**NTNU – Trondheim**
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Science and Technology

# Effects of copepod density and water exchange on the egg production of *Acartia tonsa* Dana (Copepoda: Calanoida) feeding on *Rhodomonas baltica*

Erik Salveson

Marine Coastal Development
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Supervisor: Yngvar Olsen, IBI
Co-supervisor: Jan Ove Evjemo, IBI

Norwegian University of Science and Technology
Department of Biology

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## Summary

The nutritional value of the live feed is crucial in marine larviculture. Species like *Artemia* sp. and rotifers have normally been used as live feed for the fish larvae, but limitations in the nutritional value has led to an increased need for copepods as live feed, because of the better nutritional quality. The high nutritional value of the copepods has given increased survival, growth, improved pigmentation, and general normal development when used for feeding of fish larvae.

In this study, the objective was to investigate the effect of different copepod densities and the water exchange rate $day^{-1}$ on the egg production of *Acartia tonsa* feeding on *Rhodomonas baltica*. It was a main task to find the best conditions for a maximum egg production in a large scale production system. The egg production under 3 different copepod densities (5, 8-10 and 20-53 ind/ml) and with water exchange rates of 100 and 500 % $day^{-1}$ were tested. In addition, other variables like ammonia, temperature (20 °C), pH (8), salinity (24-34 ‰), dissolved $0_2$ (> 5 mg/L) were measured and controlled if possible. Hatching success, female ratio, eggs per female and different consumption tests were measured.

The results showed that in Trial 1, with a 100 % water exchange $day^{-1}$, the best egg production occurred in the tanks with the lowest initial copepod density (LD tanks) (mean 2.6 mill eggs $day^{-1}$ $tank^{-1}$ for day 12-22 after transfer from the hatching tanks (All days mentioned will be days after transfer from the hatching tanks)). The medium copepod density (MD) and high copepod density (HD) tanks had an egg production of 1.7 and 1 mill eggs $day^{-1}$ $tank^{-1}$ (mean, day 11-22), respectively. It is important to notice that when the egg production started the copepod densities were more or less the same. At day 11, there were no statistical differences between the copepod density in the treatments (5, 8.5 and 9 ind/ml for the LD, MD and HD tanks, respectively). The highest average for the three replicates in the HD tanks were 1.5 million eggs (day 19), which was considerably lower than the highest average of the LD tanks with a production of 4.1 mill eggs on day 21.

In the second trial, the water exchange $day^{-1}$ was set to 500 %, there were no statistical differences between the egg productions of the tanks ($P>0.05$). The production of eggs in the MD tanks was stable close to 2 mill eggs $tank^{-1}$ $day^{-1}$ during the entire trial. The egg production in the HD tanks varied some more, but there were no statistical differences between the MD and HD tanks during the trial ($P>0.05$). The HD tanks had a stable egg production close to 2 mill eggs $tank^{-1}$ $day^{-1}$ during the last 5-6 days, as the MD tanks.

In Trial 3 a water exchange of 500 % was used, in addition, extra copepods were added in order to maintain the initial copepod density. The copepods lost by sampling and natural mortality were replaced (experience showed that about 10 % of the total tank volume needed to be replaced $day^{-1}$). Due to algae concentrations close to 0 the first 11 days, the egg production was low for both the MD and the HD tanks. The MD tanks remained at this egg production level the entire trial, but the HD

tanks showed some peaks in egg production, corresponding to drops in the copepod density that led to an increase in the algae concentration. The highest peak was on day 20-21, with an egg production of 2.7 million eggs $day^{-1}$, at a copepod density of 6-8 ind/ml.

The optimal egg production (mean for the LD tanks were 4.1 mill egg $day^{-1}$ $tank^{-1}$, day 21) with a water exchange of 100 % occurred at a copepod density between 2 and 4 ind/ml. For the HD tanks in Trial 2, where the water exchange was 500 %, there were on some occasions high egg production (5.7 and 4.6 mill eggs $day^{-1}$ $tank^{-1}$) at a density of 4.33-7.33 ind/ml. For the rest of that trial, the egg productions were lower than in the LD tanks in Trial 1 (mean for day 11-22 was 2.6 mill eggs $day^{-1}$ $tank^{-1}$). The high production (2.7 mill eggs $day^{-1}$ $tank^{-1}$, day 21) of Tank 3 in Trial 3 where the water exchange was 500 % occurred when the copepod density was between 6-8 ind/ml. The conditions were in other words about the same as for the highest production in Trial 2. In conclusion, the highest egg production occurred with low copepod densities, about 2-4 ind/ml with 100 % water exchange and between 4-8 ind/ml in 500 % water exchange $day^{-1}$.

The salinity in the Trials remained more or less the same during all the trial, and showed no direct influence on the mortality or the egg production in the tanks. The pH levels were close to 8 in all the tanks during all the trials, influencing the amount of reactive ammonia in the tanks

The total ammonia concentrations were generally too high in this experiment. An increased water exchange lowered the ammonia concentration in Trial 2 and 3. In Trial 1, with water exchange rate of 100 % $day^{-1}$ the total ammonia concentration was as high as 7.1 mg/L $NH_3$-N a period in the HD tanks. The LD, MD and HD tanks in this trial had an average ammonia concentration of 2.5 ± 0.1, 4.2 ± 0.5 and 5.9 ± 0.4 mg/L $NH_3$-N, respectively. Using a water exchange rate of 500 % $day^{-1}$ (with no extra copepods added) the average total ammonia concentrations were 0.5 ± 0.1 and 1.2 ± 0.3 for the MD and HD tanks, respectively. Using the same water exchange rate, but adding copepods during the trial to maintain the initial copepod density, the ammonia concentration increased to 0.9 ± 0.2 and 2.1 ± 0.5 mg/L $NH_3$-N (mean ±SE) for the MD and HD tanks, respectively.

The feed utilization and food consumption rate per copepod were calculated on 3 different days in Trial 3, showing utilization between 58-90 % and a consumption rate between 57-82 % of its own body weight $day^{-1}$.

The hatching success in the HD tanks (Trial 3) were 46.1 % which was statistically lower ($P<0.05$) than the mean of the MD tanks (64.8 %).

The female ratio in Trial 1 increased with the copepod density (LD < MD < HD), 35 %, 58.6 %, 76 % for the LD, MD and HD tanks, respectively. In Trial 2 the female ratio for the MD and the HD tanks were 67 % ± 9 % and 74 % ± 10 %, respectively. The female ratio in Trial 3 showed more or less no difference between the tanks, the MD and the HD tanks had an average of 76.5 % ± 6 % and 77.5 % ± 5 %, respectively

# Contents

# 1 Introduction

## 1.1 Production of fish larvae

In marine aquaculture one of the main challenges is the rearing of fish larvae. The development over the last years has lead to a great increase in the number of fish in a farm and the number of fish species used for farming (Kjørsvik et al., 2007). Every species has its own nutritional requirements (lipids, protein and micronutrients) and environmental parameters to fulfill the optimal rearing conditions. These requirements will also change during the development. A newly hatched cod larvae has for instance other nutritional requirements than a three weeks older cod juvenile (Kjørsvik et al., 2007).

Different fish species has different reproductive strategies, which is a crucial factor to consider for rearing of a species. Some fish, like for instance salmonides, have a strategy that involves few, but highly developed eggs (Kjørsvik et al., 2007). The eggs of these fish species are big, and the larvae are well developed with a yolk sac that will sustain the fish larvae with energy a few weeks post hatch. The hatched larvae have high survival rate and they are able to start feeding on formulated feed straight away.

Most marine fish produce small pelagic eggs distinctively different from the eggs of salmonides. They have a quantitative strategy producing large amounts of eggs where the hatched larvae are less developed. The larvae lack functioning mouth, eye pigments and differentiated fins (Kjørsvik et al., 2007) and the survival rate of the larvae are quite low if they don't get the optimal nutrition at the correct time. When the larvae have catabolized their yolk sac, they are too small to start feeding on formulated diets, like small pellets, and are therefore dependent on live prey organisms in the beginning. The feeding response is probably triggered by the smell and the movement of the live organism. The transition when the yolk reserves are nearly depleted and the larvae must start with external feeding is called the mixed feeding period (Kamler, 1992). Most of the marine species brought into aquaculture has a quantitative strategy, which involves many, but small eggs. Examples of this are cod, Atlantic halibut and turbot, but there are many other important aquaculture species as well, explaining why a sufficient understanding of the needs of fish larvae is important. Optimal production is essential for the economic aspect in aquaculture. Since the accurate conditions in the early stages of the fish are crucial for the further development, a lot of research and money is spent in optimizing this part of the production process.

## 1.2 Life feed

The live feed organisms, with its smell, shape and behavior, triggers the instinct of the fish larvae to start feeding (Conceição et al., 2010). To have formulated feed particles small enough to fit the mouth of the fish is a problem. Formulated feeds at that size have a tendency to disintegrate almost immediately, which will make it unavailable for the fish. On the other hand, fish will not eat feed particles that have sedimented to the bottom. There are a few important aspects when choosing the correct feed. First of all, the size of should not exceed the size of the fish mouth, which is an example of the match and mismatch hypothesis (Gotceitas et al., 1996). The optimal size of the feed must be fed to the larvae at the correct time during the development. There are different species of live feed organisms used in aquaculture this far and the most important are the brine shrimp (*Artemia* sp.) and rotifers (*Brachionus* sp.)(Conceição et al., 2010 ; Evjemo & Olsen, 1997 ; Evjemo et al., 2003).

The brine shrimp *Artemia* sp. was for the first time used as food for fish larvae in 1940 (Rollefsen, 1940). The live food technology did not improve much the following years and it was not before the Japanese scientists started using the rotifer, *Brachionus plicatilis*, some further important development occurred. They soon found out that the composition of essential nutrients in *Artemia* sp. and rotifers where not adequate for the needs of the fish larvae. Since most fish larvae have high requirements for highly unsaturated n-3 fatty acids (n-3 HUFA) techniques for enriching live feed HUFA's through their diets was developed later, and allowed significant improvement of the nutritional value (Olsen et al., 2007).

The production of fish larvae is therefore a multidisciplinary challenge. The environmental conditions in combination with physical-chemical environment must be satisfactory for a normal larval development. In addition, the nutritional regime has to fulfill the requirements of the larvae. The interaction of the larvae with the bacterial flora has to be managed as well (Olsen et al., 2007). During the 1990s, the relationship between normal non-pathogenic bacteria and the fish larvae was seen as the next key point to understand in larval rearing.

In the marine food web fish larvae like halibut, turbot and cod, generally live on different sizes of various zooplankton species (Evjemo et al., 2003 ; Olsen, 2007). Intensive fish production is still dependent on live feed organisms in the early larval stages. This is especially important for the altricial type of fish larvae that are still underdeveloped when the yolk sac is catabolized (Conceição et al., 2010).

## 1.3 Copepods

Copepods are small crustacean found in almost all aquatic environments. They are most abundant in the marine habitat, but many species also occupy the freshwater or estuarine environment as well. Copepods represent an important food source for a number of fish species. Most copepod species live in the benthic environment. The copepods that live in the pelagic zone are dominated by the order Calanoida, which makes up the most of the total plankton biomass. The parasitic species of copepods and the copepods that live in symbiosis with other animals counts for about one third of the total marine copepods species (Støttrup, 2003). The orders of copepods that are most interesting for aquaculture are the Calanoida, Harpacticoida and Cyclopioda. The Harpacticoida make up more than 50 % of the total copepod species. They are primarily benthic, free living organisms (Støttrup, 2003 ; Conceição et al., 2010). The Cyclopioda dominates in the freshwater environment, but is found all over the water column in the marine environment as well.

Most copepods have a sexual reproduction and shed their eggs directly into the water masses. The number of eggs female$^{-1}$ day$^{-1}$ varies from just a few to more than 50. Various species of the free-spawning *Acartia* can for example produce between 11 and 50 eggs female$^{-1}$ day$^{-1}$ (Støttrup, 2003)(Støttrup, 2003). During the entire spawning period, a female can produce more than 1200 eggs. *Calanus* species can produce even more, ranging from 15 to 230 eggs female$^{-1}$ day$^{-1}$, giving a total of 3800 eggs per female in the spawning period (Støttrup, 2003 ; Mauchline, 1998).

### 1.3.1 *Acartia tonsa* Dana

The copepod *Acartia tonsa,* of the genus calanoid copepods is a marine species with a cosmopolitan distribution. This free-spawning species is typically found in estuarine and marine areas, and is often the dominating copepod species, especially in the subtropical and temperate regions (Peck & Holste, 2006 ; Mauchline, 1998). *A. tonsa* has 6 nauplii stages before it reaches the copepodite stage. To reach the adult stage, it has to go through another 6 copepodite stages. Like other crustaceans, this is done by moulting. The eggs of *A. tonsa* are between 70-80 µm in diameter, and the hatching time range between 24 and 48 hours at 20 °C (Mauchline, 1998 ; Marchus et al., 2007). Adult individuals are easily recognizable by their long antennae, often longer than the total body length of the copepod.

*A. tonsa* has a sexual reproduction. The male species of *A. tonsa* uses their right antenna to grasp a female and releases a spermatophore on the urosome to fertilize her. The females live longer than the males and this might influence the composition of male and females in a population (Parrish & Wilson, 1978).

*Acartia* feed mostly on phytoplankton, but may as well eat rotifers, ciliates and their own eggs particularly when the abundance of other feed is low (Mauchline, 1998 ; Heinle, 1970). This is an important aspect that has to be considered in an intensive cultivation of *A. tonsa*.

### 1.3.2 Copepod production

The problem by using copepods as live feed organisms has been their availability. In nature there are natural blooms of phytoplankton in the spring/summer and autumn that is triggered by the light (Olsen, 2007). This phytoplankton bloom is followed by a zooplankton bloom and only in these periods the copepods can be utilized as live food for marine larvae. In other words, it is not optimal to time the rearing of fish larvae to periods when there are zooplankton available in the sea. The use of copepods as live feed has come from capturing in the sea (McEvoy, 2008), therefore it has been difficult to utilize the copepod fully as a source of nutrients. Copepods have to some extent been reared in a semi intensive production in closed bays or lagoons, but this method is in the same way only applicable seasonably, and is therefore only available in the summer when there is enough light to drive the natural processes in the sea.

There has been great progress in cultivating copepods in intensive systems, but there are still major challenges. The production of copepods are quite expensive compared to the production of *Artemia* and rotifers. Better technology and systems are needed to reduce the cost. The most important factor is still to have a predictable copepod production making them available at any time. This is crucial if a marine hatchery wants to invest in the use of copepods.

When using copepods as food, the different stages in the development can be utilized. Small fish larvae can for instance ingest the first nauplii stages in accordance with the match and mismatch theory (Gotceitas et al., 1996). The nutritional value of newly hatched copepod nauplii is optimal, and a further enrichment of n-3 HUFA is unnecessary.

The egg production is a bottle neck in intensive cultivation of copepod. This involves both the availability and storing of the eggs and also transport of the eggs. The eggs should be stored at stable temperatures close to 2°C. It is important to control on all the current variables involved in order to have an intensive production. The copepod density giving the highest egg production is very important to determine. It is very interesting for the aquaculture industry involved with the rearing of marine species like cod and other whitefish to get a more effective cultivation of copepods, like for example *Acartia tonsa*. Also within the aquarium industry, the copepods are evaluated as a great resource in first feeding.

## 1.4 Nutritional value

The nutritional value of *Artemia* and rotifers are not adequate and both organisms must be enriched with emulsified lipids to fulfill the nutritional needs of the fish larva, which are dependent on n-3 HUFA (highly unsaturated n-3 fatty acid), important components of phospholipids (PL) (Izquierdo, 1996). Fish are as other animals to some extent able to synthesize fatty acids (FA), but the essential fatty acids (EFA) cannot be synthesized by fish and must be provided through the food. The EFA are

synthesized by phytoplankton, which in turn is eaten by the zooplankton. In other words, one has to enrich the *Artemia* and rotifers to make them suitable for feed for the fish larvae. It is the highly unsaturated n-3 fatty acids (HUFA) like Docosahexaenoic acid (DHA, 22:6n-3) and Eicosapentaenoic acid (EPA, 20:5n-3) that are considered to be most important (Conceição et al., 2010 ; Evjemo et al., 2003). Since *Artemia* catabolizes DHA, the time after enriching before being fed to the fish larvae has to be as short as possible to minimize the losses.

The nutritional value of copepods has in contrast to that of *Artemia* and rotifers a much more suitable composition as feed for fish larvae. Several of the coastal copepod species have high contents of both DHA and EPA (Evjemo et al., 2003 ; Evjemo & Olsen, 1997). Studies have shown better survival, growth rate, improved pigmentation and less skeletal deformities for fish larvae when copepods are used, compared to *Artemia* and rotifer. (Støttrup et al., 1986 ; Evjemo et al., 2003 ; Evjemo & Olsen, 1997 ; van der Meeren et al., 2008). The stress tolerance is also greatly improved when copepods are used compared to enriched rotifers (Kraul et al., 1993). For halibut the use of copepods greatly enhanced the pigmentation and eye migration (Shields et al., 1999). Copepods contain high amounts of DHA and EPA and represent the natural food organism from the marine food web. It is not only the amount of EFAs that is important, the ratio between them are also very significant. (Evjemo et al., 2003 ; Shields et al., 1999 ; Sargent et al., 1999 ; Bell et al., 2003). When enrichment diets for *Artemia* and rotifers are produced the nutritional composition of copepods should be an important reference with respect to the lipids, proteins, etc.

The difference in where the DHA is stored in the organism is important. In copepods DHA is mainly found in the phospholipids. Enriched rotifers may have the same content of DHA, but here they are mainly not found in the phospholipids, but they are incorporated in the TAG's (triacylglyceride) or as free fatty acids (FFA) (Coutteau & Mourente, 1997). So even though both species should have the same amount of DHA, it seems as the copepods PL content give effect on the fish. They are in other words more readily available in the phospholipids for growth and pigmentation (Izquierdo et al., 2000 ; Gisbert et al., 2005).

## 1.5 Copepod feeding behavior

The algal diet of copepods will influence the egg production (Støttrup & Jensen, 1990 ; Donaghay, 1985). The size, quality and quantity of the algal cells influences on the ingestion rate of the copepods. The functional response describes the ingestion rate as a function of the food concentration. The relationship between food concentration and ingestion rate increase near-linear up to a maximum level ($I_m$) from where there is no further increase of the ingestion rate even if the food concentration increases (Støttrup & Jensen, 1990 ; Paffenhofer, 1976 ; Paffenhöfer & Harris, 1976 ; Frost, 1977). This is the saturation level and a response like this is typical for filter feeders like the copepods. For

*Acartia tonsa*, there is a sigmoid response to the algal concentration (Støttrup & Jensen, 1990). At low concentrations (below 1.0 $\mu$g chl *a*/l) the ingestion rate was found to be close to zero. As the concentration of algae increased, there was an exponential increase in feeding rate in the beginning, until it continued with a more linear increase. A maximum ingestion rate was found beyond a chlorophyll-a concentration of 10 $\mu g\ L^{-1}$ (Reeve & Walter, 1977).

The size of the food particles also influences the feeding behavior of *A. tonsa*. Small particles are filtered in the normal way, but with larger particles *A. tonsa* show a hunting behavior, a typical example of switching (Price & Paffenhöfer, 1985 ; Kiørboe et al., 1996). The optimal size of the feed particles for adult *A. tonsa* is about 15-25 µm (2-4% of the body length), while smaller stages prefer smaller particles (Støttrup & Jensen, 1990 ; Berggreen et al., 1988). There are also examples of a copepods ability to discriminate between the qualities of the food. They have the ability to avoid non food particles (Støttrup & Jensen, 1990 ; Donaghay & Small, 1979 ; Huntley et al., 1986). This is an advantage when it is known that the egg production is influenced by the food quality (Donaghay, 1985).

Studies have shown significant differences in the egg production, growth and time of maturity when different algal diets have been used. This is related to the fatty acid content in the diet. DHA will strongly influence the egg production whereas another important EFA, Arachidonic acid (ARA, 20:4n-6), is affecting the hatching (Evjemo et al., 2008). Støttrup and Jensen (1990) found that the algae *Isochrysis* and *Rhodomonas* (ca. 7.5 µm ESD; equivalent spherical diameter) gave much higher egg production because of their lipid high content (Støttrup & Jensen, 1990 ; Jónasdóttir & Kiørboe, 1996 ; Evjemo et al., 2008).

## 1.6 Water quality

The water quality is essential for all aquatic organisms, and to control the ammonia concentrations in the water is vital. Ammonia is toxic and has the un-ionized formula $NH_3$. When the pH is low, the ammonia gets ionized forming ammonium ($NH_4^+$) which is basically harmless for aquatic organism. The temperature and the ionic strength are also influencing the activity of ammonia. Below is the chemical equation of the relationship between ammonia and ammonium.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

When the pH is low, the reaction is driven to the right and if the pH is high it is driven to the left. If the pH is close to 6 there is, dependent on the temperature, almost no ammonia present. When pH is 8, there is 3.82 % $NH_3$ available and at pH 9, there is 28.4 % $NH_3$ (temperature of 20°C) (Jepsen et al., 2013 ; Emerson et al., 1975). The temperature will also influence the direction of the equation. If the

temperature is low, it will go to the right and the opposite when the temperature is high. A high pH and a high temperature are more toxic to an organism than the same pH would be at a low temperature. The un-ionized version of ammonia ($NH_3$) is in this paper named "reactive ammonia" and the "total ammonia" is the total of both the ionized and un-ionized version.

According to Jepsen et al. (2013) the lowest concentration of ammonia where adult *A. tonsa* is effected 1.8 mg $NH_3$ $L^{-1}$. The exposure over time was also registered, giving a 50 % lethal concentration for adults at 2.37 (24 hours), 0.972 (48 h) and 770 mg $NH_3$ $L^{-1}$ (72 h) (Jepsen et al., 2013). The lowest non harmful concentration of ammonia for copepods is 0.4 – 0.477 mg $NH_3$ $L^{-1}$ for a 24 hour exposure (Buttino, 1994 ; Jepsen et al., 2013).

## 1.7 Aim of study

The objective of this thesis was to describe the influence of copepod density and water exchange rate on the production of eggs from the copepod, *Acartia tonsa*, in an intensive rearing system. This is important for optimizing the production in large scale intensive systems.

Sub goals:

- Water quality at the different copepod densities.
- Feed requirements in a high density culture system

Research questions are:

- What is the optimal density for egg production and is this density possible to maintain at an adequate effort?
- What is the amount of feed required and are the quality of the water an important factor here?
- Is it possible to sustain a high density production using *Rhodomonas baltica* as feed, or is it too difficult to produce large enough amount of algae in the traditional way?
- Will high water exchange rate flush away too much of the algae meant for feed and in that way be making it too ineffective?

# 2 Materials and methods

## 2.1 Experimental setup

The different experiments were conducted with the copepod, *Acartia tonsa* Dana. The copepods were fed continuously with *Rhodomonas baltica*, and maintained at a density above 30 000 cells/ml (Skogstad, 2010 ; Berggreen et al., 1988). Tanks were modified to make an overflow system where the water volume was kept constant at 80 liter. Different variables of the water quality, like pH, salinity, temperature, dissolved oxygen (DO) and ammonia was measured and controlled. A photoperiod of 24 hours light was used.

The experiment was divided in three egg production trials.

Trial 1:

- Copepod densities: 5, 16 and 53 ind/ml. 3 replicates of each density (total of 9 tanks) with a water exchange rate of 100 percent.

Trial 2

- Copepod densities: 10 and 20 ind/ml. 3 replicates of each density (total of 6 tanks) with a water exchange rate of 500 percent.

Trial 3

- Copepod densities: 8 and 43 ind/ml. 3 replicates of each density (total of 6 tanks) with a water exchange rate of 500 percent. The difference from Trial 2 is that copepods were added during the trial to maintain the initial density.

### 2.1.1 Equipment used

Figure 1 and 2 shows the setup of the experiment. There were 3 replicates of the three treatments, giving a total of 9 experimental. The tanks were connected to an overflow system which made the water over a certain level to flow out of the tanks securing a continuous water flow through the system. In Trial 1, the water exchange (100 %) was added together with the algae into the copepod tanks. The water exchange in Trial 1 was 80 liter day $^{-1}$ and during Trial 2 and 3 this amount was 400 liters day$^{-1}$, this means a continuous flow of approximately 55 ml and 278 ml per minute, respectively.

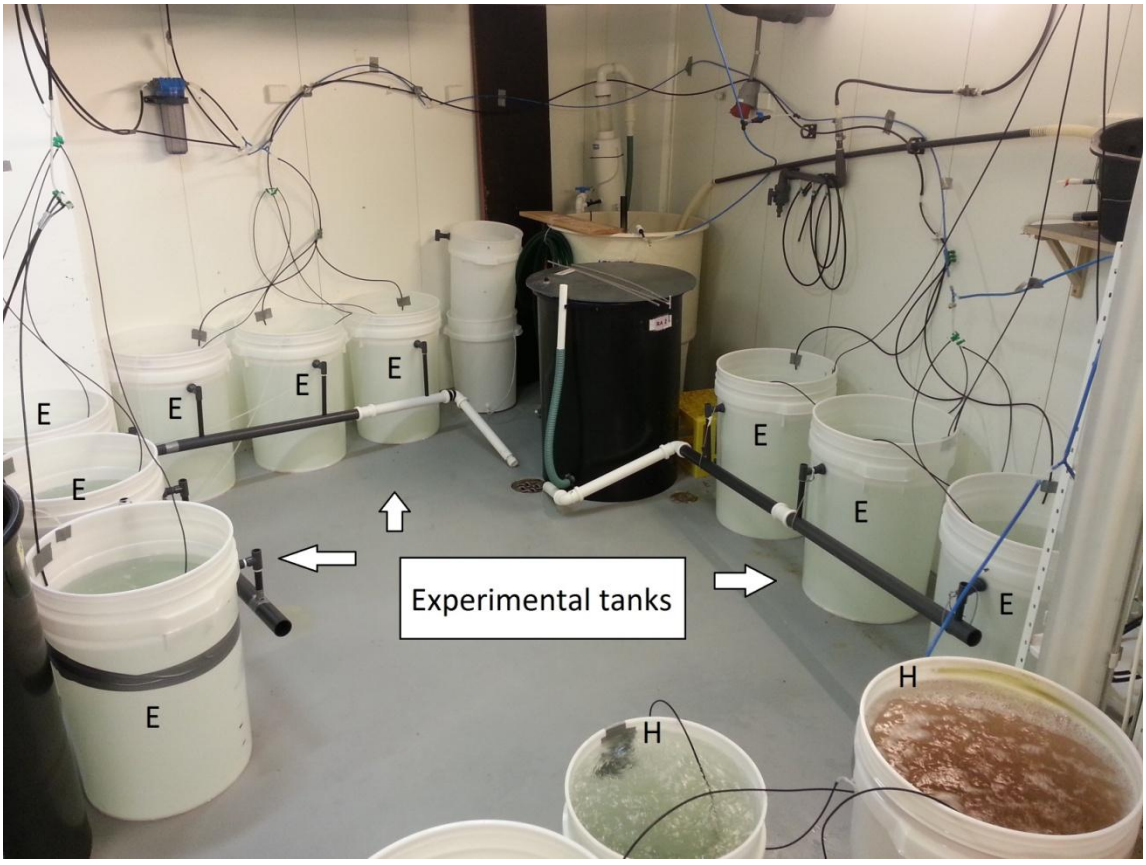


**Figure 1.** The experimental tanks (E) used in the experiment and a two hatching tanks in the front (H). The experimental tanks were connected to a drainage system (black and white pipes leading from each tank to the drain). The aeration and water exchange system is also visible, leading to each experimental and hatching tank (Photo by Erik Salveson).

The peristaltic pump (Watson-Marlow pumps, 520S Drive 220 RPM, Falmouth, Cornwall TR11 4RU, UK) allowed feeding to all the tanks (Figure 3). Several algae reservoirs (200 L) were connected to the pump simultaneously, because of the extensive feeding and water flow.

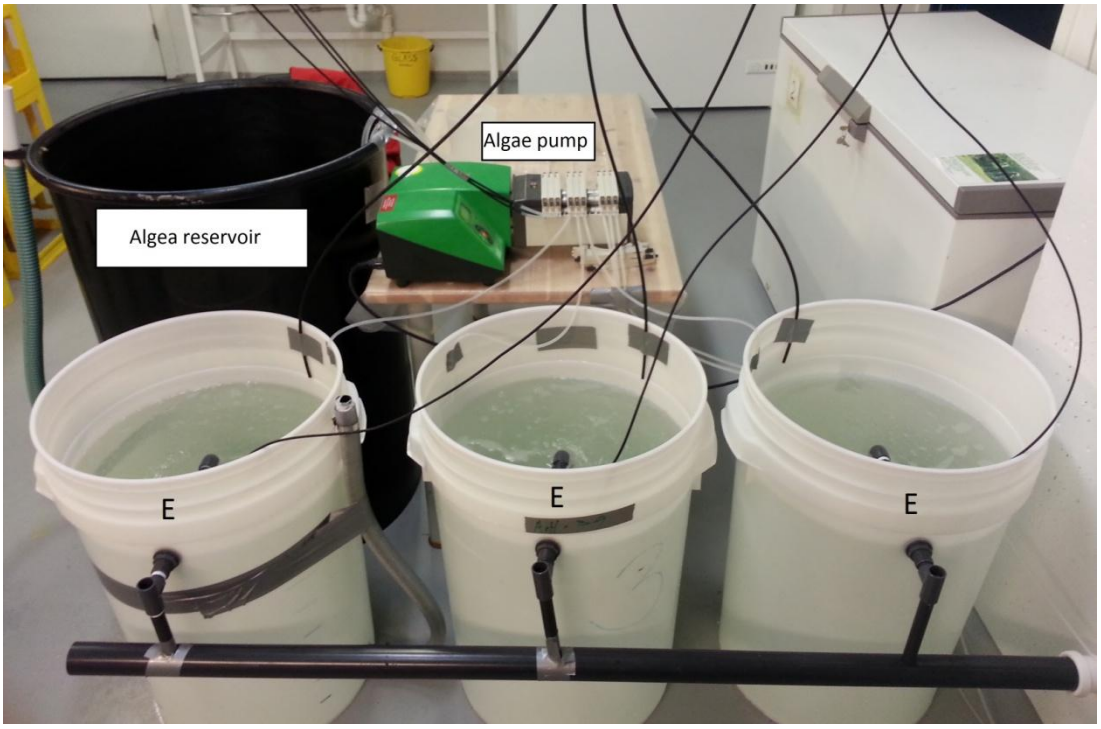


**Figure 2.** The experimental tanks were connected with a drainage system, leading the outlet water to the sewer. The algae reservoir contained the copepod feed pumped the different tanks. The white tubes were used for transporting the algae and the black tubes entering from the upper part of the picture were for aeration and inlet water (photo by Erik Salveson).

Plankton nets (SEFAR AG, Filtration Solution, 10m sefar petex 0.7-64/45, Hinterbissaustrasse 12 Werk 2/Warenannahme CH-9410 Heiden) with a mesh size of 64 µm were used in the overflow system. A filter is shown in Figure 3 (B). The mesh size of 64 µm was regarded to be adequate, since the copepods eggs were about 70-80 µm (Marchus et al., 2007). In this way, neither eggs nor copepods were lost. PVC tubes (10 cm diameter) were used for making the frame of the filters. They were cut in adequate size and polished. A 64 µm mesh was glued on (PeViCol®Nordcoll, PVC-lim til rør – og fittings, Nordcoll A/S Egeskovvej 12, DK-3490 Kvistgård) (Figure 3, A).

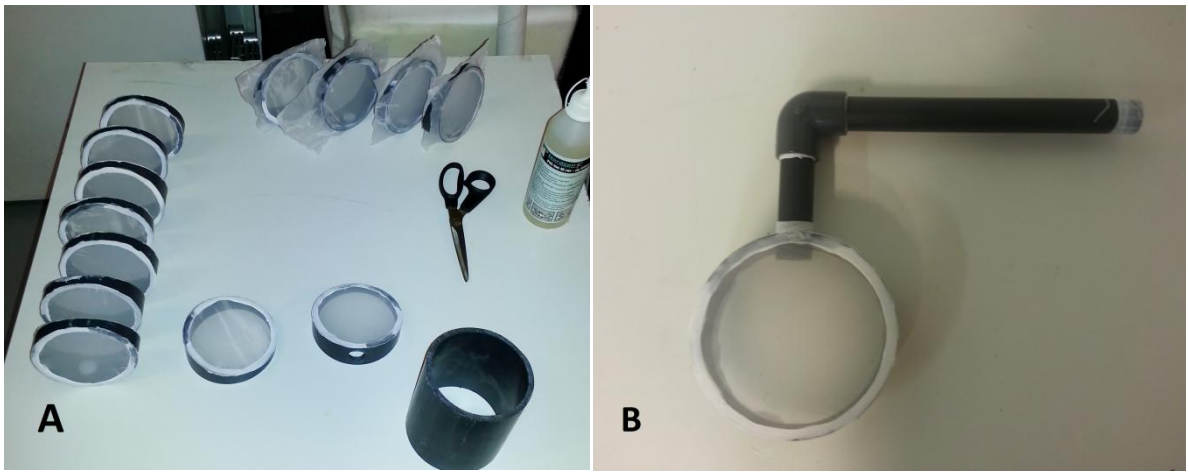


**Figure 3.** Equipment for the filters (A). A PVC tube was cut to form the frame of the filter and a mesh was glued on. A finished filter used in the experimental tanks (B) (Photo by Erik Salveson).

In every tank the aeration system generated a circulation and added oxygen to the water. Air stones were positioned above the floor in the tanks, at about 1/3 from the bottom of the tank (Figure 4). The air stones were common air diffusers used for aquariums (Karlie®, Heimtierbedarf GmbH•D-33181 Haaren).

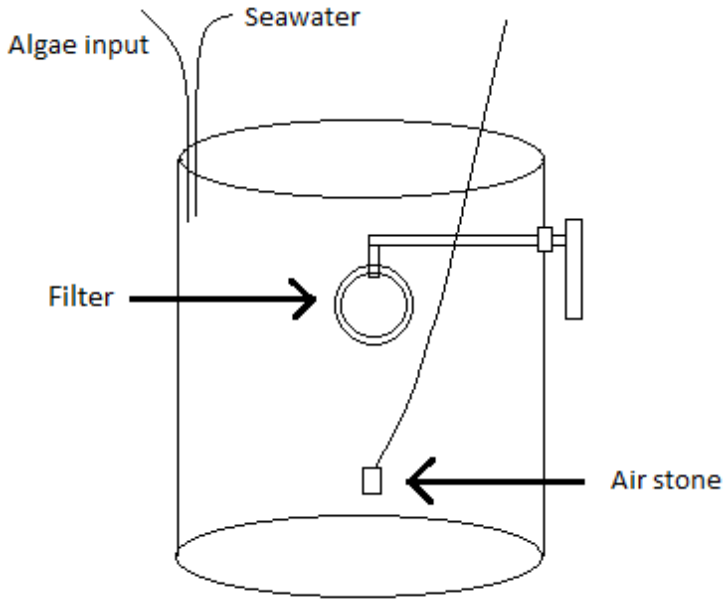


**Figure 4.** Experimental tank with air stone, tubes for algae- and seawater supply and filter system.

The seawater for both algae and copepod production was pumped from 70 meters depth from the Trondheim Fjord. The seawater was sand filtered, heated to 20 °C and filtered through a 1 µm mesh. The salinity of the seawater used for the copepods was 25 ‰ and lower than the salinity of the seawater used for algae (34 ‰). The reason for 25 ‰ salinity in the intake-water is related to the salinity for maximum egg production documented by Holste & Peck, 2006. This difference in salinity between the intake-water and algal culture influenced the salinity in the experimental tanks.

### 2.1.2 Hatching of *Acartia tonsa* eggs

The eggs used in this experiment were produced at SINTEF/NTNU Sealab, originating from the copepod *A. tonsa* Dana (Clone DFH.AT1). The eggs were hatched in 100 liter tanks filled with 80 liter of seawater (25 ‰) (Figure 5). An amount of 20 ml eggs were added to each tank (Appendix 3) and hatched after 48 hours. The newly hatched nauplii were fed *R. baltica* and grown for 5 days in Trial 1 and 2 and 11 days in Trial 3, before transferred to the experimental tanks.

**Figure 5.** Tanks used for hatching *A. tonsa* eggs. The hatching was performed under heavy aeration and continuous light for 48 hours in seawater (25 ‰) (photo by Erik Salveson).

### 2.1.3 Setup of the different egg production trials

Table 1 shows an overview of the treatments and copepod densities used in the experiments. More details are included for each trial below the table.

A total of three different copepod densities were used during the trials:

- Low copepod density tanks (LD tanks)
- Medium copepod density tanks (MD tanks)
- High copepod density tanks (HD tanks)

**Table 1.** Matrix showing the densities of copepods (ind/ml) and the treatments in the different trials, three replicates of each density.

| | Treatment | LD tanks (ind/ml) | MD tanks (ind/ml) | HD tanks (ind/ml) |
|---|---|---|---|---|
| **Trial 1:** | 100% water exchange | 5±0,5 | 16 ± 1,5 | 53±5 |
| **Trial 2:** | 500% water exchange | | 10±5 | 20 ± 0,5 |
| **Trial 3:** | 500% water exchange + adding of copepods | | 8±1 | 43±10 |

***Egg production trial 1***

The water exchange rate was 100 % per day, and was added together with the algal culture.
In the first trial 3 different densities of *A. tonsa* were used (Table 1), with 3 replicates of each density. The hatching of the eggs is explained in section 2.1.2. At day 5 the nauplii were transferred to the 9 experimental tanks.

***Egg production trial 2***

The water exchange rate in this setup was 500 % per day.

In this second trial, 2 different densities of *A. tonsa* were used (Table 1), with 3 replicates of each density. At day 5 the nauplii were transferred to the 6 experimental tanks.

***Egg production trial 3***

The water exchange rate was 500 %. To compensate for the copepods that had died or being lost due to sampling methods extra copepods were added during the trial in order to maintain the initial copepod density. Experience showed that about 10 % of the total tank volume needed to be replaced $day^{-1}$.

The same densities as in the previous trial were used again (Table 1), with 3 replicates of each density. The transfer of the copepods took place at day 11 after hatching when the copepods were at the last copepodite stage.

## 2.2 Daily routines of the experiment:

1. The algal density was measured every morning by filtering a small sample from each tank through a 64 µm mesh sieve into a bottle. The filter removed all eggs, copepods and larger particles that could interfere with the counting. The samples were taken with a cylindrical rod (1 cm in diameter) from 3 locations in each tank. A coulter counter (Multisizer ™Coulter Counter (capillary diameter 100 µm), Beckman Coulter Inc., USA) was used for counting.
2. Dissolved oxygen (DO) (measured with YSI ODO Digital Professional Series), pH (measured with pH/mV-meter, WTW pH 315i, Germany), salinity and temperature were measured daily.
3. The samples used for counting the density of *A. tonsa* in the tanks were obtained by using a cylindrical rod (1 cm in diameter) taking 5 smaller samples from different locations in each tank. A large pipette (5ml) was used to take out 3 ml for counting. From 9 samples the highest and lowest numbers were deleted.
4. The egg production was measured daily from when the egg production started.
5. Maintenance like washing filters and calibrating the water exchange rate was done every day. The pump where adjusted when needed to ensure adequate algae densities in the tanks. This was based on the daily counting of the algae densities in the tanks.

For every second or third day, the concentration of ammonia was measured (see below). During the last trial, the algal consumption and the hatching success was also measured.

### 2.2.1 Ammonia

Measurements of ammonia were done every second or third day. The instrument used was the Hach DR/890, Portable Datalogging Colorimeter. The ammonia level exceeded the level for the colorimeter to function, so the samples were diluted 10 times before measuring.

The measurement involved a 2 step reaction with the reagents Ammonia Salicylate Reagent and Ammonia Cyanurate Reagent (HACH®, Permachem® reagents for 10 mL sample).

Step 1:

- Ammonia Salicylate Reagent was added to the diluted 10 ml samples of the tanks. The reaction time was three minutes.

Step 2:

- The next reagent was added, Ammonia Cyanurate Reagent, with a reaction time of 15 minutes. Thereafter the samples were put in the colorimeter for reading the ammonia levels.

### 2.2.2 Algal consummation and losses

The ingestion rate of the copepods was estimated 3 times during the last trial. This was done to determine how the water exchange rate affected the amount of feed lost in the outlet water. The culture counter (Multisizer ™Coulter Counter (capillary diameter 100 µm), Beckman Coulter Inc., USA) was used to measure the alga density in the outlet water from each tank and the density of the total seawater and algae mixture supplied to the tanks.

### 2.2.3 Egg harvesting, washing and counting.

In order to harvest the eggs, they had to sediment to the bottom of the tanks. The height of the water column in the tanks was 52 cm and by using a sinking velocity of ≈ 0.02 cm $s^{-1}$(Miller & Marcus, 1994) the eggs needed at least 45 min to reach the bottom. The sedimentation time was therefore set to 60 min and the eggs were harvested by siphoning the bottom with a T-shaped rod. Every tank had its own bucket where the siphoned eggs and the other substances that came along were collected.

The next step was washing the eggs. This was performed by filtering the content in the buckets through a 120 and a 100 µm mesh sieve. In this process, most of the bigger particles like dead copepods, feces and aggregations of algae were removed, while the eggs (diameter 70-80 µm (Marchus et al., 2007)) went through the filter. The last filter had a 64 µm mesh size and this collected and concentrated all the eggs. During this process the eggs were washed with seawater until the blue color of the eggs appeared, making the eggs ready for storing in a refrigerator at 2 °C (SANYO Medi-Cool pharmaceutical refrigerator, model MPR-311D[H]). The eggs were collected in 28 ml bottles filled with oxygen deficient seawater (bubbled with nitrogen).

To calculate the daily egg production in the tanks a small sample of 0.5 ml was taken from every bottle. The bottles were shaken carefully before sampling, making the eggs as homogenous as possible. These 0.5 ml samples with eggs were diluted 40 times. From each sample, 7 drops of 100 µl

each were counted for eggs and the highest and lowest number was deleted. In the tanks with the highest egg production the samples were 0.25 ml instead of 0.5 ml. These samples were in addition diluted 80 times.

### 2.2.4 Hatching success of *A. tonsa* eggs

The hatching success of the eggs was tested during Trial 3. Close to 100 fresh eggs were harvested and washed the same day and thereafter set for hatching. These 100 eggs were placed in a Petri dish, filled with seawater (25 ‰) and sealed with Parafilm. The samples were kept at 21°C under continuously light to hatch for 48 hours. A stereomicroscope (Leica MZ $12_5$) was used to count the hatched nauplii and unhatched eggs fixed with Phytofix (Lugol's solution). Making the counting more accurate, a peristaltic pump (Watson-Marlow pumps, 120S/DV 200RPM, Falmouth, Cornwall TR11 4RU, UK) was used for removing the already counted eggs and nauplii. The hatching success (48-hours) was thereafter calculated using the Equation 1.

$$\text{Hatching success, 48 hours (\%)} = (N_{\text{hatched nauplii}}/N_{\text{eggs}})*100 \quad (1)$$

## 2.3 Production of microalgae (*Rhodomonas baltica*)

The production of *Rhodomonas baltica* (Clone NIVA 5/9 Cryptophycea: Pyrenomonadales) was performed in cylindrical tanks (160 -200 liters (Plexiglas, 40 cm in diameter)) and plastic bags of 300 liters. The algae were grown in seawater (34 ‰), at temperature between 20-22 °C and pH ranging from 7.5 to 8.3 (measured with pH/mV-meter, WTW ph 315i, Germany). Fluorescent tubes (6, GE Polylux XL 830 F58W) were used for a continuous illumination at 3 different sides around each tank. The tanks were aerated and added $CO_2$ (1-2 %).

The production was started with 10 liters of intermediate culture, at a density of 2-3 x $10^6$ cells per $mL^{-1}$. This constituted for at least 10 percent of the volume of the started tank. The culture was added 1 ml Conwy medium (modified from Walne (1974), Appendix 1), per liter seawater. After reaching stationary phase (density of approximately 1.2 mill cells $ml^{-1}$), the tank was divided in two, and an additional tank was filled. This was done until 9 tanks were filled (4 tanks of 160 liters and 5 of 200 liters). An additional plastic bag of 300 liters was used as well. Every day, 40-50 percent of the production was harvested and replaced by seawater and Conwy medium and run continuous. The oldest tanks were constantly washed and replaced and at least one tank was washed and replaced every day by either splitting another tank, or using the stem culture to start a new.

The seawater used in this production was sand filtered, heated to 20 °C, filtered through a 1 µm mesh, chlorinated and dechlorinated. The chlorination and dechlorination took place in two tanks of 700 and 1000 liters in the algae room. The chlorination was done by adding 25 ml chlorine per 100 liters seawater, without aeration for a minimum of 5 hours. For dechlorination, 3 grams of sodium thiosulphate pentahydrate ($Na_2S_2O_3$) per 25 ml chlorine was used. This was done under heavy aeration for a minimum of 5 hours (Hoff & Snell, 1997).

## 2.4 Statistics

All the data were collected in Microsoft Office Excel for Windows (Microsoft Inc.) and calculations of average and standard error were done here. This data was further on used in SigmaPlot for Windows Version 12.3 (Systat Software, Inc) for the making of graphs and figures. All of the tables were made in Microsoft Office Excel. The normality of the data was tested with the test of Professor Scott Guth at Mt. San Antonio College, uploaded in Microsoft Office Excel. The normalized data was further on analyzed by running ANOVA test in Microsoft Office Excel to figure out the statistical significance with the P value of 0.05.

# 3 Results

## 3.1 Copepod density

Figure 6 shows the copepod densities in the experimental tanks for the 3 trials. The LD tanks in Trial 1 showed a stable, slowly declining copepod density, during the entire trial. The same was found for the MD tanks in Trial 1 and 2 as well, but at day 7-9 a more distinct reduction of the copepod density occurred. A similar reduction was found in the HD tanks in Trial 1 and 2 on the same days. Up to day 9 there were a significant difference in copepod density between the LD, MD and HD tanks during Trial 1 and between the MD and HD tanks in Trial 2 ($P<0.05$), but from day 10 all the tanks had dropped close to the same density, 4.3 ± 0.7 and 4.0 ± 1.5 ind/ml, in Trial 1 and 2, respectively (mean ± SE).

The constant addition of copepods in Trial 3 led to a mean copepod density in the HD tanks of 32.5 ± 4.0 ind/ml (mean ± SE) up to day 15. At this point no more copepods were added and the density decreased rapidly. The MD tanks in Trial 3 showed a constant copepod density of 10.0 ± 1.5 ind/ml (mean ± SE) through the entire trial.

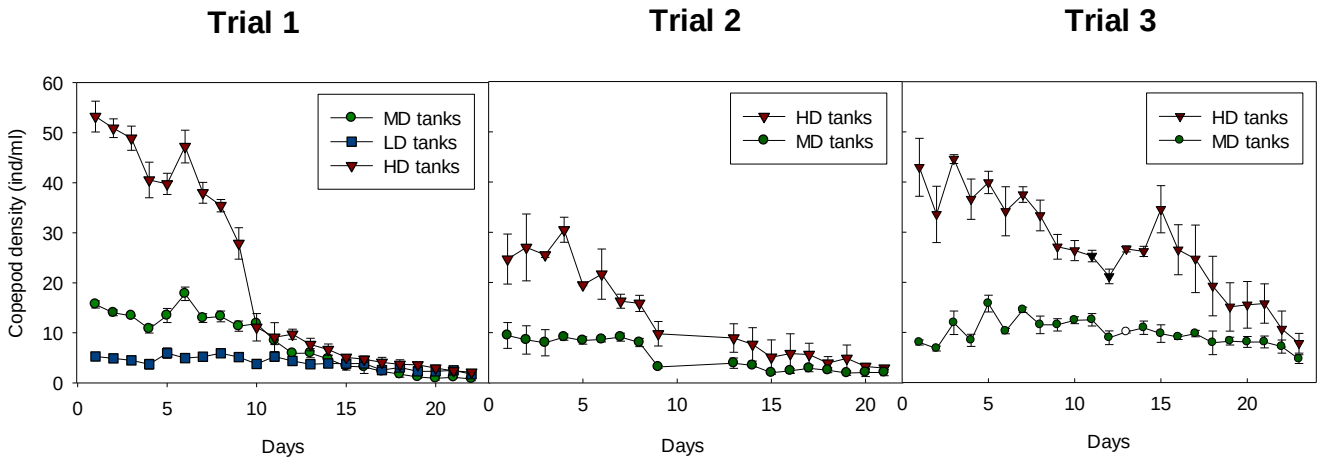


**Figure 6.** The copepod density (ind/ml) for all the experimental tanks in Trial 1, 2 and 3 (Mean ± SE).

## 3.2 Algae density

The algae densities in the tanks for Trial 1, 2 and 3 are shown in Figure 7. Trial 1 started with a high algae density for all the treatments. This was followed by a period of algae density below the saturation level (30 000 ind/ml) of the copepods up till day 8-9 (Marchus et al., 2007 ; Skogstad, 2010). After day 9, the algae density increased for all the tanks and remained above the saturation level the entire trial. The average algae number in Trial 1 for the LD, MD and HD tanks were from day 10-21, 56000 ± 20000, 91000 ± 19000 and 72000 ± 18000 cells/ml (mean ± SE), respectively.

The algae density in the MD tanks in Trial 2 decreased from the start, and increased thereafter continuously for two weeks before it stabilized at a level from day 15 of 41000 ± 3000 cells/ml (mean ± SE). The HD tanks in Trial 2 showed a similar trend, but the algae level was below the saturation level of the copepods until day 9. Besides from a significant drop in algae density on day 15, the mean ± SE from day 14 were 57000 ± 7000 cells/ml (day 15 excluded).

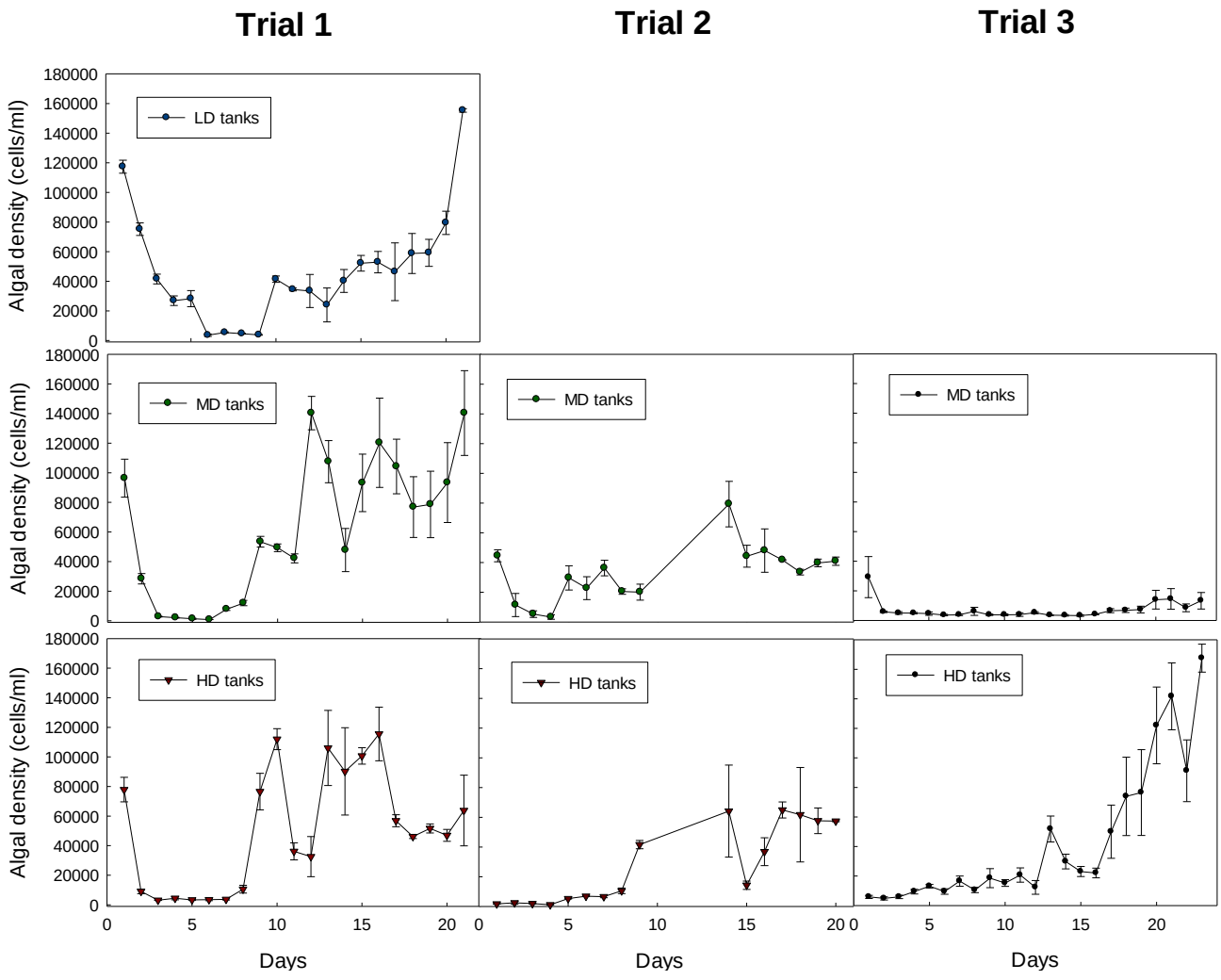


**Figure 7.** Algae density in the different experimental tanks in Trial 1, 2 and 3 (Mean ± SE).

The algae density in the MD tanks in Trial 3 were low almost through the entire trial (6200 ± 2000 cells/ml, mean ± SE), with a few days at the end where the density increased to a level close to the saturation level for one of the tanks. The algae density in the HD tanks increased during the entire trial, but remained below the saturation point until day 17, except a peak at day 13.

## 3.3 Egg production

Figure 8 shows the egg production in the experimental tanks during Trial 1, 2 and 3. The egg production in Trial 1 increased for all the tanks during the first week before it leveled off. The LD, MD and HD tanks had an egg production of 2.6 ± 0.4, 1.8 ± 0.5 and 1 ± 0.2 mill eggs day$^{-1}$ from day 12-22, respectively (mean ± SE). Even though the egg production tended to increase with decreasing copepod density (LD>MD>HD), there were no statistical differences (P>0.05) between the treatments due to the great variations, but the trend was quite apparent. The maximum egg production in an LD tank was close to 5 mill eggs day$^{-1}$.

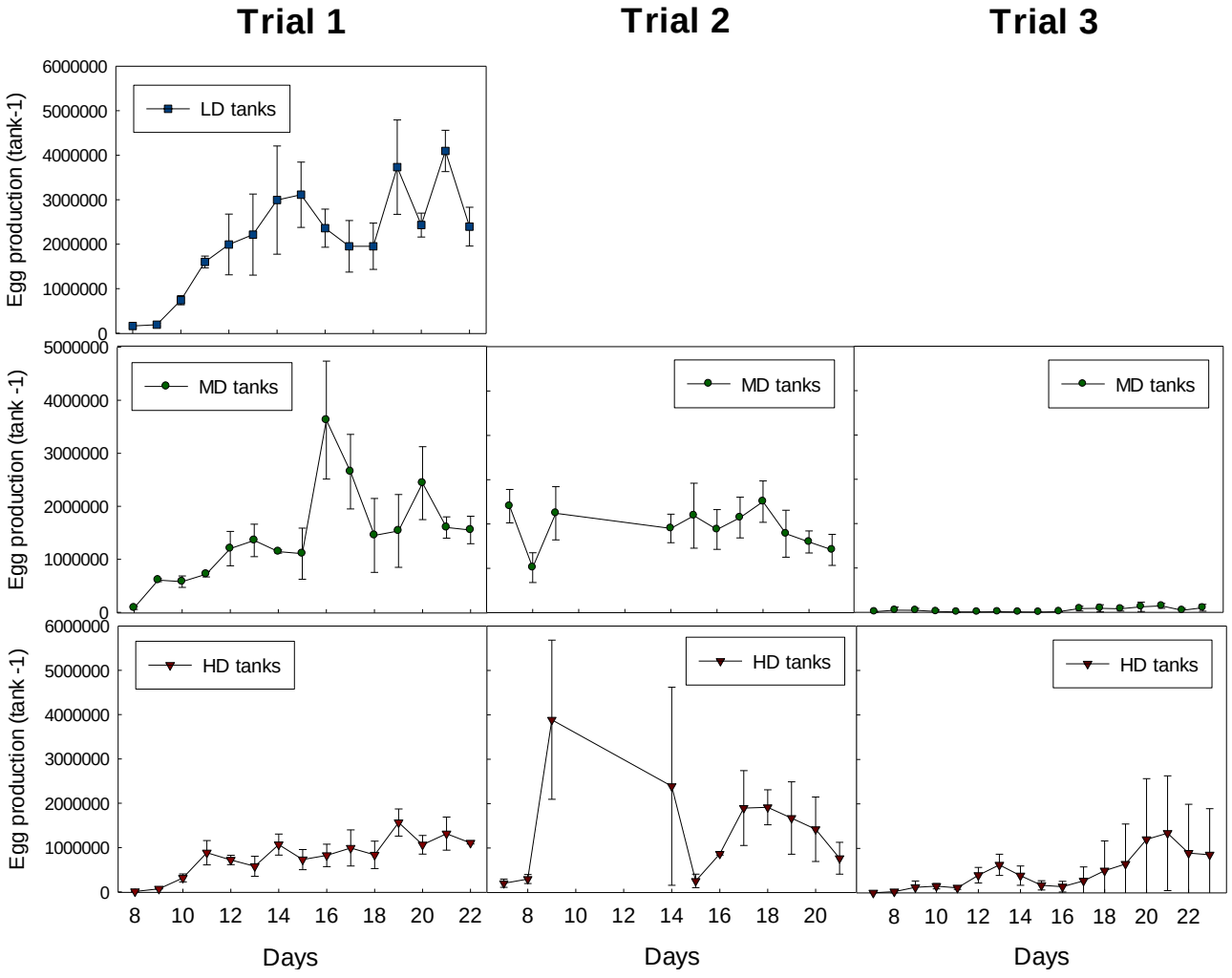


**Figure 8.** Egg production (eggs tank$^{-1}$ day$^{-1}$) in the different experimental tanks in Trial 1, 2 and 3 (Mean ± SE).

The egg production was more stable in the MD tanks (1.6 ± 0.4 mill eggs day$^{-1}$, mean ± SE) than in the HD tanks in Trial 2, where there were great variability between the days and tanks. The egg production was stabilized during the last 6 days (1.4 ± 0.4 mill eggs day$^{-1}$, mean ± SE).

In Trial 3 the egg production was low the first 7 days for both the treatments (HD and MD tanks). In the MD tanks, the egg production was low during the entire trial, 45000 ± 25000 eggs day$^{-1}$ (mean ± SE). For the HD tanks in Trial 3, Tank 3 showed a small peak in the egg production at day 9, where after it showed same variability up to day 16, beyond which it increased rapidly the next days, reaching relatively high productivity at day 21 (2.78 mill eggs). The average for Tank 3 for day 17- 23 was 1.9 mill eggs day$^{-1}$ (Mean ± SE). The other HD tanks in Trial 3 showed a lower egg production, but some of the trends were the same as in Tank 3, with variations between day 9 – 16. At the end of Trial 3, Tank 1 showed a small increase in egg production as well (1 mill eggs on day 21).

## 3.4 Female ratio of *A. tonsa* in the experimental tanks

The female ratios during the three trials are shown in Figure 9.

The average female ratio of the LD, MD and HD tanks in Trial 1 were 35.8 ± 1.2 %, 58.7 ± 6.5 % and 76.1 ± 3.0 %. The proportion of female *A. tonsa* increased accordingly with increasing total copepod density in the tanks. There were no statistical difference between the MD tanks and the HD tanks ($P>0.05$) in Trial 1, but a statistical difference were found between the LD tanks and the MD tanks and between the LD tanks and the HD tanks ($P<0.05$).

In Trial 2 the MD and the HD tanks showed a female ratio of 67.3 ± 3.7 % and 75.0 ± 4.2 % (mean ± SE), respectively. There was no significant difference between the treatments ($P>0.05$).

The MD and the HD tanks of Trial 3 showed a female ratio of 76.5 % ± 1.3 % and 77.5% ± 1.1 % (mean ± SE), respectively. The female ratio was more or less the same during the entire trial, and there were no significant difference between the MD and the HD tanks ($P>0.05$).

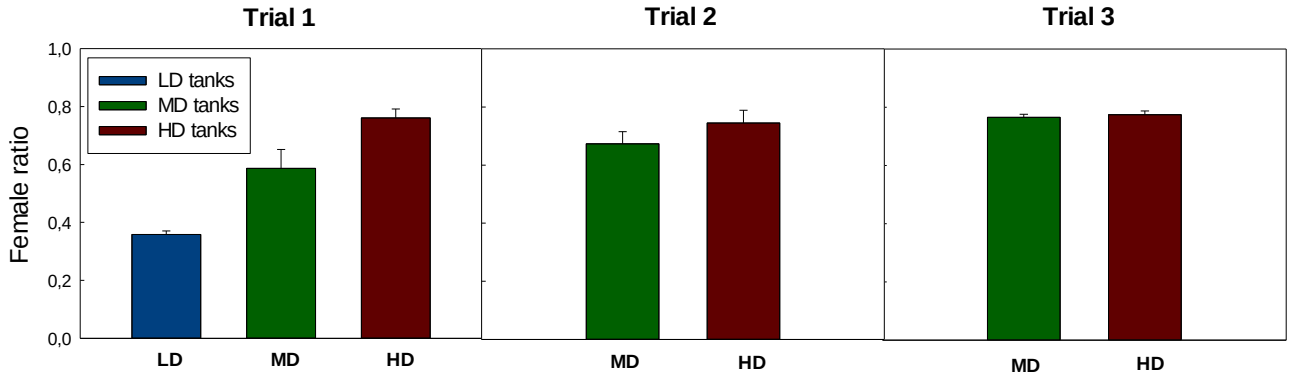


**Figure 9.** Female proportion of *A. tonsa* in the different treatments during Trial 1, 2 and 3 (Mean ± SE).

## 3.5 Eggs per female

Eggs per female on day 15 in Trial 1 are shown in Table 2. The highest egg productions were found in the LD tanks, intermediate production in the MD tanks and the lowest egg production in the HD tanks.

**Table 2.** Eggs per female on day 15 in Trial 1

| | **Eggs per female** |
|---|---|
| **LD tanks** | 28,2 |
| **MD tanks** | 7,08 |
| **HD tanks** | 2,03 |

Table 3 shows the eggs production female$^{-1}$ day$^{-1}$ at 6 different days in Trial 2. The egg production showed the same variation with time. The MD tanks showed higher egg production values than the HD tanks (mean ± SE).

**Table 3.** Eggs per female during Trial 2.

| | **MD tanks** | **HD tanks** |
|---|---|---|
| | Eggs per female | Eggs per female |
| **Day 15** | 22,82 | 1,06 |
| **Day 16** | 16,01 | 2,59 |
| **Day 17** | 13,84 | 6,26 |
| **Day 18** | 20,77 | 7,42 |
| **Day 19** | 14,02 | 5,35 |
| **Day 21** | 11,25 | 3,72 |
| **Mean ± SE** | 16,4 ± 1,8 | 4,4 ± 1,0 |

Table 4 shows eggs per female from day 6 to 23 in Trial 3. Compared to the MD tanks in Trial 2, the egg production in Trial 3 was very low for both the HD and MD tank, except for Tank 3, one replicate of the HD tanks, which is shown separately in the right column of the table. The egg production in Tank 3 increased strongly the last days of the experiment, showing a maximum at day 22. The egg production female$^{-1}$ day$^{-1}$ in the HD tanks ranged between 0.001-1.6, while for the MD tanks it ranged between 0.01 and 0.32 eggs female$^{-1}$day$^{-1}$.

**Table 4.** Eggs per female from day 6 to 23 in the Trial 3, MD and HD tanks, Tank 3 (HD) has its own column.

| | MD tanks | HD tanks | Tank 3 (HD) |
|---|---|---|---|
| | Eggs per female | Eggs per female | Eggs per female |
| **Day 6** | 0,04 | 0,005 | 0,02 |
| **Day 7** | 0,01 | 0,001 | 0 |
| **Day 8** | 0,06 | 0,015 | 0,03 |
| **Day 9** | 0,06 | 0,085 | 0,21 |
| **Day 10** | 0,03 | 0,099 | 0,15 |
| **Day 11** | 0,01 | 0,077 | 0,11 |
| **Day 12** | 0,01 | 0,292 | 0,36 |
| **Day 13** | 0,02 | 0,376 | 0,5 |
| **Day 14** | 0,01 | 0,234 | 0,37 |
| **Day 15** | 0,01 | 0,079 | 0,15 |
| **Day 16** | 0,03 | 0,086 | 0,22 |
| **Day 17** | 0,14 | 0,168 | 0,79 |
| **Day 18** | 0,21 | 0,401 | 2,37 |
| **Day 19** | 0,14 | 0,768 | 4,42 |
| **Day 20** | 0,23 | 1,171 | 6,25 |
| **Day 21** | 0,28 | 1,352 | 5,33 |
| **Day 22** | 0,09 | 1,309 | 9,74 |
| **Day 23** | 0,32 | 1,646 | 7,29 |
| **Mean ± SE** | 0,09 ± 0,02 | 0,45 ± 0,13 | 2,13 ± 0,72 |

## 3.6 Hatching success

Figure 10 shows the average hatching percent of eggs at day 14, 17 and 23 for all the experimental tanks in Trial 3. The mean value of the HD tanks (46.1 ± 5.0 %), was statistically lower ($P < 0.05$) than the mean of the MD tanks (64.8 ± 4.0 %).

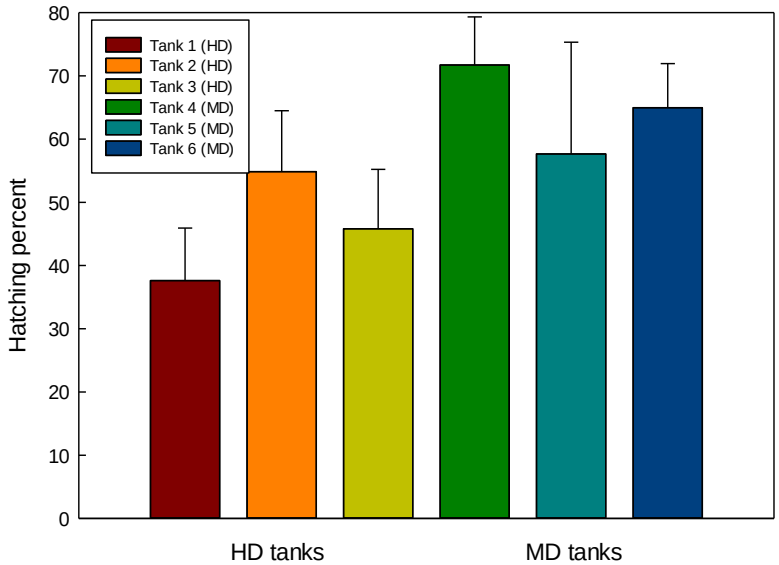


**Figure 10.** The average hatching percent at the three days of hatching (day 14, 17 and 23) for all the experimental tanks in Trial 3 (mean ± SE).

## 3.7 Feed utilization

A special study of the input and losses of feed was undertaken for Trial 3 to estimate the consumption of the copepod. The concentrations of algae in the water that was pumped into (inlet) and out of the tanks (outlet) were measured, and the differences were calculated.

Figure 11 shows the amount of algae (cells/ml) going into, out of and being consumed by the copepods at day 14, 16 and 21. The graph labeled "consumed" is the difference between the inlet and the outlet. In both the MD and the HD tanks, the amounts of algae consumed were declining during the trial. At day 14 and 16 in the HD tanks, the difference between the inlet and consumed was small, meaning that the feed utilization was high. On day 21 the difference had increased, which means that the utilization had decreased. In the MD tanks the difference between the inlet and consumed were small during the entire trial. There was a significant difference in the algae consumed between day 14 and day 21 of the HD tanks ($P<0.05$).

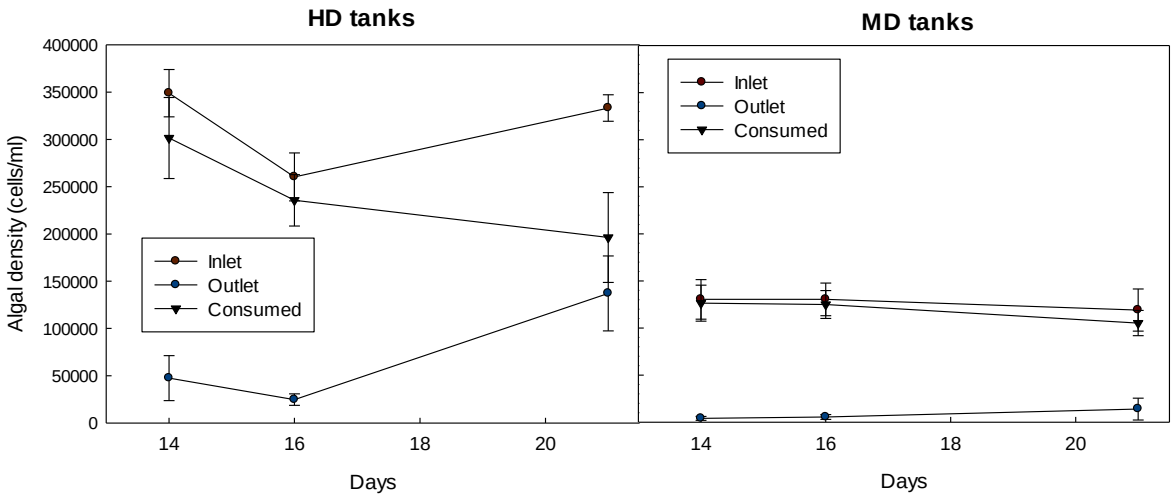


**Figure 11.** Amount of algae in inlet, outlet and being consumed in the MD tanks (right) and the HD tanks (left) in Trial 3 on day 14, 16 and 21 (Mean ± SE).

Figure 12 gives an overview of the feed utilization of the HD tanks in Trial 3 where the percentages of supplied food going into the tanks, leaving the tanks and the part being consumed by the copepods are shown together with the copepod biomass, the concentration of ammonia and egg production. As revealed in the figure, 58-90 % of the algae were consumed each day (14, 16 and 21), and 10-42 % were accordingly lost.

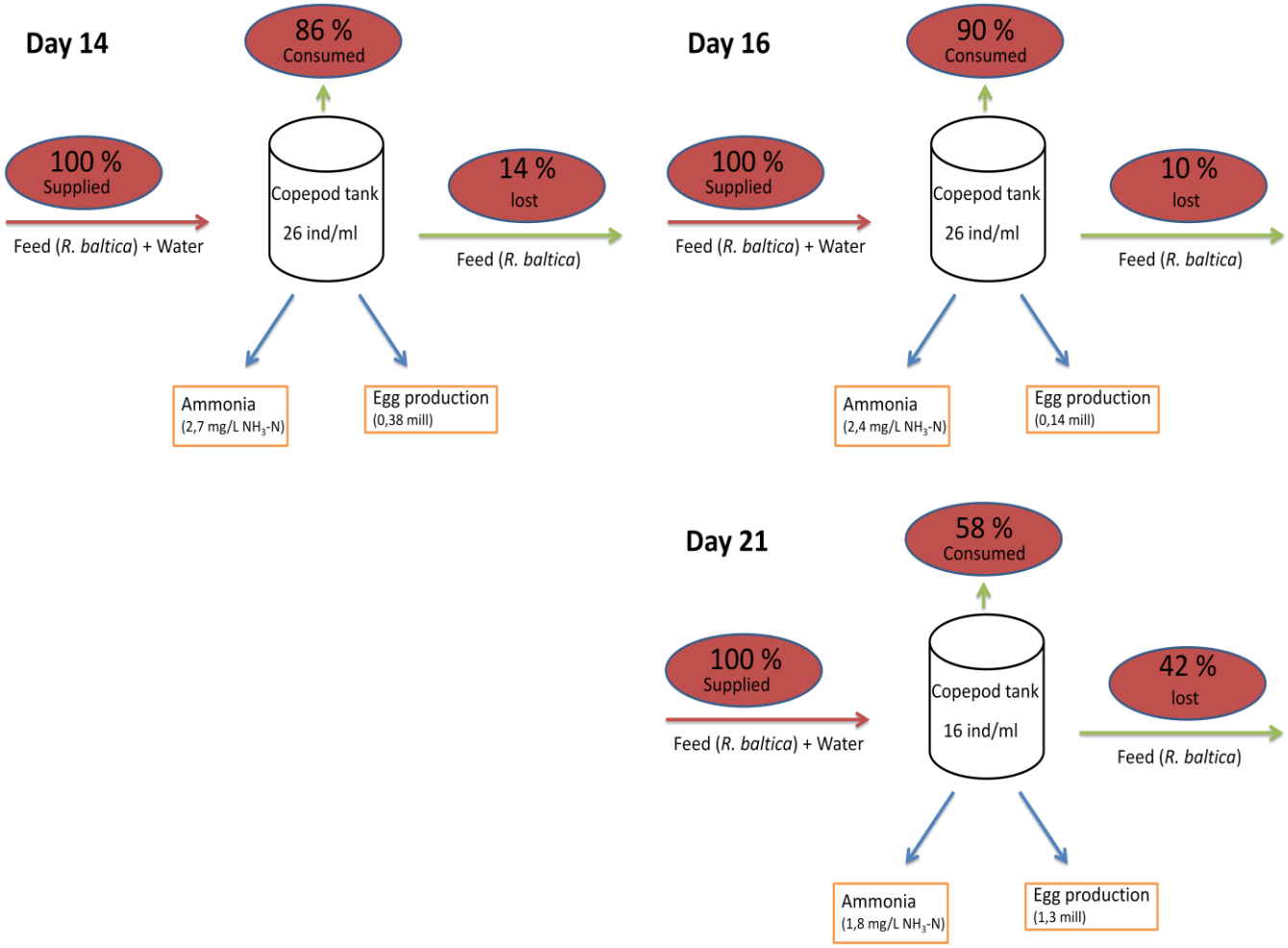


**Figure 12.** Feed supply, losses and consumption on day 14, 16 and 21 in Trial 3 expressed in terms of percent of supplied food (Red oval circles), concentration of ammonia, mean copepod density and egg production. Red arrows indicate: Total water into tank (500 % water exchange day$^{-1}$), green arrow indicate: Total water out of the tank (= amount going into the tank) and algae consumed by copepods, blue arrow: Effects of the system (Copepod survival, ammonia and egg production in the tanks).

### 3.7.1 Specific feeding rate of copepods

The carbon content of Rhodomonas baltica is 37 (pg C cell-1) (Kiørboe et al., 1985 ; Støttrup & Jensen, 1990). The carbon content of Acartia tonsa is 32.8-48.8 % of its dry weight (C content = 40 % of dry weight used further) (Ambler, 1985 ; Mauchline, 1998 ; Parsons et al., 1983). With a dry weight of 7 µg DW $ind^{-1}$ (Ikeda et al., 2001), the carbon content per individual will be 2,8 µg C $ind^{-1}$. The carbon content in algae consumed by copepods per day was calculated (Table 5), assuming that there were no algae lost through sedimentation.

**Table 5.** Number of algae consumed by the HD tanks (mean) and for the individual copepod per min. Total carbon content of the algal cells consumed by copepods a day.

| Day | Copepod density (ind/ml) | Total number of copepods (mean HD tanks) | Total number of algae consumed $min^{-1}$ (mean HD tanks) | Number of algae consumed $copepod^{-1}$ $min^{-1}$ (mean HD tanks) | Total carbon content algae consumed $copepod^{-1}$ (µg $day^{-1}$) (mean HD tanks) | Percentage of total bodyweight in carbon consumed ($day^{-1}$) |
|---|---|---|---|---|---|---|
| **14** | 26,2 | $2,09*10^6$ | $83,8*10^6$ | 40.0 | 2,1 | 75 % |
| **16** | 26,5 | $2,12*10^6$ | $65,5*10^6$ | 30,9 | 1,6 | 57 % |
| **21** | 15,8 | $1,26*10^6$ | $54,5*10^6$ | 43,3 | 2,3 | 82% |

## 3.8 Ammonia

The ammonia concentrations in the three experimental trials are shown in Table 6 and 7 and in Figure 13.

Table 6 shows that the ammonia at day 11 in Trial 1 (100 % water exchange $day^{-1}$) for the LD, MD and HD tanks. For the HD tanks the ammonia level increased during the entire trial, starting at 5.2 and ending at 7.1 mg/L $NH_3$-N. The MD tanks had a stable ammonia level at day 11-13 before it decreased on day 17. The ammonia levels for the LD tanks were stable during the days of measuring.

**Table 6.** Ammonia concentration, mg/L $NH_3$-N, in the HD, MD and LD tanks (mean ± SE) at day 11, 12, 14 and 17 in Trial 1. r gives the reactive fraction of the total ammonia ($NH_3$).

| | **Day 11** | **Day 12** | **Day 14** | **Day 17** | **Mean** |
|---|---|---|---|---|---|
| | NH3-N ± SE | NH3-N ± SE | NH3-N ± SE | NH3-N ± SE | NH3-N ± SE |
| **HD tanks** | 5.2 ± 0.4 (r=0.20 ± 0.02) | 5.5 ± 0.3 (r=0.21 ± 0.01) | 6.0 ± 0.6 (r=0.23 ± 0.02) | 7.1 ± 0.5 (r=0.27 ± 0.02) | 5.9 ± 0.4 (r=0.23 ± 0.02) |
| **MD tanks** | 4.9 ± 0.2 (r=0.19 ± 0.008) | 5.2 ± 0.1 (r=0.20 ± 0.004 | 4.0 ± 1.3 (r=0.15 ± 0.05) | 2.8 ± 0.3 (r=0.11 ± 0.01) | 4.2 ± 0.5 (r=0.16 ± 0.02 |
| **LD tanks** | 2.4 ± 0.2 (r=0.10 ± 0.008) | 2.7 ± 0.2 (r=0.10 ± 0.008) | 2.5 ± 0.3 (r=0.10 ± 0.01) | 2.5 ± 0.2 (r=0.10 ± 0.008) | 2.5 ± 0.1 (r=0.10 ± 0.004) |

Table 7 shows the ammonia concentration in the MD and HD tanks in the Trial 2 where a water exchange of 500 % was used. Both the MD and HD tanks followed the same pattern, peaking at day 9.

**Table 7.** Ammonia concentration of the HD tanks and MD tanks (mean ± SE) in Trial 2 on day 7, 9 and 16. r gives the reactive fraction of the total ammonia ($NH_3$).

| | **Day 7** | **Day 9** | **Day 16** | **Mean** |
|---|---|---|---|---|
| | NH3-N ± SE | NH3-N ± SE | NH3-N ± SE | NH3-N ± SE |
| **HD tanks** | 1.1 ± 0.05 (r=0.04 ± 0.002) | 1.8 ± 0.05 (r=0.07 ± 0.002) | 0.7 ± 0.2 (r=0.03 ± 0.008) | 1.2 ± 0.3 (r=0.05 ± 0.01) |
| **MD tanks** | 0.4 ± 0.1 (r=0.02 ± 0.004) | 0.8 ± 0.03 (r=0.03 ± 0.001) | 0.5 ± 0.1 (r=0.02 ± 0.004) | 0.5 ± 0.1 (r=0.02 ± 0.004) |

Figure 13 shows the ammonia concentration in the different copepod tanks in Trial 3 where the water exchange was 500 %. The ammonia level of the MD and HD tanks increased from day 1, peaking at day 11 (3.0 ± 0.3 and 0.11 ± 0.01 mg/L $NH_3$-N total and reactive ammonia, respectively) and 14 (1.6 ± 0.3 and 0.06 ± 0.0 mg/L $NH_3$-N total and reactive ammonia, respectively), respectively. Thereafter the ammonia level decreased till the termination of the trial. The mean of the MD and HD tanks during the trial was 0.9 ± 0.2 (0.03 ± 0.008 mg/L $NH_3$-N reactive ammonia) and 2.1 ± 0.5 (0.08 ± 0.02 mg/L $NH_3$-N reactive ammonia) mg/L $NH_3$-N (mean ±SE)

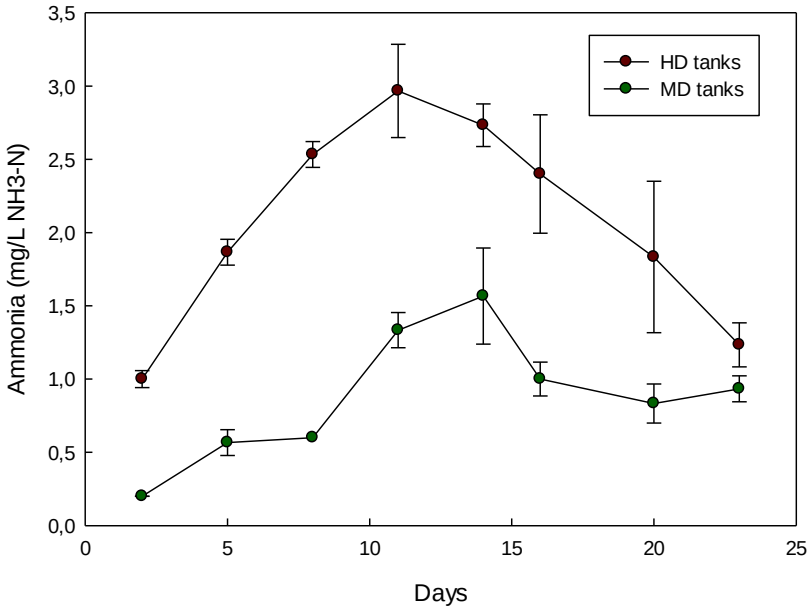


**Figure 13.** Ammonia concentration for the MD and the HD tanks measured in Trial 3 (mean ± SE).

## Ammonia excretion:

Table 8 shows an example of the estimated ammonia production per individual copepod and for the mean of the HD tanks during Trial 3.

**Table 8.** Ammonia copepod$^{-1}$ and total ammonia production tank$^{-1}$ h$^{-1}$ at 22°C.

| | **Temp (°C)** | **Ammonia (µg N ind$^{-1}$ h$^{-1}$)** | **Total number of copepods on day 14 (Mean HD tanks)** | **Total ammonia excretion in the tank (mg N tank$^{-1}$ h$^{-1}$)** | **Total ammonia excretion in the tank (mg N tank$^{-1}$ day$^{-1}$)** |
|---|---|---|---|---|---|
| ***A.tonsa*** | 22 | 0,006 ± 0,0038 | 2.09 million | 12.6 | 302 |

(Ikeda et al., 2001)

## 3.9 pH

The pH values measured in the experimental tanks are shown in Table 9. The pH in the tanks were measured daily and, but because of just small variations from day to day the results are shown as the average for the treatments for the entire trials. The pH for all the tanks of the different trials varied between a minimum of 7.77 and a maximum of 8.09, with an average of 7.97 for all the tanks.

**Table 9.** pH in Tank 1, 2 and 3 in Trial 1, 2 and 3 (mean ± SE), n gives numbers of measurements.

| | **LD tank** | **MD tanks** | **HD tanks** |
|---|---|---|---|
| | pH (mean ± SE) | pH (mean ± SE) | pH (mean ± SE) |
| **Trial 1** | 8.04 ± 0.04 (n=21) | 7.99 ± 0.02 (n=21) | 7.97 ± 0.01 (n=21) |
| **Trial 2** | | 8.09 ± 0.01 (n=20) | 7.98 ± 0.08 (n=20) |
| **Trial 3** | | 7.96 ± 0.02 (n=22) | 7.77 ± 0.06 (n=22) |

## 3.10 Salinity

The salinities in the LD, MD and HD tanks were varying during entire Trial 1 (Figure 14). All of the tanks followed the same pattern, but the salinity in the tanks increased with increasing copepod density. The salinity in the MD and HD tanks in Trial 2 were on average 25 ± 1‰. In Trial 3, the salinity in the MD and the HD tanks were 25‰ ± 1 ‰ and 27 ‰ ± 1 ‰, respectively

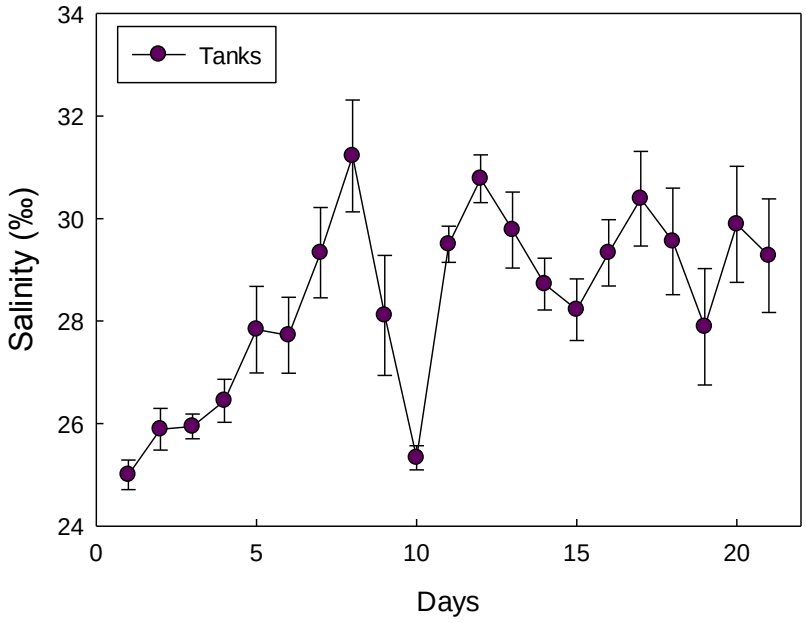


**Figure 14.** Salinity as an average of the LD, MD and HD tanks in Trial 1 (mean ± SE).

# 4 Discussion

## 4.1 Experimental trials

A decline in the copepod density due to mortality is normal. The stable reduction in the copepod density in the LD tanks during Trial 1 can be explained by copepods lost through sampling and natural mortality. In the major parts during all the trials the copepod had a similar, stable reduction in the density in all the experimental tanks.

The sampling methods used to measure the ammonia, algae and copepod density will all contribute to the stable loss of copepods. These samplings had a great impact in the copepod density, because this equaled a substantial part of water each day. The method contributing to the most substantial reduction of copepods was the harvesting of eggs. In the siphoning of eggs from the bottom, copepods were siphoned along as well. The majority of the copepods siphoned were already dead of natural causes, but there were always substantial parts alive in the siphoned water.

Following the copepod density in the MD and HD tanks in Trial 1 and 2, a distinct reduction occurred on day 7-9. This reduction was too extensive to be explained by sampling and normal mortally alone. This decline came as a consequence of the water quality in the tanks, as the ammonia levels increased in the tanks were above lethal levels, leading to enhanced mortality.

The water exchange rate is the key factor for maintaining control with the water quality. At a too low water exchange rate, the ammonia accumulated in the tanks (Jepsen et al., 2013). The amount of reactive ammonia is dependent on the pH in the water (Emerson et al., 1975), and there is a threshold at pH 8 where the fraction of toxic ammonia will start to increase, e.g. at pH 8, 3.82 % of the ammonia will be in the form of $NH_3$, and 28.4 % at pH 9 and temperature 20°C. The exact fraction of reactive ammonia is also dependent on the temperature. The pH for the different trials ranged between 7.77 and 8.09, with an average of 7.97 for all the tanks. The pH level in Trial 1, 2 and 3 indicated that there was toxic ammonia in all tanks ($NH_3$).

In Trial 1 where the water exchange rate was 100 % $day^{-1}$, the ammonia concentrations were much higher than in Trial 2 and 3 where an exchange rate of 500 % $day^{-1}$ was used. The average total ammonia concentration in the LD, MD and HD tanks in Trial 1 were 5.9 ± 0.4, 4.2 ± 0.5 and 2.5 ± 0.1 mg/L $NH_3$-N (mean ± SE), respectively, while in Trial 2 the MD and HD tanks showed values of 1.2 ± 0.3 and 0.5 ± 0.1 mg/L $NH_3$-N (mean ± SE), respectively. In the HD tanks in Trial 3 there was a peak in ammonia production on day 11 (3.0 ± 0.3 mg/L $NH_3$-N, mean ± SE) and in the MD tanks there was a peak on day 14 (1.6 ± 0.3 mg/L $NH_3$-N, mean ± SE).

These numbers are in the total ammonia available. Since the pH were close to 8 in all the tanks in all the trials, will the percentage of 3.82 showed by Emerson et al. (1975) at pH 8 and temperature of 20°C be used. The portion of reactive ammonia is relatively low at these conditions, compared to

the total ammonia available. The lowest non harmful concentration of ammonia for copepods is 0.4 – 0.477 mg $NH_3$ $L^{-1}$ for a 24 hour exposure (Buttino, 1994 ; Jepsen et al., 2013). An exposure time of several day and up to weeks as in these trials, would even a reactive ammonia concentration of 0.10 mg/L $NH_3$-N, that was measured in the LD tanks in Trial 1 (mean), assumingly be harmful for the copepods. In the two trials with 500 % water exchange $day^{-1}$, the reactive ammonia concentrations were even lower, but periods with higher values up to 0.1 mg/L $NH_3$-N occurred. The major parts of the total ammonia in the tanks are in form of the ionized version. Though it is the reactive version of ammonia that is considered toxic, the ionized version is not totally risk free, and having these high concentrations may have shown an increased effect on the mortality of the copepods (Thurston et al., 1981).

In order to maintain the initial high density of copepods in the tanks in Trial 3, extra copepods were added during the course of the experiment. The amount added per day varied according to the mortality rate, but even though large quantities of copepods were added daily, the copepod density in the HD tanks slowly decreased. Experience showed that about 10 % of the total tank volume needed to be replaced $day^{-1}$. At day 15, no more copepods were added and the copepod density in the HD tanks decreased as expected the next days. As the copepod density decreased, the ammonia level decreased as well. The ammonia levels were high during the entire trial, and it was clear that the long time exposure of ammonia were lethal for the copepods.

At day 1 in Trial 1 the algae density was well above the saturation level of the copepods of 30 000 cells/ml. When the algae density is above the saturation level, there is optimal feeding condition for the copepods to support both growth and egg production. The first day in the MD tanks in Trial 2 and 3 the algae density was above the saturation level ass well, but as the copepods grew the ingestion rate increased, which in turn led to a decrease in the algae density. The algae densities in all the experimental tanks in all the trials were below the saturation point up till day 10-15. As a result of the decrease in the copepod densities in the different tanks and trials, it was easier to maintain a high concentration of algae in the tanks. After the copepod density had dropped, the algae densities remained above the copepods saturation level of 30 000 cells/ml. The quality of the algae may have varied from day to day.

The problem of maintaining high enough algae densities were most obvious in Trial 3 where the water exchange rate was 500 % and the copepod densities were maintained at a high level during the trial. This led to a higher demand of algae than was available. More than 600 L of algae were pumped into the tanks every day. Full effort was made to sustain the HD tanks with sufficient algae. Therefore were the algae densities in the MD tanks low the entire run, giving very little egg production.

The egg production was directly related to the amount of feed available, and variations in the algae density did in turn result in variations in the egg production. In trial 1, and to some extent in Trial 2, the egg production started at a point when the densities of the copepods were practically the

same in all the tanks. Even though there were no significant differences in the egg production in these trials ($P<0.05$), there was a clear trend of a higher egg production in the tank with a lower initial copepod density than in the tanks with a higher initial density (LD>MD>HD).

From day to day there were variations in the egg production inn all the tanks in all the trials. This was mainly due to the feed availability. At the end of all the trials, when the density of the copepod approached 2-3 ind/ml, it was much easier to achieve a sufficient algae density in the tanks, and the egg production became more stable.

An example of the egg production following the feed availability is apparent in the HD tanks in Trial 3. At day 12-14 and 20-23, there were maxima in the algae density in the tanks, and the egg production increased correspondingly. The reason for this increase in the algae density was because of a decrease in the copepod density the same days. The highest egg production in Trial 3 was obtained at day 20/21 (2.7 mill eggs). At this point the copepod density ranged between 6 and 8 ind/ml.

Another example of the opposite occurred in the HD tanks in Trial 2. From day 14 to 15 there was a major drop in algae density (below 20 000) cells/ml) followed by a drop in the egg production. Normally there is a 1-2 day delay between changes in the algae density will be visible on the egg production, but a quick response like in this example, might be caused by the copepods eating their own eggs when the algae density below the saturation level (Heinle, 1970). These major changes in food concentration will, in other word, consequently affect the egg production.

To summarize Trial 3, the egg production was low as long as the copepod density was high and the algae concentration was low. At once the copepod density decreased, the algae density and the egg production increased. According to the results of Skogstad (2010), a reduction in the algae density to 20 000 cells/ml should result in just a slightly reduced egg production (not more than 30 %), but at day 15/16, the amount of algae available were close to 20 000 cells/ml and the egg production was low (0.15 mill eggs $day^{-1}$, mean ± SE). This may indicate an increased importance of maintaining algae densities above the saturation level of the copepods in high density cultures.

## 4.2 Salinity

The salinity difference between the trials and the individual experimental tanks were related to the water salinity going into the tanks. The salinity in the intake water and in the algae culture was 25 ‰ and 34 ‰, respectively.

In Trial 1 the water exchange was 100 % a day, which implied that most of the water was added with the algal culture. This caused a gradual increase in the salinity in the tanks through the entire trial, starting at 24-26 ‰ for all the tanks and ended up at 34 ‰ for the HD tanks and close to 27 ‰ for the LD and the MD tanks.

At day 10 in Trial 1, there was a major drop in the salinity in all the tanks, reducing it to 24-26 ‰. The reason for this was insufficient amount of algae culture available (35 ‰), which implied

that the water from the intake (25 ‰) provided the entire water exchange and the salinity level dropped. This might have contributed to the drop in the copepod density that occurred night to day 10. As a consequence of the drop in algae available, the water exchange rate decreased, which might have contributed to an even higher concentration of ammonia that were documented in this experiment.

In Trial 2 the water exchange rate was increased to 500 % per day. This implied that the major portion of the water came from the intake where the salinity was 25 ‰ and the result of this was a salinity of approximately 25 ‰ ± 1 ‰ through the entire trial.

In the last Trial (3), the copepod density in the HD tanks were high for a longer time than in the previous trials. In order to compensate for this, a larger amount of feed were needed in these tanks, which in turn meant a higher portion of water origination from the algae water with a salinity of 35 ‰. The salinities in the HD tanks were therefore close to 27 ‰ during the entire trial. The conditions in the MD tanks were more or less the same as for the tanks in Trial 2.

## 4.3 Ammonia excretion of the copepods

The entire mean production for the HD tanks of day 14 in Trial 3 was calculated to be 302 mg N $day^{-1}$. The measured ammonia level that day was 2.73 mg/L NH3, which gives a total of 218 mg NH3 for the 80 L tank (mean HD tanks). The measured value was therefore smaller than the calculated value. In this case there was a 500 % water exchange $day^{-1}$ that had an effect on the ammonia level in the tanks. A measured level lower than the calculated level was therefore expected.

Another factor that influenced the concentration of ammonia was the algae fed to the copepods. Algae take up ammonia and will in that way reduce the concentration. The source of ammonia in the tanks was the excretion products of the copepods. The growth medium of the algae might have had an influence, but the medium did not contain any ammonia elements.

A negative effect of a high ammonia level, apart from being lethal to copepods, is that it might have had a negative effect on the egg production (Sullivan & Ritacco, 1985 ; Buttino, 1994). A lower ammonia level in the tanks might likely have led to a higher egg production in this experiment.

These calculations cannot blindly be trusted, because of many different factors that may have an effect on the result, like temperature, feed availability, age of the copepods, etc, but they give a hint of an expected result.

## 4.4 Feeding

The differences in the amount of algae going into the tanks and going out of the tanks were assumed to represent feeding by copepods. Some alga will always be lost to sedimentation, but this was assumed to be low in this thesis.

There was a reduction in algae consumption between day 14 to day 16 (Figure 11). The consumption should be more or less the same these days because the copepod density had practically not changed (26 and 27 ind/ml, respectively). The reduction was therefore connected to a reduction in the amount of algae in the inlet the same day.

Between day 16 and 21, the algae densities in the inlet was again increased and the decrease in the consumption this day was due to the decrease in the copepod density in the tanks. The reduction in the MD tanks was insignificant ($P>0.05$), but the small reduction observed occurred because of the equally small reduction in the copepod density in these tanks.

The amount of alga losses on the different days indicates how efficient the feed is utilized. The greater the losses were, the less efficient the copepods had been in consuming the feed. This is clearly shown comparing day 14 and 16. On day 16, when the density of algae in the inlet had decreased, the copepods were more efficient than at day 14, when more algae were available.

In Figure 12, the amounts of algae consumed in the tanks were used to calculate the total feed utilization on day 14, 16 and 21. A feed utilization of 86 and 90 % is high (day 14 and 16, respectively), and will suggest that the copepods get insufficient amounts of feed. On day 21 the copepods consumed 58 % of the feed available. Since the copepod density had decreased from 27 ind/ml on day 16 to 16 ind/ml on day 21, the amount of algae available per copepod increased. To be able to utilize the feed more optimally than 58 %, the copepod density should be somewhere between 16 and 27 ind/ml, given the same amount of feed.

The total carbon content of the algae consumed per day by a copepod equaled 75 %, 57 % and 82 % for day 14, 16 and 21, respectively, of the total carbon content in a copepod (Table 5). These results fit well with the expected values according to literature. *A. tonsa* consume from 6 to 360 % of its own bodyweight a day (Støttrup, 2003 ; Mauchline, 1998), depending on the feed and stage. The result for day 21 (82 %) seemed to be around the maximum consumption capacity of the copepods fed *R.baltica* at the current copepod densities, since at this day the total feed utilization were at its lowest (58%).

## 4.5 Female ratio and eggs per female of *A. tonsa*

According to the increased copepod density in the different tanks in Trial 1, the female ratio increased as well, with rations in LD < MD < HD. The tanks with the lowest total copepod density were dominated by males (35 % females), while the tanks with the highest copepod densities were dominated by females (67-74 % females). Between the LD tanks and the MD and HD tanks there were

statistical difference in female ratio ($P<0.05$), but there were no statistical difference between the MD and the HD tanks in any trial ($P>0.05$). The female ratios varied between 67-78 % for all the MD and HD trials, concluding the female dominance in the high density cultures in these trials.

The egg production per female is estimated as the result of the total egg production per female in the tanks. In Trial 1 the egg production was quite high for the LD tanks, and the egg production per female was therefore high as well (28.2 eggs female$^{-1}$). The egg production was still low compared to Skogstads (2010) maximum results of 45.5 eggs female$^{-1}$ day$^{-1}$. Støttrup et al. (1986) used low density cultures (<100 eggs L$^{-1}$) with an average production of 25.5 eggs female$^{-1}$ day$^{-1}$. The egg productions in the MD tanks were much lower (7.08 eggs female$^{-1}$). On the other hand, if this test had been done on day 16, the egg production would have been much higher, since the total egg production increased from 1.1 mill eggs tank$^{-1}$ at day 15 to 3.6 mill egg tank$^{-1}$ on day 16. This variation was likely caused by an increase in the algae density at day 16, which could support an increased egg production. The female ratio was unfortunately not calculated that day. Using the same sex ratio showed at day 15, for calculating the eggs per female on day 16, the result would be 24 mill egg female$^{-1}$day$^{-1}$. This was a clear indication of how the egg production varied from one day to another.

For the MD tanks in Trial 2, the eggs per female were varying between 11.3 and 22.8 with a mean of 16.4 eggs female$^{-1}$ day$^{-1}$. The eggs per female for the HD tanks were lower, varying between 1.06 and 7.42 with a mean of 4.4 eggs female$^{-1}$ day$^{-1}$. The egg production and female ratio were more or less the same in this trial and the difference in egg production per female were therefore a result of the higher copepod density in the HD tanks, which means fewer eggs per female.

The egg ratios per female were as expected low for both of the treatments in Trial 3. Especially the MD tanks had very few eggs per female. This was related to the low feed concentration in these tanks.

The copepod density in Tank 3 (one replicate of the HD treatments, Trial 3) dropped from 27.5 – 11 ind/ml on day 15 – 17. This led to an increased feed concentration above the saturation level the rest of the trial, which in turn led to an increased egg production (up to 2.7 mill tank$^{-1}$, day 21). The result was an egg production per female day$^{-1}$ that increased from 0.15 to 9.74 egg female$^{-1}$ day$^{-1}$ at day 15 to day 22, respectively. The value represents still a low egg production, compared to the production in the LD tanks in Trial 1 which had an egg production more than 5 mill tank$^{-1}$ several days and an average more than 4 mill tank$^{-1}$.

## 4.6 Hatching success

The results of the hatching success showed significant variations between the different experimental tanks and also within different days of the same tank in Trial 3. There was a significant difference ($P<0.05$) between the hatching success in the MD (64.8 ± 4.0 %, mean ± SE) and the HD tanks (46.1 ±

5.0 %, mean ± SE). Statistically this is not the same results as Peck and Holste (2006) found in their experiments (Peck & Holste, 2006), they concluded that there were no effect of the copepod density on the hatching success. Though there is a slight statistical difference, the difference was not very big. This difference cannot be explained by the food availability because the food concentration in the HD tanks were higher than in the MD tanks (details earlier in the discussion), but the reason might be because of a lower ammonia concentration in the MD tanks than in the HD tanks.

## 4.7 What is the optimal copepod density for egg production?

Following the egg production in the different tanks in Trial 1, it can be concluded that the LD tanks were the tanks with the highest average egg production $day^{-1}$. At the time when the egg production in the different tanks started, the copepod densities were at more or less the same level for both the LD tanks (4 ind/ml), MD tanks (12 ind/ml) and the HD tanks (11 ind/ml). A stable copepod density at a low level, like the LD tanks will lead to a more stable, high production of eggs (mean for day 12-22 was 2.6 mill eggs $day^{-1}$ $tank^{-1}$), compared to MD (mean for day 11-22 was 1.7 mill eggs $day^{-1}$ $tank^{-1}$) and HD tanks (mean for day 11-22 was 1mill eggs $day^{-1}$ $tank^{-1}$). The highest average for the three replicates in the HD tanks were 1.5 million eggs (day 19), which is considerably lower than the highest average of the LD tanks with a production of 4.1 mill eggs in day 21.

The HD tanks in Trial 2 had two days with considerable higher egg production, compared to the rest of this Trial. On day 9, Tank 1 (HD) showed an egg production of 5.7 mill eggs while Tank 2 (HD) produced 2.1 mill eggs. The result in Tank 1 seemed high compared to the rest, but the algae density that day was quite high as well. At this day the copepod density of Tank 1 was 7.3 ind/ml. The next counting day (day 14) the density had decreased to 4.33 ind/ml, but the egg production remained high (4.6 mill eggs $day^{-1}$) at an algae density > 90 000 cells/ml. The rest of this trial, the egg production in the HD tanks was close to 2 mill eggs $tank^{-1}$ $day^{-1}$. The MD tanks showed a stable egg production close to that level during the entire trial.

For the third trial there was a high egg production in Tank 3 (HD) of 2.7 million eggs $day^{-1}$ at day 20-21 with an average between day 17- 23 of 1.9 mill eggs $day^{-1}$ (Mean ± SE). The copepod density ranged between 6-8 ind/ml, which was twice the density as for the tanks in Trial 1. When the algae concentrations additionally were above the saturation level of the copepods, one should expect a higher egg production at this point.

The reason for this high egg production in the HD tanks in Trial 2 and Tank 3 (HD) in Trial 3 compared to the egg production in the HD tanks of Trial 1 was probably because of the different water exchange rate. In Trial 2 and 3, the water exchange was 500 % $day^{-1}$, and this may have diminished the effect that the initial high copepod density may have had on the water quality and in that way increased the egg production.

## 4.8 Conclusions and future perspectives

High density cultures of copepods for egg production are not sustainable in the way it was performed in this project. Due to the high copepod density a key factor is optimal water quality. The water exchange per day should have been higher, but this would have affected the amount of algae needed, because lots of algae were flushed out with the outlet water before getting consumed by the copepods.

- The main challenge in this experiment was achieving high enough algae concentrations in the experimental tanks. And as long as there was insufficient amount of algae available for the copepods, the algae loss due to the water exchange was very low. Sustaining the copepods with algae concentrations above the saturation level led to greater loss of algae through the outlet water. For a small scale production like this, more than 600 liter of algae was needed per day for all the tanks combined. In a larger production there might be an even bigger problem sustaining the copepods with algae.
- The pH levels were close to 8 in all the tanks during all the trials, influencing the amount of reactive ammonia.
- The ammonia levels were generally high in this experiment. An increased water exchange lowered the ammonia level in Trial 2 and 3. It seemed at these high copepod densities, if one will solely use the water exchange to control the ammonia level, the water exchange rate per day should be much higher.
- The salinity in the tanks remained more or less the same during all the trials and showed no direct influence on the mortality or the egg production in the tanks.
- The food utilization and food consumption rates per copepod were calculated during the last trial, showing a utilization of between 58-90 % during 3 different days and a consumption rate between 57-82 % of its own body weight $day^{-1}$.
- The highest egg production took place at low copepod densities
    - Copepod densities of 2-4 ind/ml and 100 % water exchange $day^{-1}$ showed an average of 2.6 mill eggs $day^{-1}$ $tank^{-1}$ and maximum production close to 5 mill eggs $day^{-1}$ $tank^{-1}$.
    - Copepod densities of 4-7 ind/ml and 500 % water exchange $day^{-1}$ showed an average close to 2 mill eggs $day^{-1}$ $tank^{-1}$, and maximum productions of 5.7 and 4.6 mill eggs $day^{-1}$ $tank^{-1}$.
    - Copepod densities of 6-8 ind/ml and 500 % water exchange $day^{-1}$ showed an average of 1.9 mill eggs $day^{-1}$ $tank^{-1}$ and maximum production of 2.7 mill eggs $day^{-1}$ $tank^{-1}$.

Making an optimal intensive copepod egg production system might be to introduce a pulse-wise exchange of water, using high water exchange rate while no feed is added. This high intensity water

exchange could for instance be carried out a couple of times a day, while the remainder of the day the water exchange should be low. In the periods with low water exchange, sufficient amount of algae should be fed to the copepods for optimal growth and egg production. The high copepod densities in this experiment were the main reason for the problem achieving adequate algae densities in the experimental tanks. A more optimal copepod density might therefore be between 10-15 ind/ml. The egg production should definitely be tested with optimal amounts of feed at these copepod densities. There might be a better suitable feed in a large scale production, ensuring a more stable and available source of feed for the copepods. Algae pasta might be a solution.

Continuous harvesting of eggs is also an aspect that should be considered. Periods of heavy feeding and continuous harvesting might result in a higher total egg production in the tanks.

A better control of the ammonia in the water is crucial for having an intensive copepod production. The water exchange will probably not be sufficient to regulate the ammonia concentration in a large scale production. There are different methods available for this. The use of a biofilter might solve the problem, but this again might be an expensive investment. A solution can be using pH control, keeping a low pH and in that way reducing the reactive ratio of ammonia in the tanks.

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# Appendix 1 – Conwy medium

The medium is slightly modified from Walne. A smaller amount of manganese chloride is used than in the original recipe.

| | |
|---|---|
| $NaNO_3$ (Sodium Nitrate) | 100.0gr |
| Na-EDTA (EDTA disodium salt) | 45.0gr |
| $H_3BO_3$ (Boric Acid) | 33.6gr |
| $NaH_2PO_4 \cdot 2H_2O$ (Sodium Phosphate, monobasic) | 20.0gr |
| $FeCl_3 \cdot 6H_2O$ (Ferric Chloride, 6-hydrate) | 1.3gr |
| $MnCl_2 \cdot 4H_2O$ (Manganous Chloride, 4-hydrate) | 0.136gr |
| Vitamin $B_1$ (Thiamin HCl) | 0.1gr |
| Vitamin $B_{12}$ (Cyanocobalamin) | 0.05gr |
| Trace Metal Solution * | 1ml |
| Distilled water | 1000ml |

(Note: use 1 ml Conwy medium/liter of seawater)

| Trace Metal Solution * | |
|---|---|
| $ZnCl_2$ (Zinc Chloride) | 2.1gr |
| $CoCl_2 \cdot 6H_2O$ (Cobalt Chloride, 6-hydrate) | 2.1gr |
| $(NH_4)_6Mo_7O_{24} \cdot 6H_2O$ (Ammonium Molybdate, 4-hydrate) | 2.1gr |
| $CuSO_4 \cdot 5H_2O$ (Copper Sulphate) | 2.0gr |
| Distilled water | 100ml |

(Note: acidify with 1 M HCl until solution is clear)

# Appendix 2 – Hatching success

**Table 10.** Hatching success of eggs at day 14, 17 and 23 during the experimental Trial 3.

| Dag 14 | Unhatched eggs | Hatched nauplii | Total (eggs + nauplii) | Hatching percent |
|---|---|---|---|---|
| **1 (HD)** | 65 | 21 | 86 | 24,4 |
| **2 (HD)** | 35 | 32 | 67 | 47,8 |
| **3 (HD)** | 80 | 47 | 127 | 37,0 |
| **4 (MD)** | 17 | 24 | 41 | 58,5 |
| **5 (MD)** | 16 | 9 | 25 | 36,0 |
| **6 (MD)** | 22 | 23 | 45 | 51,1 |
| | | | | |
| **Dag 17** | | | | |
| **1 (HD)** | 42 | 23 | 65 | 35,4 |
| **2 (HD)** | 28 | 21 | 49 | 42,9 |
| **3 (HD)** | 50 | 28 | 78 | 35,9 |
| **4 (MD)** | 5 | 28 | 33 | 84,8 |
| **5 (MD)** | 6 | 23 | 29 | 79,3 |
| **6 (MD)** | 13 | 36 | 49 | 73,5 |
| | | | | |
| **Day 23** | | | | |
| **1 (HD)** | 31 | 35 | 66 | 53,0 |
| **2 (HD)** | 12 | 34 | 46 | 73,9 |
| **3 (HD)** | 17 | 31 | 48 | 64,6 |
| **4 (MD)** | 16 | 41 | 57 | 71,9 |
| **5 (MD)** | | | | |
| **6 (MD)** | 22 | 52 | 74 | 70,3 |

# Appendix 3 – Hatching of eggs for the experiment

1mL of copepod eggs = 500 000 individual eggs.
Hatching percent (HS) and daily mortality rate was taken into account.

**Trial 1:**
Number of copepods needed:
A HS of 50 % for fresh eggs were calculated:
A HS of 50 % for fresh eggs was calculated:
This gives a need of at least 25 440 000 eggs (12 720 000*2).
mL of eggs needed were: 25 440 000 eggs/500 000 eggs/ml = 50.88 mL

To be on the safe side, 3 tanks of 20 mL eggs were hatched for Trial 1.

**Table 11.** Total need of copepods in Trial 1. All of the tanks were 80 L and 3 replicates of each tank were used.

| | **LD tanks (>3 ind/ml)** | **MD tanks (>10 ind/ml)** | **HD tanks (>40 ind/ml)** | **Total need** |
|---|---|---|---|---|
| **Need for 1 tank: 80L * ind/ml** | 240 000 | 800 000 | 3 200 000 | 4 240 000 |
| **Times 3 replicates** | 720 000 | 2 400 000 | 9 600 000 | 12 720 000 |

**Trial 2:**
In this trial, the three LD tanks were not used. This gives a total need of 12 000 000 individuals. The reduction is quite insignificant, so the same amount of eggs were hatched in this trial.

**Trial 3:**
In this trial, the same amount of egg was needed for an initial filling of the tanks, but in this trial extra copepods had to be hatched for refilling when the density dropped during the project. So an extra 60 mL of eggs were hatched, giving it a total of 120 mL eggs in this run.