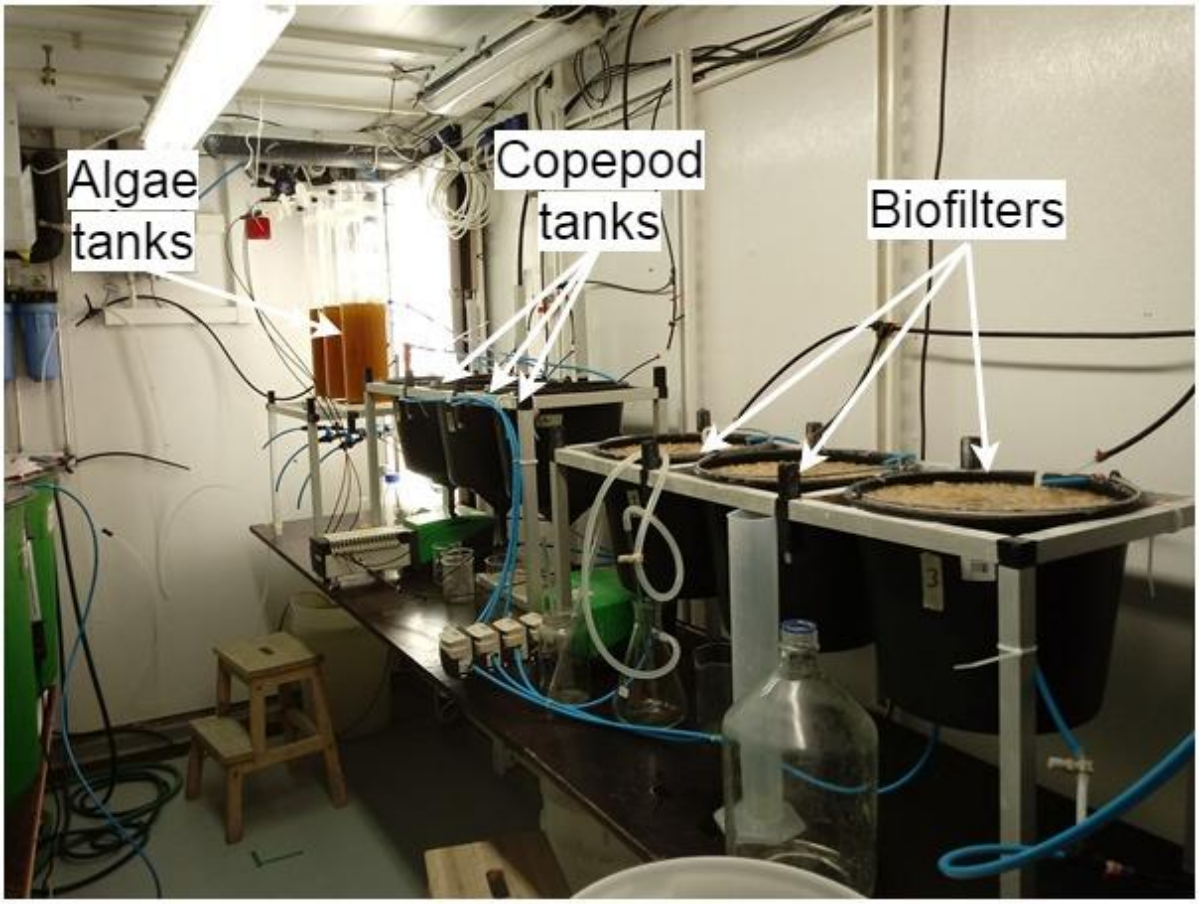

Figure 16 - Arrangement of the algae tanks, copepod tanks and biofilters.

All tanks were connected with 8 mm polyurethane tubing with the exception of the tube leading from the copepod tank to the algae tank, where a 4 mm PE tubing was used to minimize the retention time in the tube.

Each copepod tank was equipped with a sieve which had a 64 µm mesh size. Figure 17 shows the actual sieve on side A, and a schematic of its placement on side B. The sieve was made from an acrylic tube (12,5 cm diameter) and a nylon sieve which was glued on with an acrylic glue (Acrifix 1s 0116, Evonik, Germany). The sieve was attached to a PP plastic pipe which was screwed to the bottom of the tank with a fitting.

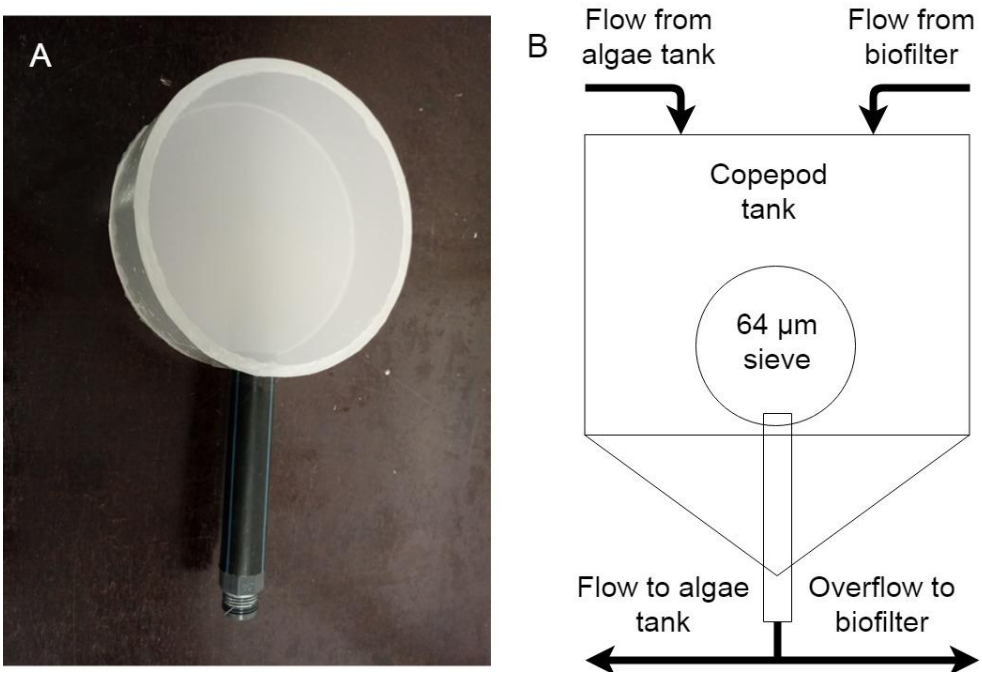

Figure 17 – A. The sieve in the copepod tank B. Side view of the copepod tank showing the placement of the sieve.

The biofilters had an upwelling water circulation. Figure 18 shows a picture of the biofilter on side A and biofilter schematics and water circulation on side B. In the middle of the biofilter was a 14 cm pipe made of PE plastic with three 8 cm legs, creating an opening on the bottom. Aeration was added in the middle of the pipe, and the air bubbles pushed the water up the pipe, forming an upwelling. On the side of the pipe was a rough sieve, with a 1 cm mesh size, made from PE which covered the outlet of the biofilter and kept bigger particles from going into the outflow. An 8 mm Adaptor (Straight adaptor, John Guest, UK) was screwed in the bottom of the tank and connected with a tube which led to the copepod tank. Each biofilter had 7 litres of bio media (Bimedia, Krüger Kaldnes, Norway).

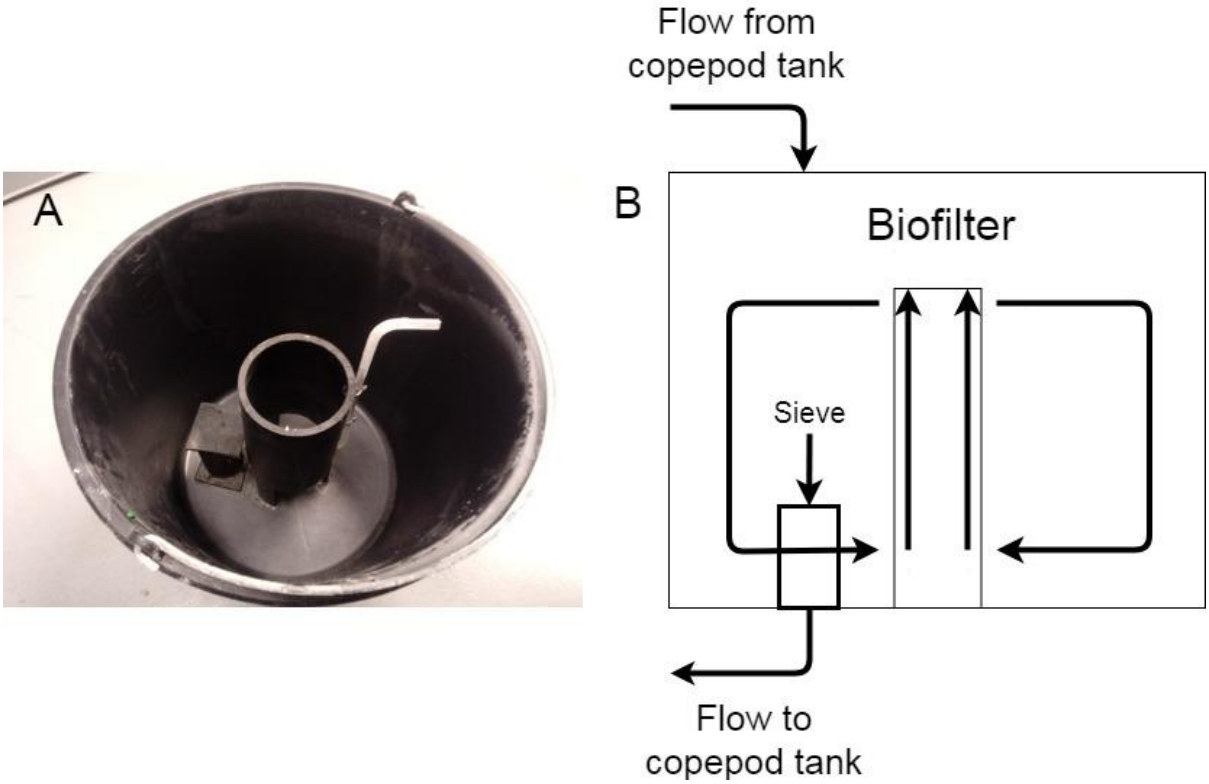

Figure 18 – A. Shows the biofilter B. Shows the water circulation schematics and placement of the sieve.

The algae tanks were made of an acrylic plastic tube (150 x 630 mm) and an acrylic plate which was glued on the bottom of the tube (Acrifix 1s 0116, Evonik, Germany). Figure 19 shows the algae tank and the fittings attached to it. An 8 mm straight adaptor (Straight adaptor, John Guest, UK) was fitted into the middle of the tank as an overflow and a valve (Shut off valve, John Guest, UK) was attached to the bottom of the tank.

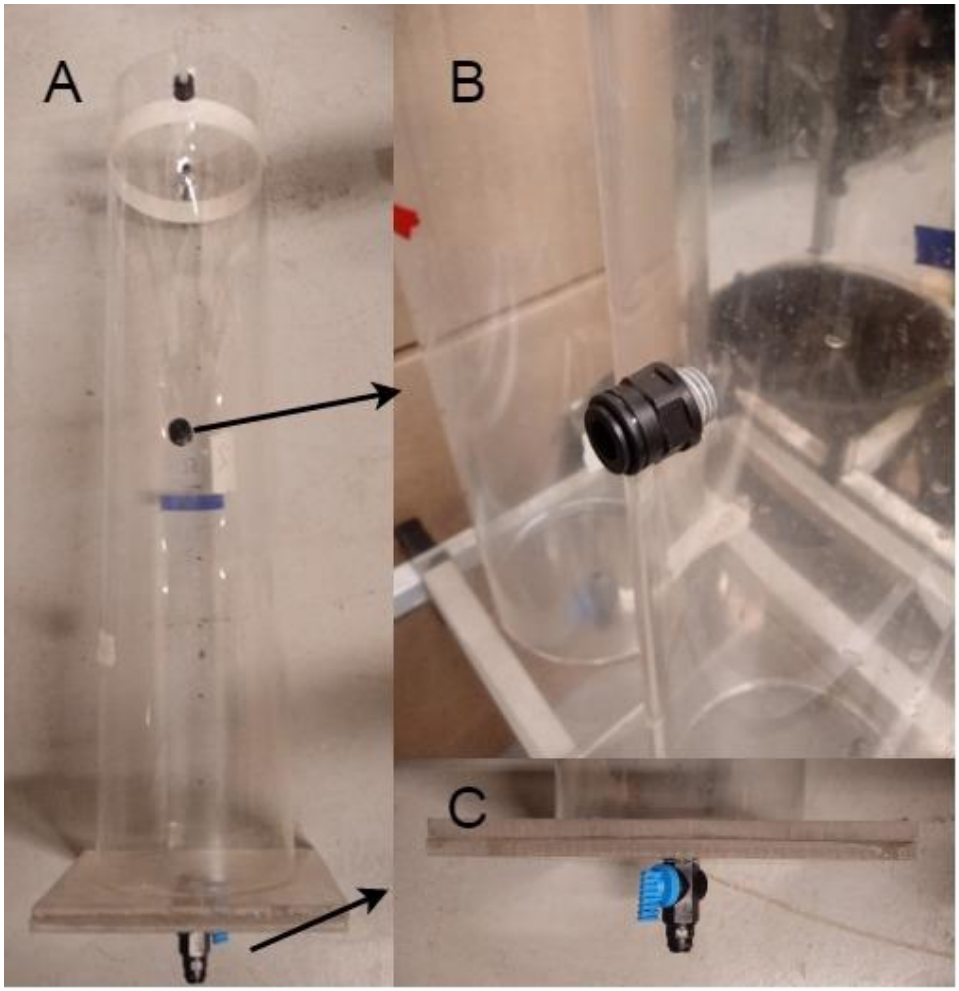

Figure 19 – A shows the whole algae tank, B shows the inserted fitting which acted as the overflow from the tank and C shows the bottom of the tank and the valve inserted into the bottom.

Figure 20 shows the schematics of the algae tanks. Three valves were used for each tank, one for the inlet of the air and CO_2, another for the bottom of the tank and a third for the outlet for samples. To connect all three valves an 8 mm polyurethane tubing was used and one tee connector (Equal tee, John Guest, UK). The algae cultures had continuous light from three fluorescent tubes (Cool white 36W/840, Osram, Germany) stacked vertically behind them.

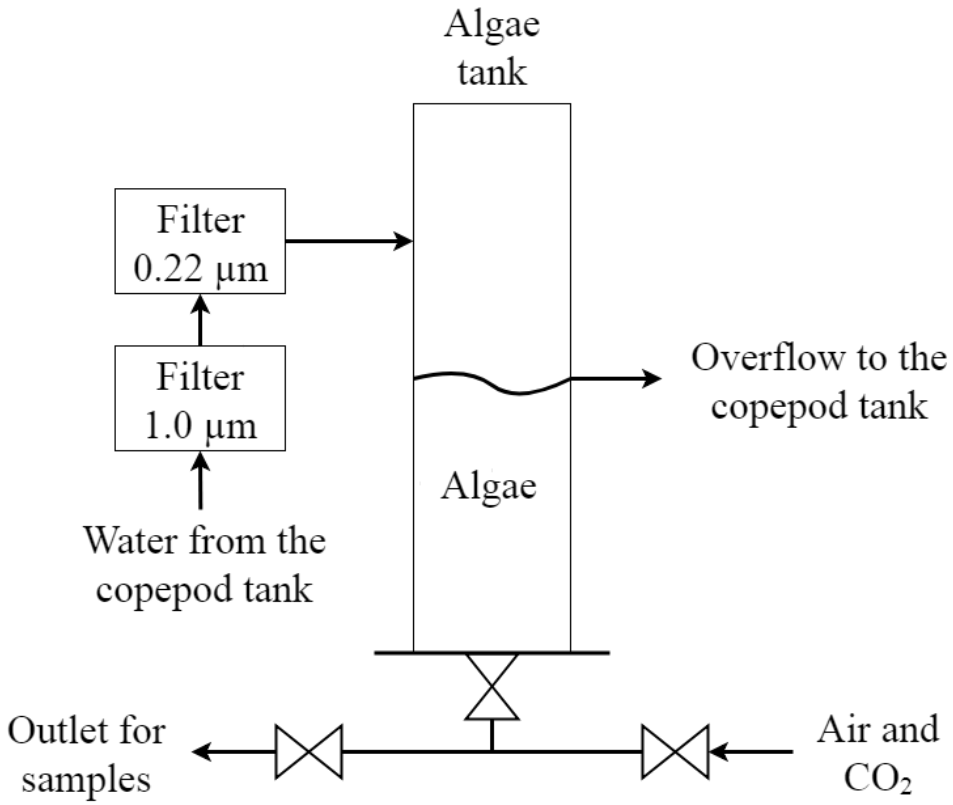

Figure 20 – The algae tank and the placement of three valves between the algae tank, air and CO_2 and the outlet.

2.2.3 Rearing of *A. tonsa* and microalgae

On day zero, copepod eggs (5 ml) were incubated in 15 L of seawater in cylindrical tank with a flat bottom (25 L container, RD plastics, Holland) at temperature of 18°C with heavy aeration to prevent the eggs from settling on the bottom. On day 2, after 48 hours, the hatched nauplii were divided into the copepod tanks at densities of ~50 individuals ml^{-1}.

The seawater used in the copepod tanks and the biofilters was collected from Trondheimsfjord, 800 m from the shore and at 70 m depth. The water was passed through a sandfilter to take out >70 µm particles and kept in a 4.5 m^3 biofilter for over 24 h, for microbial maturing the water. The temperature in the copepod tanks was kept at 18-19°C and salinity ~30 ppt.

The copepods were continously fed microalgae and it was aimed to have the algae density in copepod tanks above or close to saturation levels, giving them sufficient feeding.

To improve water quality, the copepod tanks and the biofilters were cleaned daily by siphoning the bottom of the tanks. As copepods were siphoned with the water from the copepod tanks, the siphoned water was put in a beaker, letting the debris to settle down for approximately 1 hour. Most of the water and the copepods were carefully put back into the copepod tanks and the debris on the bottom was thrown away. Deionized water was added into the system to keep

salinity stable as evaporation occurred over time. Approximately 1 L day^{-1} was added to each biofilter and 100 ml day^{-1} to each algae tank.

From stock cultures of the microalgae, new microalgae cultures for the copeponics systems were prepared. More information about the stock cultures is found in chapter 0. The algae tanks were disinfected by chlorination before algae was added to them. The tanks had a total volume of 10 L and were filled with tap water, 4 ml of hypochlorite (10-13%) was added into each tank and left over 8 hours. The tanks were emptied and rinsed with tap water to clean out the remaining chlorine. All tubes and fittings connected to algae tanks were collected into a 25 L bucket (25 L container, RD plastics, Holland) which was filled with tap water and 9 ml of hypochlorite (10-13%) was added and left over 8 hours. The fittings and tubes were rinsed with tap water.

Conway medium was added to the algae tanks (1 ml L^{-1}) as a nutrition source when necessary. As the system matured, nitrate (NO_3) started to build up and the addition of Conway medium was decreased.

2.3 Cultures of microalgae

The algae cultures were mainly overseen by Dag Altin at BioTrix AS. The microalgae were cultivated in 10 L round flat bottom flasks (Flat bottom flask 10 L, Schott Duran, Germany) (Figure 21). The seawater used to cultivate the microalgae was collected from Trondheimsfjord, 800 m from the shore and at 70 m depth. The water passed through a sandfilter to take out >70 µm particles and a cartridge filter (Micro-Klean, Cuno, USA) to take > 5 µm particles. 90 L of the seawater was collected to a 100 L container and 10 L of deionized water added to lower the salinity to approximately 30 ppt. The seawater was chlorinated with 37.5 ml of hypochlorite (10-13%) for over 8 hours and dechlorinated with 4.6 g sodium thiosulfate over 8 hours with aeration.

A modified version of Conway medium (Walne, 1974) was used as a nutrition source (1.5 ml L^{-1}) (full recipe in Appendix V). The algae were aerated with air and CO_2 (2350 ppm) and the cultures had continuous light from three fluorescent tubes (Cool white 44777, Biltema, Norway) stacked horizontally behind them.

In the nutritional study, *R. baltica* was cultivated in two flasks and 4 litres of algae were harvested from each bottle every other day. *T. lutea* was also cultivated by the same method. *C. weissflogii* was cultivated in one flask and 3 L were harvested every day. The harvesting was done around the same time of each day to keep the same phase each time, late exponential phase.

When preparing the copeponics systems, approximately 6 litres of the microalgae species used at the time was harvested from the cultures and divided into each algae tank. The algae were then further grown over few days up to 5 litres at a high density, filling up the algae tanks. Table 4 shows the microalgae species and origin of their stock culture.

Table 4 – Species of microalgae used and the source of their stock culture.

Name	Source
Rhodomonas baltica	NIVA-5/91
Tisochrysis lutea	Unknown
Conticribra weissflogii (Also known as: *Thalassiosira weissflogii*)	CCAP 1085/18

Figure 21 - Microalgae cultivated in 10 L round flat bottom flasks. From left to right, *R. baltica*, *C. weissflogii* and *T. lutea*. The cultures are approximately the same colour as the same time they were harvested at.

2.4 Sampling and analyses

In this chapter the time of sampling, methods and equipment used, and analyses performed are described. In the study of the nutritional value of *A. tonsa*, samples for total lipids and fatty acids analysis, length and carbon analyses were taken twice. The first sampling was done when >50% of *A. tonsa* reached developmental stage C1 as it is a common stage for *A. tonsa* to be used as a live feed for various marine fish species. The second sampling was done in the end of the study, after 14 whole days from egg incubation. For *A. tonsa* reared on *R. baltica,* >50% reached the developmental stage C1 at day 7, and *A. tonsa* reared on *T. lutea* and *C. weissflogii* reached developmental stage C1 at day 8.

2.4.1 Biomass of copepods, density, and length

When samples of the copepods were taken, it was important to equalize the distribution before taking the sample which was achieved by stirring the water in the symbol 8. Copepods often have patchy distribution in the water column and if this method was not followed it resulted in inaccurate sampling which did not represent the whole population within the tank. A small cylinder was used to stir the water and to take a column sample.

Density measurements were performed using the droplet method where the number of copepods were counted in twelve 100 µl droplets. The highest and the lowest numbers counted were discarded and the other ten were summed together to give an estimation of number of copepods in each ml. The sample was poured between two 30 ml beakers before each droplet was taken from the sample to equalize the distribution of the copepods within the sample. A lugol solution was added into the droplets and number of copepods counted under a stereoscope (Leica MZ 12.5, Meyer, USA).

For length measurements, copepods were collected on a 60 µm sieve (Falcon Cell strainer, Corning, USA) and put in an anaesthetic (Finquel MS-222, Argent, USA). The copepods were then put on a slide under a microscope (Eclipse E200, Nikon, Japan). Pictures were taken of the first 70 - 140 copepods encountered with a camera (Axiocam ERc 5s, Zeiss, Germany) which was attached to the microscope. The length of the copepods was measured with the program Image J, a java-based image processing program.

Figure 22 – *A. tonsa* nauplii and copepodid. A shows the total length of a nauplii and B shows the cephalothorax length of a copepodid.

The length was transformed into carbon weight using Equation 1 for nauplii and Equation 2 for copepodid (Berggreen et al., 1988).

Equation 1 $$W = 3.18 * 10^{-6} L^{3.31}$$

Equation 2 $$W = 1.11 * 10^{-5} L^{2.92}$$

W is the individual weight in ng carbon, L is the total length of the nauplii and the cephalothorax length of the copepodid in µm.

The specific growth rate was calculated using Equation 3.

Equation 3 $$SGR = \frac{(\ln W_f - \ln W_i)}{t}$$

Where W_i is the initial weight and the W_f is the final weight and t is the time between the measurements.

Diameter and density measurements were also done on the microalgae species using a Coulter counter (Multisizer 3 Coulter counter, Beckman Coulter, USA).

2.4.2 Water quality parameters

In the copeponics study, water quality samples were taken daily from each tank and different parameters measured. Temperature, algae cell density, pH, salinity, and oxygen were measured in the samples in that order. Table 5 shows the measured parameters and the instruments used for measuring.

In the nutritional study, the water quality parameters were not followed regularly as it was performed in an FTS where these parameters were more stable compared to the copeponics system.

Table 5 - The measured parameters and the instruments used.

Parameter	**Instrument**
Temperature (°C)	DIGI-Sense Dual J-T-E-K Thermocouple Thermometer, Cole-Parmer Instrument Co., USA.
Algae cell density (cells ml^{-1})	Multisizer 3 Coulter counter, Beckman Coulter, USA.
pH	pH 3210, WTW GmbH, Germany.
Salinity (ppt)	YSI-3100, YSI, USA.
Oxygen (%)	YSI Model 59, YSI, USA.
Nitrogen (TAN, NO_2-N, NO_3-N)	DR/890 Portable Colorimeter, HACH, USA.
CO2 (ppm)	Extech CO250, Extech, USA.
Light intensity (µmole $m^{-2}s^{-1}$)	Quantum Flux, Apogee, USA.

To measure algae cell density, a Coulter counter was used. Multisizer 3 Coulter Counter measured with the program multisizer 3 where 1000 µl were measured three times under a current of -800 A with a capillary of 100 µl and the electrolyte was set to seawater. The Coulter counter can only give accurate results if counted cells are between 500 – 60,000 cells ml^{-1}. In most cases the algae cell density was higher than that, and therefore the samples needed to be diluted before they were counted. The samples were diluted and put into a beaker which was placed into the coulter counter for measuring. The results from the coulter counter are multiplied by the dilution factor to get back the original content.

Equation 4
$$\frac{\text{Density}_{\text{Sample}}}{\text{d}} = \text{Density}_{\text{beaker}}$$

Equation 5
$$\text{Density}_{\text{Sample}} = \text{d} * \text{Density}_{\text{beaker}}$$

The $\text{Density}_{\text{Sample}}$ and $\text{Density}_{\text{beaker}}$ are in cells ml^{-1} while *d* is the dilution factor.

2.4.3 Nitrogen

In the copeponics study the dynamics of the nitrogenous waste products, Total Ammonia Nitrogen (TAN), and nitrate-nitrogen (NO_3-N), were measured with a DR/890 Portable Colorimeter, HACH, USA. Nitrate-nitrogen measurements were calibrated for Cl^- interference. Nitrate-nitrogen was measured using cadmium reduction method, high range (0 – 30 mg L^{-1}), and TAN was measured using salicylate method, low range (0 – 0.50 mg L^{-1}). Samples were prepared by taking 50 ml water samples from each tank and centrifuged (Universal 32, Hettich, Germany) at 3000 rpm for 3 min to settle the algae and other debris on the bottom. The water was filtered through a 25 mm GF/C glass microfiber filters (Whatman International Ltd., England) and analysed.

The measurements with the gave the concentration of nitrogen, and not the concentration of ammonia and nitrate. Equation 6 and Equation 7 were used to convert the concentration from nitrogen to concentration of ammonia and nitrate.

Equation 6
$$\mathrm{NO_3\ mg/L} = \mathrm{NO_3\text{-}N\ mg/L} * \frac{\mathrm{NO_3\ 62.004\ g/mol}}{\mathrm{N\ 14.007\ g/mol}}$$

.

Equation 7
$$\mathrm{NH_3\ mg/L} = \mathrm{NH_3\text{-}N\ mg/L} * \frac{\mathrm{NH_3\ 17.0094\ g/mol}}{\mathrm{N\ 14.007\ g/mol}}$$

Calculations derived from Clément and Merlin (1995) were used to calculate the unionized ammonia from the measured ammonia.

Equation 8
$$\%\ \text{unionized}\ \mathrm{NH_3} = \frac{100}{1 + 10^{(\mathrm{pKa - pH})}}$$

Where

Equation 9
$$\mathrm{pKa} = 0.09018 + \frac{2729.92}{T}$$

And T is the temperature (°K).

2.4.4 Carbon analysis

To prepare the samples, copepods were collected on a 60 µm sieve (Falcon Cell strainer, Corning, USA) and cleaned with 0.22 µm filtered seawater. For each sample 10-15 copepods were randomly picked and filtered on 25 mm GF/F glass microfibre filter (Whatman International Ltd., England). Three samples were taken from each copepod tank during each sampling. The sample filters were put in a six well plate and stored at -20°C.

All samples were prepared further. Figure 23 shows the setup and equipment used. All equipment had been cleaned with 96% ethanol and conditions for the filters were kept sterile. The filter was taken up with a tweezer and placed on a small metal board (Figure 23, C), the filter was punctured with a special tool (Figure 23, D) to reduce the size of the filter. The filter was rolled up and placed in a tin capsule (Tin capsules for solids 5x9 mm, Säntis, Switzerland)

(Figure 23, B), moulded into a small ball, and placed into a 96-well plate (Figure 23, A). The well plate was stored at -20°C until the samples were analysed. The night before the samples were analysed, they were put in a heating cabinet at 60°C to dry the samples. The samples were analysed by Kjersti Andersen using vario EL CUBE, Elementar, Germany.

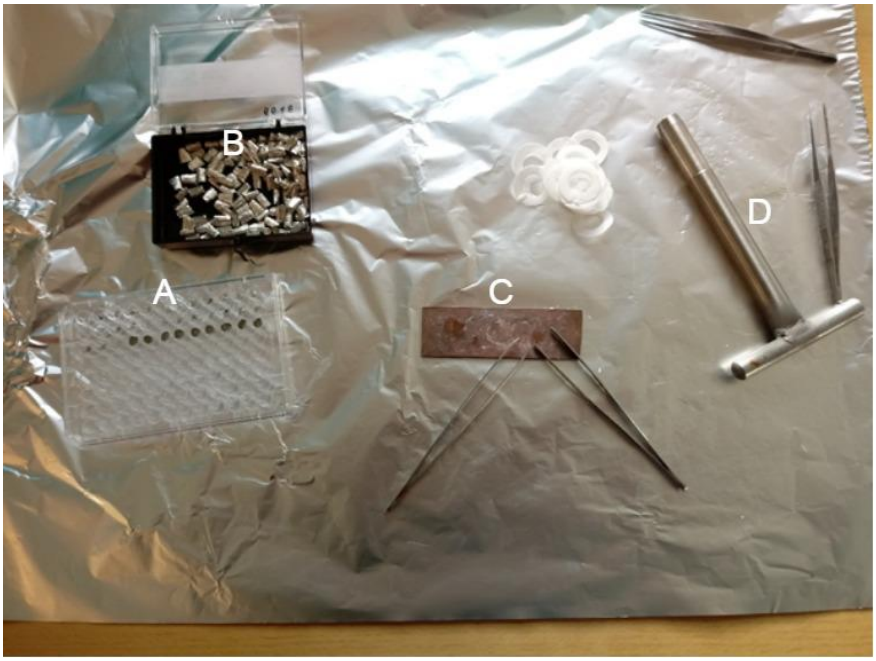

Figure 23 – The setup for preparing carbon samples. A a 96- well plate, B tin capsules, C metal board and tweezers, D the tool used to puncture the filters.

2.4.5 Total lipids and fatty acids

For total lipids and fatty acid analyses, biomass of algae and copepods was harvested. The copepod biomass was harvested without regard to stages. When collecting the copepods without getting additional debris in the sample, the copepod tanks were left to stand without aeration for approximately 20 min, the water and copepods were siphoned from the top and collected into a clean bucket, but the debris was left on the bottom. This was done twice to make sure no debris was in the sample. The copepods were collected into a 64 µm sieve and rinsed with 0.22 µm filtered seawater. They were washed in 10 ppt 0.22 µm filtered seawater to lower the salts in the sample as it can interfere with the fatty acid analysis. The sieve and copepods were dried by laying the sieve on a paper, the copepods were collected and put into a 5 ml tube. The tube was kept on ice while more samples were collected and filled with nitrogen gas and put into storage at -80°C.

The algae biomass was concentrated by centrifuging (Universal 32, Hettich, Germany) in four 50 ml tubes for 3 min at 3000 rpm. The water was removed, and more algae was added to the tube, and centrifuged again for 3 min at 3000 rpm. This was repeated five times, or until sufficient biomass was collected. The concentrated algae in the bottom of the tube was collected and placed in a 5 ml tube. The tube was filled with nitrogen gas and put into storage at -80°C.

To prepare samples for total lipids and fatty acids analyses, the samples were first freeze dried. Around 10 mg dry weight (DW) of each algae sample and 5 mg DW of each copepod samples were weighed and moved into a 20 ml vial.

A. Total lipids analysis

The method used for extracting lipids was a modified version of Bligh and Dyer (1959) and details of the modification are given below. The step by step procedure of the method is shown in Figure 24.

The algae samples were digested with an enzyme to make the lipids more easily extracted. To each algae sample, 0.7 ml 0.1 M Tris HCl (pH 7.5 at 50°C) was added and heated up to 98°C for 10 min with a tight screw cap. The vials were cooled to 50°C and 0.1 ml of a freshly made protease solution (10 mg Streptomyces griseus from Sigma Aldrich per ml of 0.1 M Tris HCl) was added to each vial. The vials were incubated at 50°C for 1 h (Jakobsen et al., 2008).

The vials were put on ice and 2 ml of methanol and 1 ml of chloroform with internal standard were added to each vial. The samples were homogenized with a small mixer (T10 basic ULTRA-TURRAX, IKA, USA) for 1 min. 1 ml of chloroform was added to the vial and homogenized for 20 s, 1 ml of dH_2O was added to the vial and again homogenized for 20 sec. During these procedures the vials were always kept on ice. Next the vials were centrifuged for 10 min at 4000 rpm (Universal 32, Hettich, Germany) and two layers formed. The added chloroform extracted the lipids from the sample and formed the lower layer in the vial. Around 1.5 ml of the lower layer was put into a new vial with a pipette. Of the 1.5 ml, 0.5 ml were put into a pre-weighed glass vial and the chloroform was evaporated with nitrogen (N-EVAP 5085, Organomation, USA). The vials were put into a vacuum desiccator for two hours and weighed. The total lipids weighed was multiplied with 4 as only one fourth of the sample was being weighed (0.5 ml of the total 2 ml). The 1 ml remaining was used for fatty acid methyl esterification (FAME) which is described below.

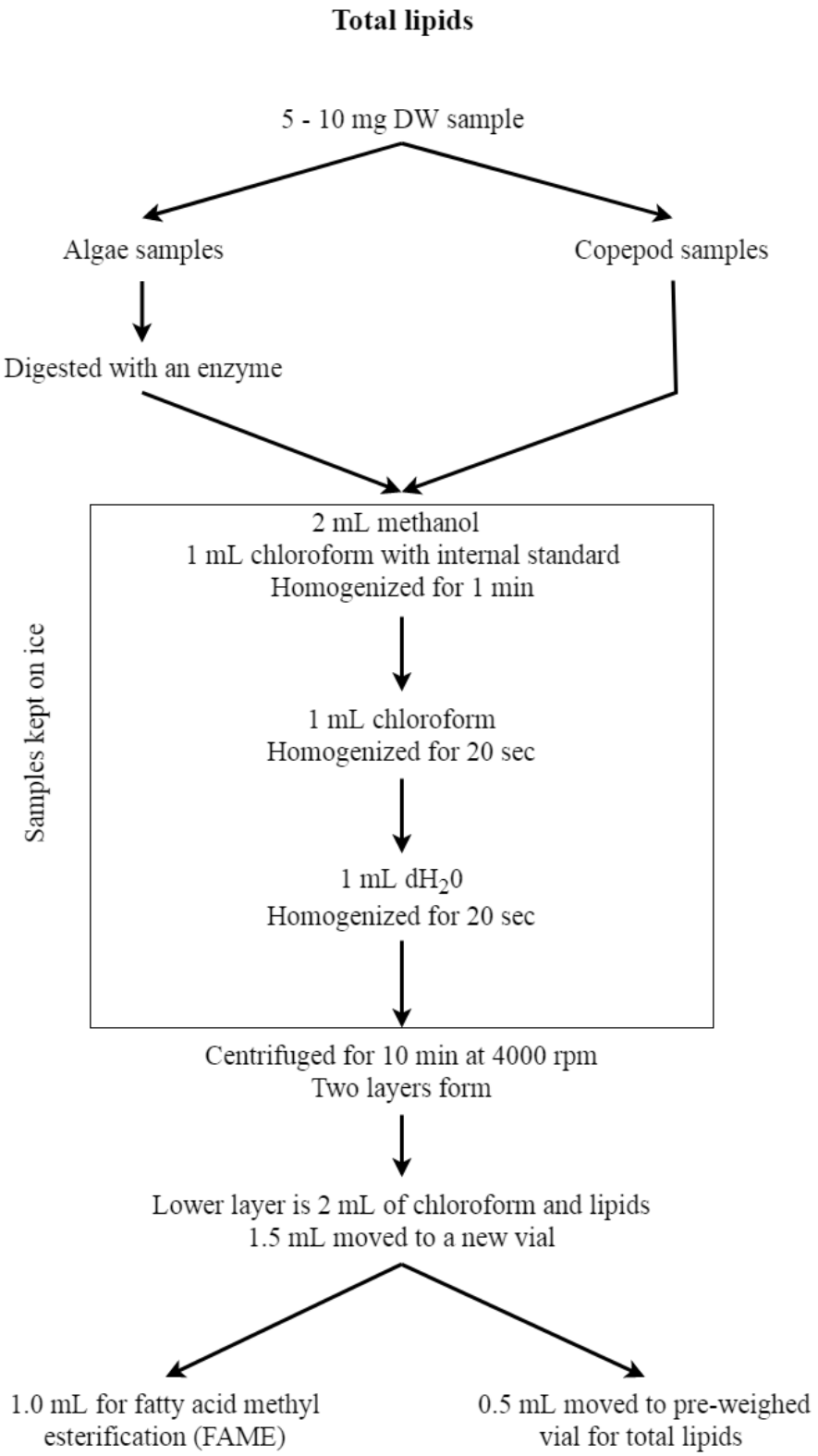

Figure 24 – Step by step procedure of the modified method used for extraction of lipids.

B. Fatty acid methyl esterification (FAME)

A known concentration of an internal standard (C23:0) was added to each sample to have a reference point within the sample. The concentration of internal standard was ~12% of the total lipids content in the sample. The algae samples were estimated to have 15% total lipids content and the copepod samples were estimated to have 11% total lipid content.

The method used for FAME was a modified version of the Metcalfe et al. (1966) method, the step by step procedure is shown in Figure 25. The 1 ml sample was evaporated with nitrogen (N-EVAP 5085, Organomation, USA) and a small pellet remained in the vial. 1 ml of 0.5N NaOH-methanol was added, the vial was vortexed and heated for 15 min at 100°C. 2 ml of BF_3-methanol was added to the vial, vortexed and heated for 5 min at 100°C. 1 ml of isooctane was added to the vial, vortexed and heated at 100°C for 1 min. The vials were cooled on ice between the heating and adding of new chemical.

3 ml of saturated NaCl in dH_2O and 0.5 ml of isooctane were added to the vial, it was vortexed and centrifuged for 3 min at 4000 rpm (Universal 32, Hettich, Germany). Two layers formed in the vial and the top layer was put into a new vial with a pipette. 0.5 ml of isooctane was added to the vial, vortexed and centrifuged for 3 min at 4000 rpm (Universal 32, Hettich, Germany). This was repeated twice more. The collected sample was transferred to a small gas chromatography (GC) vial and analyzed with the GC Agilent Technologies 7890B GC system. Further information about GC settings can be found in Appendix IV.

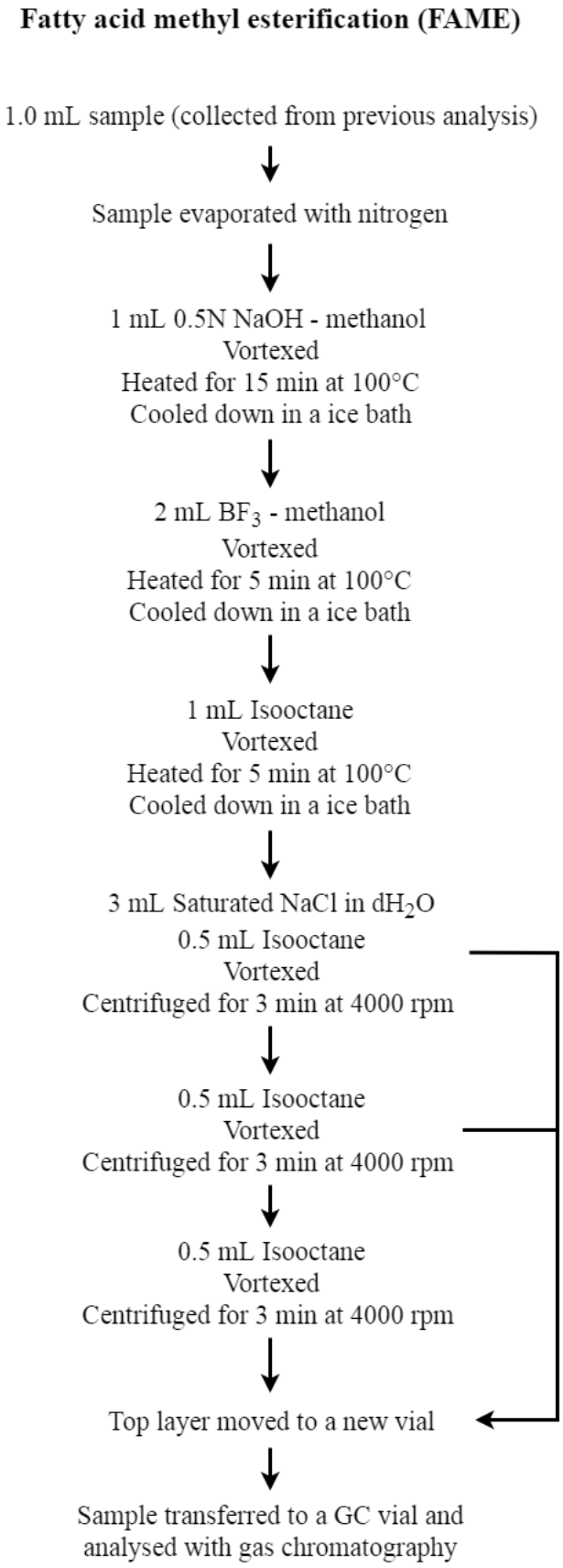

Figure 25 - Step by step procedure of the modified method of fatty acid methyl esterification (FAME).

2.5 Statistics

All statistics were performed using Sigmaplot (Sigmaplot 14.0, Systat Software Inc., USA). When group data sets were compared, the normality distribution was tested, if the data sets passed the normality test a One-Way ANOVA was run, and if it did not pass a Repeated Measure One-Way ANOVA was run. If a significant difference ($P < 0.05$) was found between the groups a Tukey's multiple comparison post was run.

Data is given as mean ± standard deviation (SD) if nothing else is denoted and level of significance was set to $p < 0.05$.

3. Results

3.1 Nutritional value of *A. tonsa*

3.1.1 Density and survival

Figure 26 shows the density of individuals ml^{-1} (ind. ml^{-1}) and survival rate (%) as a function of time for *A. tonsa* reared on the three different monoalgal diets. At day 2 the hatched nauplii were collected from the hatching tank and moved to the copepod tanks at the average densities of 58, 46 and 50 ind. ml^{-1}, for copepods reared on *R. baltica*, *T. lutea* and *C. weissflogii*, respectively. These numbers were set as the initial densities.

At day 7 the survival rate of *A. tonsa* reared on *R. baltica* dropped down to 57%. At day 8 the survival rate of *A. tonsa* reared on *T. lutea* and *C. weissflogii* dropped down to 71% and 38%, respectively. At day 7 and 8, the density of *A. tonsa* reared on *C. weissflogii* was significantly lower than the other two. At day 13 the survival rate of *A. tonsa* reared on *R. baltica*, *T. lutea* and *C. weissflogii* dropped down to 35%, 38% and 29%, respectively, without any significant differences between the groups ($p>0.05$).

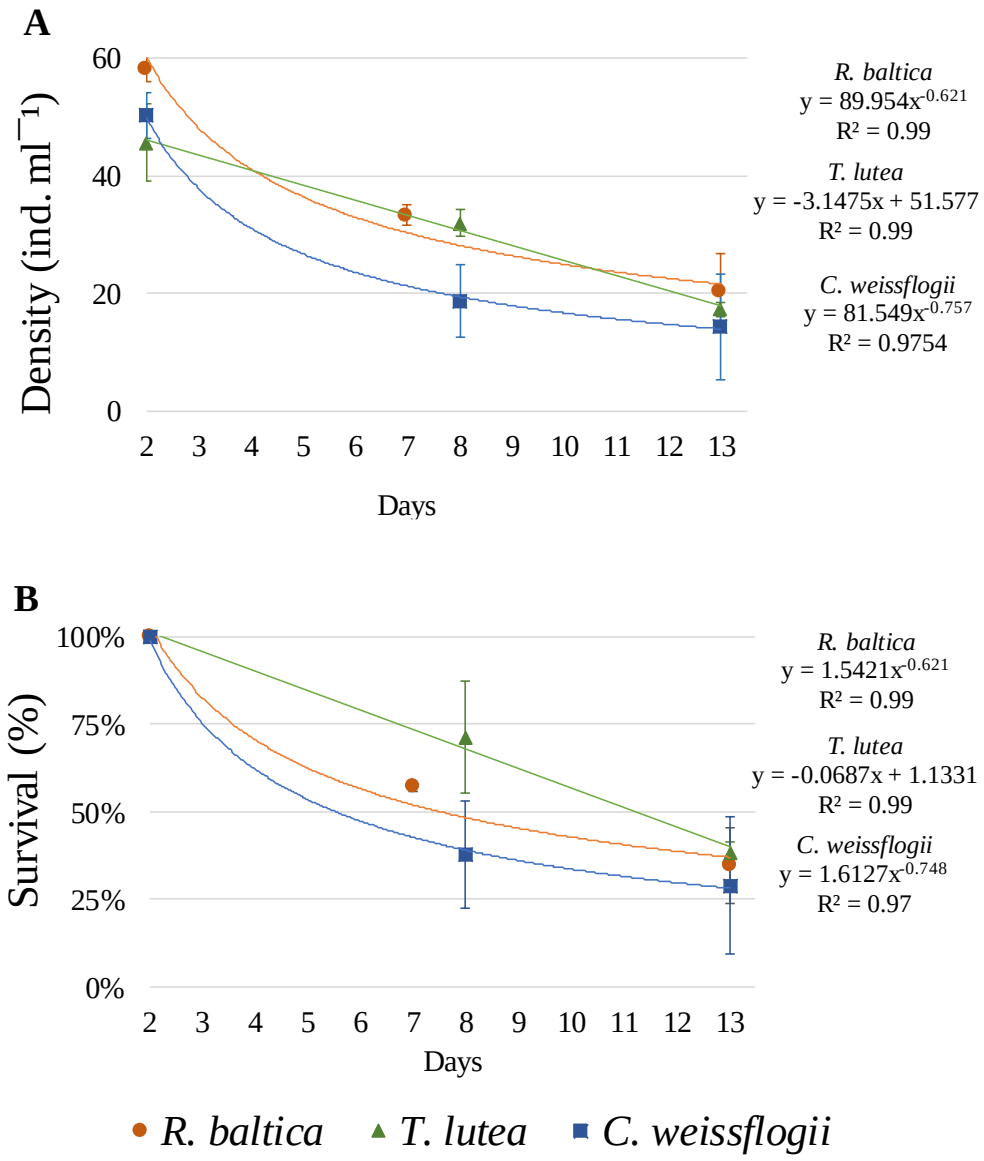

Figure 26 – (A) Density (ind. ml⁻¹) and (B) survival (%) as a function of time for *A. tonsa* reared on *R. baltica*, *T. lutea*, and *C. weissflogii*. The values presented are averages from three replicates and error bars present ± SD (n = 3).

3.1.2 Length and carbon content

Figure 27 shows changes in length (µm individual^{-1}) and weight in carbon content (µg C individual^{-1}) calculated from length measurements as a function of time. At day 2 the average length of individuals was 100 µm and average carbon content 0.11 µg C ind.$^{-1}$. At day 7, the length of *A. tonsa* reared on *R. baltica* were 283 µm and average carbon content of 0.23 µg C ind.$^{-1}$. At day 8, *A. tonsa* reared on *T. lutea* and *C. weissflogii* were 281 and 290 µm at average length and average carbon content of 0.22 and 0.23 µg C ind.$^{-1}$, respectively. No significant difference was observed in length or carbon content between groups at day 7 and 8. At day 13, no significant differences were observed in the length of *A. tonsa* reared on *R. baltica* and *T.*

lutea which were 595 and 611 µm, respectively. Corresponding no significant differences were found for the average carbon content of the same groups, being 1.49 and 1.63 µg C ind.$^{-1}$, respectively. Significantly lower individual length and carbon content was found in the groups reared on *C. weissflogii*, being 304 µm and 0.41 µg C ind^{-1}, respectively.

Additionally, diameter measurements were made on the microalgae species using the Coulter counter. *R. baltica* measured between 5.5 – 9.5 µm, on average 7.0 µm, *T. lutea* measured between 3.5 – 6.0 µm, on average 4.3 µm, and *C. weissflogii* measured between 8.5 – 15.0 µm, on average 11.0 µm.

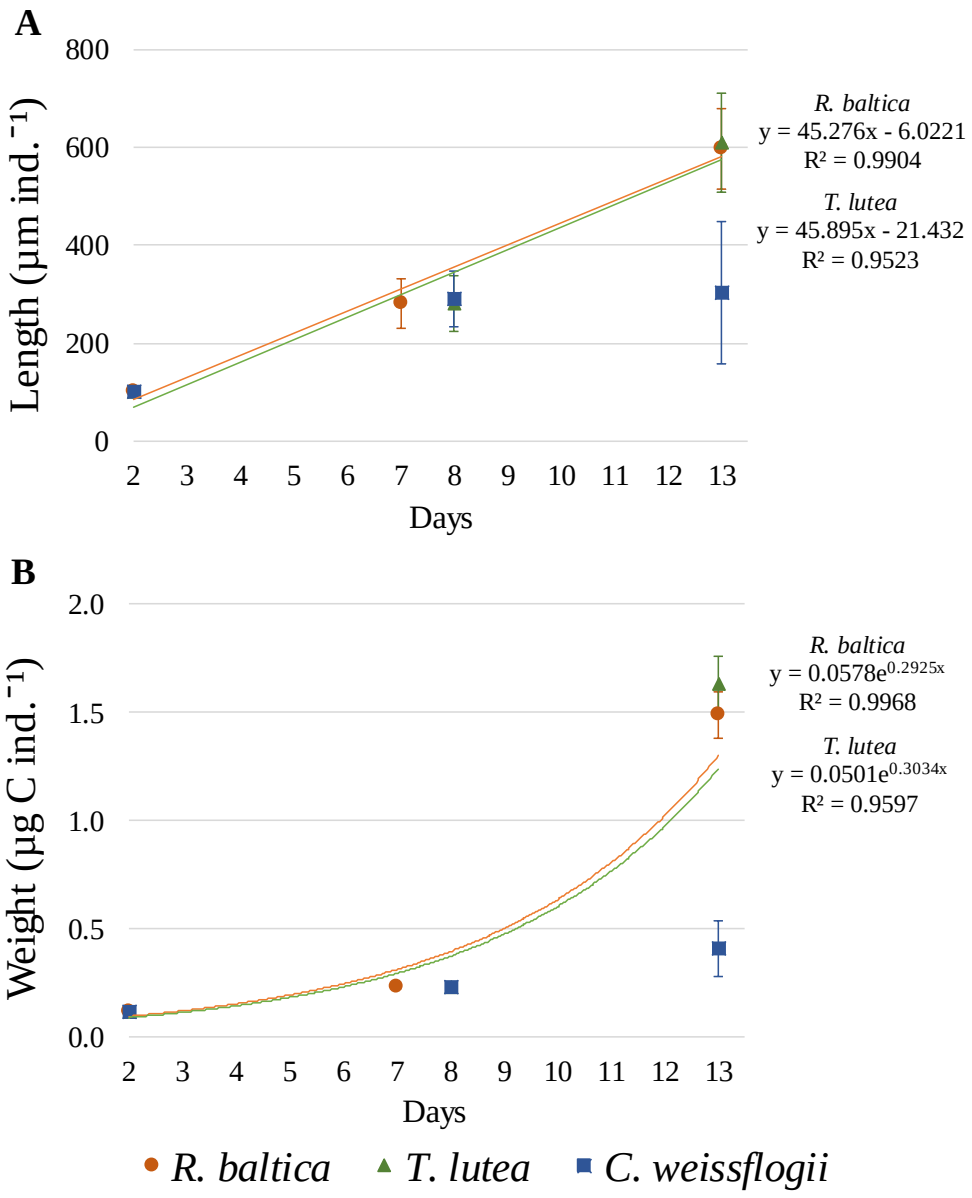

Figure 27 – (A) Length (µm ind.$^{-1}$) and (B) weight in carbon content (µg C ind.$^{-1}$) as a function of time for *A. tonsa* reared on *R. baltica*, *T. lutea*, and *C. weissflogii*. The values presented are averages from three replicates and error bars present ± SD (n = 3).

3.1.3 Carbon content from analysis

Figure 28 presents the weight in carbon content of *A. tonsa* from carbon analysis as a function of time. At day 7, significantly higher carbon content was found in *A. tonsa* reared on *R. baltica* being 0.68 µg C ind.$^{-1}$ than in *A. tonsa* reared on *T. lutea* and *C. weissflogii* at day 8, being 0.47 and 0.45 µg C ind.$^{-1}$, respectively. At day 13, the carbon content of *A. tonsa* reared on *R. baltica*, *T. lutea* and *C. weissflogii* were significantly different from each other, being 1.54, 1.75 and 0.68 µg C ind.$^{-1}$, respectively.

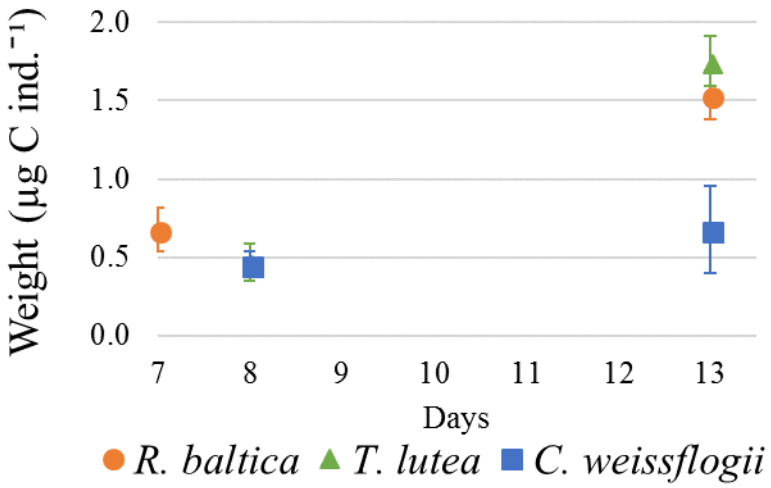

Figure 28 – Weight in carbon content (µg C ind^{-1}), from carbon analysis, as a function of time for *A. tonsa* reared on *R. baltica*, *T. lutea*, and *C. weissflogii*. The values presented are averages from three replicates and error bars present ± SD (n = 3).

3.1.4 Specific growth rate

The specific growth rates (SGR) based on calculated individual carbon content of *A. tonsa*, were calculated for time periods day 2 – 7 and day 7 – 13 for use of *R. baltica*, and for time periods day 2 – 8 and day 8 – 13 for use of *T. lutea* and *C. weissflogii* as well as for day 2 - 13 for all diet groups (Table 6). During the time period day 2-7 and day 2 - 8, no significant differences in the SGR of *A. tonsa* were found between the groups reared on *R. baltica*, *T. lutea* and *C. weissflogii*, being 0.15, 0.14 and 0.15 day^{-1}, respectively. During the time period day 7 - 13 and day 8 - 13, significantly higher SGR was found for *A. tonsa* reared on *R. baltica* and *T. lutea* than on *C. weissflogii*, being 0.31, 0.33 and 0.09 day^{-1}, respectively. During the time period day 2 - 13, significantly higher SGR of *A. tonsa* was found in groups reared on *R. baltica* and *T. lutea* than on *C. weissflogii*, being 0.24, 0.24 and 0.12 day^{-1}, respectively.

Table 6 - Specific growth rate (SGR) (day^{-1}) at different time periods for *A. tonsa* reared on *R. baltica*, *T. lutea*, and *C. weissflogii*. The values presented are averages from three replicates for each treatment ± SD (n = 3), values marked with "*" are significantly different.

	Specific growth rate		
Time period	**Day 2 - 7**	**Day 7 - 13**	**Day 2 - 13**
R. baltica	0.15 ± 0.00	0.31 ± 0.03	0.24 ± 0.01
Time period	**Day 2 - 8**	**Day 8 - 13**	**Day 2 - 13**
T. lutea	0.14 ± 0.01	0.33 ± 0.02	0.24 ± 0.01
C. weissflogii	0.15 ± 0.01	0.09 ± 0.07 *	0.12 ± 0.03*

* Significant difference

3.1.5 Developmental stage distribution

Figure 29 shows developmental stage distribution (nauplii, copepodid and adult) as a function of time. All treatments consisted of 100% nauplii at day 2. At day 7, *A. tonsa* reared on *R. baltica* had 59% copepodid and 41% nauplii, and at day 13, 96% copepodid and 4% adults. At day 8, *A. tonsa* reared on *T. lutea* had 57% copepodid and 43% nauplii, and at day 13, 90% copepodid, 9% adults and 1% nauplii. At day 8, *A. tonsa* reared on *C. weissflogii* had 67% copepodid and 33% nauplii, and at day 13, 58% nauplii, 40% copepodid and 2% adults.

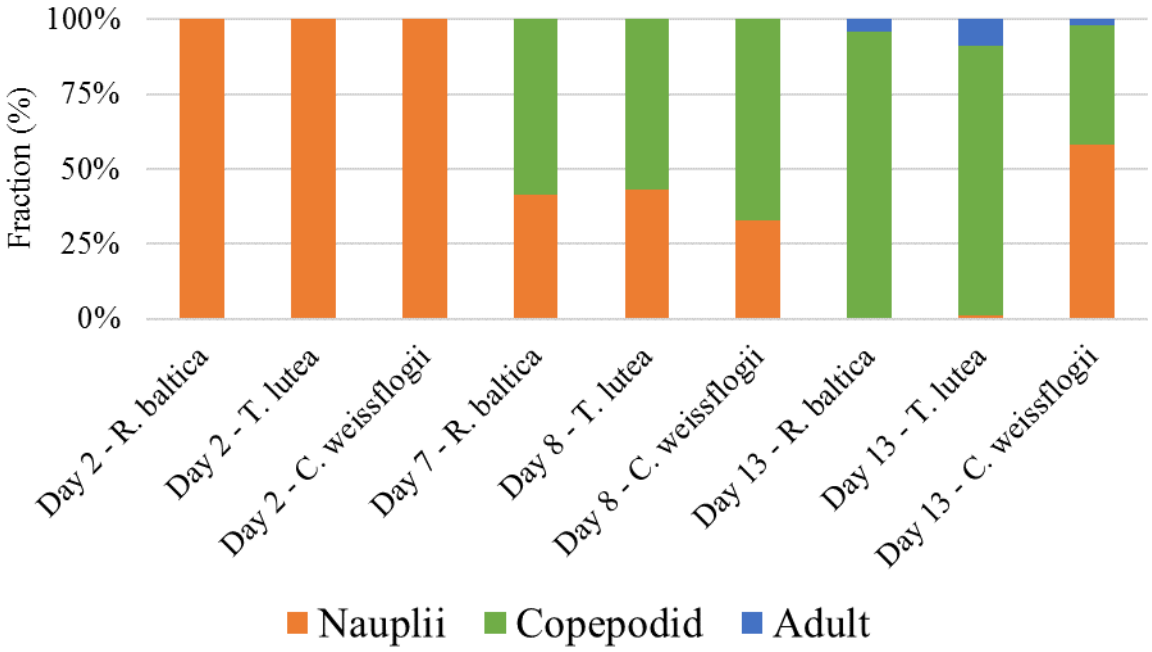

Figure 29 – Developmental stage distribution (nauplii, copepodid, adult) as a function of time for *A. tonsa* reared on *R. baltica*, *T. lutea* and *C. weissflogii*. The values presented are averages from three replicates and the error bars present ± SD (n = 3).

3.1.6 Total lipids

Figure 30 shows total lipid content (TL) (µg mg^{-1} DW) in *R. baltica*, *T. lutea* and *C. weissflogii* and of *A. tonsa* reared on those microalgae species. A significant difference in total lipid content was observed between *R. baltica*, *T. lutea* and *C. weissflogii* with a content of 168, 292 and 114 µg mg^{-1} DW, respectively. At days 7 and 8, a significant difference was observed in *A. tonsa* reared on *R. baltica* and *T. lutea*, which had the TL content of 162 and 195 µg mg^{-1} DW, respectively. At day 13, a significant difference was observed between *A. tonsa* reared on *R. baltica* and *C. weissflogii*, which had the total lipid content of 108 and 197 µg mg^{-1} DW, respectively.

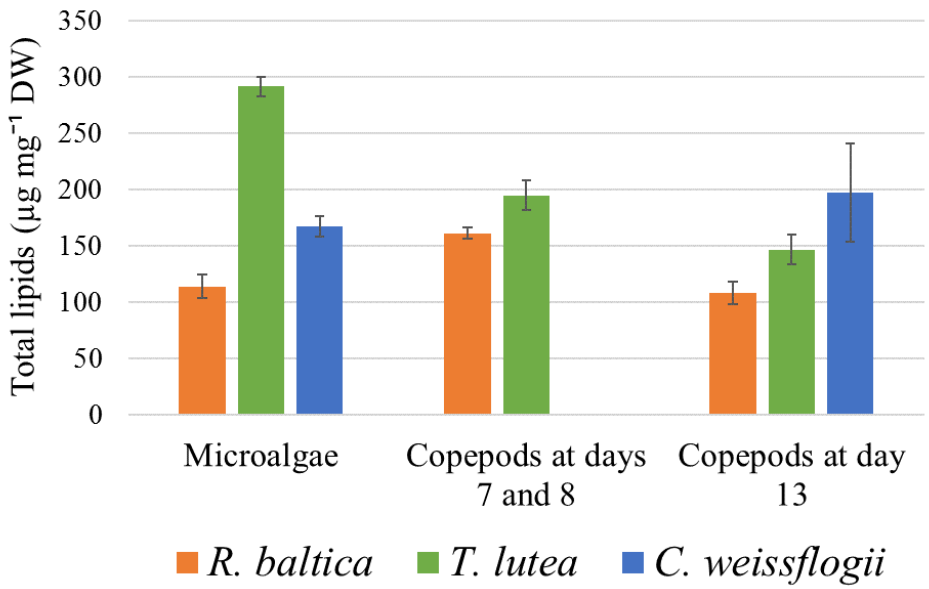

Figure 30 – Total lipids (µg mg^{-1} DW) in *R. baltica*, *T. lutea* and *C. weissflogii* and *A. tonsa* reared on those microalgae species at day 7 and 8 and at day 13. The values presented are averages from three replicates and error bars present ± SD (n = 3). Data for treatment *C. weissflogii* at days 7 and 8 was missing.

3.1.7 Fatty acid composition and ratio

Table 7 shows the concentration of fatty acids (mg g^{-1} DW) and DHA/EPA, EPA/ARA and n-3/n-6 ratios in *R. baltica*, *T. lutea* and *C. weissflogii* and in *A. tonsa* reared on those microalgae species, at day 7, 8 and 13.

The groups total FAs, SFAs, MUFAs, PUFAs and the HUFAs; DHA, EPA and ARA were compared for significant difference. Significant difference was observed in the microalgae species for all groups of fatty acids except in MUFAs and DHA, where there was no significant difference found between *R. baltica* and *C. weissflogii*. There was also no significant difference between *R. baltica* and *T. lutea*, and between *T. lutea* and *C. weissflogii* in regard to content of ARA.

For *A. tonsa* reared on *R. baltica* and *T. lutea* at day 7 and 8, a significant difference was observed within all groups of fatty acids. When copepods at day 13 were compared, *A. tonsa* reared on *R. baltica* was a significantly lower in total FAs, SFAs and MUFAs than *A. tonsa* reared on *T. lutea* and *C. weissflogii*. When PUFAs were compared, *A. tonsa* reared on *R. baltica* was significantly lower than *A. tonsa* reared on *T. lutea.* When the groups of HUFAs; DHA, EPA and ARA, were compared between diets there was a significant difference within all HUFAs and diets.

The ratio of DHA/EPA for the microalgae *R. baltica* and *A. tonsa* reared on *R. baltica* at day 7 and day at 13 was 1.1, 1.4 and 2.2, respectively, the EPA/ARA ratio was 3.2, 3.6 and 6.5, respectively, and the n-3/n-6 ratio was 1.9, 8.7 and 5.8, respectively.

The ratio of DHA/EPA for the microalgae *T. lutea* and *A. tonsa* reared on *T. lutea* at day 8 and at day 13 was 27.0, 11.4 and 10.5, respectively, the EPA/ARA ratio was 2.0, 2.2 and 3.7, respectively, and the n-3/n-6 ratio was 2.1, 2.1 and 4.1, respectively.

The ratio of DHA/EPA for the microalgae *C. weissflogii* and *A. tonsa* reared on *C. weissflogii* at day 13 was 0.2 and 0.7, respectively, the EPA/ARA ratio was 0.0 and 232.2, respectively, and the n-3/n-6 ratio was 33.7 and 73.2, respectively.

Table 7 – The concentration of fatty acids (mg g^{-1} DW) in *R. baltica*, *T. lutea* and *C. weissflogii* and in *A. tonsa* reared on those microalgae species at days 7, 8 and 13. Values of 0.0 are < 0.05 mg g^{-1} DW. The values presented are averages from three replicates ± SD (n=3). Data for the diet of *C. weissflogii* at day 8 is missing.

	Microalgae			***A. tonsa***				
				R. baltica		***T. lutea***		***C.weissflogii***
	R. baltica	***T. lutea***	***C. weissflogii***	**Day 7**	**Day 13**	**Day 8**	**Day 13**	**Day 13**
C14:0		5.4 ± 0.1	1.6 ± 0.1	0.9 ± 1.0	0.5 ± 0.0	4.5 ± 0.1	2.2 ± 0.4	1.2 ± 0.3
C16:0	1.2 ± 0.2	2.5 ± 0.0			1.5 ± 0.0	3.1 ± 0.0	2.4 ± 0.1	3.0 ± 0.4
C17:0					0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.2 ± 0.6
C18:0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.9 ± 0.5	0.6 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
C20:0					0.6 ± 0.0	2.0 ± 1.7	1.6 ± 0.3	
C22:0				0.1 ± 0.1				
Total SFAs	1.2 ± 0.3	8.0 ± 0.2	1.6 ± 0.1	1.9 ± 1.7	3.4 ± 0.1	10.2 ± 1.9	6.7 ± 0.8	6.0 ± 1.4
C14:1n5	0.0 ± 0.0	0.1 ± 0.0				0.0 ± 0.0		
C16:1n7	0.0 ± 0.0	1.8 ± 0.1	0.0 ± 0.1			2.1 ± 0.1	0.8 ± 0.2	2.7 ± 0.9
C18:1n9	0.0 ± 0.0	2.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	2.6 ± 0.1	1.4 ± 0.2	0.2 ± 0.1
C18:1n7	0.3 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
C20:1n9						0.0 ± 0.0	0.0 ± 0.0	
Total MUFAs	0.3 ± 0.1	4.7 ± 0.1	0.2 ± 0.1	0.5 ± 0.3	0.1 ± 0.0	5.1 ± 0.2	2.5 ± 0.5	3.4 ± 1.1
C18:2n6	2.5 ± 0.5	3.6 ± 0.0	0.1 ± 0.1		0.7 ± 0.0	4.4 ± 0.1	1.7 ± 0.3	0.0 ± 0.0
C20:2n6	0.0 ± 0.0	0.0 ± 0.1			0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
C20:4n6 ARA	0.3 ± 0.1	0.1 ± 0.0		0.7 ± 0.4	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Total n-6	2.9 ± 0.6	3.7 ± 0.1	0.1 ± 0.1	0.7 ± 0.4	1.1 ± 0.0	4.8 ± 0.2	2.0 ± 0.3	0.1 ± 0.1
C18:3n3	3.3 ± 0.3	4.3 ± 0.2	0.3 ± 0.0		0.9 ± 0.0	4.0 ± 0.1	1.9 ± 0.3	0.2 ± 0.2
C20:5n3 EPA	1.0 ± 0.2	0.1 ± 0.0	4.2 ± 0.2	2.5 ± 1.4	1.8 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	5.2 ± 0.9
C22:6n3 DHA	1.0 ± 0.1	3.3 ± 0.1	0.9 ± 0.0	3.5 ± 2.0	3.8 ± 0.0	5.7 ± 0.0	5.4 ± 0.1	3.4 ± 0.1
Total n-3	5.2 ± 0.6	7.7 ± 0.3	5.5 ± 0.2	6.0 ± 3.5	6.6 ± 0.1	10.2 ± 0.2	7.9 ± 0.5	8.7 ± 1.2
Total PUFAs	8.1 ± 1.2	11.4 ± 0.4	5.6 ± 0.3	6.7 ± 3.9	7.7 ± 0.1	15.0 ± 0.3	9.9 ± 0.8	8.8 ± 1.3
Total FAs	9.6 ± 0.9	24.1 ± 0.2	7.4 ± 0.2	9.2 ± 0.8	11.2 ± 0.1	30.3 ± 2.2	19.0 ± 2.0	18.1 ± 3.4
DHA/EPA	1.1 ± 0.1	27.0 ± 8.5	0.2 ± 0.0	1.4 ± 0.0	2.2 ± 0.1	11.4 ± 0.3	10.5 ± 0.1	0.7 ± 0.1
EPA/ARA	3.2 ± 1.2	2.0 ± 0.5		3.6 ± 0.1	6.5 ± 0.2	2.2 ± 0.1	3.7 ± 0.4	232.2 ± 38.7
n-3/n-6	1.9 ± 0.5	2.1 ± 0.1	33.7 ± 3.3	8.7 ± 0.2	5.8 ± 0.2	2.1 ± 0.0	4.1 ± 0.4	73.2 ± 8.1

3.2 Copeponics

In total, six experiments were performed, three with the use of *R. baltica*, one with *T. lutea* and two with *C. weissflogii*. For a number of reasons most of the experiments could not be completed. The results from the experiments with the use of *R. baltica* will be presented here. The experiments using *T. lutea* and *C. weissflogii* did not perform well and will be treated in the discussion chapter.

3.2.1 First experiment – *R. baltica*

The 1st experiment with *R. baltica* ran for 9 days in total and was then stopped due to loss of copepods.

A. Density of *A. tonsa*

Table 8 shows the density (ind. ml^{-1}) of *A. tonsa* reared on *R. baltica*. At day 2, *A. tonsa* was moved from the hatching tank to the copepod tanks at ~36 ind. ml^{-1}. The density measured on average 17 and 0 ind. ml^{-1} at day 5 and 9, respectively.

Table 8 - Density of *A. tonsa* (ind. ml^{-1}) on day 2, 5 and 9. The values are averages from three replicates ± SD (n = 3).

Day	Copepod density (ind. ml^{-1})
2	36 ± 0.8
5	17 ± 6.2
9	0 ± 0.0

B. Water quality

Table 9 shows the water quality parameters pH, salinity (ppt), oxygen (%) and temperature (°C). All of the parameters were relatively stable throughout the study, seen by the low SD. The pH in the algae tanks, copepod tanks and biofilters was on average 8.3, 7.8 and 7.8, respectively. The salinity in the algae tanks, copepod tanks and biofilters was on average 34.9, 34.2 and 34.3 ppt, respectively. The oxygen in the copepod tanks and biofilters was on average 91% and 89.2%, respectively. The temperature in the algae tanks, copepod tanks and biofilters was on average 22.5, 20.6 and 19.4°C, respectively.

Table 9 - Water quality parameters, pH, salinity (ppt), oxygen (%) and temperature (°C) measured in algae tanks, copepod tanks and biofilters. The values are averages of three replicates throughout the experiment ± SD (n = 3).

	pH	Salinity (ppt)	Oxygen (%)	Temperature (°C)
Algae tanks	8.3 ± 0.2	34.9 ± 0.3		22.5 ± 0.1
Copepod tanks	7.8 ± 0.0	34.2 ± 0.1	91.3 ± 1.3	20.6 ± 0.3
Biofilters	7.8 ± 0.0	34.3 ± 0.1	89.2 ± 1.2	19.4 ± 0.1

C. Harvested algae and Conway medium

Table 10 shows the volume used of Conway medium (ml day^{-1}) and volume of algae harvested (L day^{-1}) each day of the experiment from each replicate. The algae harvested was increased from 0.5 L day^{-1} to 2 L day^{-1}, at day 0 to day 3, and from day 4 to day 9, 0.5 L day^{-1} were harvested. In total for each replicate, 7.7 ml of Conway medium were added, and 8.7 litres of algae harvested, giving the average Conway medium use 0.88 ml L^{-1}. The Conway medium contains 16.48 mg ml^{-1} of dissolved inorganic nitrogen (DIN), and in total 125.5 mg of DIN was added to each system.

Table 10 - The volume of algae harvested (L day^{-1}), volume of Conway medium added (ml day^{-1}) and dissolved inorganic nitrogen (DIN) content in Conway medium (mg).

Day	Algae harvested (L day^{-1})	Conway medium (ml day^{-1})	DIN in Conway (mg)
0	0.5	0.0	0.0
1	1.0	0.7	10.7
2	2.0	1.0	16.4
3	2.0	2.0	32.8
4	0.5	2.0	32.8
5	0.5	0.5	8.2
6	0.5	0.5	8.2
7	0.5	1.0	16.4
8	0.5	0.0	0.0
9	0.5	0.0	0.0
Total	8.7	7.7	125.5

D. Algae density

Figure 31 shows the algae density (cells ml^{-1}) of *R. baltica* in the (A) algae tanks, (B) copepod tanks and (C) biofilters for each day of the experiment. The algae density was relatively stable in the algae tanks, from day 0 to day 4 the density was ~ 2 million cells ml^{-1}, and from day 5 to day 9, increases up to ~2.5 million cells ml^{-1}. The algae density in the copepod tanks at day 0 was ~22,000 cell ml^{-1} and steadily increase to ~160,000 cells ml^{-1} at day 4. The algae density rapidly increases from day 6 to day 9, reaching a maximum of ~560,000 cells ml^{-1}. The algae density in the biofilters was relatively stable throughout the experiment, on average ~54,000 cells ml^{-1}, with the exception of higher density on day 6 and 9 where the algae density reached ~ 200,000 cells ml^{-1}.

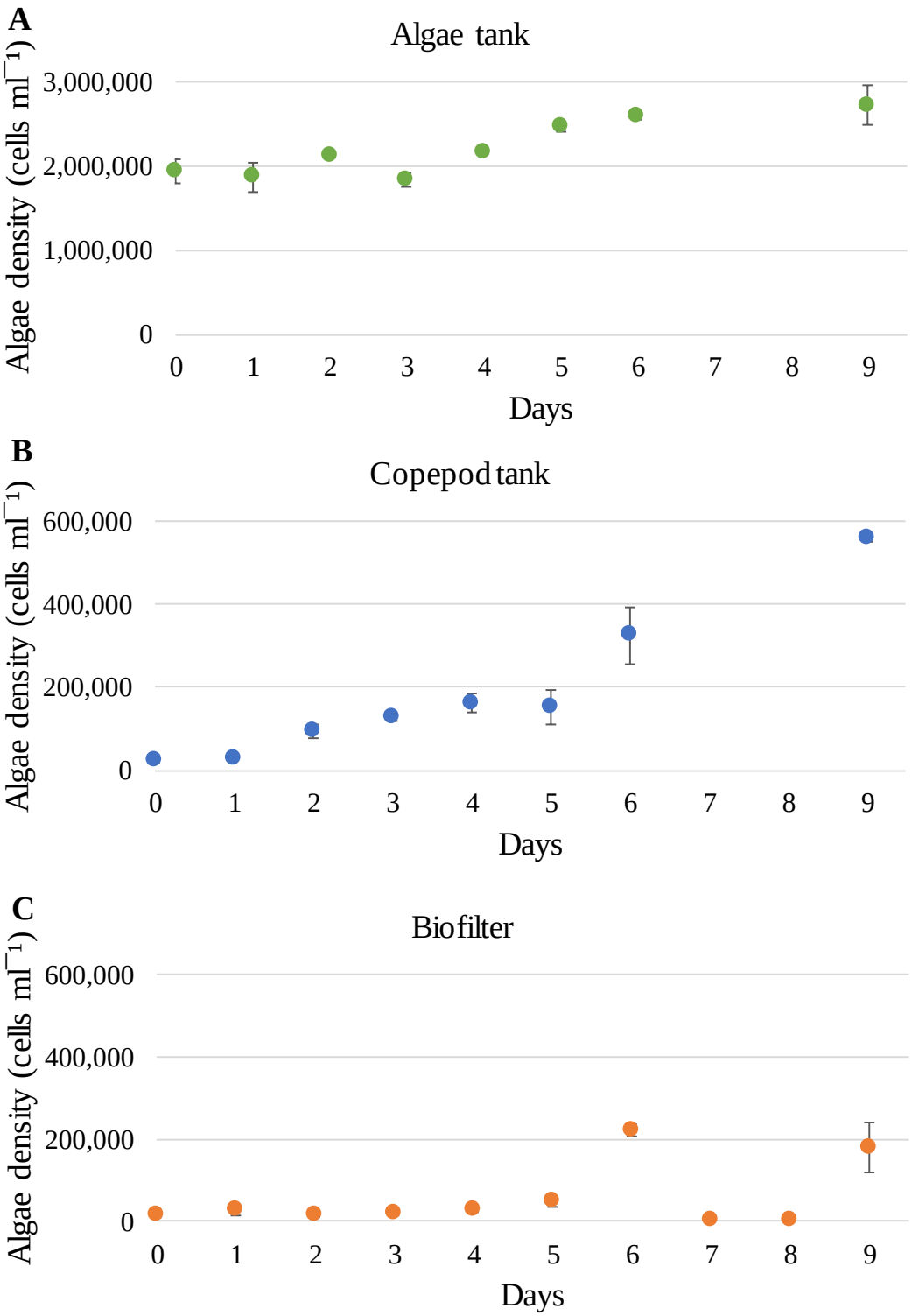

Figure 31 – Algae density (cells ml^{-1}) of *R. baltica* as a function of time in the (A) algae tanks, (B) copepod tanks and (C) biofilters. The values presented are averages from three replicates and error bars present ± SD (n = 3).

3.2.2 Second experiment – *R. baltica*

The 2nd experiment with *R. baltica* ran for the planned 14 day period.

A. Density of *A. tonsa*

Table 11 shows the density (ind. ml^{-1}) of *A. tonsa* reared on *R. baltica*. At day 3, the copepods were moved from the hatching tank to the copepod tanks at ~32 ind. ml^{-1}. The density lowered steadily and measured on average 22, 16, 11 and 4 ind. ml^{-1} at day 6, 11, 12, and 14, respectively.

Table 11 - Density of *A. tonsa* (ind. ml^{-1}) on day 3, 6, 11, 12 and 14. The values are averages from three replicates ± SD (n = 3).

Day	Copepod density (ind. ml^{-1})
3	32 ± 2.1
6	22 ± 2.4
11	16 ± 0.9
12	11 ± 0.8
14	4 ± 0.8

B. Water quality

Table 12 shows the water quality parameters pH, salinity (ppt), oxygen (%) and temperature (°C). Most of the parameters were relatively stable throughout the study, seen by the low SD. The pH in the algae tanks, copepod tanks and biofilters was on average 8.2, 7.7 and 7.8, respectively. The salinity in the algae tanks, copepod tanks and biofilters was on average 30.0, 29.8 and 29.5 ppt, respectively. The oxygen in the copepod tanks and biofilters was on average 82.1% and 90.5%, respectively. The temperature in the algae tanks, copepod tanks and biofilters was on average 20.7, 18.6 and 17.6°C, respectively.

Table 12 - Water quality parameters, pH, salinity (ppt), oxygen (%) and temperature (°C) measured in algae tanks, copepod tanks and biofilters. The values are averages of three replicates throughout the experiment ± SD (n = 3).

	pH	Salinity (ppt)	Oxygen (%)	Temperature (°C)
Algae tanks	8.2 ± 0.1	30.0 ± 1.0		20.7 ± 0.3
Copepod tanks	7.7 ± 0.2	29.8 ± 0.8	82.1 ± 11.0	18.6 ± 0.1
Biofilters	7.8 ± 0.1	29.5 ± 0.8	90.5 ± 5.7	17.6 ± 0.4

C. Harvested algae and addition of Conway medium

Table 13 shows the volume used of Conway medium (ml day^{-1}) and volume of algae harvested (L day^{-1}) each day of the experiment from each replicate. From day 2 to day 5, the volume of harvested algae was increased from 0.5 L day^{-1} to 1.5 L day^{-1}. From day 10 to day 13, 1.5 L day^{-1} were harvested. At day 10 the volume of Conway medium was reduced to ~0.5 ml for

each litre of algae harvested. In total for each replicate, 11.6 ml of Conway medium were added, and 13.2 litres of algae harvested, giving the average Conway medium use 0.88 ml L^{-1}. In total 190.7 mg of DIN was added to each system with Conway medium and the total DIN in each system measured on average 3.5 mg.

Table 13 – The volume of algae harvested (L day^{-1}), volume of Conway medium added (ml day^{-1}), dissolved inorganic nitrogen (DIN) content in Conway medium (mg) and total DIN content measured in the system.

Day	Algae harvested (L day^{-1})	Conway medium (ml day^{-1})	DIN content in Conway medium (mg)	Total DIN content in system (mg)
0	0.0	0.0	0.0	-
1	0.0	0.0	0.0	-
2	0.5	3.0	49.2	6.4
3	0.5	0.5	8.2	3.2
4	0.5	0.5	8.2	1.9
5	0.8	0.5	8.2	-
6	1.0	0.8	12.3	1.6
7	1.3	1.0	16.4	-
8	1.3	1.3	20.5	4.2
9	1.3	1.3	20.5	3.8
10	1.5	0.6	10.3	3.2
11	1.5	0.8	12.3	4.2
12	1.5	0.8	12.3	3.3
13	1.5	0.8	12.3	-
Total	13.2	11.6	190.7	

D. Algae density

Figure 32 shows the algae density (cells ml^{-1}) of *R. baltica* in the (A) algae tanks, (B) copepod tanks and (C) biofilters for each day of the experiment. The algae density was relatively stable in the algae tanks, on average ~1.9 million cells ml^{-1}. The algae density in the copepod tanks at day 2 was ~90,000 cell ml^{-1} and steadily increased to ~200,000 cells ml^{-1} at day 4. The algae density drops down to ~80,000 cells ml^{-1} at day 5, and steadily increases again to ~200,000 cells ml^{-1} over the next three days, to day 8. Between day 8 and day 12, the algae density was relatively stable ~200,000 cells ml^{-1}. At day 13, the algae density increases up to ~270,000 cells ml^{-1}. The algae density in the biofilters was relatively stable throughout the experiment and was on average ~85,000 cells ml^{-1}.

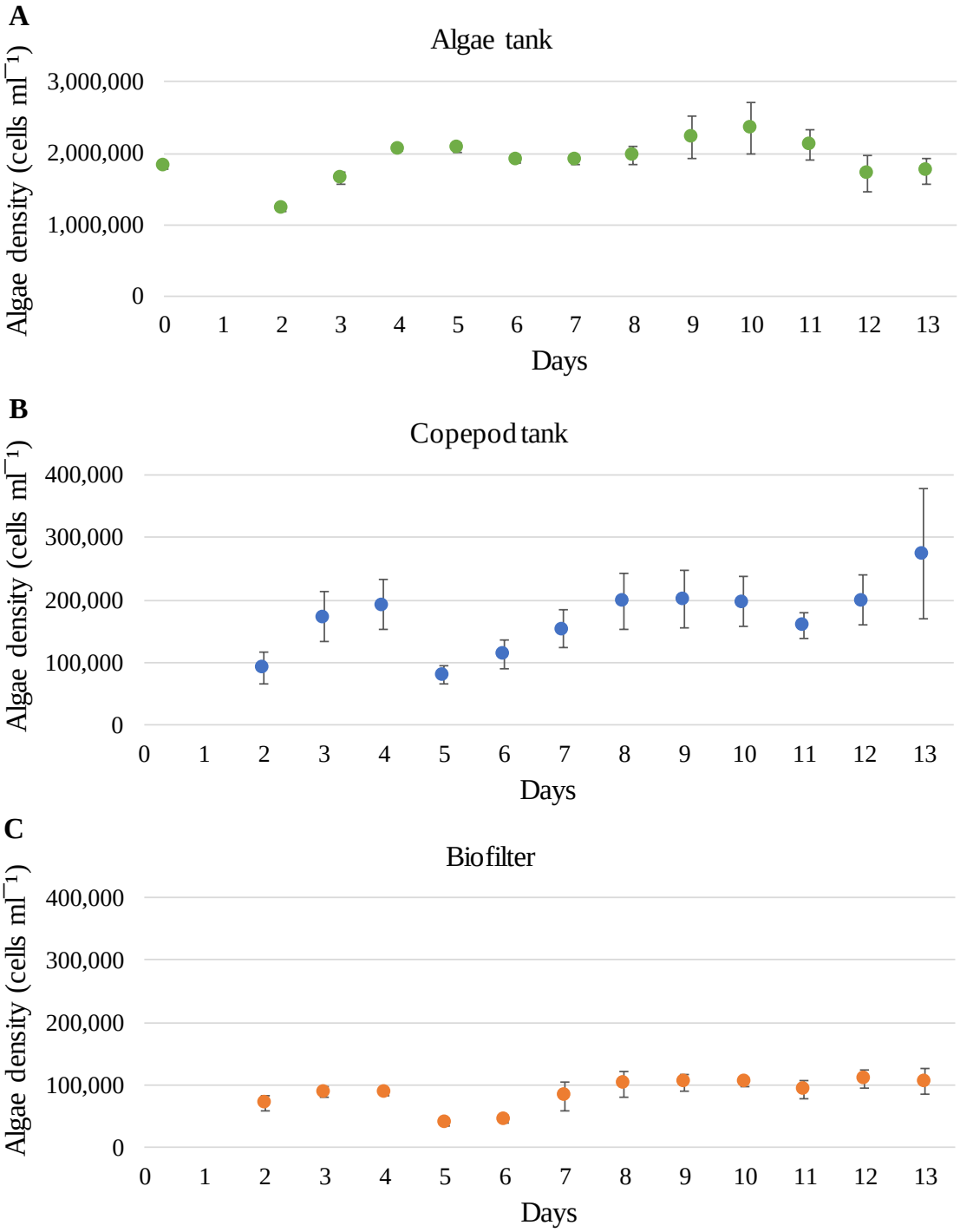

Figure 32 - Algae density (cells ml^{-1}) of *R. baltica* as a function of time in the (A) algae tanks, (B) copepod tanks and (C) biofilters. The values presented are averages from three replicates and error bars present ± SD (n = 3).

E. Nitrate and ammonia

Figure 33 shows the concentrations of nitrate and TAN in mg L^{-1} as a function of time in the (A) algae tanks, (B) copepod tanks and (C) biofilters. TAN concentrations were relatively low in all tanks throughout the experiment and measured on average 0.02, 0.03 and 0.01 mg L^{-1} in the algae tanks, copepod tanks and biofilters, respectively. The nitrate concentrations in the algae tanks at day 2 was on average 8.3 mg L^{-1}, and steadily decreased to 2.1 mg L^{-1} at day 4. Between day 4 and day 8 the nitrate concentration increases up to 6.9 mg L^{-1}. From day 8 to day 12, the nitrate concentration fluctuates between ~5 and ~10 mg L^{-1}. The nitrate concentration in the copepod tanks and biofilters followed similar trends as in the algae tanks, with the exception of having lower concentration from day 8 and 12, between ~3.5 – 5.5 mg L^{-1}.

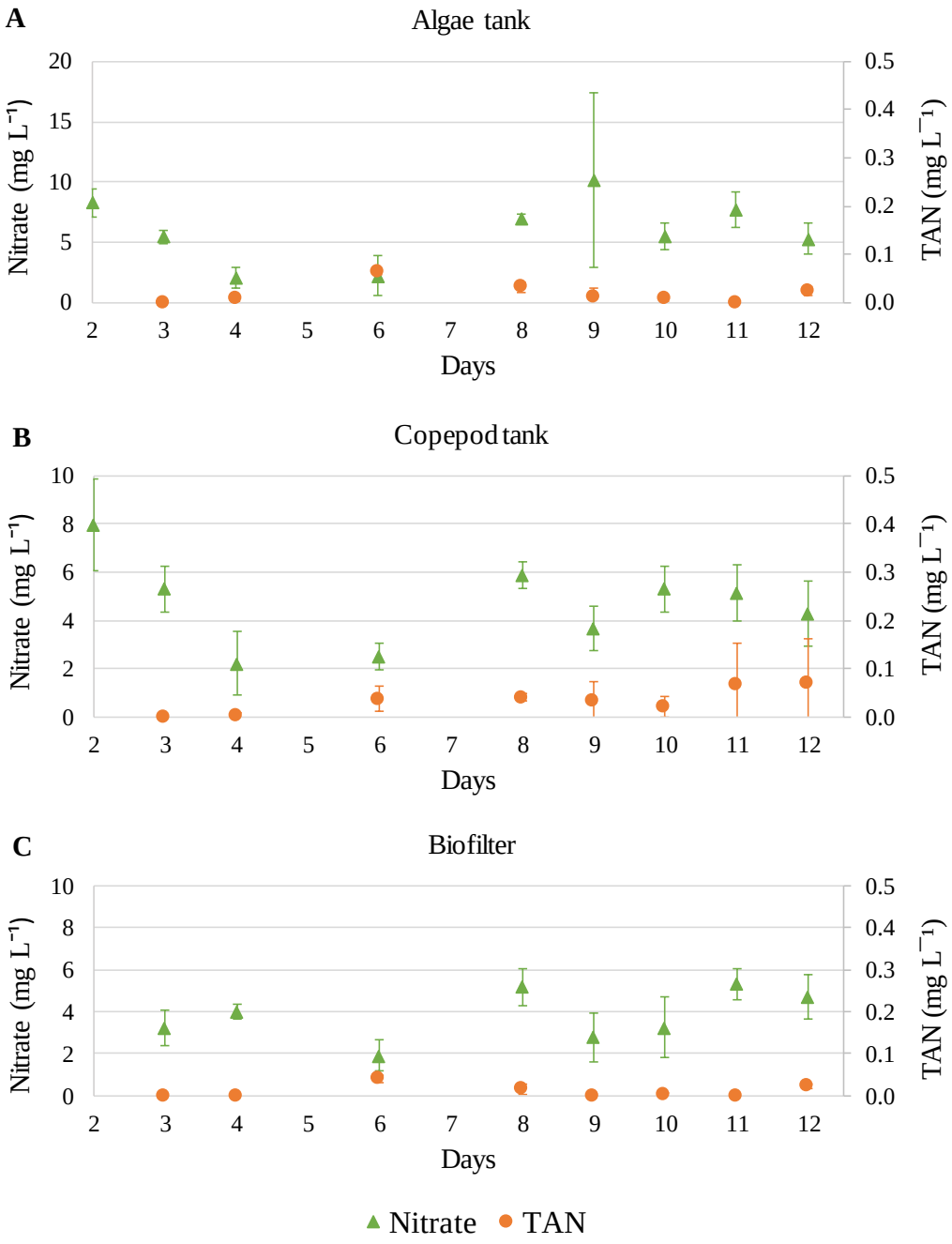

Figure 33 – Concentration of nitrate (mg L^{-1}) and total ammonia nitrogen (TAN) (mg L^{-1}) as a function of time in the (A) algae tanks, (B) copepod tanks and (C) biofilters. The values presented are averages from three replicates and error bars present ± SD (n = 3).

3.2.3 Third experiment – *R. baltica*

The 3rd experiment with *R. baltica* ran for 15 days. At day 8, replicates 2 and 3 were stopped due to loss of copepods and therefore the data after day 8 apply only to replicate 1.

A. Density of *A. tonsa*

Table 14 shows the density (ind. ml^{-1}) of *A. tonsa* reared on *R. baltica* in each copepod tank (CT) on day 1, 8 and 15. At day 1 the copepods were moved from the hatching tanks to the CTs at ~55 ind. ml^{-1}. At day 8, the densities in CT 2 and CT 3 measured zero and no copepods were found alive. At day 15, replicate 1 had a density of 33 ind. ml^{-1}.

Table 14 - Density of *A. tonsa* (ind. ml^{-1}) in each replicate on day 1, 8 and 15. The "-" sign stands for missing data.

	Copepod density (ind. ml^{-1})		
Day	CT 1	CT 2	CT 3
1	55	53	56
8	-	0	0
15	33	-	-

B. Water quality

Table 15 shows the water quality parameters pH, salinity (ppt), oxygen (%) and temperature (°C). All of the parameters were relatively stable throughout the study, seen by the low SD. The pH in the algae tanks and copepod tanks was on average 7.9 and 7.8, respectively. The salinity in the algae tanks and copepod tanks was on average 31.0 and 30.6 ppt, respectively. The oxygen in the copepod tanks was on average 89.5%. The temperature in the algae tanks and copepod tanks was on average 20.8 and 18.1°C, respectively.

Table 15 - Water quality parameters, pH, salinity (ppt), oxygen (%) and temperature (°C) measured in algae tanks and copepod tanks. The values are averages of three replicates throughout the experiment ± SD (n = 3).

	pH	Salinity (ppt)	Oxygen (%)	Temperature (°C)
Algae tanks	7.9 ± 0.2	31.0 ± 0.1		20.8 ± 0.1
Copepod tanks	7.8 ± 0.1	30.6 ± 0.1	89.5 ± 1.2	18.8 ± 0.2

C. Developmental stage distribution

Figure 34 shows the developmental stage distribution of *A. tonsa* at day 15 for replicate 1. Nearly ~60% of the copepods were in stage C4, ~20% in stage C5 and ~10% in stage C3. A fraction of the group was in the nauplii stage, ~2%.

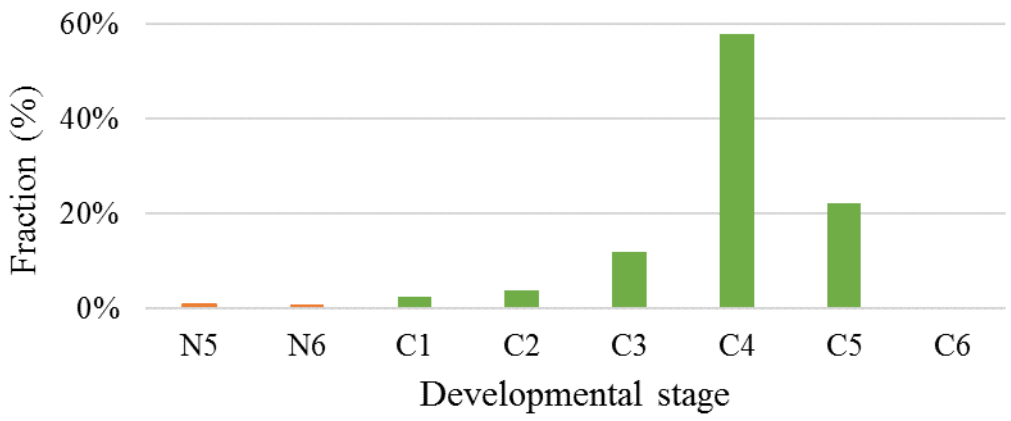

Figure 34 - Developmental stage distribution (%) of *A. tonsa* reared on *R. baltica*, from developmental stage N5 to C6 at day 15 for replicate 1.

D. Harvested algae and addition of Conway medium

Table 16 shows the volume of algae harvested (L day^{-1}), volume of Conway medium added (ml day^{-1}), DIN content in Conway medium (mg) and total DIN content measured in the system for each day of the experiment. At day 8, replicates 2 and 3 were terminated due to loss of copepods and therefore data after day 8 only apply to replicate 1. From day 2 to 7, the volume of algae harvested was increased from 0.5 L day^{-1} up to 2.5 L day^{-1}. From day 9 to day 14, the volume of algae harvested was 2.0 L day^{-1}. In total for replicate 1, 13.5 ml of Conway medium was used, and 21.5 litres of algae were harvested, giving the average Conway medium use 0.63 ml L^{-1}. In total, 221.4 mg of DIN was added to each system with Conway medium and the total DIN in each system measured on average 53.8 mg.

Table 16 – The volume of algae harvested (L day^{-1}), volume of Conway medium added (ml day^{-1}), dissolved inorganic nitrogen (DIN) content in Conway medium (mg) and total DIN content measured in the system.

Day	Algae harvested (L day^{-1})	Conway medium (ml day^{-1})	DIN content in Conway medium (mg)	Total DIN content in system (mg)
2	0.5	0.5	8.2	-
3	0.5	0.5	8.2	29.2
4	1.5	1.0	16.4	-
5	2.0	1.5	24.6	-
6	2.5	2.5	41.0	16.2
7	2.5	2.5	41.0	-
8	0.0	0.0	0.0	38.7
9	2.0	0.0	0.0	48.1
10	2.0	3.0	49.2	104.7
11	2.0	0.0	0.0	85.7
12	2.0	0.0	0.0	-
13	2.0	2.0	32.8	-
14	2.0	0.0	0.0	-
15	-	-	-	97.4
Total	21.5	13.5	221.4	

E. Algae density

Figure 35 shows the algae density (cells ml^{-1}) of *R. baltica* as a function of time in the (A) algae tanks and (B) copepod tanks. At day 8, replicates 2 and 3 were terminated due to loss of copepods and therefore the data after day 8 apply only to replicate 1. Between days 2 and 4, the algae density in the algae tank was ~2.0 million cells ml^{-1}. At day 4, the algae were diluted as the author deemed the algae culture to be entering the death phase, that explains the two data points on day 4, where the lower data point is the density after dilution. From day 4 to day 8, the algae density increases from ~1.0 million cells ml^{-1} up to ~1.6 million cells ml^{-1}. From day 10 to 14, the algae density was stable ~1.0 million cells ml^{-1} but dropped down to ~600,000 cells ml^{-1} on day 15. The algae density in the copepod tanks steadily decreased from ~200,000 cells ml^{-1} at day 2 down to ~20,000 cells ml^{-1} at day 7. At day 8, the algae density increases to ~220,000 cells ml^{-1} with a ± SD of 150,000 cells ml^{-1}. This was due to loss of copepods within two of the replicates, which lead to a build-up of uneaten algae cells. From day 10 to 15, the algae density was very low, between 1,000 to 6,000 cells ml^{-1}.

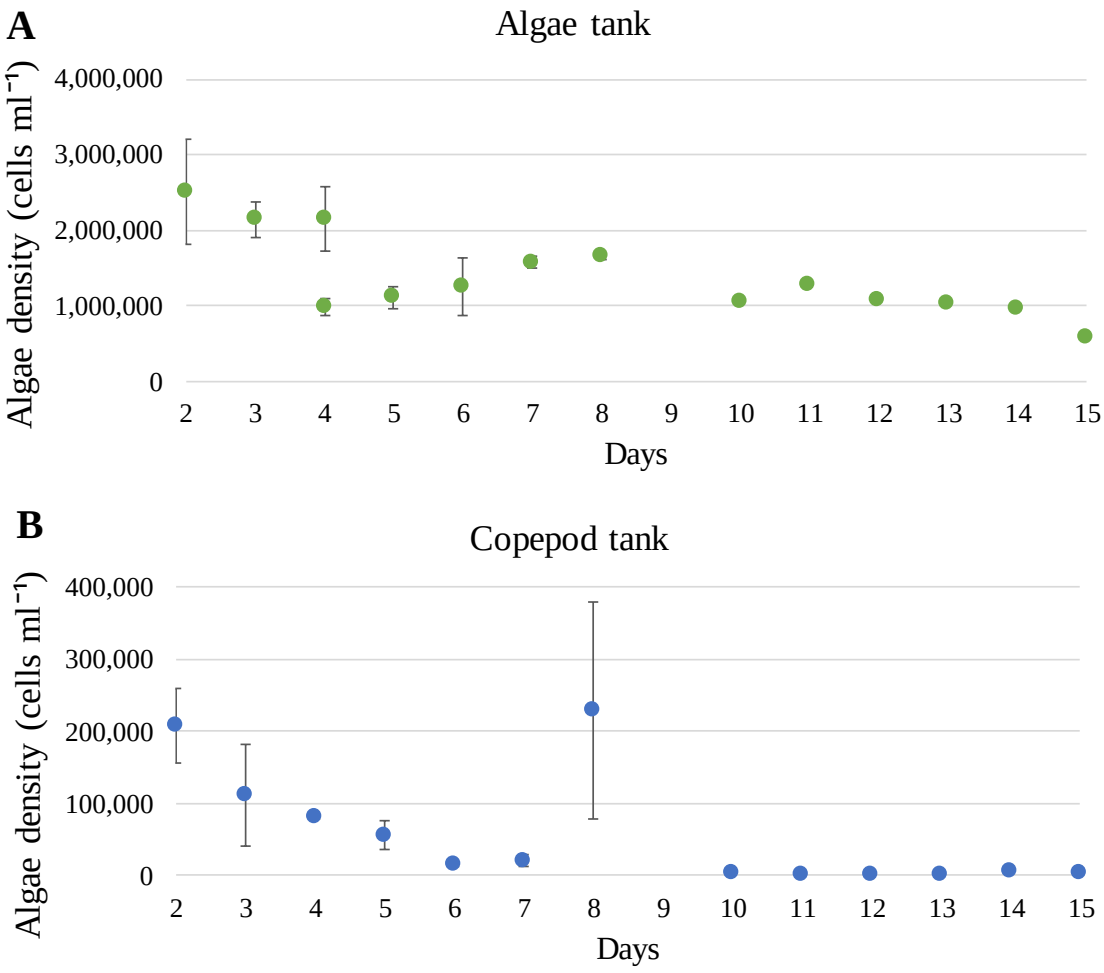

Figure 35 – Algae density (cell ml^{-1}) of *R. baltica* as a function of time in the (A) algae tanks and (B) copepod tanks. The values presented from day 2 to day 8 are averages from three replicates, from day 10 to day 15 the value is from one replicate, error bars present ± SD (n = 3).

F. Nitrate and ammonia

Figure 36 shows the concentration of nitrate and TAN in mg L^{-1} as a function of time. At day 8, replicates 2 and 3 were stopped due to loss of copepods and therefore the data after day 8 apply only to replicate 1. The TAN concentration measured relatively low throughout the study, on average 0.01, 0.30 and 0.32 mg L^{-1} in the algae tanks, copepod tanks and biofilter, respectively. The TAN concentration in measured highest on day 8, on average 0.58 and 0.32 mg L^{-1} in the copepod tanks and biofilters, respectively.

The nitrate concentration in the algae tanks was relatively low from day 3 to day 8, measuring on average 2.7 mg L^{-1}. At day 10, the nitrate concentration spikes up to ~22 mg L^{-1} and decrease down to 7.1 mg L^{-1} at day 11. At day 15, the nitrate concentration measured 0.9 mg L^{-1}. The nitrate concentration in the copepod tanks measured 0.87 mg L^{-1} at day 3, and 0.40 mg L^{-1} on day 6. From day 6 to day 15, the nitrate concentration steadily increases on average by 1.8 mg

L^{-1} each day, reaching a maximum nitrate concentration on day 15, 17.7 mg L^{-1}. The nitrate concentration in the biofilters followed the same trend as in the copepod tanks, however the concentration was overall higher, and measured highest 17.3 mg L^{-1} on day 15.

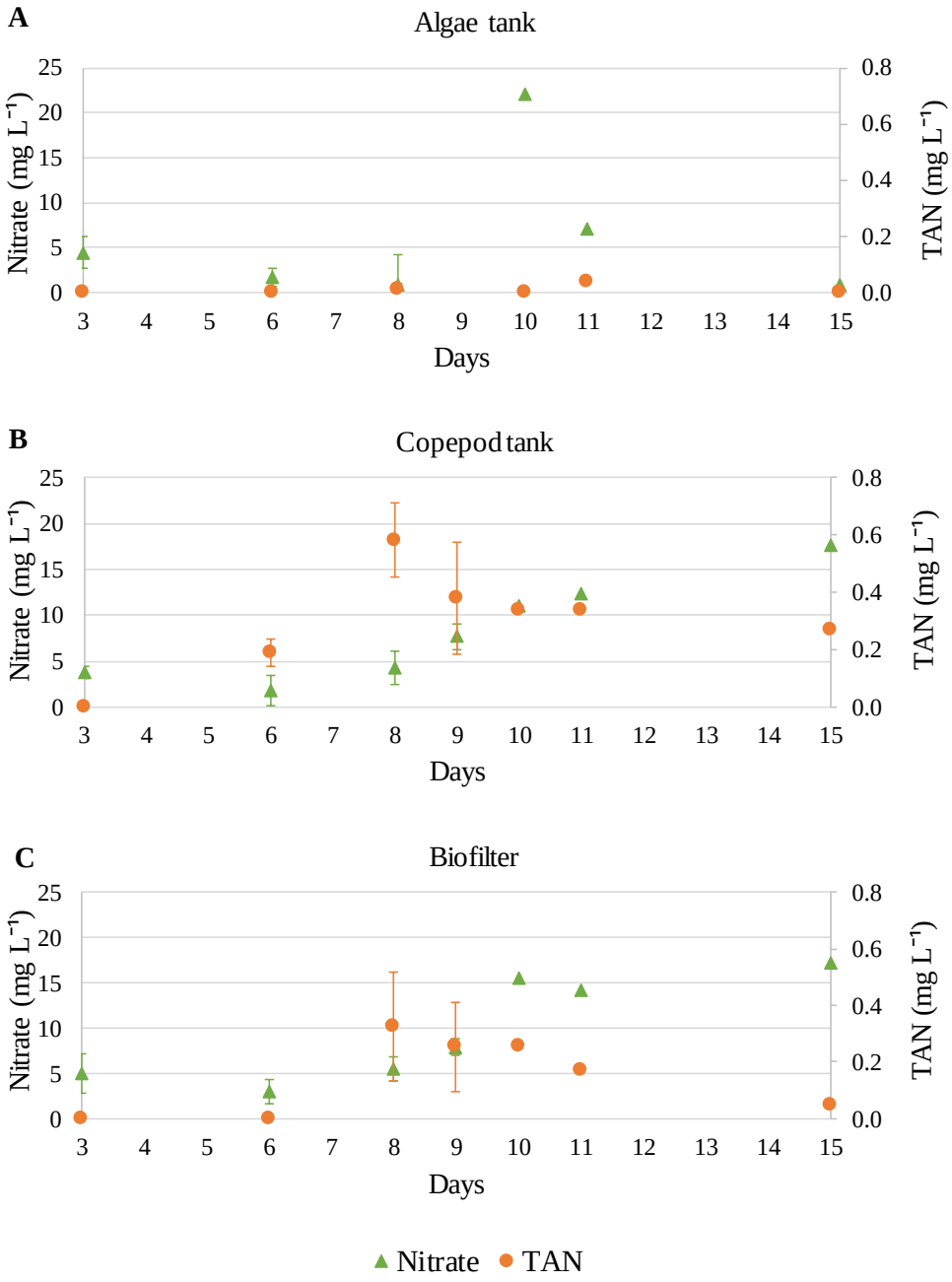

Figure 36 – Concentration of nitrate (mg L^{-1}) and total ammonia nitrogen (TAN) (mg L^{-1}) as a function of time in (A) algae tanks, (B) copepod tanks and (C) biofilters. The values presented from day 3 to day 9 are averages from three replicates, from day 10 to day 15 the value is from one replicate, error bars present ± SD (n = 3).

Figure 37 shows the concentration of unionized ammonia (NH_3) in µg L^{-1} for each CT. The NH_3 concentration steadily increases in all replicates from day 3 to day 8. At day 8, the NH_3 concentration measured 9.9, 15.8 and 11.6 µg L^{-1} for CT1, CT2 and CT3, respectively. At day 9, CT1 has two data points, the author first measured 16.3 µg L^{-1} and then lowered the pH level, which lowered the NH_3 concentration down to 11.1 µg L^{-1}. From day 10 to 15, the NH_3 was relatively stable and measured on average 6.0 µg L^{-1}.

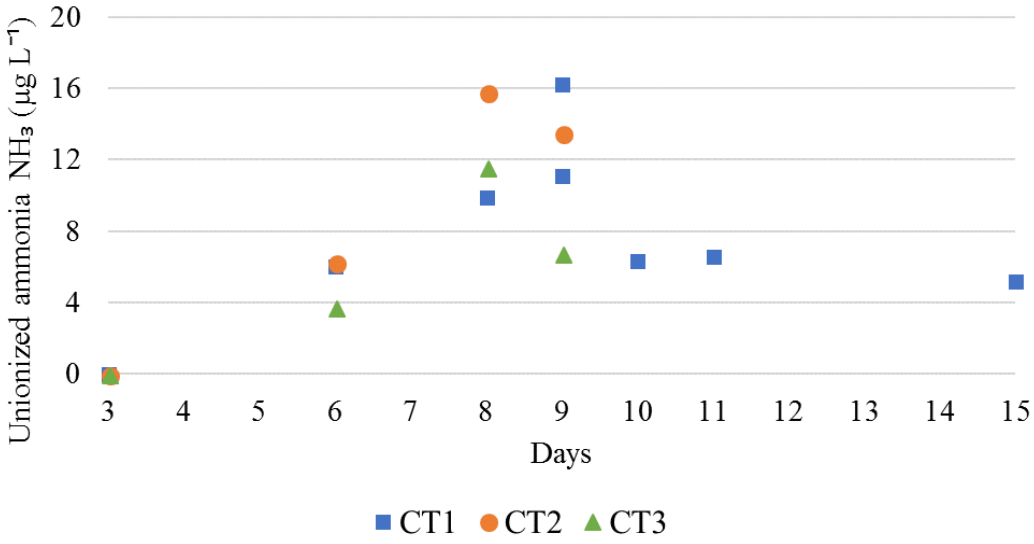

Figure 37 – Concentration of unionized ammonia (NH_3) (µg L^{-1}) as a function of time in the copepod tanks (CT). The values presented are from each replicate from day 3 to day 9, and from day 10 to day 15 the values are from one replicate.

4. Discussion

4.1 Nutritional value of *A. tonsa*

Density, survival, and biomass growth

The cultures of *A. tonsa* showed a steady mortality rate when reared on *R. baltica* 5.9% ± 0.9% day^{-1}, *T. lutea* 5.6% ± 0.3% day^{-1} and *C. weissflogii* 6.5% ± 1.6% day^{-1}. Similar mortality rates have been previously reported, 4% - 13% day^{-1} (Støttrup et al., 1986, Medina and Barata, 2004, Drillet et al., 2006, Drillet et al., 2008, Skogstad, 2010).

The copepod tanks were never siphoned to collect the debris from the bottom of the tanks during the study and this might have affected the water quality. Although the copepod tanks had high water exchange (100% day^{-1}) it is possible that the collected debris might have affected the growth and survival of the copepods.

The length and weight in carbon content of *A. tonsa* reared on all three diets showed a steady increase from day 2 to day 7 and 8. *A. tonsa* reared on *R. baltica* and *T. lutea* showed a weight increase in exponential regression and length increase in linear regression. *A. tonsa* reared on *C. weissflogii* did not follow those trends and had significantly lower growth than *A. tonsa* reared on *R. baltica* and *T. lutea*. *A. tonsa* reared on *C. weissflogii* showed little increase in both weight and length from day 8 to day 13, indicating that the culture conditions were not optimal. The culture conditions of *A. tonsa* in all three diets were the same, except for the diet itself. It is therefore likely that the reason for different growth and survival was because of the microalgae, *C. weissflogii*.

The microalgae species. *R. baltica* and *T. lutea* both have flagellates and were mobile while *C. weissflogii* was a diatom and only moves with the water current. This could have affected the suitability of the algae as diet for *A. tonsa*. There was also a size difference between the species, the diameter of *R. baltica* measured between 5.5 – 9.5 µm, on average 7.0 µm, *T. lutea* measured between 3.5 – 6.0 µm, on average 4.3 µm, and *C. weissflogii* measured between 8.5 – 15.0 µm, on average 11.0 µm. As *C. weissflogii* was bigger and non-mobile it sedimented faster than the other species, possibly making it less available to the copepods. Furthermore, it is also possible that the biggest cells of *C. weissflogii* were too large for *A. tonsa* to capture. Berggreen et al. (1988) looked at the food size spectra and growth of *A. tonsa* and concluded that the upper food size limit increased with development, 10 to 14 µm for NII to NIII, and 250 µm for adults. In this study *C. weissflogii* measured up to 15.0 µm in diameter, indicating that they were too large for *A. tonsa* nauplii. However, *A. tonsa* has been reared successfully on *C. weissflogii*, giving normal growth and development rates (Roman, 1984, Støttrup et al., 1999, Ismar et al., 2008). Ismar et al. (2008) showed that *C. weissflogii* gave high naupliar survival and fast development, but slow copepodid development. In this study, *C. weissflogii* gave high naupliar development, but low survival rate. Few copepodid were seen in the end of the study, indicating that *C. weissflogii* did not support copepodid development.

The SGR of *A. tonsa* in the study was between 0.09 – 0.33 day^{-1}, depending on the diet and the time period calculated from. The SGR for *A. tonsa* reared on *R. baltica* and *T. lutea* calculated from day 7 to 13 was ~33 day^{-1}, almost double the SGR calculated from day 2 to 7, ~15 day^{-1}, indicating faster growth between the days 7 and 13. For *A. tonsa* reared on *C. weissflogii* the SGR calculate from day 2 to 7 was ~15 day^{-1}, which was nearly double the SGR from day 7 to 13, ~0.09 day^{-1}, indicating poor growth between days 7 to 13.

The SGR calculated from the whole period was 0.24, 0.24 and 0.12 day^{-1} for treatments *R. baltica*, *T. lutea*, *C. weissflogii*, respectively. Berggreen et al. (1988) and Skogstad (2010) reported higher SGR from 0.45 – 0.51 day^{-1}, which was nearly double compared to this study. Jepsen et al. (2017) had similar finding as in this study, SGR 0.19 – 0.20 day^{-1} at full saturation levels, the low SGR was speculated to be due to an airlift in the cultivation units to keep the algae suspended which disrupted the growth of the copepods. This might also have been the case in this study, aeration was added to the tanks to keep the algae suspended and mixed.

Carbon analysis

The carbon content was analysed as well as calculated from the length of the copepods. The carbon results from the length calculations and carbon analysis differ quite a lot and were compared in Appendix II. The results from the length calculations were 2-3 times lower than from the carbon analysis at days 7 and 8, however at day 13 the difference was not as significant. This difference might be due to the sample size in the carbon analysis, where average number of copepods were 12 for each sample. Using only 12 copepods to represent the whole population might not give accurate results, whereas the number used in the length calculation were between 70-140 copepods and might give more accurate results. Both results were presented as they both give information of the growth and nutritional content.

The carbon content of *A. tonsa* has been reported in the literature (Jones et al., 2002, Saba et al., 2009, Saba et al., 2011) and was compared to the results from this study in Appendix II. Similar results of carbon content were found in this study as in the literature when developmental stages were considered. The other studies mainly report numbers from adults, copepodid or nauplii but not individual stages. When the first samples were taken for carbon analysis, at day 7 and 8, the main stages were N6 and C1 and the average carbon content from all treatments was 0.53 µg C $ind.^{-1}$, not unlike what Jones et al. (2002) reported 0.88 µg C $ind.^{-1}$ for copepodid stages. The diets of *R. baltica* and *T. lutea* resulted in similar stages at day 13, mainly C4 and C5, and the average carbon content was 1.64 µg C $ind.^{-1}$, which was between the numbers of adult and copepodid that Jones et al. (2002) and Saba et al. (2009) reported, although Saba et al. (2011) reported numbers up to 5.9 µg C $ind.^{-1}$ for adults.

Developmental stage distribution

The developmental stage distribution at day 7 and 8, in all diets, had above 50% copepodid stage. *A. tonsa* reared on *R. baltica* developed fastest to the copepodid stage, at day 7, which was one day ahead *A. tonsa* reared on *T. lutea* and *C. weissflogii* that developed to copepodid at day 8.

The developmental time for *A. tonsa* reared on *R. baltica*, *T. lutea* and *C. weissflogii* was 0.81, 0.81 and 0.48 stage day^{-1}, respectively. The developmental time on *A. tonsa* reared on *R. baltica* and *T. lutea* were similar to developmental time found by other studies, 0.8 - 0.9 stage day^{-1} (Berggreen et al., 1988, Leandro et al., 2006, Skogstad, 2010). It is interesting to note that Medina and Barata (2004) showed that the developmental time was significantly delayed depending on densities, at day 12 post egg incubation, the densities 5, 10 and 20 ind. ml^{-1} had the mean percentages of adults of 93%, 66% and 10%, respectively. In this study the density if ~50 ind. ml^{-1} was used, which might have affected the developmental time to some extent.

In cultures of *A. tonsa* reared on *C. weissflogii* the fraction of nauplii increases from day 8 to day 13. It is not probable that the number of nauplii increased, one explanation might be selective mortality, the survival rate dropped from ~38% to ~29% from day 8 to day 13, and the majority of dying copepods were in the copepodid stage, bringing the ratio of nauplii up. Another reason might be that unhatched eggs were in the copepods tanks and had delayed hatching, increasing the number of nauplii. However, a precaution was taken not take any unhatched eggs when the nauplii were moved from the hatching tank to copepod tanks. The eggs were allowed to settle on the bottom of the hatching tank and the nauplii were siphoned from the top. It is therefore unlikely that any unhatched eggs were in the copepod tanks. This change in nauplii ratio was seen in all the replicates of *C. weissflogii*. A study by Carotenuto et al. (2002) concluded that the copepod *Temora stylifera* was not able to develop to adult stage on diatoms, dying without passing the nauplii stage or on early copepodid stage, not unlike as in this study.

Total lipids and fatty acid composition

A significant difference was found between the total lipids (TL) content of the microalgae species *R. baltica*, *T. lutea* and *C. weissflogii*, ~11%, ~29% and 17%, respectively. Similar results of TL content for the microalgae species has been found in the literature and were compared in Appendix II. The microalgae *T. lutea* had nearly three times more TL than *R. baltica* and when *A. tonsa* reared on *R. baltica* and *T. lutea* at day 7 and 8 were compared, they had a TL content of ~16% and ~20%, respectively. This suggests that the TL of *A. tonsa* at day 7 and 8 stabilizes around 16 - 20% when fed full saturation levels. At day 13, *A. tonsa* reared on *R. baltica* and *T. lutea* have decreased their TL down to ~11% and ~15%, respectively, lowering the TL content by 5% in both diets. The TL content of *Acartia sp*. has been reported between 6 – 24% (McKinnon et al., 2003, Barroso et al., 2013, Betancor et al., 2017b, Betancor et al., 2017a) which was within the findings of this study.

The fatty acid (FA) composition varied markedly between the three microalgae species. *T. lutea* had noticeably the highest content in most groups, however lacked EPA, giving a high DHA/EPA ratio of ~27. *R. baltica* had low contents of SFAs and MUFAs but high content of PUFAs and a DHA/EPA ratio of ~1. *C. weissflogii* also had low contents of SFAs and MUFAs and high content of PUFAs, but had higher content of EPA than DHA, giving a very low DHA/EPA ratio of ~0.2. Similar fatty acid composition of the three microalgae species has been found in other studies (Kurmaly et al., 1989, Arendt et al., 2005, Pratoomyot et al., 2005, Huerlimann et al., 2010) and comparison from their results and from this study can be found in Appendix II.

When the FA composition of *A. tonsa* reared on the three microalgae species was compared, it shows that it was flexible and can be manipulated through the diet. The results clearly show that there was a correlation between the FA composition of the microalgae and *A. tonsa* reared on them. This has also been demonstrated by Støttrup et al. (1999) where *A. tonsa* was cultivated with four different monoalgal diets; *R. baltica*, *Isochrysis galbana*, *Heterocapsa triquetra* and *C. weissflogii*. The results of FA composition from this study and from Støttrup et al. (1999) were compared and can be found in Appendix II.

The highest content of DHA was measured in *A. tonsa* reared on *T. lutea* at day 8 (5.7 µg mg^{-1} DW), EPA measured highest in *A. tonsa* reared on *C. weissflogii* at day 13 (5.2 µg mg^{-1} DW) and ARA measured highest in treatment *R. baltica* at day 7 (0.7 µg mg^{-1} DW). However, research has shown that the ratio of FAs was more important for growth and development than the individual FA contents (Rodriguez et al., 1997, Sargent et al., 1999). Better growth, survival and development was yielded by using live prey with the similar ratios as was found in the yolk sack or eggs of marine fish larvae, DHA/EPA ratio of ~2 and EPA/ARA ratio of 4-8 (Rodriguez et al., 1997, Sargent et al., 1999).

The most similar ratios as in the marine fish eggs, ex. cod and halibut eggs, was found in *A. tonsa* reared on *R. baltica* at day 13, where the DHA/EPA ratio was ~2 and EPA/ARA ratio was ~6. *A. tonsa* reared on *T. lutea* had much higher DHA/EPA ratio of ~10 due to low EPA contents and EPA/ARA ratio of 2 - 4. At day 13, *A. tonsa* reared on *C. weissflogii* had high contents of DHA (3.5 mg g^{-1} DW) and EPA (5.2 mg g^{-1} DW), giving a low DHA/EPA ratio of ~0.7 and only trace contents of ARA were measured (0.02 mg g^{-1} DW) resulting in a high EPA/ARA ratio of ~230. Results from FA composition of the microalgae *C. weissflogii* show that no content of ARA was measured, which evidentially explains the low levels of ARA measured in *A. tonsa* reared on *C. weissflogii*. This was different from what Støttrup et al. (1999) found, where both the nauplii and adult *A. tonsa* reared on *C. weissflogii* had relatively high contents of ARA. This difference in ARA content of *C. weissflogii* could be due to the culture conditions of the microalgae.

Although the FA composition of the copepods and the microalgae were similar, they were not the same. *A. tonsa* accumulates or metabolises different FAs and the contents also change depending on the developmental stage. In *A. tonsa* reared on *R. baltica* it was noticeable that SFAs accumulate over time, but the MUFAs and PUFAs were relatively stable. The HUFAs,

DHA, EPA and ARA have accumulated at day 7 and at day 13, EPA and ARA have decreased. This could be an indication that *A. tonsa* uses EPA and ARA for their development between day 7 and 13.

A. tonsa reared on *T. lutea* had accumulated FAs at day 8, mainly SFAs, PUFAs, and DHA and small contents of EPA and ARA. Between day 8 and day 13, the content of SFAs, MUFAs and PUFAs lowers, but the DHA and EPA contents stay the same and only trace contents of ARA were measured. This could be an indication that *A. tonsa* used SFAs, MUFAs and PUFAs for their development between day 8 and 13.

A. tonsa reared on *C. weissflogii* had at day 13 accumulated FAs in all groups, SFAs, MUFAs, and PUFAs. DHA accumulated to higher levels than found in *C. weissflogii*, but the content of EPA was similar as in *C. weissflogii*. In this study, the microalgae *C. weissflogii* produced no, or only traces of ARA. However, diatom often have high percentage of ARA (up to 4%) (Guedes and Malcata, 2012), and literature has shown ARA levels for *C. weissflogii* of 0.1 – 0.8% of total FAs (Pratoomyot et al., 2005, Arendt et al., 2005). The lack of ARA in the diet of *A. tonsa* might have caused the low development and survival, but ARA has shown to be essential for the development of many species (Sargent et al., 1993, Sargent et al., 1997).

The results show that *A. tonsa* will accumulate the HUFAs, especially the DHA and EPA if the microalgae contain them. The DHA and EPA accumulated up to 4 times of the concentration that was found in the microalgae. When the PUFAs were compared in all treatments at day 13, the contents were similar ~8 - 10 µg mg^{-1} DW, and also the DHA content 3.5 - 5.5 µg mg^{-1} DW. This gives the assumption that *A. tonsa* accumulates PUFAs and DHA to a certain content but metabolizes them if they exceed certain concentration.

The results show that at day 13, *A. tonsa* reared on *R. baltica* gave high survival and growth rate and had the most suitable DHA/EPA and EPA/ARA ratios required by marine fish larvae. The author therefor recommends using *R. baltica* as a feed for *A. tonsa* and harvesting at day 13. In future studies, it might be interesting to look at the FA composition of more developmental stages and diets with more than one microalgae species. By doing so, it would be possible to tailor the FA composition of *A. tonsa* for optimum nutrition for marine fish larvae

4.2 Copeponics

The purpose of running a copeponics system was to reduce nutrients and water required to cultivate copepods. A number of problems were encountered with the cultivation of microalgae and copepods in the system and here the main problems will be discussed.

Hydrogen sulphide and aeration

The 1st experiment with *R. baltica* ran for 9 days and had to be terminated due to loss of copepods in all replicates. A build-up of organic matter in replicate 3 lead to an oxygen deprived layer on the bottom of the copepod tank. It has been long known that some bacteria species can adjust themselves to anaerobic conditions to use naturally found sulphide (S^{-2}) in the water instead of oxygen (O_2), and give off hydrogen sulphide (H_2S) as a metabolite (Elion, 1927). Hydrogen sulphide is toxic to most aerobic organisms and therefore not desirable in nearly all cultivation systems. A review of toxicity of hydrogen sulphite was done by Wang and Chapman (1999), for further information. It is therefore speculated that hydrogen sulphite was the reason for death of the copepods in replicate 3, and this was supported by a strong egg rotten smell which hydrogen sulphite gives off.

To prevent too much build-up of organic matter on the bottom of the copepod tanks, aeration was increased in replicates 1 and 2. This increase in aeration created strong water currents within the copepod tanks, which likely slammed the copepods to the walls of the tank, disintegrating the copepods.

From this the author recommends that the cultivation tanks to be siphoned regularly to prevent the build-up of organic matter and have moderate aeration, such to not create too strong water current within the cultivation tank.

Contamination in algae cultures

In the experiments with *C. weissflogii* and *T. lutea*, the algae cultures got contaminated with other organisms which affected the microalgae growth in a negative way. In the 1st experiment with *T. lutea*, an unidentified heterotrophic flagellate was found in the culture. In the 1st experiment with *C. weissflogii* the stock culture was contaminated with *R. baltica* which overtook the culture as it had superior growth rate. In the 2nd experiment with *C. weissflogii* the culture got contaminated of an unidentified ciliate species. Ciliates naturally graze upon microalgae in nature (Rosetta and McManus, 2003) and can often be problematic in mass cultivation of microalgae (Wang et al., 2013).

There were two main possibilities where the contamination in the algae cultures came from, either from poor handling of equipment or from inadequate filtration from the copepod tanks to the algae tanks. In the first five experiments, a smaller filter (Acro 50 vent filter, VWR, USA) was used between the copepod tank and the algae tank, which had lower filtration area and a

pore size of 1 µm. The author believed this filter to have been too small and was changed out for the sixth experiment, or 3rd experiment with *R. baltica*, for two filters as described in the materials and methods. However, the new filters and improved handling of equipment kept the algae culture clear of contamination in the last experiment only until day 10, where an unidentified organism was spotted.

To keep algae cultures from getting contaminated, the author recommends excessive caution when handling equipment used for the algae cultures. Choosing filters with adequate pore size and filtration area was critical for keeping other organisms and debris from entering the algae cultures and contaminating them.

Toxic ammonia

In the 3rd experiment with *R. baltica*, higher levels of TAN were measured within the systems than in previous experiments. This was possibly the reason for the low survival in two of the replicates, but density measurement at day 8 for replicate 2 and 3 showed 0 ind. ml^{-1}.

Dissolved TAN in aqueous solution takes on two forms, ammonium ion (NH_4^+) and unionized ammonium (NH_3). The relative content of the two forms is controlled by pH and temperature (Erickson, 1985). The unionized form is more toxic as it is uncharged and lipid soluble, and therefore crosses biological membranes more readily than the charged NH_4^+ (Milne et al., 1958), and many toxicity studies only refer to the contents of NH_3.

In this study the maximum content of NH_3 recorded in each replicate was 16.3, 15.8 and 11.6 µg NH_3 L^{-1}, in replicate 1, 2 and 3, respectively. The survival in replicates 2 and 3 was very low, most likely due to the levels of NH_3 content and long exposure time. However, in replicate 1 the maximum content recorded of NH_3 was only exposed to the copepods for a few hour period as the author lowered the pH level, bringing the content down to 11.1 µg NH_3 L^{-1}. Vu Thi Thuy (2011) showed a maximum content of 10 µg NH_3 L^{-1} for *A. tonsa* in both an FTS and a RAS, however that level of NH_3 did not seem to be affecting the mortality negatively. Other studies have indicated higher NH_3 tolerance for *A. tonsa* (Sullivan and Ritacco, 1985, Jepsen et al., 2015). A recent research by Jepsen et al. (2015) concluded that the No Observed Effect Concentration for *A. tonsa* nauplii was 30 µg NH_3 L^{-1} and for adult it was 477 µg NH_3 L^{-1}. This suggests that the levels of NH_3 measured in this study were not lethal for *A. tonsa* and the mortality was due another factor.

Another factor that might have affected the survival rate within the replicates was the difference in temperature, the average temperature was 19.4, 18.3 and 18.3 in replicate 1, 2 and 3, respectively. This was due to the system setup where replicate 1 was closest to the lights for the algae tanks, which evidently increased the temperature by ~1°C. As *A. tonsa* was cultivated with a higher temperature in replicate 1, the copepods probably grew a bit faster than in the other two replicates, resulting in a further developed copepods. The results from Jepsen et al. (2015) indicate that further developed life stages of *A. tonsa* tolerate higher contents of NH_3, which might also explain the high survival rate in replicate 1 compared to replicate 2 and 3.

The developmental stage distribution for replicate 1 at day 15 showed that most of the copepods were in stage C4, which was a lower developmental and growth rate than recorded in other studies (Berggreen et al., 1988, Leandro et al., 2006, Skogstad, 2010). At least two explanations were possible for the slower growth in this study, the copepods were not fed enough and/or levels of NH_3 affected the growth negatively. On average, the copepods were fed 200,000 cells ml^{-1} per day of *R. baltica*, which was well above the saturation levels found by Berggreen et al. (1988), indicating that a shortage of food was not an issue. That leaves the question if the NH_3 content affected the growth rate. There were none researches on the long-term effect of low level exposure of NH_3 on *A. tonsa* and NH_3 is mainly toxic in elevated concentration (Ip et al., 2001), but the low levels measured might have affected the growth rate negatively.

Reduction of nutrients and water

The main purposes of running a copeponics system was to reduce the nutrients and water needed to cultivate copepods. The average water input into the copeponics system was 1.1 L day^{-1}, and when compared to an FTS that would require ~11-12 L day^{-1}, the water consumption was reduced by ~90%. The lowest consumption of Conway medium was 0.63 ml L^{-1}, suggesting that the nutrient consumption was reduced by ~53% when compared to 1.2 ml L^{-1} as normal use.

In this study, the concentration of DIN (nitrate and ammonia) were followed, however those results on their own only show part of the picture. The DIN supplied from Conway medium and total DIN measured in the system suggests that most of the nitrogen was taken up by organisms. The DIN measured in the system gives an indication of how much of the nitrogen is cycled through the system and how much reduction of Conway medium is possible. The author recommends looking at a nitrogen budget for the system, as well as for other nutrients such as phosphor and other trace nutrients required by the microalgae. Those nutrient budgets would be give valuable information for further optimization of the system.

Copeponics system design

One of the biggest drawback of RAS when compared to FTS is the increased energy consumption (Aubin et al., 2006, Colt et al., 2008, d'Orbcastel et al., 2009), which is due to increased use of electrical equipment needed for the treatment and circulation of water. The design of the system also plays a big role in the energy consumption, for example pumps require more energy as more pressure, or the pumping height, increases (Badiola et al., 2018). Therefore the height and placement of equipment is important when designing a RAS (Badiola et al., 2018).

The copeponics system was built with simplicity in mind, with as few components as possible. By having fewer components in a system the probability of a failure was lower which decreases maintenance and work behind running the system. The tanks were placed with minimal

pumping height in mind to decrease the energy output of the pumps. The reason for using overflows in the system was to simplify it and no pumping was required. One of the biggest drawback of the systems were the lights for the algae cultures which were lit up with fluorescent tubes that were relatively high energy demanding when compared to light emitting diodes (LEDs) (Nardelli et al., 2017), which could have been used instead.

In future research the author recommends using white copepod tanks as it increases visibility and makes cleaning the tanks easier. He also recommends sizing the equipment to fit into washing machines, to make cleaning easier and more efficient, especially between experiments. Choosing the right filters, both in filter area and pore size, was critical for the function of a copeponics system as a contaminated algae culture will decrease the function of the system. The feeding regime should be adjusted to the copepod biomass and overfeeding should be kept to a minimal to avoid producing excessive nitrogenous waste.

When upscaling the copeponics system for the use of fish farmers, the author recommends using multiple tanks for each compartment; algae tank, copepod tank and biofilter. By doing so, it decreases the risk of a total system crash by having available units that can be used in case of contamination or other incidents. To get the most out of a copeponics system, water exchange rate should be kept to a minimal rate, and the water should not be switched between copepod generations. By doing so, the nitrogen within the system does not get lost and can be taken up the by microalgae.

5. Conclusion

In conclusion, the study of the nutritional value of *A. tonsa* showed that it was possible to manipulate the fatty acid composition of *A. tonsa* through the diet. The results clearly show a correlation between the FA composition of the microalgae and *A. tonsa* reared on them. The survival and growth rate of *A. tonsa* was highest when reared on *R. baltica* and *T. lutea*. At day 13, the fatty acid composition of *A. tonsa* reared on *R. baltica* resulted in the most suitable HUFA ratio of DHA/EPA (~2) and EPA/ARA (~6) for marine fish larvae. From these results, the author recommends using *R. baltica* as a feed for *A. tonsa* and harvesting at day 13. In future studies, it might be interesting to look at the FA composition of more developmental stages and diets combined of more than one microalgae species. By doing so, it would be possible to tailor the FA composition of *A. tonsa* for optimum nutrition for marine fish larvae.

The copeponics system functioned when using the microalgae *R. baltica* and the results give strong indication that it was possible to reduce the water and nutrient consumption by ~90% and ~53%, respectively, compared to a traditional FTS. However, the system did not perform well while using the microalgae *C. weissflogii* and *T. lutea*. In future studies, creating a nitrogen budget, as well as a budget for other nutrients, would give valuable information for further optimization of the system.

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Appendix I

Table 17 – The concentration (µg mg-1 DW and % of total FAs) of fatty acids in the microalgae *R. baltica* and in *A. tonsa* reared on *R. baltica* at day 7 and day 13 in and % of total fatty acids. The values presented are averages from three replicates (n = 3).

	Microalgae	*A. tonsa* reared on *R. baltica* (mg g^{-1} DW)			Microalgae	*A. tonsa* reared on *R. baltica* (% of total FAs)	
	R. baltica	**Day 7**	**Day 13**		***R. baltica***	**Day 7**	**Day 13**
C14:0	0.0	0.9	0.5	**C14:0**	0%	10%	4%
C16:0	1.2	0.0	1.5	**C16:0**	13%	0%	13%
C17:0	0.0	0.0	0.3	**C17:0**	0%	0%	3%
C18:0	0.0	0.9	0.6	**C18:0**	0%	10%	5%
C20:0	0.0	0.0	0.6	**C20:0**	0%	0%	5%
C22:0	0.0	0.1	0.0	**C22:0**	0%	1%	0%
Total SFAs	1.2	1.9	3.4	**Total SFAs**	13%	21%	30%
C14:1n5	0.0	0.0	0.0	**C14:1n5**	0%	0%	0%
C16:1n7	0.0	0.0	0.0	**C16:1n7**	0%	0%	0%
C18:1n9	0.0	0.1	0.0	**C18:1n9**	0%	1%	0%
C18:1n7	0.3	0.4	0.1	**C18:1n7**	3%	5%	1%
C20: 1n9	0.0	0.0	0.0	**C20: 1n9**	0%	0%	0%
Total MUFAs	0.3	0.5	0.1	**Total MUFAs**	3%	6%	1%
C18:2n6	2.5	0.0	0.7	**C18:2n6**	26%	0%	6%
C20:2n6	0.0	0.0	0.1	**C20:2n6**	0%	0%	1%
C20:4n6	0.3	0.7	0.3	**C20:4n6**	3%	8%	2%
Total n-6	2.9	0.7	1.1	**Total n-6**	30%	8%	10%
C18:3n3	3.3	0.0	0.9	**C18:3n3**	34%	0%	8%
C20:5n3	1.0	2.5	1.8	**C20:5n3**	10%	27%	16%
C22:6n3	1.0	3.5	3.8	**C22:6n3**	10%	39%	34%
Total n-3	5.2	6.0	6.6	**Total n-3**	54%	66%	59%
Total PUFAs	8.1	6.7	7.7	**Total PUFAs**	84%	73%	69%
DHA/EPA	1.0	1.4	2.2	**DHA/EPA**	1.0	1.4	2.2
EPA/ARA	3.0	3.6	6.5	**EPA/ARA**	3.0	3.6	6.5
n-3/n-6	1.8	8.7	5.8	**n-3/n-6**	1.8	8.7	5.8
Total FAs (mg g^{-1} DW)	9.6	9.2	11.2	**Total FAs (mg g^{-1} DW)**	9.6	9.2	11.2
Total lipids (mg g^{-1} DW)	114.2	161.5	108.2	**Total lipids (mg g^{-1} DW)**	114.2	161.5	108.2

Table 18 - The concentration (µg mg^{-1} DW and % of total FAs) of fatty acids in the microalgae *T. lutea* and in *A. tonsa* reared on *T. lutea* at day 8 and day 13 in and % of total fatty acids. The values presented are averages from three replicates (n = 3).

	Microalgae	*A. tonsa* reared on *T. lutea* (mg g^{-1} DW)			Microalgae	*A. tonsa* reared on *T. lutea* (% of total FAs)	
	T. lutea	**Day 8**	**Day 13**		***T. lutea***	**Day 8**	**Day 13**
C14:0	5.4	4.5	2.2	**C14:0**	22%	15%	12%
C16:0	2.5	3.1	2.4	**C16:0**	10%	10%	13%
C17:0	0.0	0.2	0.1	**C17:0**	0%	1%	0%
C18:0	0.1	0.4	0.4	**C18:0**	0%	1%	2%
C20:0	0.0	2.0	1.6	**C20:0**	0%	7%	9%
C22:0	0.0	0.0	0.0	**C22:0**	0%	0%	0%
Total SFAs	8.0	10.2	6.7	**Total SFAs**	33%	34%	35%
C14:1n5	0.1	0.0	0.0	**C14:1n5**	1%	0%	0%
C16:1n7	1.8	2.1	0.8	**C16:1n7**	8%	7%	4%
C18:1n9	2.4	2.6	1.4	**C18:1n9**	10%	9%	7%
C18:1n7	0.3	0.4	0.3	**C18:1n7**	1%	1%	1%
C20: 1n9	0.0	0.0	0.0	**C20: 1n9**	0%	0%	0%
Total MUFAs	4.7	5.1	2.5	**Total MUFAs**	20%	17%	13%
C18:2n6	3.6	4.4	1.7	**C18:2n6**	15%	15%	9%
C20:2n6	0.0	0.2	0.1	**C20:2n6**	0%	1%	1%
C20:4n6	0.1	0.2	0.1	**C20:4n6**	0%	1%	1%
Total n-6	3.7	4.8	2.0	**Total n-6**	15%	16%	10%
C18:3n3	4.3	4.0	1.9	**C18:3n3**	18%	13%	10%
C20:5n3	0.1	0.5	0.5	**C20:5n3**	1%	2%	3%
C22:6n3	3.3	5.7	5.4	**C22:6n3**	14%	19%	29%
Total n-3	7.7	10.2	7.9	**Total n-3**	32%	34%	42%
Total PUFAs	11.4	15.0	9.9	**Total PUFAs**	47%	50%	52%
DHA/EPA	25.5	11.4	10.5	**DHA/EPA**	25.5	11.4	10.5
EPA/ARA	2.0	2.2	3.7	**EPA/ARA**	2.0	2.2	3.7
n-3/n-6	2.1	2.1	4.0	**n-3/n-6**	2.1	2.1	4.0
Total FAs (mg g^{-1} DW)	24.1	30.3	19.0	**Total FAs (mg g^{-1} DW)**	24.1	30.3	19.0
Total lipids (mg g^{-1} DW)	291.7	195.2	147.0	**Total lipids (mg g^{-1} DW)**	291.7	195.2	147.0

Table 19 - The concentration (µg mg^{-1} DW and % of total FAs) of fatty acids in the microalgae *C. weissflogii* and in *A. tonsa* reared on *C. weissflogii* at day 8 and day 13 in and % of total fatty acids. The values presented are averages from three replicates (n = 3).

	Microalgae *C. weissflogii*	*A. tonsa* reared on *C. weissflogii* (mg g^{-1} DW) Day 13		Microalgae *C. weissflogii*	*A. tonsa* reared on *C. weissflogii* (% of total FAs) Day 13
C14:0	1.6	1.2	**C14:0**	22%	7%
C16:0	0.0	3.0	**C16:0**	0%	17%
C17:0	0.0	1.2	**C17:0**	0%	6%
C18:0	0.0	0.5	**C18:0**	0%	3%
C20:0	0.0	0.0	**C20:0**	0%	0%
C22:0	0.0	0.0	**C22:0**	0%	0%
Total SFAs	1.6	6.0	**Total SFAs**	22%	33%
C14:1n5	0.0	0.0	**C14:1n5**	0%	0%
C16:1n7	0.0	2.7	**C16:1n7**	0%	15%
C18:1n9	0.1	0.2	**C18:1n9**	2%	1%
C18:1n7	0.0	0.4	**C18:1n7**	0%	2%
C20: 1n9	0.0	0.0	**C20: 1n9**	0%	0%
Total MUFAs	0.2	3.4	**Total MUFAs**	3%	19%
C18:2n6	0.1	0.0	**C18:2n6**	1%	0%
C20:2n6	0.0	0.0	**C20:2n6**	0%	0%
C20:4n6	0.0	0.0	**C20:4n6**	0%	0%
Total n-6	0.1	0.1	**Total n-6**	1%	0.5%
C18:3n3	0.3	0.2	**C18:3n3**	4%	1%
C20:5n3	4.2	5.2	**C20:5n3**	57%	29%
C22:6n3	0.9	3.4	**C22:6n3**	13%	19%
Total n-3	5.5	8.7	**Total n-3**	74%	48%
Total PUFAs	5.6	8.8	**Total PUFAs**	75%	49%
DHA/EPA	0.2	0.6	**DHA/EPA**	0.2	0.6
EPA/ARA	-	319.7	**EPA/ARA**	-	319.7
n-3/n-6	51.0	102.6	**n-3/n-6**	51.0	102.6
Total FAs (mg g^{-1} DW)	7.4	18.1	**Total FAs (mg g^{-1} DW)**	7.4	18.1
Total lipids (mg g^{-1} DW)	167.8	197.5	**Total lipids (mg g^{-1} DW)**	167.8	197.5

Table 20 – Developmental stage distribution and average length (µm) of *A. tonsa* reared on *R. baltica* from three replicate copepod tanks at day 7 and day 13.

Treatment *R. baltica* - Day 7

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	1	1%	121	N1	0	0%		N1	0	0%	
N2	0	0%		N2	0	0%		N2	2	2%	136
N3	1	1%	174	N3	5	4%	162	N3	1	1%	150
N4	3	2%	184	N4	2	2%	193	N4	6	5%	192
N5	11	9%	210	N5	9	7%	211	N5	8	7%	207
N6	37	30%	254	N6	51	39%	245	N6	17	15%	245
C1	69	56%	312	C1	60	46%	322	C1	76	66%	318
C2	1	1%	378	C2	3	2%	368	C2	6	5%	375
C3	0	0%		C3	0	0%		C3	0	0%	
C4	0	0%		C4	0	0%		C4	0	0%	
C5	0	0%		C5	0	0%		C5	0	0%	
C6	0	0%		C6	0	0%		C6	0	0%	

Treatment *R. baltica* - Day 13

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	0	0%		N1	0	0%		N1	0	0%	
N2	0	0%		N2	0	0%		N2	0	0%	
N3	0	0%		N3	0	0%		N3	0	0%	
N4	0	0%		N4	0	0%		N4	0	0%	
N5	0	0%		N5	0	0%		N5	0	0%	
N6	0	0%		N6	0	0%		N6	0	0%	
C1	0	0%		C1	0	0%		C1	1	1%	347
C2	4	4%	374	C2	6	6%	394	C2	4	4%	384
C3	6	6%	450	C3	8	9%	467	C3	1	1%	469
C4	25	25%	541	C4	34	37%	540	C4	19	19%	545
C5	59	58%	641	C5	44	47%	626	C5	71	70%	648
C6	7	7%	752	C6	1	1%	785	C6	5	5%	756

Table 21 - Developmental stage distribution and average length (µm) of *A. tonsa* reared on *T. lutea* from three replicate copepod tanks at day 8 and day 13.

Treatment *T. lutea* - Day 8

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	0	0%		N1	0	0%		N1	1	1%	122
N2	0	0%		N2	0	0%		N2	0	0%	
N3	1	1%	169	N3	7	5%	156	N3	5	4%	162
N4	3	2%	193	N4	9	6%	192	N4	4	3%	191
N5	14	10%	210	N5	17	12%	207	N5	8	6%	213
N6	47	32%	242	N6	35	24%	238	N6	31	24%	235
C1	74	51%	323	C1	69	48%	322	C1	81	62%	322
C2	7	5%	370	C2	7	5%	390	C2	1	1%	402
C3	0	0%		C3	1	1%	424	C3	0	0%	
C4	0	0%		C4	0	0%		C4	0	0%	
C5	0	0%		C5	0	0%		C5	0	0%	
C6	0	0%		C6	0	0%		C6	0	0%	

Treatment *T. lutea* - Day 13

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	0	0%		N1	0	0%		N1	0	0%	
N2	0	0%		N2	0	0%		N2	0	0%	
N3	0	0%		N3	0	0%		N3	0	0%	
N4	0	0%		N4	0	0%		N4	1	1%	194
N5	1	1%	220	N5	0	0%		N5	0	0%	
N6	1	1%	289	N6	0	0%		N6	0	0%	
C1	2	2%	301	C1	1	1%	328	C1	0	0%	
C2	1	1%	383	C2	5	7%	391	C2	2	2%	391
C3	6	6%	449	C3	4	5%	460	C3	4	4%	463
C4	28	28%	538	C4	9	12%	522	C4	21	20%	548
C5	56	55%	641	C5	48	65%	648	C5	65	63%	649
C6	6	6%	770	C6	7	9%	779	C6	11	11%	793

Table 22 - Developmental stage distribution and average length (µm) of *A. tonsa* reared on *C. weissflogii* from three replicate copepod tanks at day 8 and day 13.

Treatment *C. weissflogii* - Day 8

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	0	0%		N1	0	0%		N1	0	0%	
N2	0	0%		N2	0	0%		N2	1	1%	138
N3	2	1%	171	N3	1	1%	155	N3	6	4%	163
N4	9	6%	190	N4	5	4%	183	N4	6	4%	189
N5	17	12%	212	N5	11	8%	209	N5	9	6%	208
N6	23	16%	241	N6	18	13%	244	N6	32	23%	240
C1	87	60%	320	C1	86	63%	319	C1	79	56%	315
C2	7	5%	390	C2	16	12%	381	C2	7	5%	379
C3	0	0%		C3	0	0%		C3	1	1%	424
C4	0	0%		C4	0	0%		C4	0	0%	
C5	0	0%		C5	0	0%		C5	0	0%	
C6	0	0%		C6	0	0%		C6	0	0%	

Treatment *C. weissflogii* - Day 13

Copepod tank 1				Copepod tank 2				Copepod tank 3			
Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length	Stage	Number	Number %	Avg. length
N1	0	0%		N1	0	0%		N1	0	0%	
N2	0	0%		N2	1	1%	137	N2	1	1%	133
N3	5	7%	163	N3	24	21%	164	N3	9	8%	165
N4	13	17%	189	N4	32	28%	189	N4	21	18%	188
N5	15	20%	211	N5	18	16%	208	N5	11	9%	208
N6	12	16%	247	N6	7	6%	242	N6	8	7%	255
C1	18	24%	321	C1	8	7%	306	C1	9	8%	321
C2	0	0%		C2	11	10%	389	C2	14	12%	385
C3	1	1%	475	C3	5	4%	464	C3	20	17%	452
C4	2	3%	540	C4	4	4%	528	C4	15	13%	536
C5	6	8%	625	C5	4	4%	618	C5	8	7%	640
C6	4	5%	761	C6	0	0%		C6	0	0%	

Table 23 – Algae density (cell ml^{-1}) and algae volume (μm^3 $cell^{-1}$ and μm^3 ml^{-1}) in each copepod tank in treatment *R. baltica*, *T. lutea* and *C. weissflogii* before adding freshly cultivated algae into the tanks, from day 3 to day 12.

Treatment *Rhodomonas baltica*

	Copepod tank 1			Copepod tank 2			Copepod tank 3		
	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml
Day 3	16,984	185	3,146,800	8,996	174	1,569,600	21,664	181	3,927,200
Day 4	10,124	182	1,841,600	6,888	172	1,185,600	12,348	182	2,243,200
Day 5	16,716	179	2,986,800	12,784	177	2,262,000	16,136	178	2,864,800
Day 6	47,744	173	8,280,000	41,976	173	7,264,000	66,704	175	11,672,000
Day 7	71,608	167	11,936,000	77,704	166	12,864,000	101,880	172	17,528,000
Day 8	66,700	164	10,954,000	73,660	165	12,160,000	56,020	161	9,024,000
Day 9	7,980	171	1,361,340	7,200	163	1,171,300	8,740	149	1,303,300
Day 10	2,912	139	405,944	1,632	142	231,320	2,552	168	427,792
Day 11	916	146	133,988	1,276	130	165,948	626	143	89,646
Day 12	1,620	164	265,556	1,656	164	271,128	1,876	161	302,796

Treatment *Tisochrysis lutea*

	Copepod tank 1			Copepod tank 2			Copepod tank 3		
	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml
Day 3	123,812	53	6,616,000	129,868	54	7,048,000	122,880	54	6,584,000
Day 4	104,400	51	5,336,000	106,856	52	5,552,000	99,196	51	5,016,000
Day 5	236,320	49	11,664,000	236,704	50	11,936,000	190,760	50	9,616,000
Day 6	424,272	49	20,680,000	395,424	49	19,336,000	350,072	53	18,624,000
Day 7	528,260	47	24,820,000	528,160	57	30,160,000	570,680	47	26,660,000
Day 8	619,540	45	28,180,000	643,980	47	30,180,000	690,760	47	32,180,000
Day 9	133,220	42	5,658,000	134,120	43	5,756,000	180,520	43	7,732,000
Day 10	45,440	41	1,872,800	64,080	41	2,633,600	129,944	40	5,179,200
Day 11	6,092	39	239,736	6,200	37	227,636	5,212	43	224,920
Day 12	19,404	43	825,200	17,248	44	751,600	22,364	43	958,400

Treatment *Conticribra weissflogii*

	Copepod tank 1			Copepod tank 2			Copepod tank 3		
	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml	Cells/ml	Volume/cell	Volume/ml
Day 3	17,124	866	14,836,000	19,876	977	19,424,000	18,524	870	16,112,000
Day 4	18,528	884	16,372,000	23,824	870	20,736,000	19,688	856	16,848,000
Day 5	27,524	823	22,660,000	34,868	839	29,256,000	29,720	865	25,716,000
Day 6	41,256	849	35,016,000	42,904	841	36,064,000	52,648	920	48,416,000
Day 7	23,064	813	18,752,000	28,656	817	23,408,000	33,728	838	28,256,000
Day 8	40,980	817	33,500,000	54,920	881	48,360,000	60,300	830	50,060,000
Day 9	50,480	693	34,980,000	51,520	794	40,920,000	49,000	788	38,620,000
Day 10	38,048	763	29,040,000	31,688	785	24,880,000	32,296	774	25,000,000
Day 11	25,010	775	19,388,000	6,508	741	4,824,000	5,222	743	3,878,000
Day 12	30,272	851	25,752,000	9,772	762	7,444,000	8,196	741	6,072,000

Table 24 – The algae density (cell ml^{-1}) and algae volume (μm^3 $cell^{-1}$) from the harvested algae cultures of *R. baltica*, *T. lutea* and *C. weissflogii* from day 2 to day 12.

	R. baltica		C. weissflogii		T. lutea	
	Cells/ml	**Volume/cell**	**Cells/ml**	**Volume/cell**	**Cells/ml**	**Volume/cell**
Day 2	3,265,600	185	704,000	1,080	14,677,200	49
Day 3	4,146,800	185	672,800	1,061	13,360,400	49
Day 4	2,641,600	187	644,400	1,051	13,429,200	49
Day 5	4,075,600	185	667,600	1,028	13,198,400	47
Day 6	3,540,000	185	794,800	827	12,758,800	47
Day 7	3,543,200	172	784,800	675	11,925,600	47
Day 8	3,752,400	184	836,800	793	12,994,400	56
Day 9	3,446,800	188	852,800	861	13,111,600	44
Day 10	3,375,600	186	878,800	782	12,511,200	46
Day 11	3,345,200	190	841,600	811	12,367,200	42
Day 12	3,114,000	185	993,200	780	13,536,000	43

Appendix II

Table 25 - The fatty acid composition (% of total FAs) of the microalgae *R. baltica*, *T. lutea* and *C. weissflogii* compared to the literature. The data with microalgae names marked with "*" are from this study. The data from this study are averages from three replicates (n = 3).

	R. baltica	***R. baltica***	***R. baltica***
SFAs	12.6%	20%	17 - 39%
MUFAs	3.3%	8%	8.7 - 15.9%
PUFAs	84.1%	73%	45 - 75.2%
ARA (C20:4n6)	3.3%	3%	0.5 - 1.1%
EPA (C20:5n3)	10.0%	12%	4.1 - 12.6%
DHA (C22:6n3)	10.4%	8%	1.6 -4.7%
DHA/EPA	1.1	0.7	0.2 - 0.9
EPA/ARA	3.2	3.9	6.6 - 18.5
Source	-	(Kurmaly et al., 1989)	(Huerlimann et al., 2010)

	T. lutea	***T. lutea***	***Isochrysis sp.***
SFAs	33.0%	31.0%	23.7 - 31.2%
MUFAs	19.6%	16.0%	19.1 - 33.1%
PUFAs	47.4%	44.0%	35.7 - 54.3%
ARA (C20:4n6)	0.3%	0.2%	0 - 0.2%
EPA (C20:5n3)	0.5%	1.0%	0.3 - 0.9%
DHA (C22:6n3)	13.7%	8.0%	8.2 - 15.0%
DHA/EPA	27.0	7.6	14.4 - 27.3
EPA/ARA	2.0	5.9	0 - 6.0
Source	-	(Arendt et al., 2005)	(Huerlimann et al., 2010)

	C. weissflogii	***C. weissflogii***	***C. weissflogii***
SFAs	22.2%	24.6%	35.7%
MUFAs	2.6%	32.2%	25.0%
PUFAs	75.3%	33.8%	21.4%
ARA (C20:4n6)	0.0%	0.1%	0.8%
EPA (C20:5n3)	57.4%	16.7%	8.4%
DHA (C22:6n3)	12.7%	1.3%	1.9%
DHA/EPA	0.2	0.1	0.2
EPA/ARA	0.0	139.2	10.5
Source	-	(Pratoomyot et al., 2005)	(Arendt et al., 2005)

Table 26 – Comparison of the fatty acid composition of *A. tonsa* from this study and the literature (Støttrup et al., 1999). The comparison is of the nauplii and adult *A. tonsa* reared on *R. baltica*, *C. weissflogii*, *T. lutea* and *Isochrysis galbana*. Data from this study is marked with "*".

	Nauplii	***Day 7**	**Adult**	***Day 13**
	R. baltica	***R. baltica***	***R. baltica***	***R. baltica***
SFAs	20.8%	20.6%	25.0%	30.5%
MUFAs	8.8%	6.0%	6.8%	0.8%
PUFAs	68.0%	73.4%	65.4%	68.7%
Total n-3	63.2%	65.9%	63.5%	58.6%
Total n-6	4.8%	7.6%	1.9%	10.1%
n-3/n-6 ratio	13.2	8.7	33.4	5.8
ARA (C20:4n6)	0.7%	7.6%	0.4%	2.5%
EPA (C20:5n3)	14.2%	27.0%	13.4%	15.9%
DHA (C22:6n3)	28.5%	38.9%	36.8%	34.4%
DHA/EPA	2	1	3	2
EPA/ARA	20	4	34	6

	Nauplii	***Day 8**	**Adult**	***Day 13**
	I. galbana	***T. lutea***	***I. galbana***	***T. lutea***
SFAs	23.7%	33.4%	26.6%	35.0%
MUFAs	15.9%	16.9%	14.2%	13.1%
PUFAs	54.9%	49.7%	55.5%	51.9%
Total n-3	46.2%	33.7%	45.6%	41.7%
Total n-6	8.7%	16.0%	9.9%	10.3%
n-3/n-6 ratio	5.3	2.1	4.6	4.1
ARA (C20:4n6)	0.2%	0.8%	0.2%	0.0%
EPA (C20:5n3)	6.8%	1.7%	3.7%	2.7%
DHA (C22:6n3)	30.3%	18.9%	25.6%	28.8%
DHA/EPA	4	11	7	11
EPA/ARA	34	2	19	4

	Nauplii	**Adult**	***Day 13**
	C. weissflogii	***C. weissflogii***	***C. weissflogii***
SFAs	26.5%	25.5%	32.7%
MUFAs	9.6%	10.6%	18.3%
PUFAs	60.7%	45.3%	49.0%
Total n-3	56.1%	40.4%	48.6%
Total n-6	4.6%	4.9%	0.4%
n-3/n-6 ratio	12.2	8.2	73.2
ARA (C20:4n6)	0.3%	0.3%	0.1%
EPA (C20:5n3)	23.2%	20.8%	28.6%
DHA (C22:6n3)	28.5%	14.3%	19.0%
DHA/EPA	1	1	1
EPA/ARA	77	69	232

Table 27 - Comparison of total lipids (% of DW) of the microalgae *R. baltica*, *T. lutea* and *C. weissflogii* from this study and the literature. Data from this study is marked with "*".

Microalgae species	**Total lipids (% of DW)**	**Source**
**C. weissflogii*	16.8%	-
C. weissflogii	20.4%	(Roman, 1984)
C. weissflogii	~25%	(Griffiths and Harrison, 2009)
**T. lutea*	29.2%	-
I. galbana	28.4%	(Nanton and Castell, 1999)
I. galbana	28.4%	(Nanton and Castell, 1998)
I. galbana	22.0%	(Griffiths and Harrison, 2009)
**R. baltica*	11.4%	-
R. baltica	4.3%	(Kurmaly et al., 1989)
R. baltica	12.1%	(van Houcke et al., 2017)

Table 28 – Comparison of carbon content (µg C ind^{-1}) of *A. tonsa* from this study, carbon analysis, carbon content calculated by length from (Berggreen et al., 1988) and from the literature.

Treatment	Carbon analysis (µg C $ind.^{-1}$)	Calculated carbon (µg C $ind.^{-1}$)	Source
Day 7 - *R. baltica*	0.68	0.23	-
Day 8 - *T. lutea*	0.47	0.22	-
Day 8 - *C. weissflogii*	0.45	0.23	-
Day 13 - *R. baltica*	1.54	1.49	-
Day 13 - *T. lutea*	1.75	1.63	-
Day 13 - *C. weissflogii*	0.68	0.41	-

Stage	Carbon (µg C $ind.^{-1}$)	Source
Adult	5.90	(Saba et al., 2011)
Adult	3.10	(Saba et al., 2009)
Adult	2.60	(Saba et al., 2009)
Adult	2.80	(Jones et al., 2002)
Copepodid	0.84	(Jones et al., 2002)
Nauplii	0.11	(Jones et al., 2002)

Appendix III

Table 29 – Detailed overview of measured parameters in the algae tanks in the 1st experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *R. baltica*

Algae tank 1

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml)
0	1,768,000	530	9.37E+08				
1	1,691,400	1,021	1.73E+09	8	35.3	22.2	0.65
2	2,115,800	2,002	4.23E+09	8.39	34.8	22.2	1
3	1,892,200	2,002	3.79E+09	8.38	34.1		2
4	2,169,200	530	1.15E+09	8.45	34.5	22.1	2
5	2,417,600	530	1.28E+09	8.42	34.6	22.7	0.5
6	2,638,800	530	1.40E+09	8.42	34.6	22.6	0.5
7		530					1
8		530					
9	2,964,200	530	1.57E+09	8.44	35.4	22.4	

Algae tank 2

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml)
0	1,922,400	530	1.02E+09				
1	1,835,200	1,021	1.87E+09	7.97	34.9	22.5	0.65
2	2,161,600	2,002	4.33E+09	8.16	35.1	22.6	1
3	1,902,400	2,002	3.81E+09	8.14	34.5		2
4	2,165,800	530	1.15E+09	8.2	34.8	22.3	2
5	2,463,800	530	1.31E+09	8.24	36.2	22.5	0.5
6	2,549,600	530	1.35E+09	8.23	35.8	22.8	0.5
7		530					1
8		530					
9	2,496,400	530	1.32E+09	8.15	35.1	22.4	

Algae tank 3

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml)
0	2,129,200	530	1.13E+09				
1	2,098,000	1,021	2.14E+09	8.63	34.6	22.7	0.65
2	2,113,600	2,002	4.23E+09	8.46	34.8	22.5	1
3	1,733,000	2,002	3.47E+09	8.41	34.1		2
4	2,181,800	530	1.16E+09	8.53	34.3	22.4	2
5	2,531,200	530	1.34E+09	8.53	34.4	22.8	0.5

Table 30 - Detailed overview of measured parameters in the copepod tanks and biofilters in the 1st experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *R. baltica*
Copepod tank 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml⁻¹)
0	16,574	7.84	35.2	20.4		35
1	26,500	7.91	35.5	21.2	96	
2	85,000	7.89	34.1	21.2		
3	136,960	7.72	33.8	21.1		
4	173,440	7.8	33.6	20.9	87	
5	206,040	7.79	33.7	20.9		19
6	394,900	7.83	33.6	21		
7						
8						
9	552,400	7.78	33	20.8		0

Copeponics 1st experiment - *R. baltica*
Biofilter 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)
0	10,848	7.73	35.7	19.6	
1	14,500	7.76	35.7	19.8	91
2	12,840	7.76	34.3	19.6	
3	19,020	7.76	34	19.7	
4	33,780	7.78	34	19.2	85
5	63,120	7.83	33.5	19.2	
6	207,800	7.85	33.4	19.4	
7					
8					
9	119,000	7.78	32.8	19.5	

Copepod tank 2

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml⁻¹)
0	26,914	7.81	35.5	19.9		37
1	30,485	7.79	35.6	20.3	95	
2	117,620	7.8	34.3	20.5		
3	122,580	7.71	34	20.4		
4	129,500	7.71	34.1	20.3	86	
5	103,520	7.72	34.1	20.2		24
6	255,500	7.78	33.8	20.9		
7						
8						
9	564,800	7.73	32.8	20.6		0

Biofilter 2

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)
0	11,581	7.77	36.4	19.2	
1	20,940	7.71	35.7	19.3	93
2	13,720	7.79	34.6	19.4	
3	16,700	7.82	34.2	19.4	
4	22,220	7.83	34.3	19.1	88
5	33,680	7.86	33.8	19.4	
6	234,000	7.84	33.5	19.4	
7					
8					
9	239,000	7.75	33	19.7	

Copepod tank 3

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml⁻¹)
0	24,852	7.8	35.8	19.8	94	36
1	29,300	7.77	35.8	20.2		
2	79,400	7.78	34.2	20.3		
3	122,500	7.7	33.8	20.4	90	
4	180,980	7.73	33.9	20.1		
5	142,680	7.68	33.8	20.7		9

Biofilter 3

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)
0	15,351	7.79	36.6	19.3	
1	42,480	7.68	35.6	19.6	93
2	12,400	7.77	34.3	19.7	
3	15,060	7.74	34	19.6	
4	27,220	7.8	34.1	19.2	85
5	39,840	7.74	33.8	19.2	

Table 31 - Detailed overview of measured parameters in the algae tanks in the 2nd experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *R. baltica*

Algae tank 1

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0	1,830,000					20.7					
1											
2	1,283,800	530	6.80E+08	7.72	31.6	19.8	3			9.3	2.1
3	1,755,600	530	9.30E+08	8.02	31.8	20.8	0.5	0.00	0	4.9	1.1
4	2,017,400	530	1.07E+09	7.92	31.2	20.8	0.5	0.01	0.01	0.9	0.2
5	2,007,000	775	1.55E+09	7.99	30.6	20.8	0.5				
6	1,896,400	1,021	1.94E+09	7.91	30.2	20.8	0.75	0.07	0.06	0.0	0
7	1,952,800	1,266	2.47E+09	7.88	29.3	20.8	1				
8	2,138,600	1,266	2.71E+09	7.95	29.6	20.9	1.25	0.05	0.04	6.6	1.5
9	2,638,400	1,266	3.34E+09	8	29.5	20.9	1.25	0.04	0.03	20.4	4.6
10	2,859,400	1,512	4.32E+09	7.91	29.9	20.8	0.625	0.01	0.01	5.8	1.3
11	2,363,600	1,512	3.57E+09	7.98	29.4	20.8	0.75	0.00	0	5.8	1.3
12	2,071,400	1,512	3.13E+09	7.92	29	20.6	0.75	0.02	0.02	6.2	1.4
13	1,958,800	1,512	2.96E+09	7.83	29.9	20.7	0.75				
14											

Algae tank 2

Day	Cells/ml	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0	1,865,000					21					
1											
2	1,177,400	530	6.24E+08	8.11	31.5	19.7	3			6.6	1.5
3	1,550,000	530	8.21E+08	8.43	32.2	20.9	0.5	0.00	0	5.3	1.2
4	2,070,800	530	1.10E+09	8.47	31.1	21	0.5	0.01	0.01	2.2	0.5
5	2,150,000	775	1.67E+09	8.44	30.7	20.9	0.5				
6	1,867,400	1,021	1.91E+09	8.35	30.1	21	0.75	0.06	0.05	2.7	0.6
7	1,812,400	1,266	2.29E+09	8.38	29.5	21	1				
8	1,897,000	1,266	2.40E+09	8.37	29.3	20.9	1.25	0.02	0.02	6.6	1.5
9	2,002,800	1,266	2.54E+09	8.41	29.5	21	1.25	0.00	0	4.9	1.1
10	2,112,600	1,512	3.19E+09	8.46	29.4	20.9	0.625	0.01	0.01	4.0	0.9
11	2,148,600	1,512	3.25E+09	8.39	29	21.1	0.75	0.00	0	8.0	1.8
12	1,619,400	1,512	2.45E+09	8.39	28.9	20.7	0.75	0.04	0.03	6.2	1.4
13	1,772,400	1,512	2.68E+09	8.35	29.3	21	0.75				
14											

Algae tank 3

Day	Cells/ml	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0	1,757,200					20.7					
1											
2	1,216,400	530	6.45E+08	7.95	31.4	19.7	3			8.9	2
3	1,665,800	530	8.83E+08	8.21	31.9	20.7	0.5	0.00	0	6.2	1.4
4	2,089,000	530	1.11E+09	8.18	31.1	20.8	0.5	0.00	0	3.1	0.7
5	2,087,400	775	1.62E+09	8.2	29.8	20.8	0.5				
6	1,979,000	1,021	2.02E+09	8.14	29.9	20.7	0.75	0.06	0.05	4.0	0.9
7	1,927,400	1,266	2.44E+09	8.16	29.4	20.8	1				
8	1,863,800	1,266	2.36E+09	8.15	29.3	20.7	1.25	0.02	0.02	7.5	1.7
9	2,025,200	1,266	2.56E+09	8.21	29.4	20.7	1.25	0.00	0	5.3	1.2
10	2,086,200	1,512	3.15E+09	8.2	29.3	20.7	0.625	0.00	0	6.6	1.5
11	1,835,200	1,512	2.77E+09	8.19	28.9	20.7	0.75	0.00	0	9.3	2.1
12	1,472,400	1,512	2.23E+09	8.17	28.8	20.5	0.75	0.01	0.01	3.5	0.8
13	1,530,400	1,512	2.31E+09	8.15	29	20.8	0.75				
14											

Table 32 - Detailed overview of measured parameters in the copepod tanks in the 2nd experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *R. baltica*

Copepod tank 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual ml⁻¹)	Average length (µm)
0	181,800			19.2							
1											
2	117,600	7.87	30.8	19.2				6.6	1.5		210
3	225,000	7.84	30.6	19	91	0.0	0	6.2	1.4	32	
4	218,800	7.91	30.1		91.1	0.0	0.01	2.7	0.6		
5	97,400	7.87	29.8	18.9	92.5						
6	137,800	7.67	30.4	19.1		0.1	0.05	1.8	0.4	22	
7	193,400	7.62	30	19.1							
8	210,200	7.57	30.1	19.2		0.0	0.03	6.6	1.5		283
9	262,200	7.57	29.8	18.6		0.1	0.09	3.5	0.8		
10	242,200	7.59	29.4	18.9	82.3	0.1	0.05	5.8	1.3		
11	160,400	7.59	29	19	81.3	0.2	0.19	5.8	1.3	15	409
12	246,000	7.42	29	18.9	63.3	0.2	0.2	6.2	1.4	12	
13	200,400	7.75	28.9	18.9	69.8						
14										3	542

Copepod tank 2

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual ml⁻¹)	Average length (µm)
0	165,600			18.6							
1											
2	98,800	7.93	31.2	18.6				6.6	1.5		158
3	164,800	7.85	31.3	18.4	93	0.0	0	4.0	0.9	30	
4	222,800	7.82	31		92.4	0.0	0	0.4	0.1		
5	79,600	7.81	30.8	18.9	93.9						
6	118,000	7.62	30.5	18.6		0.1	0.06	3.1	0.7	25	
7	145,800	7.61	30	18.4							
8	246,600	7.58	29.6	18.3		0.1	0.05	5.8	1.3		239
9	193,000	7.56	29.3	18.4		0.0	0.01	4.9	1.1		
10	203,000	7.61	29	18.6	83.4	0.0	0.01	6.2	1.4		
11	184,200	7.61	28.6	18.6	84.2	0.0	0	6.2	1.4	17	335
12	203,200	7.49	28.7	18.6	71.6	0.0	0.01	3.1	0.7	10	
13	420,800	7.56	28.8	18.3	67.8						
14										4	424

Copepod tank 3

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual ml⁻¹)	Average length (µm)
0	120,200			18.6							
1											
2	58,000	7.95	31.2	18.4				10.6	2.4		163
3	127,800	7.86	31.1	18.3	91.4	0.0	0	5.8	1.3	35	
4	135,200	7.83	30.4		92.9	0.0	0	3.5	0.8		
5	61,600	7.81	30.3	18.4	93.6						
6	83,600	7.58	30	18.4		0.0	0	2.7	0.6	19	
7	120,400	7.51	29.7	18.6							
8	138,400	7.44	29.3	18.1		0.0	0.04	5.3	1.2		285
9	148,400	7.38	29	18.2		0.0	0	2.7	0.6		
10	146,000	7.49	28.6	18.3	86.5	0.0	0	4.0	0.9		
11	132,400	7.48	28.3	18.4	78.5	0.0	0.01	3.5	0.8	15	416
12	148,600	7.35	28.4	18.3	60.8	0.0	0	3.5	0.8	11	
13	200,800	7.63	28.4	18.3	62.5						
14										5	487

Table 33 - Detailed overview of measured parameters in the biofilters in the 2nd experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *R. baltica*

Biofilter 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0				17.4					
1									
2	54,600	7.81	30.6	17.3				10.6	2.4
3	99,000	7.89	30.1	17.2	94	0.0	0	2.7	0.6
4	92,000	7.84	29.7		93	0.0	0	4.4	1
5	45,000	7.85	29.7	17.4	97				
6	37,200	7.76	30.4	17.9		0.0	0.04	0.9	0.2
7	114,400	7.71	29.9	18.1					
8	90,600	7.73	29.8	17.1		0.0	0	5.3	1.2
9	117,400	7.8	29.4	17.4		0.0	0	2.2	0.5
10	106,800	7.73	29	17.8	88.7	0.0	0	4.9	1.1
11	82,200	7.76	28.4	18.2	91.7	0.0	0	5.3	1.2
12	102,800	7.59	29.1	17.7	76.2	0.0	0.02	6.2	1.4
13	129,600	7.9	28.7	16.9	92.2				
14									

Biofilter 2

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0				17.4					
1									
2	73,200	7.9	31.5	17.4				11.1	2.5
3	82,000	7.89	30.7	17.3	94	0.0	0	4.4	1
4	84,000	7.9	30.9		95.1	0.0	0	3.5	0.8
5	31,400	7.86	31	17.6	97.7				
6	43,600	7.74	30.3	18.1		0.0	0.03	2.2	0.5
7	62,200	7.73	29.6	18.1					
8	130,600	7.68	29.2	18.6		0.0	0.02	6.2	1.4
9	106,600	7.83	29.2	17.3		0.0	0	4.4	1
10	109,200	7.74	28.8	17.8	90.3	0.0	0.01	3.5	0.8
11	113,800	7.7	28.3	18.1	89.7	0.0	0	4.4	1
12	128,200	7.61	29	17.8	73.3	0.0	0.03	4.0	0.9
13	107,000	7.91	28.6	16.8	91.7				
14									

Biofilter 3

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0				17.4					
1									
2	81,800	7.91	30.8	17.4				15.1	3.4
3	83,200	7.91	30.4	17.3	94	0.0	0	2.7	0.6
4	84,600	7.9	30.1		95.6	0.0	0	4.0	0.9
5	42,000	7.88	29.9	17.4	97.2				
6	51,600	7.71	29.9	18.1		0.1	0.05	2.7	0.6
7	69,400	7.75	29.3	18.2					
8	82,200	7.69	28.9	17.9		0.0	0.03	4.0	0.9
9	85,200	7.74	28.6	17.9		0.0	0	1.8	0.4
10	92,600	7.73	28.4	17.8	88.8	0.0	0	1.3	0.3
11	79,600	7.7	27.9	18.1	85.4	0.0	0	6.2	1.4
12	96,200	7.7	28.3	18.2	85.6	0.0	0.02	4.0	0.9
13	78,200	7.9	28.1	16.8	90.3				
14									

Table 34 - Detailed overview of measured parameters in the algae tanks in the 3rd experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 3rd experiment - *R. baltica*

Algae tank 1

Day	Cells ml	Pumping rate (ml/day)	Cells harvested (cells/day)	pH	Salinity	Temp. (°C)	Conwy medium added (ml/day)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
2	2,102,000	500	1.05E+09	8.04	31.5	20.7	0.5				
3	2,052,200	500	1.03E+09	8.07	31.2	20.8	0.5	0.0	0	4.4	1
4	2,005,000	1,000	2.01E+09	8.08	31.3	20.8	0.5				
4	900,200	1,500	1.35E+09	8.06	31.5	20.8	1				
5	1,062,200	2,000	2.12E+09	8.09	31.3	20.8	1.5				
6	1,177,600	2,500	2.94E+09	8.11	31.1	20.8	2.5	0.0	0	1.8	0.4
7	1,490,800	2,500	3.73E+09	7.99	31.1	20.9	2.5				
8	1,604,000	0	0.00E+00	8.07	31.0	20.9	0	0.0	0.03	0.9	0.2
9	-				31.0	20.8	0				
10	1,058,200	2,000	2.12E+09	7.7	31.0	20.8	3	0.0	0	22.1	5
11	1,278,000	2,000	2.56E+09	7.76	30.9	20.8	0	0.0	0.03	7.1	1.6
12	1,085,000	2,000	2.17E+09	7.71	30.9	20.6	0				
13	1,028,400	2,000	2.06E+09	7.58	30.8	20.7	2				
14	973,200	2,000	1.95E+09	7.6	30.8	20.8	0				
15	587,000	2,000	1.17E+09	7.72	30.7	20.8		0.0	0	0.9	0.2

Algae tank 2

Day	Cells/ml	Pumping rate (ml/day)	Cells harvested (cells/day)	pH	Salinity	Temp. (°C)	Conwy medium added (ml/day)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
2	2,122,400	500	1.06E+09	8.08	31.2	21	0.5	0	0	2.2	0.5
3	1,978,400	500	9.89E+08	8.12	31.1	20.9	0.5				
4	1,820,400	1,000	1.82E+09	8.08	31.2	21	0.5				
4	949,800	1,500	1.42E+09	8.06	31	20.9	1				
5	996,600	2,000	1.99E+09	8.01	30.8	21	1.5				
6	908,800	2,500	2.27E+09	8.02	30.8	21	2.5	0	0	0.0	0
7	1,633,400	2,500	4.08E+09	8.02	30.6	21	2.5				
8	1,706,800	0	0.00E+00	8.1	30.5	20.9		0	0	0.9	0.2
9											

Algae tank 3

Day	Cells/ml	Pumping rate (ml/day)	Cells harvested (cells/day)	pH	Salinity	Temp. (°C)	Conwy medium added (ml/day)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
2	3,317,800	500	1.66E+09	8.05	31.3	20.7	0.5	0	0	5.8	1.3
3	2,405,600	500	1.20E+09	8.12	31.2	20.8	0.5				
4	2,635,800	1,000	2.64E+09	8.10	31.3	20.7	0.5				
4	1,104,000	1,500	1.66E+09	8.12	31.0	20.7	1				
5	1,286,600	2,000	2.57E+09	8.11	31.1	20.7	1.5				
6	1,670,000	2,500	4.18E+09	8.13	31.1	20.8	2.5	0	0	1.8	0.4
7	1,620,600	2,500	4.05E+09	8.11	30.9	20.8	2.5				
8	1,657,600	0	0.00E+00	8.09	30.7	20.7		0	0	6.6	1.5
9											

Table 35 - Detailed overview of measured parameters in the copepod tanks in the 3rd experiment with *R. baltica* in the copeponics system. Data is shown from each replicate.

Copeponics 3rd experiment - *R. baltica*

Copepod tank 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual ml⁻¹)
2	263,600	7.95	31.4	19.2	94.2					55
3	189,600	7.92	31.2	19.2	94.6	0.0	0	4.4	1	
4	77,400	7.93	31.4	19.1	93.9					
5	38,600	7.95	31	18.9	92.6					
6	14,952	7.95	31.2	18.9	94.2	0.2	0.15	3.1	0.7	
7	11,892	7.81	30.8	19.3	94.7					
7	62,112	7.75	30.6	19.2	92.1					
8	57,000	7.73	30.2	18.6	88.3	0.5	0.4	5.8	1.3	
9		7.87	30.2	18.9	90.7	0.6	0.5	7.1	1.6	
10	2,556	7.69	30.3	18.9	91.3	0.3	0.3	11.1	2.5	
11	1,180	7.71	30.5	18.9	90.2	0.3	0.3	12.4	2.8	
12	1,152	7.74	30.7	19.0	88.6					
13	1,096	7.71	30.5	18.9	87.6					
14	6,784	7.72	30.2	19.0	82.8					
15	4,280	7.74	30.2	19.3	83.3	0.3	0.2	17.7	4	33

Copepod tank 2

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual/ml)
2	201,200	7.92	31.6	18.6	92.3	0	0	3.1	0.7	53
3	86,800	7.93	31.5	18.4	90.2					
4	81,000	7.89	31.2	18.6	90.6					
5	51,400	7.88	30.6	18.6	90.1					
6	14,340	7.87	30.3	18.3	90.8		0.2		0	
7	28,204	7.82	30.5	18.4	89.5	0		0.0		
7	90,020	7.8	30.3	18.4	82.6					
8	340,000	7.79	30	18.6	79.2	0.7	0.6	4.9	1.1	0
9						0.4	0.3		2.1	

Copepod tank 3

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)	Density (individual/ml)
2	159,400	7.92	31.4	18.6	92.3	0	0	4.0	0.9	56
3	55,600	7.9	31.2	18.4	93.3					
4	82,800	7.96	31	18.3	94.2					
5	78,000	7.91	30.7	18.4	92.7					
6	17,976	7.87	30.5	18.3	93.5		0.1		0.5	
7	22,508	7.84	30.5	18.3	94.8	0		0.0		
7	87,492	7.82	30.2	18.2	90.3					
8	287,600	7.79	29.9	18.3	84.3	0.5	0.4	2.2	0.5	0
9						0.2	0.2		1.5	

Table 36 - Detailed overview of measured parameters in the algae tanks in the 1st experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *C. weissflogii*

Algae tank 1

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})
2	589,000	518	3.05E+08	7.92	35	20.8	2.5
3	677,400	1,037	7.02E+08	8.18	33.4	20.8	1

Algae tank 2

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})
2	576,200	518	2.99E+08	8.45	34.6	21.1	2.5
3	630,800	1,037	6.54E+08	8.46	33.3	20.8	1

Algae tank 3

Day	Cells ml^{-1}	Pumping rate (ml day^{-1})	Cells harvested (cells day^{-1})	pH	Salinity	Temp. (°C)	Conwy medium added (ml day^{-1})
2	584,000	518	3.03E+08	8.16	34		2.5
3	629,600	1,037	6.53E+08	8.26	33.3	20.7	1

Table 37 - Detailed overview of measured parameters in the copepod tanks in the 1st experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *C. weissflogii*

Copepod tank 1

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml^{-1})
2	37,600	8.08	30.1	18.6	98.4	63
3	163,800	7.95	30.8	19.2	94.7	

Copepod tank 2

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml^{-1})
2	119,400	8.03	29.9	18.2	98.2	65
3	151,400	7.98	30.7	18.7	96.1	

Copepod tank 3

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	Density (individual ml^{-1})
2	87,400	8.00	30.0	18.1	98.3	62
3	87,000	7.96	30.9	18.6	95.6	

Table 38 - Detailed overview of measured parameters in the biofilters in the 1st experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *C. weissflogii*

Biofilter 1

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)
2	115,200	7.83	29.7	17.7	93.3
3	108,400	7.83	30.5	17.8	94.4

Biofilter 2

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)
2	63,400	7.74	30.2	17.7	89.6
3	56,800	7.83	30.8	17.7	97.9

Biofilter 3

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)
2	26,800	7.79	30.1	17.7	92.1
3	11,200	7.80	30.7	17.8	95.8

Table 39 - Detailed overview of measured parameters in the algae tanks in the 2nd experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *C.weissflogii*

Algae tank 1

Day	Cells ml^{-1}	Pumping rate ($ml\ day^{-1}$)	Cells harvested ($cells\ day^{-1}$)	pH	Salinity	Temp. (°C)	Conwy medium added ($ml\ day^{1}$)	TAN ($mg\ L^{-1}$)	NH_3 - N ($mg\ L^{-1}$)	Nitrate NO_3 ($mg\ L^{-1}$)	NO_3 - N ($mg\ L^{-1}$)
1	677,800			8.03	29.8	21.3					
2	639,400	1,011	6.46E+08	8.1	29.4	21.3	3				
3	363,600	1,011	3.68E+08	8.03	29.8		3	0.05	0.04	19.5	4.4
4	497,400	1,011	5.03E+08	8.6	29.7	21.6	1				
5	400,600	1,512	6.06E+08	8.15	30.3	21.5					
9	494,400			8.57	31.9						
10	434,600			8.07	31.1						

Algae tank 2

Day	Cells ml^{-1}	Pumping rate ($ml\ day^{-1}$)	Cells harvested ($cells\ day^{-1}$)	pH	Salinity	Temp. (°C)	Conwy medium added ($ml\ day^{1}$)	TAN ($mg\ L^{-1}$)	NH_3 - N ($mg\ L^{-1}$)	Nitrate NO_3 ($mg\ L^{-1}$)	NO_3 - N ($mg\ L^{-1}$)
1	674,200			8.46	30.1	21.7					
2	608,000	1,011	6.15E+08	8.07	29.2	21.3	3				
3	416,200	1,011	4.21E+08	8.05	29.2		3	0.00	0	29.7	6.7
4	472,000	1,011	4.77E+08	8.6	29.6	21.6	1				
5	390,200	1,512	5.90E+08	8.19	30.2	21.5					
9	478,400			8.61	31.8						
10	501,400			8.06	30.7						

Algae tank 3

Day	Cells ml^{-1}	Pumping rate ($ml\ day^{-1}$)	Cells harvested ($cells\ day^{-1}$)	pH	Salinity	Temp. (°C)	Conwy medium added ($ml\ day^{1}$)	TAN ($mg\ L^{-1}$)	NH_3 - N ($mg\ L^{-1}$)	Nitrate NO_3 ($mg\ L^{-1}$)	NO_3 - N ($mg\ L^{-1}$)
1	654,000			8.15	29.9	21					
2	640,000	1,011	6.47E+08	8.15	28.9	21.2	3				
3	406,600	1,011	4.11E+08	8.04	29.2		3	0.00	0	30.5	6.9
4	495,800	1,011	5.01E+08	8.9	29.4	21.6	1				
5	387,400	1,512	5.86E+08	8.11	29.7	21.3					
9	447,800			9.05	31.7						
10				8.53	30.9						

Table 40 - Detailed overview of measured parameters in the copepod tanks in the 2nd experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *C.weissflogii*

Copepod tank 1

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual ml^{-1})
1	18,000	7.97	31.2	18.7	94.4					
2	178,200	8.05	31.5	18.4	90.6					53
3	91,200	7.96	30.5	19.2	92.6	0.01	0.01	0.9	0.2	
4	119,000	8.11	30.1	18.8	89.5					41
5	69,800	8.02	29.6	18.8	91.1					
9										
10										

Copepod tank 2

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual ml^{-1})
1	15,600	8	31.2	17.9	96.6					
2	223,400	8.05	31.3	17.6	94.5					50
3	229,200	7.93	30.8	18.4	91.7	0.00	0	3.5	0.8	
4	390,200	8	30.2	17.9	89.6					35
5	298,000	8.05	29.9	17.9	92					
9										
10										

Copepod tank 3

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual ml^{-1})
1	5,000	7.98	31.3	17.8	98.9					
2	64,000	8.05	31.7	17.5	93.4					52
3	81,200	7.83	30.9	18.2	89	0.00	0	3.5	0.8	
4	78,800	7.95	30.4	17.7	88.6					48
5	61,600	8	30.4	17.9	91					
9										
10										

Table 41 - Detailed overview of measured parameters in the biofilters in the 2nd experiment with *C. weissflogii* in the copeponics system. Data is shown from each replicate.

Copeponics 2nd experiment - *C.weissflogii*

Biofilter 1

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
1	4600	790	31.4	17	94.8				
2	140400	7.93	30.8	16.7	91.6				
3	41200	7.89	30.4	17.6	92.6	0.00	0	2.7	0.6
4	28800	7.95	29.7	17.1	90.5				
5	22400	7.97	29.3	16.8	91.1				
9									
10									

Biofilter 2

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
1	18400	7.86	31.4	17.1	95.1				
2	83000	7.91	30.8	16.7	90.8				
3	21800	7.86	30.4	17.4	91.9	0.00	0	2.2	0.5
4	57000	7.94	29.7	16.8	90				
5	24800	7.96	29.5	16.8	91.2				
9									
10									

Biofilter 3

Day	Cells ml⁻¹	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
1	32,800	7.88	31.5	17.1	97.5				
2	59,400	7.91	30.8	16.8	91.9				
3	75,200	7.84	30.5	17.4	90.6	0.00	0	0.0	0
4	60,000	7.86	29.7	17.1	87.2				
5	24,000	7.95	30.1	16.9	90.8				
9									
10									

Table 42 - Detailed overview of measured parameters in the algae tanks in the 1st experiment with *T. lutea* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *T. lutea*

Algae tank 1

Day	Cells ml⁻¹	Pumping rate (ml day⁻¹)	Cells harvested (cells day⁻¹)	pH	Salinity	Temp. (°C)	Conwy medium added (ml day⁻¹)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0	4,498,400			7.91	31.5						
1	8,077,600			7.95	31						
2	3,368,400	505	1.70E+09	7.71	31.5	21.4					
3	7,445,200	1,009	7.52E+09	8.1	31	19.9		0.0607	0.05	14.1664	3.2
4	8,134,800	1,009	8.21E+09	8.39	31	21.7	0.5				
5	9,046,400	1,009	9.13E+09	8.13	29.9	20.6	0.5	0.04856	0.04	5.7551	1.3
6	7,094,400	1,512	1.07E+10	8.1	30	21.7	1				
7	6,644,400										

Algae tank 2

Day	Cells/ml	Pumping rate (ml/day)	Cells harvested (cells/day)	pH	Salinity	Temp. (°C)	Conwy medium added (ml/day)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0	4,750,600			7.97	31.1						
1	7,835,600			8	31.3						
2	5,275,200	505	2.67E+09	7.72	31.5	21.8					
3	8,485,600	1,009	8.57E+09	8.14	31.7	19.9		0.03642	0.03	11.0675	2.5
4	8,352,400	1,009	8.43E+09	8.3	31.6	21.4	0.5				
5	9,258,800	1,009	9.35E+09	8.08	30.7	20.3	0.5	0	0	3.9843	0.9
6	6,376,400	1,512	9.64E+09	8.05	30.8	21.8	1				
7	5,458,400										

Algae tank 3

Day	Cells/ml	Pumping rate (ml/day)	Cells harvested (cells/day)	pH	Salinity	Temp. (°C)	Conwy medium added (ml/day)	TAN (mg L⁻¹)	NH_3 - N (mg L⁻¹)	Nitrate NO_3 (mg L⁻¹)	NO_3 - N (mg L⁻¹)
0	4,838,800			8.01	31.1						
1	7,915,200			8.05	31.3						
2	6,121,600	505	3.09E+09	7.75	31.5	21.6					
3	7,127,200	1,009	7.19E+09	8.15	31.8	20		0.03642	0.03	12.8383	2.9
4	7,776,000	1,009	7.85E+09	8.21	31.4	21.2	0.5				
5	8,831,200	1,009	8.91E+09	8.43	31	20.5	0.5	0.02428	0.02	6.6405	1.5
6	7,822,400	1,512	1.18E+10	8.12	31	20.8	1				
7	8,449,200										

Table 43 - Detailed overview of measured parameters in the copepod tanks in the 1st experiment with *T. lutea* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *T. lutea*

Copepod tank 1

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual/ml)
0										
1										
2	276,400	7.86	30.7	19	89.5					52
3	223,000	7.89	30	19.3	91	0	0	7.0832	1.6	
4	446,200	7.71	30	19.3	90.1					52
5	581,800	7.83	30	17.9	92.4	0	0	8.4113	1.9	
6	393,200	7.89	29.7	19.2	87.6					
7										

Copepod tank 2

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual/ml)
0										
1										
2	280,000	7.87	30.9	18.3	93.3					55
3	255,200	7.86	30	18.6	91.7	0	0	6.1978	1.4	
4	410,600	7.63	29.9	18.7	88.3					60
5	449,600	7.76	30	17.4	90.9	0	0	3.0989	0.7	
6	325,400	7.79	29.8	18.6	86.5					
7										

Copepod tank 3

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})	Density (individual/ml)
0										
1										
2	205800	7.82	30.8	18.3	89.1					50
3	253800	7.81	30.3	18.3	90.7	0	0	6.6405	1.5	
4	314600	7.69	30.4	18.4	95.9					62
5	373200	7.87	30.4	17.1	91.1	0	0	4.427	1.0	
6	370400	7.88	30.3	18.3	86.1					
7										

Table 44 - Detailed overview of measured parameters in the biofilters in the 1st experiment with *T. lutea* in the copeponics system. Data is shown from each replicate.

Copeponics 1st experiment - *T. lutea*

Biofilter 1

Day	Cells ml^{-1}	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0									
1									
2	150,200	7.82	28.1	17.9	89.9				
3	159,200	7.77	29.5	17.8	90.6	0	0	8.854	2
4	134,400	7.86	29.6	17.6	95.1				
5	207,400	7.88	29.7	16.6	93.2	0	0	4.8697	1.1
6	217,200	7.89	29.6	17.7	8.81				
7									

Biofilter 2

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0									
1									
2	132,200	7.83	27.7	17.7	88.2				
3	173,600	7.75	29.4	17.7	91.3	0	0	7.9686	1.8
4	155,000	7.85	29.5	17.6	94.7				
5	200,200	7.89	29.7	16.6	94.1	0	0	3.9843	0.9
6	182,800	7.9	29.5	17.6	88.6				
7									

Biofilter 3

Day	Cells/ml	pH	Salinity	Temp. (°C)	Oxygen (%)	TAN (mg L^{-1})	NH_3 - N (mg L^{-1})	Nitrate NO_3 (mg L^{-1})	NO_3 - N (mg L^{-1})
0									
1									
2	211,600	7.82	28.2	17.7	88.9				
3	71,600	7.73	29.9	17.8	90.2	0.085	0.07	4.427	1.00
4	99,800	7.83	29.8	17.6	99.6				
5	132,000	7.87	30.1	16.7	93.3	0	0	4.427	1.00
6	197,200	7.88	30	17.7	89.3				
7									

Appendix IV

Gas Chromatography settings

Split injections with helium as the carrying gas was used by the Gas Chromatography Agilent Technology 7890B with a flame ionization detector (FID) with three columns.

Column 1#

Front SS Inlet HE → Agilent CP7713:2

CP-WAX 52 CB

20 ºC – 250 ºC (265 ºC): 25m x 250µm x 0.2µm → PCM C He

Column 2#

PCM C HE → < Not Inventoried >

MS Restrictor

0 ºC – 325 ºC (325 ºC): 1.5m x 150µm x 0µm → MSD

Column 3#

PCM C HE → [Controlled via Column #2] < Not Inventoried >

FID Restrictor

0 ºC – 325 ºC (325 ºC): 1m x 150µm x 0µm → Front Detector FID

The program Agilent 7890B GC - Acquisition calculates the content ng μl^{-1} of the different know peaks from the internal standard in the chromatogram moreover 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 will be as followed:

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

Appendix V

Table 45 – Modified version of Conway medium (Walne, 1974).

Prep of nutrients to modified Conwy-enrichment.

	Amount:
Major Nutrients:	
$FeCl_3$ x 6 H_2O	1.300 gr
$MnCl_2$ x 4 H_2O	0.3600 gr
H_3BO_3	11.20 gr*
Na_2 EDTA x 4 H_2O	30.00 gr**
NaH_2PO_4	16.95 gr
$NaNO_3$	100.00 gr
Vitamins:	
Thiamine-HCl	0.1000 gr
Cyanocobalamine Stock	5.0 mg
Trace Metal Stock (50% strength)	2.0 ml
Deionised water to	1000 mL

Cyanocobalamine Stock	
Cyanocobalamine (vit. B_{12})	0.0500 gr
Deionised water	50 mL

Trace Metal Stock (50% strength):	
$ZnCl_2$	2.6250 gr
$CoCl_2$	2.5000 gr
$(NH_4)_6Mo_7O_{24}$ x $4H_2O$	1.1250 gr
$CuSO_4$ x $5H_2O$	2.5000 gr
De-ionised water	250 ml

*: Reduced to 1/3 of initial amount
**: Reduced to 2/3 of initial amount