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Particle characterization and influence on the nauplii and egg production of calanoid copepod (Acartia tonsa Dana) in water treatment systems with and without membrane filtration

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Abstract

The calanoid copepod *Acartia tonsa* has been demonstrated to be able to replace the *Artemia* or rotifer for marine fish larvae due to its excellent nutrient, wide range of temperature and salinity tolerance and capability of producing resting eggs in the life cycle. Hence, *A. tonsa* is one of the most promising copepod species for marine larviculture purposes. The present study conducted to evaluate the impacts of colloidal particles (30 nm - 1 µm) and fine solids (2 µm - 60 µm) on nauplii and egg production of the copepod *A. tonsa* in three systems: conventional recirculating system (cRAS), membrane modified recirculating system (mRAS) and flow through aquaculture system (FTAS). The initial copepod density was 75000 ind L^{-1} for investigating of growth performance and 15 ind L^{-1} for testing of egg production capacity of the copepods within 4 weeks.

The results in the present study showed a better water quality regarding nitrogenous waste products and colloidal particles concentration in the FTAS compared to the RAS systems (cRAS and mRAS) during the experiment. The survival rate, nauplii and egg production of the copepods in the FTAS were significantly higher than the RAS systems ($p < 0.05$); while in the mRAS they were not significant difference to the cRAS ($p > 0.05$). The high concentration of colloidal particles in the RAS systems during the experiment could be an indication for the significant difference of nauplii and egg production compared to the FTAS ($p < 0.05$). Nevertheless, no effects of fine solids in all systems were observed on the performance of *A. tonsa* in terms of survival, growth and development, as well as nauplii and egg production rate. An efficient removal of colloidal particles in the mRAS compared to the cRAS and FTAS was only observed after increasing the water exchange rate (to 8 times daily volume tank) from 8 days post hatching.

Abbreviations

BF-MBR	-	Hybrid Biofilm Membrane Bioreactor
cRAS	-	Conventional Recirculating Aquaculture System
C/N	-	Carbon to Nitrogen ratio
DWI	-	Daily Weight Increase
Dph	-	Days post hatching
EP	-	Egg production
FTAS	-	Flow Throught Aquaculture System
TMP	-	Trans-membrane Pressure
TAN	-	Total Ammonia Nitrogen
mRAS	-	Membrane-modified Recirculating Aquaculture System
MBR	-	Membrane Bioreactor
LC50	-	Lethal Concentration (50 % of members in a tested population is died after a specific duration)

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1. Introduction

1.1 Copepod in aquaculture

Live feed organisms are nowadays still applied in the larviculture of marine fish species due to their advantages compared to formulated feeds such as nutritional content, smell, shape and movement that trigger the start feeding behavior of fish larvae (Conceição et al., 2010). Furthermore, the biochemical and behavioral constraints of formulated feeds are also a barrier for replacing live feeds (Drillet et al., 2006). There have been many different species of live feed organism so far but the most popular, important and traditional used in marine larval rearing have been mainly brine shrimp (*Artemia spp.*) and rotifer (*Brachionus spp.*) (Conceição et al., 2010; Evjemo and Olsen, 1997). However, the nutrient composition, swimming behavior and size of these traditional live feeds are not optimal for fish larvae (Ohs et al., 2009; Payne and Rippingale, 2001b). It is necessary to have an alternative food source that can overcome these obstacles and promote adequate growth of fish larvae.

Since 1980s the utilization and cultivation of copepods as live feed has developed (Støttrup, 2007; Støttrup et al., 1986). Marine fish larvae consumes copepods as the main food source in nature and the nutrient composition of copepods is generally believed to meet the fish larval requirements (Evjemo et al., 2003; McKinnon et al., 2003; Sargent et al., 1997). Many experiments demonstrate that marine copepods, especially calanoids, can serve as the ideal feed for many marine larvae due to its excellent nutritional value compared with rotifers and *Artemia* (Hernández Molejón and Alvarez-Lajonchère, 2003; Marcus, 2007). It is reported for Atlantic halibut (*Hippoglossu shippoglossus*) (Evjemo et al., 2003; McEvoy et al., 1998), grouper (*Ephinephelus coioides*) (Toledo et al., 2007), Australian seahorse (*Hippocampus subelongatus*)

(Payne and Rippingale, 2000), pipefish (*Stigmatopora argus*) (Payne et al., 1998), pink snapper (*Pagrus auratus*) (Payne and Rippingale, 2001b) and octopus paralarvae (*Octopus vulgaris*) (Iglesias et al., 2007). A broad spectrum in sizes of nauplii and copepodite stages in many copepod species are suitable to be utilized as first feed for small larvae having small mouth gape and difficulty in ingesting of *Artemia* and rotifer (Ohs et al., 2009). Last but not least, one of the most important factors stimulating the feeding of fish larvae is the swimming behavior of copepod nauplii and copepodites; typically behaviors of jumping, pausing and another jumping, called jerky or zigzagging movement (Lavens and Sorgeloos, 1996; Marcus, 2005).

Calanoida, Hapacticoida and Cyclopoida are the three copepod species that have free living stages and are used as first feed in larviculture (Støttrup, 2007; Støttrup and Norsker, 1997). Although the benefits in the utilization of copepods were proven, it is still facing many challenges such as lack of information about biology, reproduction and response of copepods to cultivation environmental conditions, reliable rearing techniques and economically feasibility to carry out a mass production of copepods to supply a reliable and sustainable feed source for a commercial large - scaled fish larvae production (Støttrup, 2000). Water quality is generally the main reason for success or failure in cultivation of aquatic species, because poor water quality may cause negative impacts on growth, fertility, mortality and stress on cultured species (Van Rijn, 1996). The most important parameters in aquaculture are temperature, pH, dissolved oxygen, ammonia, nitrite, carbon dioxide, suspended solids, alkalinity and microbial community (Boyd and Tucker, 1998). Each water quality parameter has complex connections with others; the concentration of one parameter can be harmless in one situation, but become toxic in another (Timmons et al., 2002). Calanoid copepods have a low tolerance to poor water quality (Hoff and

Snell, 1997), they are especially sensitive to high level of ammonia (Jepsen et al., 2013; Støttrup, 2007). Difficulties in management costs and cultivation techniques are the main reasons to prevent the development of copepod mass production as live feed. However many approaches in copepod production are conducted such as cultivation of *Acartia* spp. (Schipp et al., 1999) and *Gladioferens imparipes* (Payne and Rippingale, 2001b) in intermittent continuous water flow through system, or mass cultivation of the harpacticoid copepod *Amphiascoides atopus* (Sun and Fleeger, 1995), *Tisbe holothuriae* (Støttrup and Norsker, 1997) and the calanoid copepod *Gladioferens imparipes* (Payne and Rippingale, 2001b).

1.1.1 Cultivation techniques

In extensive systems or the mesocosm technique, a mix of many wild plankton species is cultured in large fertilized areas such as lagoons, outdoor pond systems or large tanks (Conceição et al., 2010; Støttrup, 2007). The advantages of extensive systems are that they just require simple culture facilities and offer a large diversity of size and live prey species to meet the changes in developmental stages of fish larvae. These benefits are useful in case of low input source, lack of culture technical equipment and especially for new cultured species with unknown dietary requirement (Dhert et al., 1997). Cultivation of copepods in these systems can start up with either resting eggs or harvested copepods from the sea. The main composition of copepods in such systems is generally smaller species in the calanoid generas such as *Acartia* spp., *Centropages* spp., and *Temora*spp., some harpactoids (e.g. *Tisbe* spp.) and cyclopoids (e.g. *Oithona* spp.) (Støttrup, 2007; Van der Meeren and Naas, 1997). However, the main drawbacks in this system are the lack of control of physical environmental cultivation conditions and seasonal variations of available biomass and species composition. This type of outdoor system is also the pathway to introduce parasites or pathogens to the fish larvae, such as viral nervous

necrosis (Liao et al., 2001) when utilizing directly wild harvested copepods. Parasites can adhere to copepods as the intermediate host, and then be transferred to fish larvae. This affects the survival and growth rate of the cultured fish larvae (Marcogliese, 1995; Støttrup, 2000). However, an inoculation of indoor cultured microalgae and zooplankton rather than wild resources through mesocosm technique was carried out successfully for large scale of semi-intensive production of *Acartia tonsa* served as feed for European turbot larvae and golden snapper (Schipp et al., 2001).

A more controlled alternative for producing large quantity of marine copepods for larval feed is intensive systems. The advantages of intensive cultivation systems could be that they are more reliable, sustainable and less need of cultivation area than extensive systems and they may be operated independently from outdoor physical environmental conditions (Payne and Rippingale, 2001b). Either wild harvested copepods (Payne and Rippingale, 2001b; Støttrup et al., 1986; VanderLugt et al., 2009) or sediments containing resting eggs (Næss, 1996) from nature can be used to start up in intensive cultivation systems. Many options in cultivation of copepods can be conducted, such as batch, semi-continuously or recirculating systems (Payne and Rippingale, 2001b; Støttrup and Norsker, 1997; Støttrup et al., 1986). The harvest of copepod nauplii and eggs can be done manually or automatically by siphoning, plankton nets or light traps in the production cultures before feeding to the fish larvae (Payne and Rippingale, 2001a; Støttrup and Norsker, 1997; Støttrup et al., 1986). These efforts have mainly focused on dealing with the challenges of seasonal variation and possible infections of parasites in the utilization of wild harvested zooplankton, a global shortage of *Artemia* cysts (Støttrup, 2000) or mis-match of size between the mouth gape of fish larvae with the traditional preys as *Artemia* or rotifers (Payne

and Rippingale, 2001a). The intensive system requires a close and careful control of water quality and dietary input (McKinnon et al., 2003; Støttrup, 2000). Most studies for mass cultivation of copepods are carried out with species of calanoid copepods (*Acartia* spp., *Eurytemora* spp., *Centropages* spp. and *Gladioferens imparipes* (Støttrup, 2007) and the paracalanid species *Parvovalanus* spp. (McKinnon et al., 2003; Shields et al., 2005)). However, the cultivation of calanoid copepod species in high densities are difficult to operate (Støttrup, 2007). In fact, the calanoid copepod speices decreased in fecundity, increased in mortality and negative effects on the hatching success (Miralto et al., 1996; Peck et al., 2008). Even though, there are many studies operated with high ranges of densities, the cultivation of calanoid copepod with density ranges from 100 to 2000 ind L^{-1} has been often operated in the intensive systems (Støttrup, 2007; Støttrup et al., 1986). Small scale cultivation of *Acartia tonsa* with a density of 7000 ind L^{-1} has been done with low and density-independent mortality (Drillet et al., 2006). Furthermore, a culture density of about 30000 ind L^{-1} for the *Parvovalanus* spp. (Shields et al., 2005) or the harpacticod copepods (e.g. *Tisbe spp., Amphiascoides atopus* and *Tigriopus japonicas*) with the possibility of cultivating in much high density more than 100000 ind L^{-1}(Ajiboye et al., 2011) was also reported. However, the hapacticoid copepods are epibenthic animals which spend much time on the bottom, hence less available as prey for pelagic fish larvae (Fleeger, 2007; McKinnon et al., 2003; Støttrup, 2007). Even though only few studies in copepod cultivation by application of recirculating aquaculture system have reported; it is suggested that the abilities to obtain and maintain water quality in this system could be certain suitability for live feed production, especially copepod organisms (Drillet et al., 2011).

1.1.2 Recirculating aquaculture system

The recirculating aquaculture system (RAS) has been developed and applied in order to obtain a high stable water quality, to save water and to reduce possible negative environmental impacts from discharges of cultured water (Drillet, 2010; Timmons et al., 2002; Van Rijn, 1996). A typical RAS includes production tanks and compartments for water treatment that play key roles in removal and conversion of organic matter, particulates and accumulated nutrients in the system (Lekang, 2008; Timmons and Ebeling, 2007). However, there are also some drawbacks in financial costs such as initial set-up and operating costs including professional skilled labors, energy to heat/cool the culture water, pumping and back-up of power plant. Furthermore, significant challenges of disease out-break or parasite infections are also observed as well (Hutchinson et al., 2004).

The most important component in RAS is the biofilter that regulates the level of un-ionized ammonia, which is very toxic and affects the welfare of aquatic organisms (Drillet, 2010; Timmons et al., 2002; Van Rijn, 1996). The end-products of protein metabolism are un-ionized ammonia (NH_3-N) and ionized ammonia (NH_4^+-N). The sum of these ammonia forms is called total ammonia nitrogen (TAN), and the un-ionized ammonia is known to be more toxic than ionized ammonia (Timmons et al., 2002). There are two steps to convert toxic ammonia to less toxic nitrate nitrogen (NO_3-N) via nitrite nitrogen (NO_2-N) in a biofilter. In the first step, ammonia oxidizing heterotrophic bacteria (*Nitrosococcus, Nitrosospira, Nitrosolobus* and *Nitrosovibrio*) produces nitrite from amonia. In the second step, nitrite autotrophic oxidizing (nitrifying) bacteria (*Nitrobacter, Nitrococcus, Nitrospira* and *Nitrospina*) convert nitrite to nitrate (United States Environmental Protection Agency, (EPA), 1993).

First step: $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+$

Second step: $NO_2^- + 1.5\ O_2 \rightarrow NO_3^-$

Or

$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + H^+$

The nitrite nitrogen (NO_2-N) in the nitrification process is also reported to be more toxic than the nitrate nitrogen (NO_3-N) and to have a negative impact on the oxygen-carrying capacity of blood hemoglobin in the cultivated aquatic organisms (Losordo et al., 1998; Timmons and Ebeling, 2007; Timmons et al., 2002).

1.1.2.1 Particles and general impacts on water quality

Solids and nitrogen compounds (N-compounds) are two of the most important factors affecting the operation of RAS. Excreted waste products, microorganisms and uneaten feed are the main sources of organic solids, promoting the increase of microbial growth due to its biodegradable process. Particle solids are broken up and mineralized, leading to the production of N-compounds in the system. Moreover, excretion by cultivated species is also another source of N-compounds in the system (Timmons and Ebeling, 2007). There are many different forms of N-compounds, the non-polar un-ionized ammonia (NH_3-N) is concerned very toxic because it can pass through the biological membranes of cultivated aquatic species. Concentrations between 0.09 - 3.35 mg $NH_3\ L^{-1}$ is reported as the general lethal concentrations with over 50 % mortality (96h LC_{50}) of marine fish species within 96 hours of exposure (Eddy, 2005). In case of calanoid copepods, 0.91 - 2.37 mg $NH_3\ L^{-1}$ (24h LC_{50}) was observed for adult *Acartia clausii* at ambient temperature (Buttino, 1994) and *Acartia tonsa* at 17 ^oC (Buttino, 1994). Furthermore, LC_{50} of

0.97 - 1.26 mg NH_3 L^{-1} (48 h) and 0.22 - 0.77 mg NH_3 L^{-1} (72 h) at 17 ^oC for both nauplii and adult *Acartia tonsa* have been reported recently (Jepsen et al., 2013).

A solution has carried out to remove particles immediately and as gently as possible to avoid break-up and mineralization into smaller sizes. Such organic particles or compounds could be removed by different types of equipment such as drum filter, protein skimmer etc, which are normally integrated in a conventional RAS design. However, the currently used systems are only capable of removing particles larger than 40 - 60 μm, leaving fine suspended solids (particle less than 35 μm) and colloidal particles (less than 1 μm) in the production systems (Chiam and Sarbatly, 2011).

The accumulated particles cause negative impacts in the RAS such as protecting the microorganisms leading to reduced disinfection effects (Hess-Erga et al., 2008) , raising the biological oxygen demand and creating biofouling in the systems (Chiam and Sarbatly, 2011). Particles which also serve as substrates for bacteria and microorganism, will stimulate and enhance the proliferation of fast-growing (opportunistic bacteria) or slow-growing bacteria (beneficial bacteria) communities (Attramadal et al., 2012; Skjermo et al., 1997; Vadstein et al., 1993a). Furthermore, blooms of opportunistic bacteria strains can be stimulated in case of unstable water quality conditions. Opportunistic microorganism may also cause diseases in fish when they are cultivated under stressing conditions. The high particle suspended levels can affect to the function of fish gill by abrasion and clogging (Bullock et al., 1994; Timmons and Ebeling, 2007). In addition, the high concentration of particles causes some negative impacts to the survival and growth of the embryonic and larval development in common carp *Cyprinus carpio* (Martins et al., 2009b) and in Nile Tilapia *Oreochromis niloticus* (Martins et al., 2009a).

Particles are also able to affect to the nitrification kinetics (Chen et al., 2006; Guerdat et al., 2011; Zhu and Chen, 2001) and clogging of the biofilters in RAS (Eding et al., 2006). Competition between autotrophic nitrifying and heterotrophic bacteria for space, nutrients and oxygen creates a stratified biofilm (Nogueira et al., 2002) on the biological filter media, causing an inhibition of the nitrification process. With a higher maximum growth rate, about five times that of autotrophic nitrifying bacteria, heterotrophic bacteria occupies the outer layer of the stratified biofilm and covers the inner layer where nitrifying bacteria settles (Ohashi et al., 1995). Hence heterotrophic bacteria inhibits the diffusion of nitrogenous substrate and dissolved oxygen into the autotrophic nitrifying bacteria, leading to the negative impacts of the nitrification process (Chen et al., 2006). The availability and competition for organic carbon and nitrogen sources are measured by a carbon to nitrogen ratio (C/N). A high C/N ratio creates a good condition for heterotrophic bacteria to dominate in the biofilters and effectively out-compete the autotrophic nitrifying bacteria for both of oxygen and space. It has been reported that the removal rate of volumetric TAN is lower 30 % at a C/N ratio of 0.5 than at a C/N ratio of 0 (Michaud et al., 2006). Furthermore, Zhu and Chen (2001) also showed a reduction of 70 % in TAN removal rate at a C/N ratio of 1.0 or 2.0 compared to a C/N ratio of 0 in another system. Hence, to achieve the expected nitrification, the procedure of organic matter removal should be improved.

1.1.2.2 Membrane bioreactor filtration

Membrane bioreactor (MBR) filtration (Figure 1.1) is an effective technology in drinking water production and waste water treatment to remove solids such as microorganisms, colloidal and particulate material (Lesjean and Huisjes, 2008; Van Der Bruggen et al., 2003). Many studies have demonstrated the advantages of MBR compared to conventional wastewater treatment such as low footprint, good disinfection capability, high effluent quality, higher volumetric loading

and less sludge production (Judd, 2006). A combination between a biofilm reactor such as a moving bed biofilm reactor (MBBR) with a membrane filtration system (microfiltration or ultrafiltration with pore size range of 0.001 – 10 μm) to form a hybrid biofilm membrane bioreactor, BF-MBR (Leiknes and Ødegaard, 2007) has performed to improve the water treatment. The application of BF-MBR can reduce the accumulation of colloidal, fine suspended solids, and overall concentration of organic and nitrogen compounds, which affect directly the water quality, biofouling, nitrification and disinfection efficiency in the RAS. In addition, BF-MBR can stabilize the food concentration to microorganisms by stabilizing the available bacterial substrates and reducing the amount of potential opportunistic bacteria.

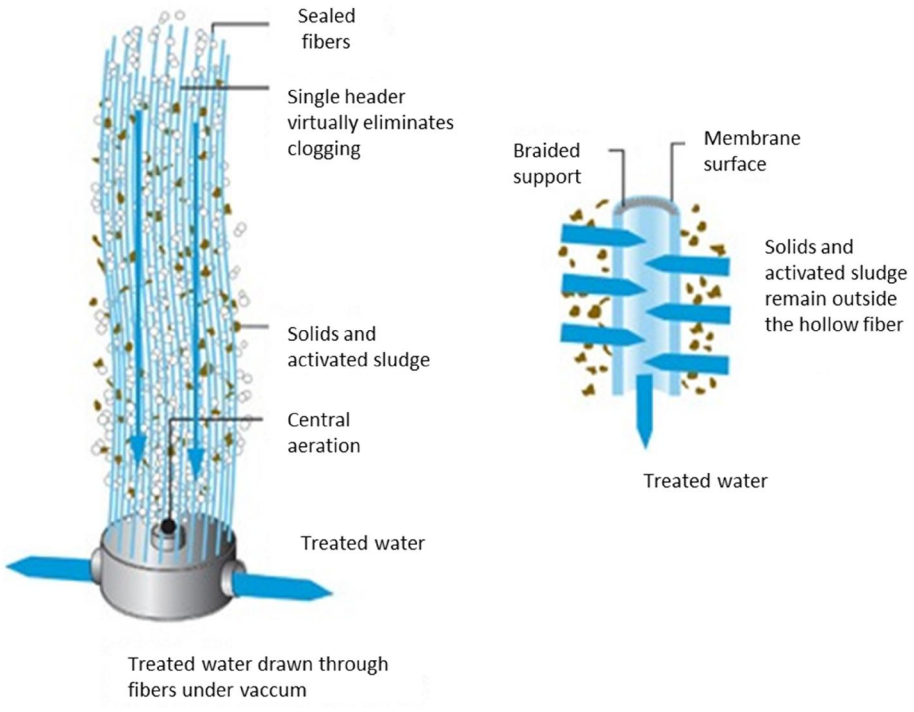

Figure 1.1. *The membrane module (PURON® MBR) operation mode. Source of pictures: Koch Membrane systems, Inc.*

However, the potential decline in filtration flux is generally the main challenge in the process of membrane filtration. The solid deposition, pore clogging by colloidal particles, absorption of soluble compounds and biofouling on the surface of or within the membrane are the main reasons for decreasing in filtration flux (Judd, 2006; Le-Clech et al., 2006; Leiknes and Ødegaard, 2007). The main contributors to the fouling of membrane are suspended solids, particle size and soluble organic contents (i.e. biopolymers, soluble microbial products and extracellular polymeric substances) (Ivanovic and Leiknes, 2012). Some solutions reduce fouling in the MBR such as bubble aeration for limiting the particle deposition and back-washing for declining the internal fouling (Bouhabila et al., 2001). Furthermore, problems of membrane fouling or clogging can be controlled by operating below critical flux, periodic back-washing and aeration (Judd, 2006). However, the instantaneous flux is considered as the main factor for increase of fouling rate in both of back-washing and relaxation (Wu et al., 2008). Some categories of fouling in the MBR are irreversible and cannot be removed easily by physical cleaning (bubble aeration or back-washing), but only by chemical methods. Moreover, neither physical nor chemical cleaning can remove the irrecoverable fouling that affect to the permeability of MBR in the long run (years).

Monitoring of trans-membrane pressure (TMP) is an effectively pathway to study the evolution of membrane fouling (Kraume et al., 2009) (Figure 1.2). TMP is the different pressures across the membrane between the feed and permeate side (membrane treated water) when water flows through the membrane. The increase of fouling will result to the raise of TMP level when the flux is kept constant. Furthermore, information of reversible and irreversible fouling is also provided through TMP monitoring, and then determining needs for back-washing or chemical cleaning.

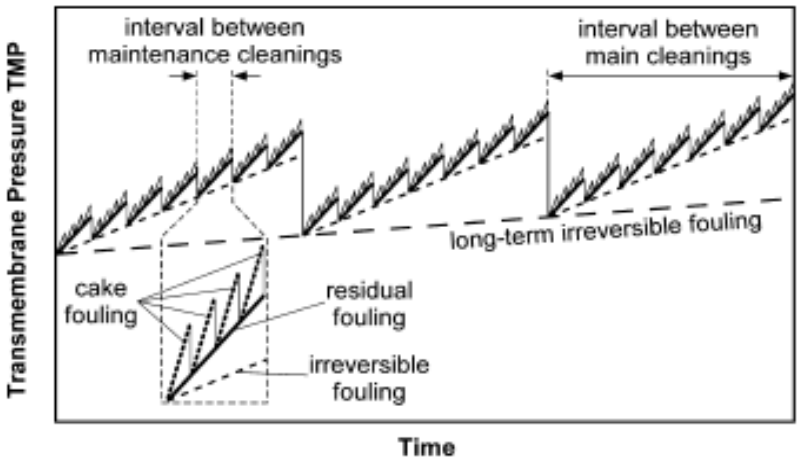

Figure 1.2. *Evolution of fouling rates under scheme during the long-term operation of full-scale MBRs (Kraume et al., 2009).*

1.2 Copepod *Acartia tonsa* Dana

Acartia tonsa (*A. tonsa*) is a pelagic calanoid copepod species (Crustacea/ Copepoda/ Calanoida/ Acartiidae) with a cosmopolitan distribution in coastal waters (Mauchline, 1998). This species is often the dominating copepod species and is typically found in estuarine and marine areas in the subtropical and tropical regions (Mauchline, 1998).

A. tonsa has a broad temperature (0 - 30 ^{o}C) and salinity (1 - 38 ppt) tolerance range (Mauchline, 1998), has a size of 1.5 mm and short generation time (13 days at 16 - 18^{o}C) (Berggren et al., 1988). To reach up to the adult stage, *A. tonsa* has to grow and molt through 6 nauplii stages and then through another 6 stages of copepodite. The ability to produce dormant eggs, including both of diapausing and quiescent non-diapausing eggs via sexual reproduction (Castro-Longoria, 2001) shows a huge benefit for maintaining the copepod population in outdoor extensive fish rearing systems (Marcus, 2005). Moreover, the concentrated harvest of *A. tonsa* eggs in intensive systems can be stored up to several months at 2 – 3 ^{o}C without affecting the viability and fitness

of next generation (Drillet et al., 2006). According to Støttrup (1986), the maintenance of *A. tonsa* in the cultivation system is easy to operate, therefore it is considered as one of the most studied live prey candidates for aquaculture purposes (Peck and Holste, 2006).

1.3 Aim of study

The main aim of this study was to evaluate the impacts of colloidal particles (30 nm – 1 µm) and fine solids (2 µm – 60 µm) on nauplii and egg production of the copepod *A. tonsa* Dana in a conventional recirculating system (cRAS), a membrane-modified recirculating system (mRAS) and a flow throught aquaculture system (FTAS).

The following hypothesis was tested in the present study:

- Efficient removal of colloidal particles and fine solids leads to improve the performance of *A. tonsa* in terms of survival, growth and development, and to increase the nauplii and egg production in the mRAS, compared to the cRAS and FTAS.

2. Materials and Methods

The study was carried out at Centre of Fisheries and Aquaculture, Norwegian University of Technology and Science (NTNU Sealab), Brattørkaia, Trondheim, Norway. All experiments were conducted from August – October 2013.

2.1 Experimental design

Two experiments with the copepod *A. tonsa* Dana were conducted to test the influences of particle characteristics, which are in different range sizes from 30 nm – 1 µm (colloidal particles) and 2 µm – 60 µm (fine solids), on the growth performance of copepods, as well as nauplii and egg production of adult copepods through the total period of 4 weeks.

Experiment 1: The first experiment was carried out for 2 weeks to study the growth performance of copepod nauplii in three different water treatment systems: a conventional recirculating aquaculture system (cRAS), a modified RAS connected a membrane filtration between two biofilters (mRAS), and a flow through aquaculture system (FTAS) (Figure 2.1). The initial densities of copepod nauplii in the cultivation tanks (4 replicates) of each system were 75 individuals ml^{-1}. The impact of particles on copepod nauplii performance was determined by studying survival, growth and developmental of nauplii during the experiment.

Experiment 2: In the second experiment, 15 randomly selected adult copepods in each cultivation tank of each water treatment system from experiment 1 were transferred into 1 L beaker of new seawater with 3 replicates per tank in the same conditions to study nauplii and egg production

within 2 weeks. Nauplii and egg production rate of copepods was calculated by daily harvesting copepod eggs and compared between water treatment systems at the end of experiment.

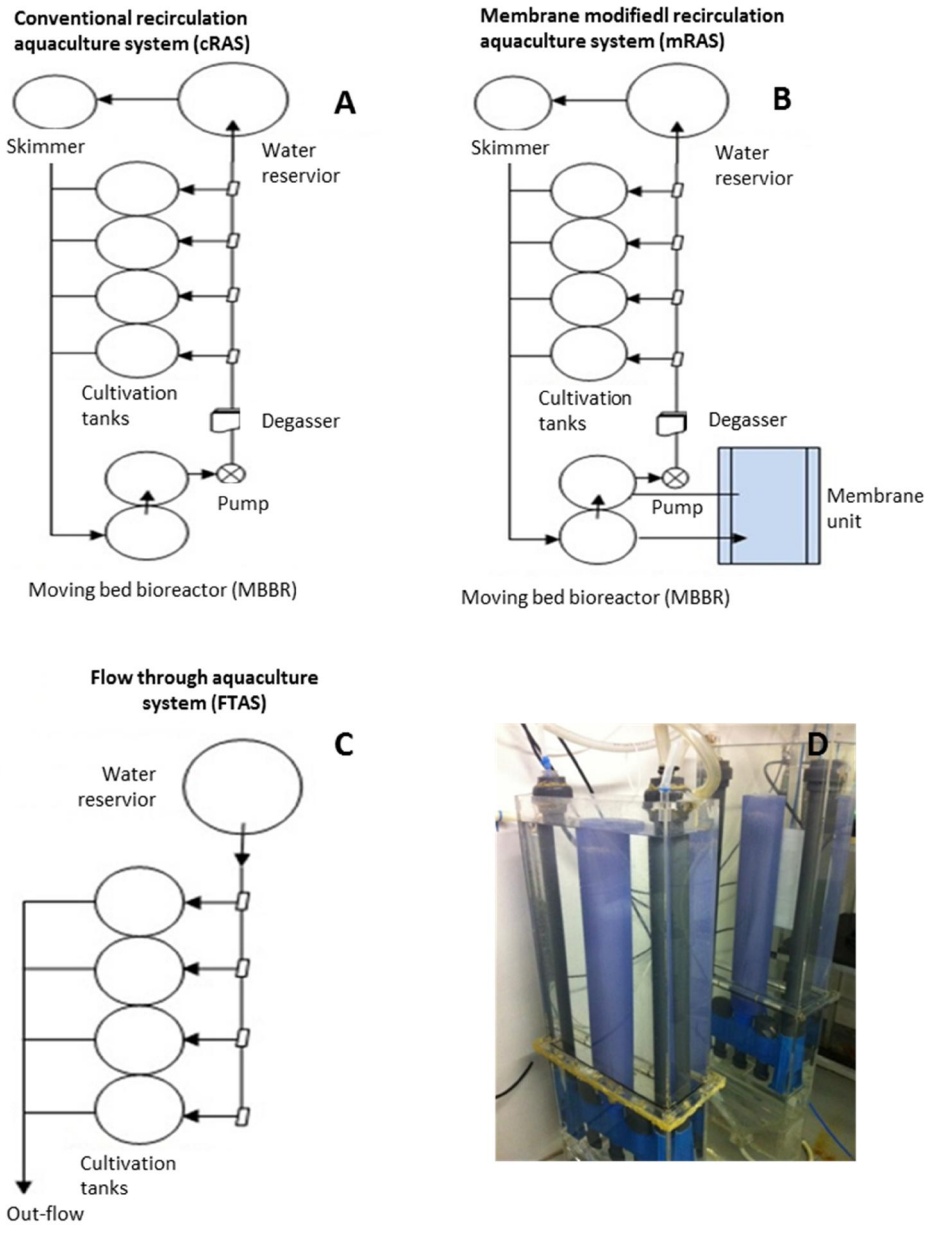

Figure 2.1.*(A) A conventional recirculating aquaculture system (cRAS), (B) A modified RAS connecting with a membrane filtration between two moving bed biofilters (mRAS), (C) A flow through aquaculture system (FTAS) and (D) Test pilot membrane module (PURON® polymer membranes, Koch Membrane System, USA).*

2.1.1 Experimental systems for cRAS, mRAS and FTAS

The cRAS and mRAS consisted of four cultivation tanks (100 L), a water reservoir (160 L), a skimmer (80 L), two biofilters in series (267 L each), and a degasser (50 L, vacuum operated). The cRAS and mRAS differed by an ultrafiltration (UF) membrane module (pore size of 50 nm) in the water treatment system of mRAS. The FTAS consisted of four cultivation tanks (100 L) with the continuously supply of seawater by gravity into the tanks from a reservoir. The outlet filters with mesh size of 40 μm were placed in center of all cultivation tanks to ensure retaining of copepod nauplii inside the tanks.

Recycling of water rate in the cultivation tanks was 3 times per day (from 0 - 3 days post hatching, dph), and increased to 5 times per day (from 4 - 7 dph) and to 8 times per day (from 8 - 14 dph). The daily water exchange of new water was 4.5 % of the total water volume in the system. The reservoir contained seawater (34 - 35 ppt) from Trondheimfjord, about 800 meters from Sealab and with an inlet at a depth of 70 m. The water was in advance filtrated through a sand filter to remove suspended particles (> 20 μm) and then aerated and vacuumed to avoid supersaturating of nitrogen gas. The temperature of supplied seawater to cultivation tanks in all systems was about 20 - 22 ^{o}C and it was maintained by an immersed heater in the biofilter and water reservoir of FTAS.

The biofilters were designed as moving bed biofilm reactors (MBBR) and filled with biofilm carriers type K1 (Anox Kaldnes), with filling fractions of two MBBRs that were 15 % for heterotrophic bacteria and 22.5 % for nitrifying bacteria and giving area of 75 and 112.5 m^2/m^3 reactor volume for biomass growth, respectively. The membrane filtration (30 L tank) in the

mRAS consisting of two modules of low pressure submerged ultrafiltration that was operated as an outside-in mode. Peristaltic pumps (Masterflex L/S, Cole-Parmer Instrument Company, Norway) was utilized to pump feed water to membrane module from heterotrophic biofilter and the membrane treated water (permeate) discharged to the nitrification biofilter. Two membrane modules (Figure 1D) (PURON® polymer membranes, Koch Membrane System, USA) with a pore size of 50 nm and an area of 0.97 m^2each was installed in 30 L tank. The constant filtration flux of 33 L m^{-2} h^{-1} was alternated with back-washing (2 times per day with 10 minutes each time) and air scouring (17 L/min) to reduce fouling on the membrane surface. The performance of the membrane unit was determined by monitoring the trans-membrane pressure (TMP) evolution over time with a pressure transducer (Standard Genspec, 4 - 20 mA, ESI Technology). A TMP of 0.3 bar was the set point indicating the need for chemical washing of the membrane unit. The membrane unit was cleaned prior to the experiment, by soaking the membranes with 1.3 g L^{-1} citric acid for 4 hours, 0.5 % hypochlorite for 8 hours and then 0.7 g L^{-1} of citric acid for 3 hours (Holan et al., 2014).

The particle retained water in the membrane module after filtration was drained daily and replaced with fresh seawater one time per day. It was carried out to minimize the leakage of smaller components through the membrane unit from hydrolyzed particles. Furthermore, solid deposition and pore clogging on the surface and within membrane were also limited to cause negative impacts on filtration flux of membrane.

2.1.2 Light, aeration and disinfection procedure

A 24 hours light regime was used during the first experiment. A weak aeration was applied in each cultivation tank to ensure sufficient oxygenation, help to distribute evenly copepod in tank,

maintain algae in suspension (Støttrup and McEvoy, 2008), avoid clogging of the outlet filter, and stratification of temperature. While, heavy aeration was applied in the biofilters to supply sufficient oxygen for bacterial growth and in live algae feeding reservoir to keep algae alive and avoid sedimentation of algae at the bottom. The metal outlet sieves in cultivation tanks were washed daily to avoid clogging of sieves from 0 - 7 dph and were replaced with nylon outlet sieves with mesh size of 64 μm at 8 dph when the outlet water flow was significantly obstructed by clogging.

2.2 Physiochemical water quality parameters

The table 2.1 shows the different physiochemical parameters of water quality that were measured during the first 14 days of experiment in different sampling days and the measurements were applied according to standard methods from manufacturer manuals.

The water samples to measure TAN, NO_2-N, NO_3-N, turbidity and particles were filtered through a 40 μm sieve to prevent the collection of copepod nauplii in the water sample, then water was sampled by a pipette with a 25 mL sterilized tube. Each tank in each system had its own sampling equipment (a 40 μm sieve and a 25 mL sterilized tube), which was disinfected by 70 % ethanol before using. For measurement of temperature, salinity, DO and pH, the instruments were placed at a fixed depth between the tank wall and outlet sieve.

For measurement of colloidal particles, 10 μm filters (Versapor® Membrane, Pall, USA) were used to remove the algae in the water sample before measuring to avoid influences of algae on the measurement process.

Table 2.1. *Physiochemical water quality parameters and method/ instrument applied.*

Parameter	Instrument	Sampling days		
		Tanks	Inlet water	Permeate
Temperature (°C)	YSU Pro ODO Digitlal Professional Series, USA	Daily		
Dissolvedoxygen (DO)	YSU Pro ODO Digitlal Professional Series, USA	Daily		
pH	pH/mV meter (VWR pH10-pen, United Kingdom)	Daily		
Salinity	Salinity Refractometer (ATC-S/Mill-E Range 0-100%, Japan)	Daily		
Total ammonia nitrogen (TAN)	DR/890 HACH Datalogging Colorimeter, HACH, USA	0, 3, 7, 11, 14	0, 3, 7, 11, 14	0, 3, 7, 11, 14
Nitrite (NO_2) and nitrate (NO_3) nitrogen	DR/890 HACH Datalogging Colorimeter, HACH, USA	0, 3, 7, 11, 14	0, 3, 7, 11, 14	0, 3, 7, 11, 14
Turbidity (NTU)	Micro 950 Turbidimeter (Palintest, United Kingdom)	0, 2, 6, 9, 13, 14	0, 5, 8, 12, 14	0, 5, 8, 12, 14
Colloidal particles (30 nm - 1 µm)	NanoSight LM10 instrument (NanoSight, Amesbury, United Kingdom)	0, 2, 6, 9, 13	0, 5, 8, 12, 15	0, 5, 8, 12, 15
Fine solids (2 µm – 60 µm)	Beckman Multisizer™3 Coulter Counter® (Beckman Coulter Inc., USA)	0, 2, 6, 7, 8, 9, 10, 11, 12, 13, 14	0, 1, 5, 8, 12, 14	0, 1, 5, 8, 12, 14

2.3 Production of *Rhodomonas baltica*

Rhodomonas baltica (*R. baltica*) was cultured semi-continuously in transparent polycarbonate plastic cylinders (160 - 200 L) and plastic bags (300 L) in an air conditioned room at 20 ^{o}C. Seawater (34 - 35 ppt) was disinfected with sodium hypochlorite (25 mL, 10 - 15 % NaOCl / 100 L of seawater) over night, then neutralized with sodium thiosulphatepentahydrate (3.2 g $Na_2O_3S_2*5H_2O$ / 25 mL NaOCl) for at least 2 hours before it was used for algae cultivation.

The aeration was placed at the bottom of plastic cylinders and bags to mix the algal volume, which were exposed to continuous illumination by six fluorescent tubes (GE Polylux XL 830 F58W). To optimize the growth rate of *R. baltica*, CO_2 (1 - 2 % of aeration) was added to the cultures and adjusted to maintain the pH in the cultures within the range of 7.5 - 8.7. Amount of 10 - 20 L of algae with a density of 2 - 3 x 10^6 cells $ml^{-1}$was added into plastic cylinders and bags for starting of algae cultivation. The growth medium (Conwy) with ratio of 1 ml/L seawater was added to culture algae (Walne, 1974).

Copepod nauplii in each cultivation tank of each system was fed daily 15 L of algae (2.3 - 3.1 x 10^6 cells ml^{-1}) by a feeding robot (Storvik Robma, Storvik AS, Norway). To ensure the saturated food concentration for optimal growth of *A. tonsa* (10^4 *R. baltica* cells ml^{-1}; Skogstad 2010), the algae concentration in the cultivation tanks was measured daily by Beckman Multisizer^{TM}3 Coulter Counter$^{®}$ instrument, considering all particles (cells ml^{-1}) with a diameter in size range of 5.333 - 9.567 µm as *R. baltica*.

2.4 The copepod *Acartia tonsa* Dana

2.4.1 Copepod eggs and cultivation of *A. tonsa*

The strain of *A. tonsa* Dana used in the current study is originally from Danmarks Fiskeriundersøgelser, Charlottenlund, Denmark. This strain was isolated in 1981 in Øresund and given the identification code as DFH.AT1 (Støttrup et al., 1986).

A. tonsa eggs were harvested by siphoning at the bottom of copepod tanks to collect eggs from an on-going broodstock culture at SINTEF. This on-going culture is cultivating with continuous light in 24 hours and slight aeration. The supplied concentration of algae is more than 50000 cells ml^{-1}. The harvested eggs are washed by seawater before keeping in 200 mL Easy Flasks (NUNC^{TM}) with sterile seawater (34 - 35 ppt) and stored in a 2 ^{o}C refrigerator (SANYO Pharmaceutical Refrigerator MPR-311D (H), Japan) for up to 3 months prior to the experiment.

Copepod eggs were hatched in 25 white polypropylene flat bottom tanks (100 L each) with strong aeration at the bottom and filled with seawater (34 - 35 ppt). Approximately 14 - 18.5 million eggs of copepods were added to each hatching tank and incubated at 20 ^{o}C with continuous light for 48 hours. To reduce differences in the development (not beyond stage 2) of newly hatched nauplii, no feeding during the hatching process was carried out. Unhatched eggs at the bottom of the hatching tanks were removed before transferring newly hatched nauplii into cultivation tanks.

A nauplii concentrator (60 L) was used to concentrate the copepod nauplii before distribution to the experimental tanks. The nauplii concentrator consisted of a white polycarbonate basket with four sides filtered frames (40 μm metal sieves) on the side wall, giving the total concentrated

volume of approximately 15 L inside the basket. After concentrating, copepod nauplii were sampled randomly in 5 different positions in the concentrators with a polycarbonate rod (0.6 cm in diameter) to measure the density by manual counting under an inverted microscope (Eclipse TE2000-S, Nikon, Japan) to ensure the equal transfer of copepod nauplii from concentrators into cultivation tanks of each system.

2.4.2 **Sampling procedures and density measurement of *A. tonsa***

In the water column, irregular distribution of copepods is caused by the differences of behaviors between nauplii and copepodite stages, such as swimming or spatial distribution (Mauchline, 1998). A 90 cm polycarbonate rod (0.6 cm in diameter) was used to stir the water in tank when taking samples in order to ensure the better distribution of copepods in the water column and to sample at different areas of tank with a fast speed, which restricted the escape of sampled copepod from the rod. Sampling for density was conducted every day. The water samples (10 - 20 mL) for measurements of copepod density and image analysis were made daily to calculate the density in the cultivation tanks.

The instantaneous rates of mortality (z day^{-1}) were calculated according to Breteler (2004) using equation 2.1.

$N_t = N_o e^{-z\,t}$ (2.1)

Where t is the time in days; N_0 and N_t are the numbers of individuals L^{-1} at time 0 and at time t, respectively.

Daily percentage mortality of a specific time interval was estimated based on the equation 2.2 (Breteler et al., 2004)

% mortality $day^{-1} = (e^{z} - 1) \times 100$ % (2.2)

2.4.2 Image analysis

After measurements of copepod density in water samples, copepods in the remaining volumes were continuously mixed and transferred to a tubular chamber (Hydro-Bios, Germany) by a pipette. The copepods were anesthetized with Tricainemethanesulfonate solution (FINQUEL, Argent chemical laboratories, Washington) and placed on the inverted microscope. The first 50 copepods in sample from each cultivation tank were photographed randomly by a camera (Digital sight DS-5Ms, Nikon, Japan) connected with an inverted microscope. The total length of nauplii and prosome length of copepodite from the 50 copepods photographed were measured by the software ImageJ (version 1.44, National Institute of Health, USA) (Figure 2.2).

The regression equations 2.3 and 2.4 (Berggren et al., 1988) were applied to convert the measured length of copepods to carbon biomass.

Nauplii

$W = 3.18 \times 10^{-6} L^{3.31}$ (2.3)

Copepodite:

$W = 1.11 \times 10^{-5} L^{2.92}$ (2.4)

Where W is the individual weight in ng C and L is the total length of nauplii or prosome length of copepodites in μm.

Daily weight increase (% DWI) was investigated to calculate the daily growth rate of copepod according to (Ricker, 1958).

$\%\ DWI = 100 \times (e^{SGR} - 1)$ (2.5)

With

$SGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$ (2.6)

Where SGR is the specific growth rate (μg day^{-1}), W_1 and W_2 are mean copepod weights at times t_1 and t_2, respectively.

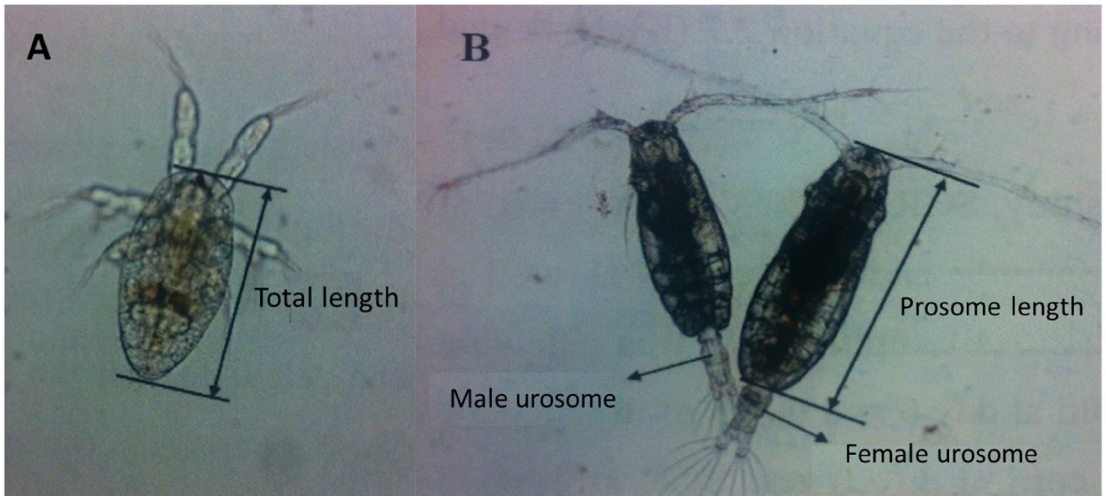

Figure 2.2. *Total length of nauplii (A) and prosome length of copepodite (B) of A. tonsa used for measurement. The male and female copepod adults are indicated by the difference of urosome as shown in the picture (Marcus, 2007). Photo. Minh Vu Thi Thuy, 2010.*

2.4.3 Egg production of *A. tonsa*

The nauplii and egg production (EP) experiment was started at 16 dph after finishing the experiment of growth performance at 14 dph in order to ensure the completed adult development of copepods. The number of 15 largest copepods was picked up randomly and then transferred into a 1 L beaker of new seawater (22 - 24 ^{o}C), with 3 replicates per tank (Figure 2.3). To ensure sufficient food for survival of copepods and compensate for sedimentation in reproduction beaker, an amount of *R. baltica* (3 x 10^4 cells mL^{-1}), at which egg production of *A. tonsa* is independent of food supply (Kiørboe et al., 1985; Skogstad, 2010) was supplied twice a day. At

the beginning, a heavy aeration was injected into beakers, hence causing a massive loss of copepods from 16 dph to 21 dph. Due to the un-identified gender of dead copepods, the data of egg production used in the current study was recorded from 22 dph to 29 dph. A weak aeration was replaced at 22 dph into each beaker by a plastic tube (2 mm in diameter) at the bottom in order to just provide sufficient oxygen for survival of copepod and keep algae in suspension. The beakers were incubated at 22 ^{o}C air conditioned room and continuous light.

The live copepods in each beaker were transferred each 24 hours of incubation by a spoon into another clean beaker that was filled with new seawater and algae *R. baltica*. Gender and number of dead copepods were identified and recorded for calculating of egg production (equation 2.7) at the end of experiment. To harvest the copepod eggs in the water volume, a mesh size of 50 μm (Figure 2.3) was used. Counting of copepod eggs then conducted under the inverted microscope.

Egg production (EP) = (number of eggs + number newly hatched nauplii) / number of adult females (eggs female $^{-1}$ day^{-1}) (2.7).

Figure 2.3. *The beakers (1 L) utilized in egg production experiment (left), equipment including the egg collector with a filter sieve 50 µm at the bottom and for transferring of the adult copepods (right). Photo: Anh Hung Phan.*

2.5 Statistical analysis

Graph Prism version 6 for Windows (GraphPad Software, Inc. San Diego, California, USA) was employed for all statistical analysis at the 95 % confidence level ($p < 0.05$). Mean ± standard error (SE) was represented in text and on graphs. $p < 0.05$ was used as the critical value for significance. Data presented in percent (% survival rate) was arcsine transformed [arcsine $(\%/100)^{0.5}$] before running statistical analysis. One-way ANOVA (Turkey`s Multiple Comparison Tests) was used to compare the means between treatments. Correlation between variables was tested by using plot regression analysis, as significant at $p < 0.05$.

3. Results

3.1 Physiochemical water quality parameters

3.1.1 Temperature, salinity, oxygen and pH

No difference was found in the parameters of pH and oxygen concentration over time between systems (Table 3.1). There were significant differences of average temperature and salinity values in the cRAS and mRAS tanks, compared to the FTAS during the experiment.

Table 3.1. *Water quality parameters (mean ± SE) in the cultivation tanks (n = 4) of the cRAS, mRAS and FTAS. Different letters mark significant differences between the treatments, $p < 0.05$.*

Parameters	cRAS	mRAS	FTAS
Temperature (^{o}C)	22.7 ± 0.1^{a}	22.7 ± 0.1^{a}	21.8 ± 0.1^{b}
Salinity (ppt)	37.2 ± 0.3^{a}	38 ± 0.2^{b}	36 ± 0.3^{c}
Oxygen concentration (mg L^{-1})	69.3 ± 0.06	7.08 ± 0.05	6.95 ± 0.09
pH	7.8 ± 0.0	7.9 ± 0.0	7.8 ± 0.0

3.1.2 Nitrogenous waste production

The concentration of total ammonia nitrogen, TAN (NH_3-N and NH_4^+-N) in the cRAS tanks showed a high fluctuation, while the concentrations in the mRAS and FTAS were stable during the experiment and were significantly lower than cRAS at 7 dph (Figure 3.1A). The TAN concentrations in the inlet water of mRAS, FTAS and permeate developed in the same trend through the experiment and were significantly lower than cRAS, at 7 dph and 11 dph (Figure 3.1B).

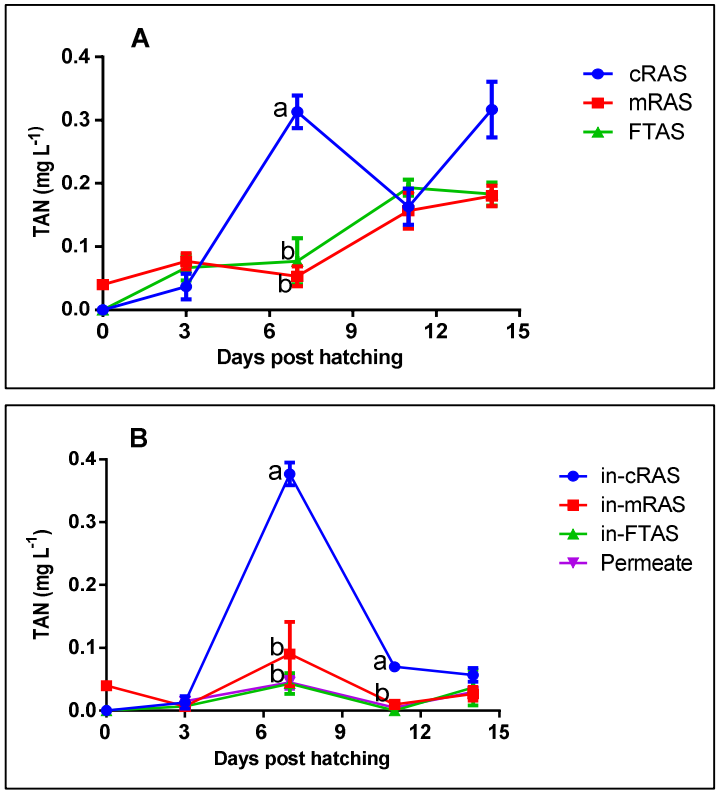

Figure 3.1. *Values of total ammonia nitrogen (TAN) (mean ± SE) (n = 4) in the cultivation tanks (A), and in the inlet water and the permeate (B). Different letters mark significant differences between the treatments, $p < 0.05$.*

The concentration of calculated un-ionized ammonia (NH_3-N) in the cultivation tanks of cRAS was significantly higher than in FTAS and mRAS tanks at 7 dph (Figure 3.2A). At 14 dph, cRAS was no longer different to mRAS but still significantly higher than FTAS. The NH_3-N concentration in the inlet of cRAS increased after 3 dph and was significantly higher than permeate, mRAS and FTAS at 7 dph and 11 dph (Figure 3.2B).

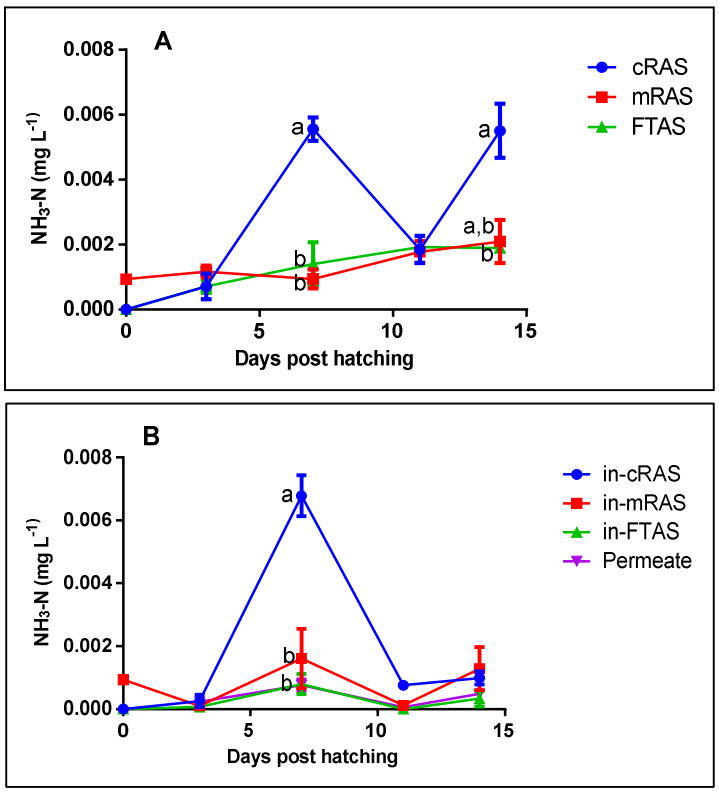

Figure 3.2. *Values of calculated un-ionized ammonia (NH_3-N) (mean ± SE) (n = 4) in the cultivation tanks (A), inlet water and permeate (B). Different letters mark significant differences between the treatments, $p < 0.05$.*

The difference in nitrite nitrogen (NO_2-N) concentrations in the cultivation tanks was statistically significant between the treatments (Figure 3.3A). Throughout the experiment, the NO_2-N concentrations of mRAS was significantly lower the cRAS but higher than the FTAS. The concentrations of NO_2-N in the inlet water of mRAS and permeate developed in the same trend during the experiment until registering significant differences at 3 dph and 11 dph (Figure 3.3B). Their concentrations were significantly lower the cRAS but higher than the FTAS throughout the experiment.

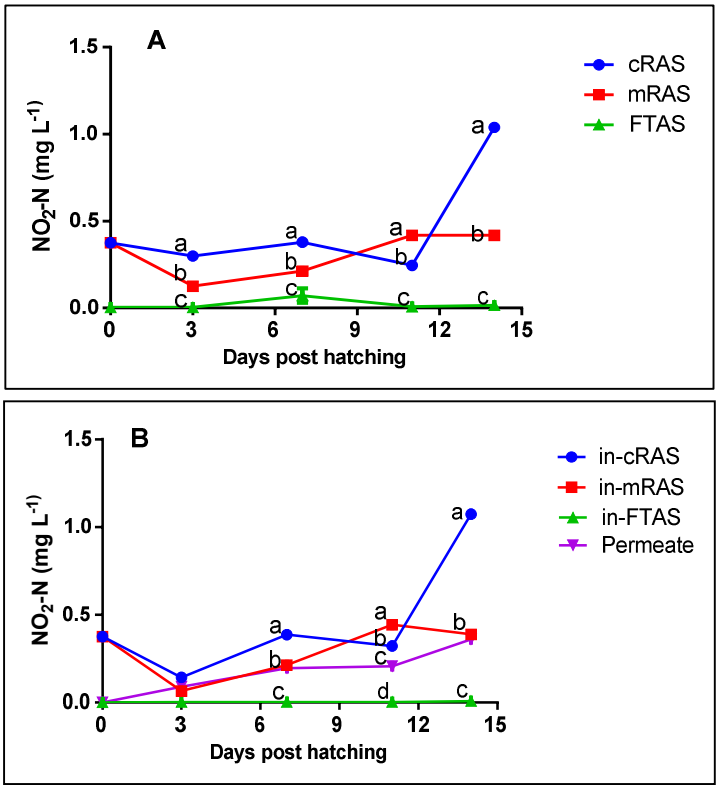

Figure 3.3. *Values of nitrite nitrogen (NO_2-N) (mean ± SE) (n = 4) in the cultivation tanks (A), inlet water and permeate (B). Different letters mark significant differences between the treatments, $p < 0.05$.*

No significant difference of nitrate nitrogen (NO_3-N) concentrations in the cultivation tanks (Figure 3.4A) and inlet water (Figure 3.4B) was found between the systems.

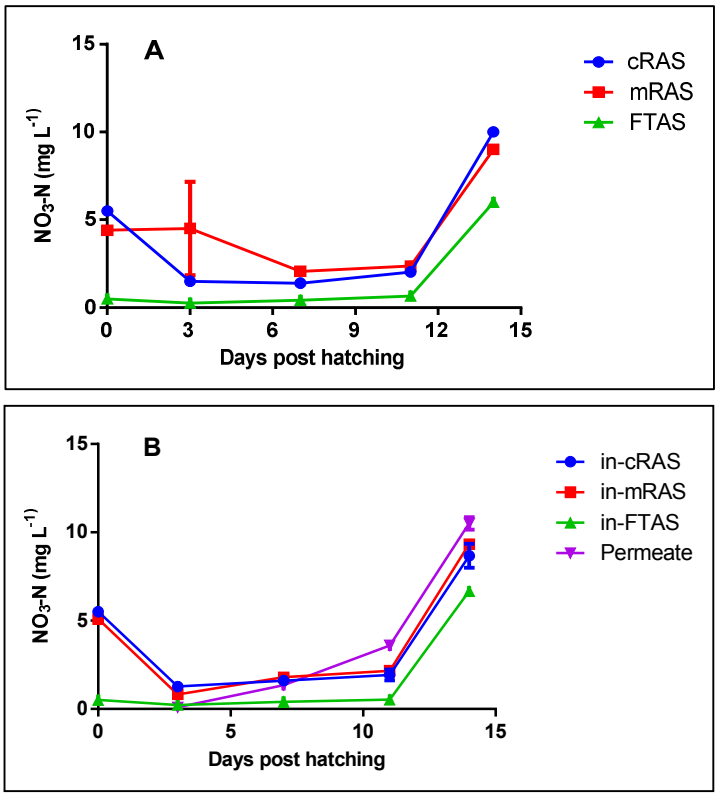

Figure 3.4. *Values of nitrate nitrogen (NO_3-N) (mean ± SE) (n = 4) in the cultivation tanks (A), inlet water and permeate (B) during the experiment. Different letters mark significant differences between the treatments, $p < 0.05$.*

3.2 Characteristics of particles

3.2.1 Turbidity

The levels of nephelometric turbidity units (NTU) increased in the cultivation tanks of all systems up to 6 dph; then cRAS and mRAS developed in the same trend until the end of experiment, whereas FTAS fluctuated highly (Figure 3.5A). The NTU levels in the inlet water of

mRAS and cRAS were similar during the experiment, only showing a significant difference at 14 dph (Figure 3.5B). While the levels of permeate and FTAS were low and stable during the experiment.

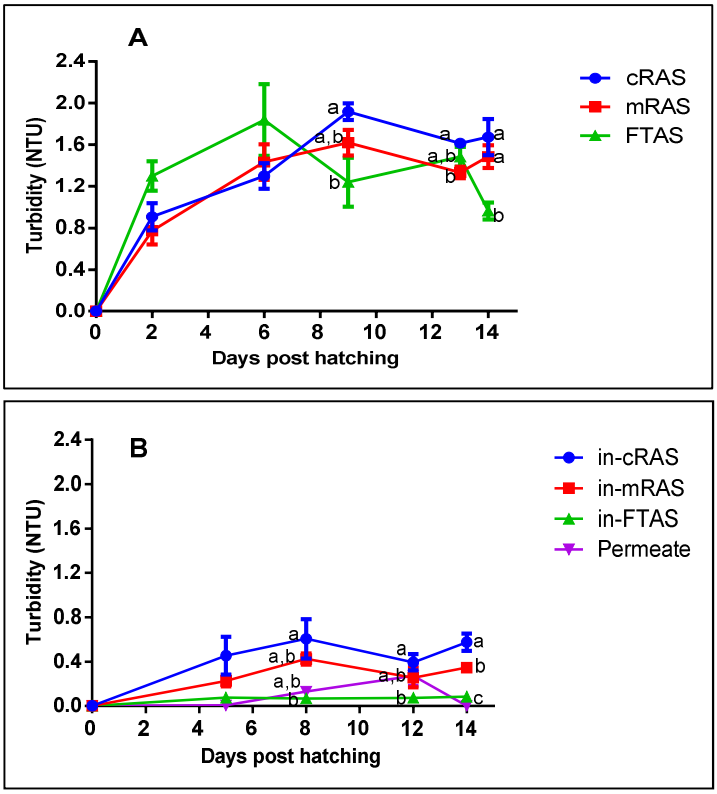

Figure 3.5. *Turbidity levels (mean ± SE) (n = 4) in the cultivation tanks (A), inlet water and permeate (B) during the experiment. Different letters mark significant differences between the treatments, $p < 0.05$.*

3.2.2 Colloidal particles (30 nm – 1 µm)

The numbers of colloid particles in the cRAS and mRAS tanks increased during the first 6 days of experiment and were significantly higher than the FTAS at 6 dph (Figure 3.6A). The particle numbers in the cRAS and FTAS increased from 6 dph; whereas mRAS was stable and started to decline from 9 dph until the end of experiment. The numbers of colloidal particles in the inlet

water of FTAS and permeate were low and stable, while cRAS and mRAS showed a similar trend and were significant differences with the FTAS and permeate throughout the experiment (Figure 3.6B).

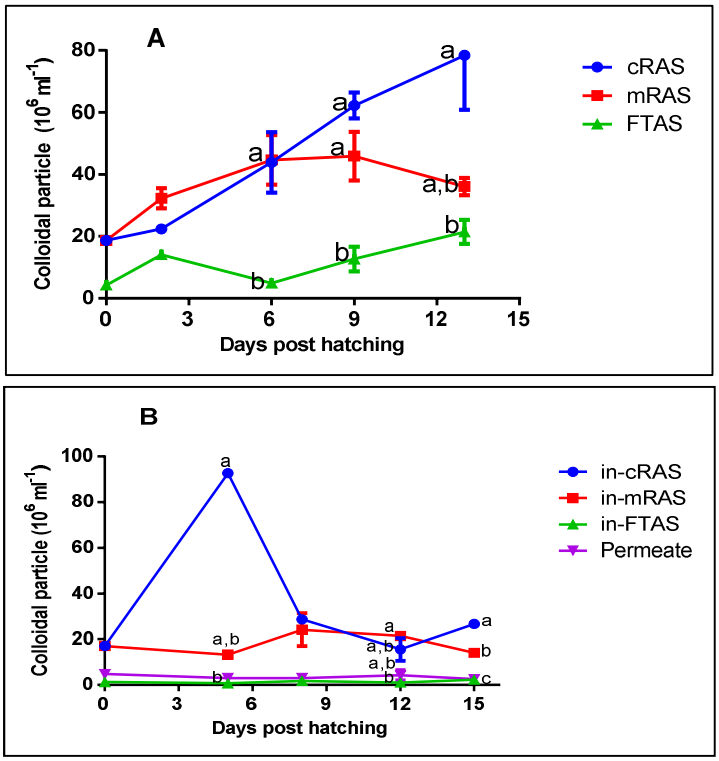

Figure 3.6. *Concentrations of colloidal particles (mean ± SE) (n = 4) in the cultivation tanks (A), inlet and permeate water (B). Different letters mark significant differences between the treatments, $p < 0.05$.*

3.2.3 Fine solids (2 µm – 60 µm)

No significant difference in the concentrations of fine solids in the cultivation tanks was found between the systems during the experiment (Figure 3.7A). The particle concentrations in the inlet water of mRAS, FTAS and permeate showed a similar trend throughout the experiment; whereas

a rapid increase of number in the mRAS was observed and significant difference with others at 5 dph (Figure 3.7B). The particle numbers of mRAS then showed a similar trend with others after 8 dph.

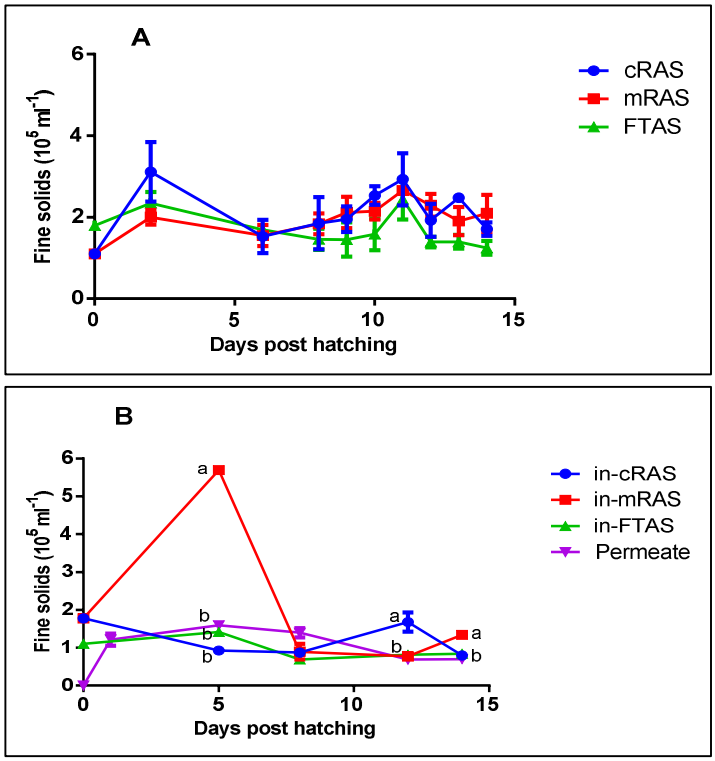

Figure 3.7. *Concentrations of fine solids (2 µm – 60 µm) (mean ± SE) (n = 4) in the cultivation tanks (A), inlet and permeate water (B). Different letters mark significant differences between the treatments, $p < 0.05$.*

3.3 Growth of copepod *A. tonsa*

3.3.1 Density and survival of *A. tonsa*

The densities of copepods in the systems decreased in the same trend during the experiment until registering a significant difference at 11 dph (Figure 3.8). However, the copepod numbers in the FTAS and cRAS tanks showed high variations between the replicates compared to the mRAS through the experiment (Appendix 1).

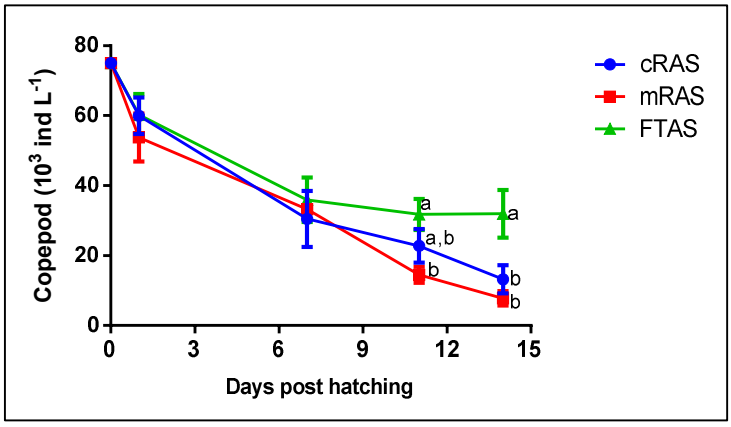

Figure 3.8. *Densities of copepod A. tonsa (mean ± SE) (n = 4) in the cRAS, mRAS and FTAS systems at selected sampling days during the experiment. Different letters mark significant differences between the treatments, $p < 0.05$.*

Throughout the first 7 days of experiment, there was no significantly difference in survival rates of copepods between the systems until registering the significant difference at 11 dph (Table 3.2). The average daily mortality rate throughout the experiment was significantly lower in the FTAS (7 %), compared to other treatments (14 - 19 %).

Table 3.2. *Survival (%) and mortality (% day^{-1}) (mean ± SE) (n = 4) rate of the copepods in the cRAS, mRAS and FTAS at selected sampling days during the experiment. Different letters mark significant differences between the treatments, $p < 0.05$.*

	Survival (%)			**Mortality (% day^{-1})**		
Experimental day	**cRAS**	**mRAS**	**FTAS**	**cRAS**	**mRAS**	**FTAS**
0	100 ± 0.0	100 ± 0.0	100 ± 0.0			
1	80 ± 7.0	71.7 ± 9.2	80.3 ± 8.0			
7	40.7 ± 10.7	44.3 ± 1.5	48 ± 8.4	14.6 ± 2.8^{a}	19.3 ± 3.5^{a}	6.9 ± 1.7^{b}
11	30.3 ± 6.4^{a,b}	19.3 ± 3.0^{a}	42.3 ± 5.9^{b}			
14	17.7 ± 5.4^{a}	10.3 ± 2.8^{a}	42.7 ± 9.1^{b}			

3.3.2 Growth of *A. tonsa*

The growth of *A. tonsa* is illustrated in terms of mean length and carbon biomass weight at selected sampling days during the experiment (Figure 3.9). Mean development of the copepod length (Figure 3.9A) in the mRAS was significantly lower than in the FTAS, while the mean carbon weight (Figure 3.9B) of the copepod was similar between the systems at the end of experiment.

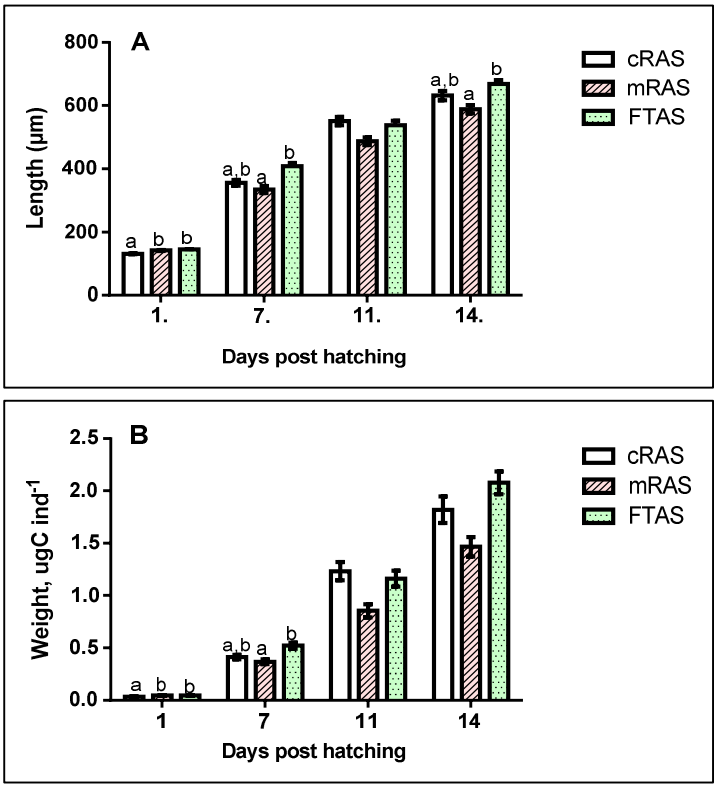

Figure 3.9. *The developments of length (A) and carbon biomass weight (B) (mean ± SE) (n = 4) of A. tonsa in different systems at the selected sampling days. Different letters mark significant differences between the treatments, $p < 0.05$.*

The growth rate of *A. tonsa* is represented in term of the daily carbon weight increase (% DWI) for different developmental stages (nauplii and copepodite) through the growth experiment (Figure 3.10). Only the statistically significant differences of DWI at the copepodite stage were found between cRAS and mRAS during the experiment.

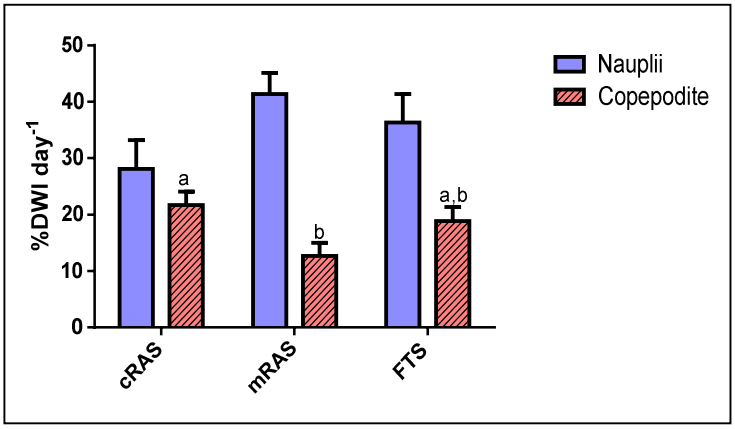

Figure 3.10. *Daily weight increase (% DWI day^{-1}) (mean ± SE) (n = 4) of A. tonsa in different developmental stages (nauplii and copepodite) in cRAS, mRAS and FTAS. Different letters mark significant differences between the treatments, $p < 0.05$.*

3.2.4 Distribution of developmental stages of *A. tonsa*

The determination of developmental stages of the copepod *A. tonsa* in the systems was based on daily measured length (Figure 3.11). The copepod nauplii in all systems dominated during the first five days of experiment. The copepodite stages were observed firstly in the FTAS at 4 dph, whereas it was recorded at 5 dph for cRAS and mRAS. Most of the copepods, about 90 % for cRAS, FTAS and 70 % for mRAS, molted from nauplii to copepodite stages at day 6. Adults were observed at 9, 10 and 11 dph in the FTAS, mRAS and cRAS, respectively. Approximately 20 % of adults was found in the cRAS and FTAS, whereas only 10 % in the mRAS at the end of experiment.

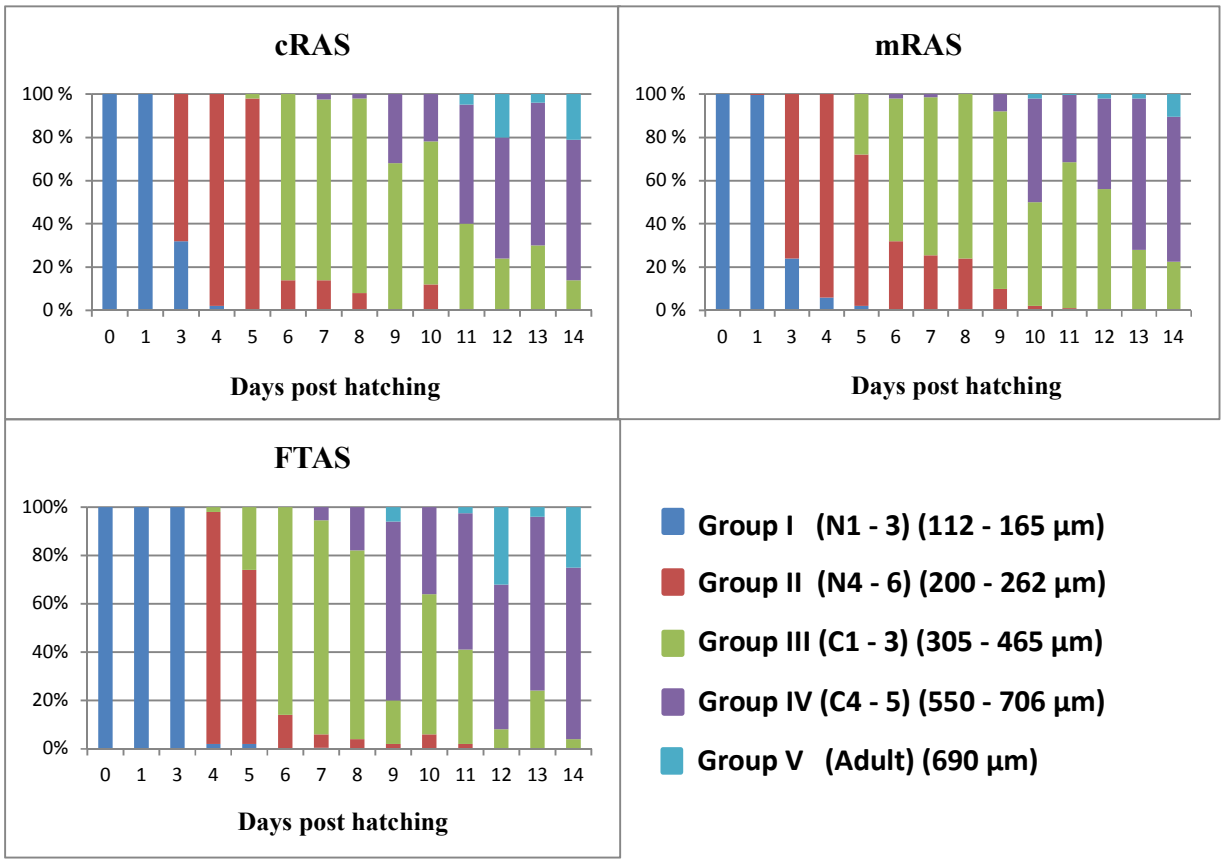

Figure 3.11. *Fractions (%) of developmental stages based on lengths of the copepod A. tonsa (n = 4) through the duration of growth experiment in the cRAS, mRAS and FTAS. The data of 2 dph is not represented.*

3.3 Egg production

The female fraction of total surviving copepods after incubation in the FTAS was significantly higher 2 – 4 times than cRAS and mRAS, respectively (Table 3.3). While no significant difference in the male fraction of total surviving copepods was found between the systems. The mean egg production (eggs female^{-1} day^{-1}) of copepods in the end of experiment was significantly higher in the FTAS (25.1 eggs female^{-1} day^{-1}), compared to the other treatments (9.8 – 11.4 eggs female^{-1} day^{-1}).

Table 3.3. *The number of incubated copepods, survival rate, sex ratio and egg production (mean ± SE) (n = 12) of the copepod A. tonsa in the cultivation systems of cRAS, mRAS and FTAS were recorded during the egg production experiment. Different letters mark significant differences between the treatments, p < 0.05.*

Number of incubated copepods	15.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0
% female of survival copepods	21.1 ± 0.9[a]	12.8 ± 0.3[a]	47.2 ± 0.8[b]
% male of survival copepods	0.0	0.0	3,9 ± 0.2
Egg production (eggs female^{-1} day^{-1})	9.8 ± 1.5[a]	11.4 ± 2.6[a]	25.1 ± 2.1[b]

The mean egg productions in the cRAS and mRAS were similar, about 10 eggs female^{-1} day^{-1}; whereas number in the FTAS was much higher than both of mRAS and cRAS during the experiment, the highest values were 72 % and 75 % at 13 dph, respectively (Figure 3.12).

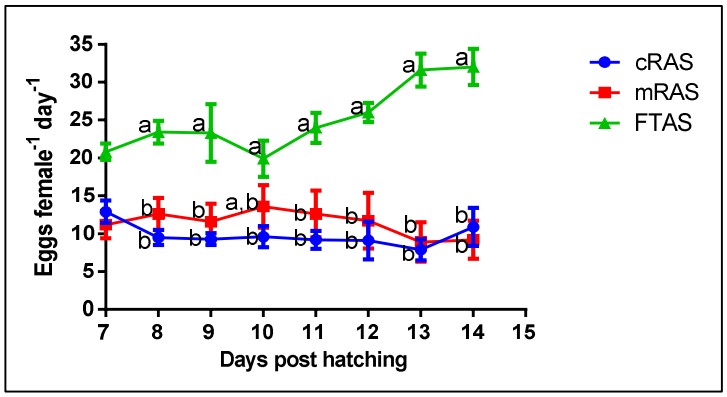

Figure 3.12. *Egg production (mean ± SE) (n = 12) of the copepod A. tonsa cultured in the cRAS, mRAS and FTAS during the experiment. Different letters mark significant differences between the treatments, p < 0.05.*

3.4 Trans-membrane pressure (TMP)

The performance of membrane filtration was monitored through the TMP during the experiment of copepod growth performance (Figure 3.13). The measured TMP value was below 0.1 bar during the experiment.

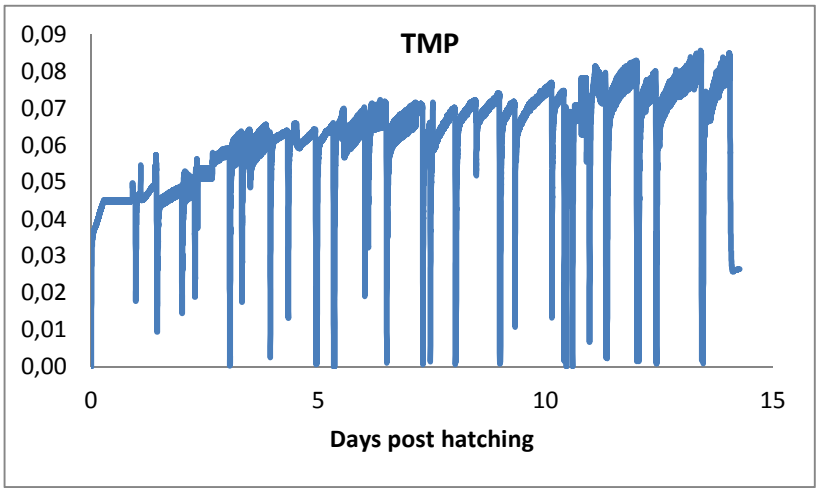

Figure 3.13. *Trans-membrane pressure (TMP) during the experiment of copepod growth performance.*

3.5 Particle size distribution and copepod performance

The correlations between the concentrations of particles (colloidal and fine solids) with the copepod growth performances (DWI and survival rate) and the nitrogenous compounds (TAN and NH_3-N) in all systems were tested by regression analysis. The survival rate, DWI and particles were not measured at the same day, so the interpolation values between sample points in some cases was conducted to run regression analysis. Only correlations between colloidal particles with survival rate and nitrogenous compounds in the cRAS were found ($p < 0.05$) (Figure 3.13 and 3.14).

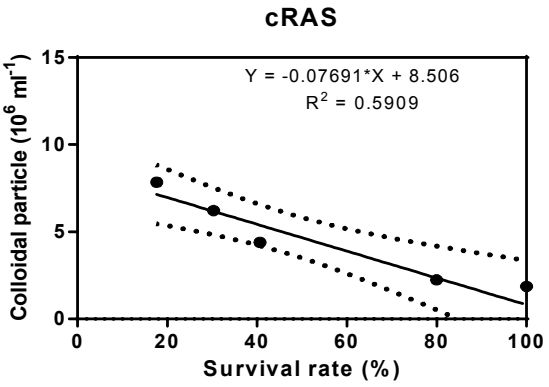

Figure 3.13. *Correlation between the collodial particles (30 nm -1 µm) and survival rate (%) of the copepods in the cRAS during the experiment. Each point in the graph presents a mean concentration of colloidal particles at a sampling day, $p < 0.05$.*

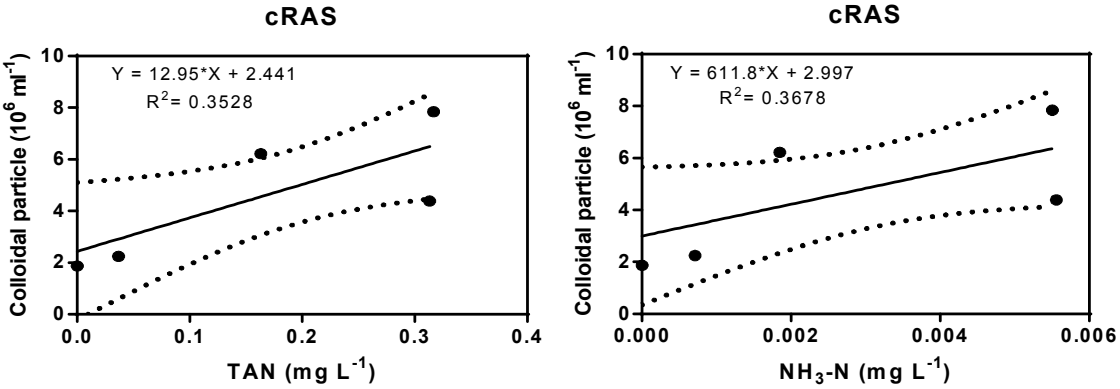

Figure 3.14. *Correlations between the collodial particles (30 nm-1 µm) with the TAN (mg L^{-1}) and NH_3-N (mg L^{-1}) of the copepods in the cRAS during the experiment. Each point in the graph presents a mean concentration of colloidal particles at a sampling day, $p < 0.05$.*

To test whether the water quality (temperature and salinity) affected to the copepod performance, the correlations between them were investigated. Only the DWI of copepod and cultivation temperature in the mRAS was correlated ($p < 0.05$) (Figure 3.15).

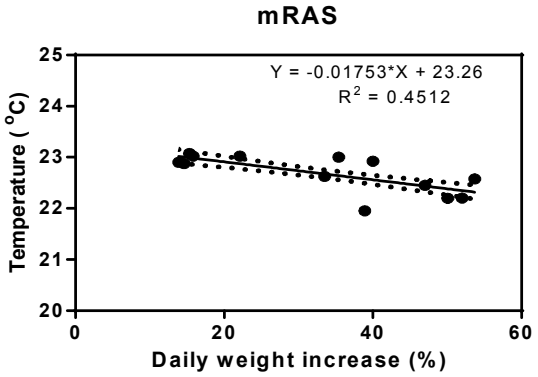

Figure 3.15. *Correlation between the cultivation temperature and daily weight increase (% DWI) of the copepods in the mRAS during the experiment. Each point in the graph presents a mean cultivation temperature at a sampling day, $p < 0.05$.*

4 Discussion

4.1 Physiochemical water quality parameters

Throughout the experiment, the RAS systems (cRAS and mRAS) always showed higher mean levels of temperature, salinity, nitrogenous waste products and particles than the FTAS. Only minor variations in parameters of oxygen concentration and pH between systems were observed (Table 3.1). The working of pumps in the RAS systems, which created heat in the systems, might have caused the difference of average temperature in cultivation tanks between the systems. In addition, the application of aeration in the biofilters led to high evaporation rate of water, resulting in higher mean salinity levels in both RAS systems than in FTAS.

The developments of TAN and NH_3-N in the inlet water of the mRAS and FTAS were similar, while in cRAS it fluctuated highly. The average concentrations of ammonia in the inlet water of mRAS, FTAS and permeate remained low - below 0.03mg L^{-1} (TAN), 0.001 mg L^{-1} (NH_3-N) and showed similar stable developments. This indicated that ammonia removal in the biofilter of mRAS operated more satisfactory during the experiment, compared to cRAS (0.1 mg L^{-1} for TAN and approximately 0.02 mg L^{-1} for NH_3-N). The accumulations of NO_2-N and NO_3-N in inlet water were observed in the RAS systems and in permeate, but not for FTAS during the experiment. The concentrations of TAN and NH_3-N in the permeate and mRAS were of similar low levels during the experiment, this could be explained as that the membrane unit reduced the amount of particle material which affected the nitrification rate in the biofilters and the development of biofilm on the surface of membrane (Pasmore, 2001; Ivnitsky, 2007) which would have some conversions of TAN into NO_2-N. However, the conversion of NO_2-N to NO_3-N in the membrane unit might not be carried out due to the short duration of experiment, which was probably not sufficient time for development of autotrophic bacteria in the membrane unit.

This led to the higher accumulation of NO_3-N in permeate than in the mRAS. Furthermore, the water in the mRAS flowed through the tank column of CO_2 degasser, where a biofilm of bacteria could develop on the surface of the packing material and thus contribute to the conversion of ammonia, while the permeate was just taken from the first biofilter, where heterotrophic bacteria dominated.

The manual feeding, low water exchange rate and the loss of water in the biofilter due to high frequent clogging of metal outlet sieve in the cultivation tanks in some of first 7 days of experiment may have resulted in an unstable operation of the biofilter in the cRAS. That probably led to the peak in the concentrations of TAN and NH_3-N at 7 dph. Furthermore, nitrification kinetic is affected by organic carbon (Chen et al., 2006; Guerdat et al., 2011; Zhu and Chen, 2001) and suspended solids, which contain both inorganic and organic material (Timmons and Ebeling, 2007). The colloidal particle numbers in the cRAS tanks were correlated to the developments of TAN and NH_3-N concentrations (Figure 3.14). This explained the peaks in the TAN and NH_3-N concentrations in the experiment of the cRAS tanks at 14 dph. At the end of the experiment, the average TAN levels in the cultivation tanks of three systems (0.10 - 0.16 mg L^{-1}) were lower than in the study of Skogstad (2010) (0.56 - 1.89 mg L^{-1}), but were higher than in the study of Minh (2011) (0.04 - 0.10 mg L^{-1}) with the same species and strain. However, the copepod density in the current study was much higher than those previous studies, 321 to 647 ind L^{-1} cultivated in FTAS (Skogstad, 2010) or 20000 ind L^{-1} cultivated in FTAS and cRAS (Minh, 2011). Moreover, the lethal concentrations (48h LC_{50}) of NH_3-N for *A. tonsa* nauplii were estimated in previous studies to be 0.18 - 0.22 mg L^{-1}(Sullivan and Ritacco, 1985) or 0.97 - 1.26 mg L^{-1} (48h LC_{50}) and 0.22 - 0.77 mg L^{-1} (72h LC_{50}) for both nauplii and adult *A. tonsa* (Jepsen et al., 2013). In the current study, the observed NH_3-N concentrations in all

systems for both of cultivation tank and inlet water below 0.006 mg L^{-1}, and should be in the safe range for species.

The concentrations of NO_2-N and NO_3-N in the cultivation tanks were significantly higher in the RAS systems than in the FTAS, which was more stable by continuous exchange of cultivation water. Effect of the nitrification process is expressed by the concentrations of NO_2-N and NO_3-N in the cultivation systems during the experiment. The accumulations of NO_2-N in the RAS systems showed a slower reaction to convert NO_2-N to NO_3-N, as is generally seen in a marine biofilter (Manthe and Malone, 1987). The present of nitrite oxidation step was recorded in the biofilter, but the accumulation of NO_3-N was still observed. Furthermore, another contribution to the concentration of NO_3-N was the unassimilated nitrate from the algal growth medium (Conwy medium) (Minh, 2011). In the current study, the average concentrations of NO_3-N in all systems were only about 4 mg L^{-1} for 2 weeks. This level was similar to one month study of Minh (2011) with the same strain and species but with lower copepod density. The considered safe of NO_2-N is less than 1.0 mg L^{-1} for most of marine fish cultivation, especially less than 0.01 mg L^{-1} for salmonids (Lucas and Southgate, 2003). Whereas the expected tolerance level of NO_3-N is below 50 mg L^{-1} for most of fish and is high as 400 mg L^{-1} in a RAS for cultivation of Tilapia (mainly *Tilapia mossambica*) and European eel (*Anguilla Anguilla*) (Otte and Rosenthal, 1979). Furthermore, the marine tropical aquarium fish such as anemonefish (*Amphiprionocellaris*) is also very sensitive to NO_3-N concentration with limit of 20 mg L^{-1} (Frakes and Hoff Jr, 1982).

Overall, a better physiochemical water quality in range of measured parameters in the mRAS compared to the cRAS was observed in both cultivation tanks and biofilters. However, the measured water quality parameters in the FTAS were better than the RAS systems. The

measured levels of TAN and NH_3-N in the permeate also demonstrated the positive effects of BF-MBR in the process of water treatment compared to the inlet water of RAS systems.

4.2 Characteristics of particles

The category of microfiltration (pore size 0.1 - 10 μm) in membrane filtration has been demonstrated as an effective approach to enhanced removal of microorganisms, colloidal particles and other particulate matter (Van Der Bruggen et al., 2003). In the current study, the application of an ultrafiltration (UF) membrane with even much smaller pore size (50 nm) was used in order to remove colloidal particles and fine solids more efficiently. The improvement of particle removal in this study was documented as a mean reduction at the end of experiment in turbidity of the inlet water in the mRAS (39 %), permeate (80 %) and FTAS (86 %) compared to cRAS. Furthermore, level of colloidal particle was lower in mRAS (48 %), permeate (91 %) and FTAS (92 %) compared to cRAS at 15 dph. The rapid increases in the colloidal particles of cRAS and fine solids of mRAS in the inlet water at 5 dph were sampling errors; only one water sample of each system was measured. As un-expected result of fine solids, the number of fine solids in permeate was not significant difference to those in the RAS systems and FTAS during the experiment; this could be caused by the particles from the development of biofilms on the permeate side of membrane. However, no available data from literature to evaluate the concentration of fine solid in the permeate after treating through the UF membrane was found.

The observed levels of turbidity and colloidal particles in the cultivation tanks of the RAS systems in the current study were not significant difference, consistent with data from Holan et al (2013) working with the lower density (9 ind ml^{-1}) of the same strain and species of copepod. This might be explained by the low water exchange rate during the first 7 days of experiment.

The colloidal particles of mRAS tanks showed a steady reduction after increasing the water exchange rate (to 8 times daily volume tank) from 8 dph, while the particle number in the cRAS and FTAS kept increasing stably. The highest reduction of 50 % in the number of colloidal particles in the mRAS tanks compared to the cRAS was observed at 13 dph. Furthermore, a mean reduction of turbidity in the mRAS tanks was 26 % compared to cRAS after 8 dph. However, the concentrations of fine solids were obtained similarly in cultivation tanks of all systems during the experiment.

The current experiment showed an improved water quality with respects of turbidity and colloidal particle in the cultivation tanks of mRAS compared to cRAS. Furthermore, the membrane unit also showed good capacity to produce a clean permeate, which was approximate to the water quality of FTAS tanks in this study.

4.3 Performance of copepod *A. tonsa*

4.3.1 Density and survival

Even though a better water quality in the cultivation tanks of mRAS than cRAS was observed during the experiment, the survival rate of cultivated copepods in the mRAS was lower than cRAS (42 %) and FTAS (77 %) at the end of experiment. High concentration of suspended solids regarding turbidity (6 – 38 NTU) levels has a significant effect on both feeding and mortality rate of copepod *Acartiella natalensis* (Carrasco et al., 2013). The aspect of turbidity levels should not have caused any negative impacts on the mortality rates in the current study as it was lower than 2 NTU in all tanks during the experiment.

The low survival rates of copepod in the RAS systems during the experiment was not readily apparent based on particle analysis; only the survival rate of copepod and colloidal particles in

the cRAS tanks was correlated (Figure 3.13). Whereas the concentration of colloidal particles decreased steadily in the mRAS and increased stably in the cRAS and FTAS, the survival rate of copepods in the RAS systems declined similarly and remained stable in the FTAS after 8 dph. High concentration of colloidal particles (50 nm polystyrene beads in concentration of 1.14×10^{12} ml^{-1}) is an indication for the mortality of copepod *Tigriopus japonicas* (Lee et al., 2013). Moreover, the colloidal particles concentration (10^8 ml^{-1}) had not negative effect to survival rate of *A. tonsa* in the study done by Holan et al (2013) with lower cultivated copepod density (9 ind ml^{-1}). The highest concentration of colloidal particles (45×10^6 ml^{-1}) in the cRAS tanks of this study will thus not probably affect to the survival and mortality rate of *A. tonsa* within the observed size interval in the present study. The developments of fine solid concentrations in the cultivation tanks of all systems were similar during the experiment. It also may not have had any negative impacts on the survival and mortality rate of copepods as reported in the study of the copepod *Tigriopus japonicas* (Lee et al., 2013) with the similar fine solid concentration (2.1×10^5 ml^{-1}). In the experiment of microbial impacts on the nauplii and egg production of copepod *A. tonsa* that was done by my colleague, (Anh, 2014) in the same study, a lower mean fraction of opportunistic bacteria (%) in the total amount of bacteria of FTAS (< 50 %) compared to the RAS systems (> 50 %) was observed during the experiment. With the dominance of opportunistic bacteria in the RAS tanks, it probably resulted to the lower 2 - 4 times survival rate of the copepods compared to the FTAS at the end of experiment.

The highest cultivated copepod density were previously observed in the range of 15000 - 33000 ind L^{-1} for nauplii *A. tonsa* in cRAS and FTAS systems, with 50 L tanks during 10 days of experiment (Minh, 2011), and 1000 - 30000 ind L^{-1} for *Parvocalanus crassirostris* and *Bestiolina similis*, the species are smaller than *A. tonsa* (Ajiboye et al., 2011; McKinnon et al.,

2003; Shields et al., 2005). The daily mortality rate in these studies was in range of 3.9 – 10 % (Drillet et al., 2008; Drillet et al., 2006; Medina et al., 2004; Støttrup et al., 1986). In the present study, the cultivated copepod density was approximately 75000 ind L^{-1}, which was 2 - 75 times higher and daily mortality rates were higher than reported in the previous studies, FTAS (6.9 ± 1.7 %), cRAS (14.6 ± 2.8 %) and mRAS (19.3 ± 3.5 %) in 2 weeks of experiment. There was a significant difference of NO_2-N concentrations between the systems but no information was found in the literature describing its effects on the development and survival rate of *A. tonsa*.

4.3.2 Growth and developmental time of copepods

Available food in the copepod cultivation tanks has been reported as strongly influence on the growth of *A. tonsa*, the optimal growth of this copepod requires a minimum food concentration of about 10^4 cells mL^{-1} or 370 µg C L^{-1} equivalently (Skogstad, 2010) and approximately 500 µg C L^{-1}(Berggren et al., 1988). Even though the manual feeding was carried out some days in the beginning of experiment due to mechanical problems, the amount of microalgae was ensured to reach the concentration for optimum growth of copepods (10^4 cells mL^{-1}) by measuring the microalgae twice a day (7 am and 10 pm). The copepods in the cultivation tanks was fed approximately 105 ml of microalgae (2.3 - 3.1x 10^6 cells ml^{-1}) each 10 minutes during the day by feeding robot. The mean weight/length of individual copepods in the cRAS was significantly lower than in the mRAS and FTAS at 1 dph, which could be explained by the uneven distribution in size of copepod nauplii at the beginning. Even though the copepods in the different systems were fed the same amount of microalgae, the ratio of fed microalgae per copepod in the mRAS was higher than FTAS. However the mean weight/length of individual copepods in the FTAS was significantly higher than in the mRAS at 7 dph and 14dph.

The DWI (%) during the nauplii stage was higher than in the copepodite stage of all systems, consistent with the reports from previous studies (Leandro et al., 2006; Minh, 2011; Skogstad, 2010) with the same strain and species. Significant differences in DWI between cRAS and mRAS were found only during the copepodite stage, even though the amounts of supplied food and copepod densities in both systems were similar during the experiment. The DWI of nauplii (0.3 - 0.34 μg day^{-1}) and copepodite stages (0.11 – 0.19 μg day^{-1}) in the current experiment were lower than results from previous studies with unsaturated and saturated food levels both of nauplii (0.39 - 0.42 μg day^{-1} (Minh, 2011) or 0.55 μg day^{-1} (Skogstad, 2010)) and copepodite stages (0.21 - 0.23 μg day^{-1} (Minh, 2011) or 0.33 μg day^{-1} (Skogstad, 2010)). Moreover, the highest growth rate of copepod *A. tonsa* was reported as 0.45 μg day^{-1} in the surplus microalgae concentration (Berggren et al., 1988). Temperature and salinity can have negative impacts on growth rates of copepod *Acartia sinjiensis* (Milione and Zeng, 2008), *Pseudocalanus newmani* (Lee et al., 2003) and *A. tonsa* (Leandro et al., 2006). Furthermore, the DWI of copepods and cultivation temperature in the mRAS in the current study were positively correlated (Figure 3.15).

The time to reach 50 % of copepodite stage in the experiment with similar systems of cRAS and FTAS at temperature of 18 - 20 ^{o}C was 6 - 7 dph (Minh, 2011; Skogstad, 2010), which was slower than development rate of copepods in the current study, 70 % (mRAS) and 90 % (cRAS and FTAS) at 6 dph at an average temperature of 22 ^{o}C. Temperature has a significant effect on growth rate of copepod *A. tonsa*, increasing incubation temperature will decrease the development time of *A. tonsa* (Leandro et al., 2006). Even though the appearance of adult copepod in the mRAS was sooner one day than cRAS and later one day than FTAS; the fraction of copepodite stage in the mRAS was higher than in the cRAS and FTAS, which probably

demonstrated a delayed moulting into the adult stage of copepods in mRAS. The significant difference in the fraction of opportunistic bacteria at 6 dph in the mRAS (86 %), compared to the cRAS (51 %) and FTAS (30 %) may have negative effects to the development of the copepods (Anh, 2014).

4.3.3 Egg production

The egg production (EP - eggs female^{-1} day^{-1}) in the copepod *A. tonsa* is high and stable during 2 - 3 weeks after maturation and then decreased in EP rate, according to (Jónasdóttir and Kiørboe, 1996; Parrish and Wilson, 1978).The EP rate in the current study of FTAS (25.1 ± 2.1 eggs female^{-1} day^{-1}) was significantly higher than in the cRAS (9.8 ± 1.5 eggs female^{-1} day^{-1}) and mRAS (11.4 ± 2.6 eggs female^{-1} day^{-1}). Differences in the cultivation conditions could be an indication to reflect the considerable ranges in EP rate of *A. tonsa* between the systems. Several of described factors affecting the EP of copepods were temperature, salinity, photo-period, adult stocking density (Peck et al., 2008; Peck and Holste, 2006), food concentration (Støttrup and Jensen, 1990) and oxygen level (Marcus et al., 2004; Sedlacek and Marcus, 2005). With the exception of temperature and salinity, other factors in the current study were controlled mostly similar for all systems, thus differences in temperature and salinity between systems could influent to the EP capacity of *A. tonsa*.

There were significant differences in concentrations of NH_3-N and NO_2-N between the systems but no information was found in the literature describing the effects of NO_2-N to the development and EP of *A. tonsa*. In a study with NH_3-N concentrations, (Buttino, 1994) found that after nine days of exposure the copepod *A. tonsa* for 0.12 mg NH_3-N L^{-1}, an increase in the EP rate of female copepod in this study was observed. The observed concentrations of NH_3-N in

all systems in the present study was below 0.006 mg L^{-1}, thus the NH_3-N concentration have not caused any difference in the EP rate between the systems.

There was a significant difference in the colloidal particles number of the FTAS compared to the RAS systems during the experiment, consistent with the difference in the EP rate between the systems (Figure 3.12). The mean concentration of colloidal particles in the FTAS showed a 3 - 4 folds decrease compared to the RAS systems, leading to a mean increase by up to 60 % of the EP rate in the FTAS. No difference in the fine solids number between the systems was found during the experiment. It is likely that the high concentration of colloidal particles in the RAS systems compared to the FTAS during the experiment may have a negative effect to the EP rate of copepod *A. tonsa*. Furthermore, the dominance of opportunistic bacteria fraction (> 50 %) in the RAS systems compared to the FTAS (< 50%) throughout the experiment also may have some effects to the EP capacity (Anh, 2014).

In the present study, the EP rate just achieved approximately 25 % for the RAS systems and 50 % for the FTAS, compared to the potential EP rate (40 - 50 eggs $female^{-1}$ day^{-1}) for *A. tonsa* in a small scale experiment (Drillet et al., 2008; Kiørboe et al., 1985; Peck and Holste, 2006). A wide range in the EP rate capacity of *A. tonsa* has been reflecting the differences in the cultivation conditions such as temperature, salinity, food concentration and photo-period. The previous studies about the EP of *A. tonsa* at 18 ^oC were also 33 - 39 eggs $female^{-1}day^{-1}$ (Skogstad, 2010) or the observed EP rates at 20 ^oC were 25 - 33 eggs $female^{-1}day^{-1}$ (Castro-Longoria, 2003; Holste and Peck, 2006; Minh, 2011).

4.4 Trans-membrane pressure (TMP)

The potential benefits of mRAS for the advanced treatment of recycle stream were readily apparent through the observed results of water quality in the current study. A major obstacle in the membrane filtration unit is the fouling behavior and control. The measured TMP value (Figure 3.13) was just below 0.1 bar during the experiment, indicating a good performance of membrane filtration and no need for a chemical cleaning. The observed results of TMP from this study could demonstrate for the feasibility and practicality of integration BF-MBR into a RAS.

5. Conclusion and future perspective

A better water quality regarding nitrogenous waste products and colloidal particles concentration in the FTAS compared to the RAS systems was observed during the experiment. The survival rate, nauplii and egg production of the copepods in the FTAS were significantly higher than the RAS systems ($p < 0.05$); while in the mRAS they were not significant difference to the cRAS ($p > 0.05$). The hypothesis " Efficient removal of colloidal particles and fine solids leads to improve the performance of *A. tonsa* in terms of survival, growth and development, and to increase the nauplii and egg production in the mRAS, compared to the cRAS and FTAS" could not be verified based on the data in the present study. The accumulation of NO_2-N in the production systems might have some effects on the growth performance of the *A. tonsa*. Temperature, salinity and high concentrations of colloidal particles in the RAS systems could be the indications for the difference of EP compared to the FTAS ($p < 0.05$). However, the concentrations of fine solids (2 µm – 60 µm) in all systems did not have any effects on the growth performances and EP capacity of the *A. tonsa*. An efficient removal of colloidal particles (30 nm - 1 µm) in the mRAS compared to the cRAS and FTAS was only observed after increasing the water exchange rate to 8 times per day from 8 dph.

For similar experiments in the future, it is important to set up the rate of recycling water in the cultivation tanks so that the MBR can operate well at the beginning of the experiment. Setting up a component of denitrifying can be an option to decline accumulation of NO_2-N in production system. An experiment to find the lethal concentration or stress of NO_2-N to copepod *A. tonsa* should be carried out. Furthermore, an experiment of hatching success of produced eggs should be investigated for further know impacts of particle characteristics on EP of copepods.

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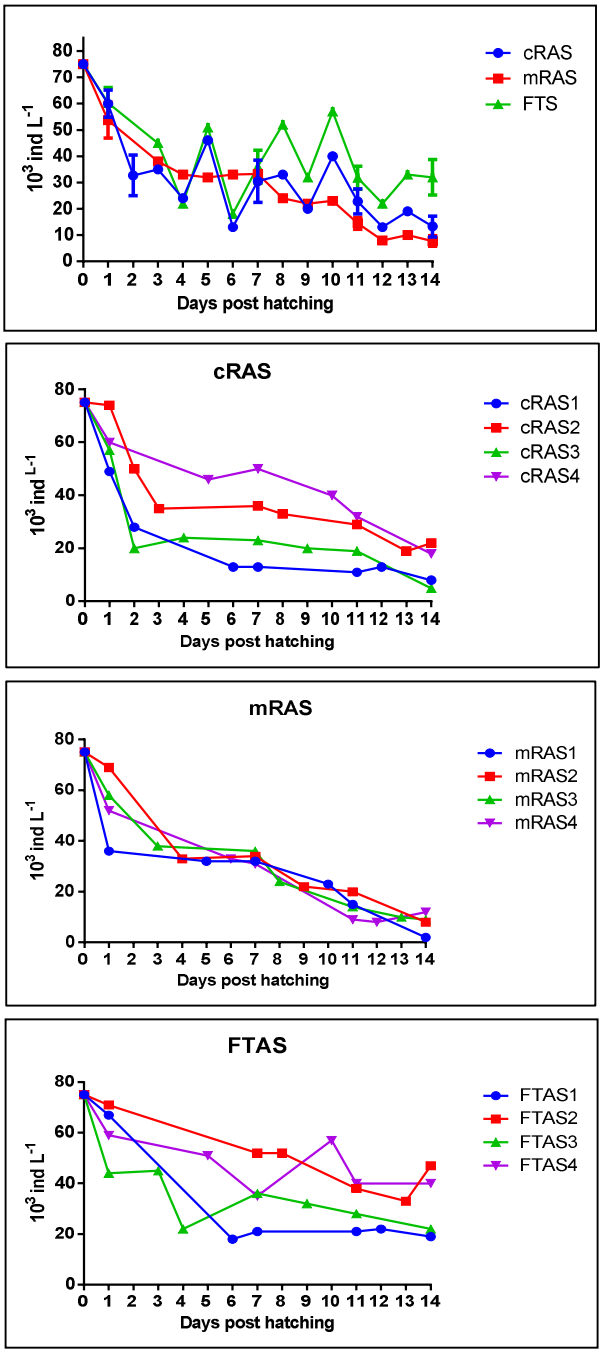
Appendix

Appendix 1. *Densities of copepods (mean ± SE) (n = 4) in the cultivation tanks of cRAS, mRAS and FTAS during the growth experiment.*



Appendix 2. *Length (µm) and calculated carbon biomass weight (µg C ind^{-1}) (mean ± SE) (n = 4) of the copepod A. tonsa from the cRAS, mRAS and FTAS during the period of growth experiment.*

Exp	Length (µm)			Weight (µg C ind^{-1})		
day	cRAS	mRAS	FTAS	cRAS	mRAS	FTAS
0	130.2 ± 1.58	130.2 ± 1.58	130.2 ± 1.58	0.03 ± 0.001	0.03 ± 0.001	0.03 ± 0.001
1	131.6 ± 1.47	142.3 ± 1.7	144.9 ± 1.58	0.034 ± 0.001	0.044 ± 0.002	0.046 ± 0.002
3	181.4 ± 2.54	190 ± 2.54	142.8 ± 2.1	0.099 ± 0.005	0.115 ± 0.005	0.045 ± 0.002
4	217.8 ± 2.82	217.4 ± 3.66	225.8 ± 2.64	0.18 ± 0.007	0.182 ± 0.01	0.201 ± 0.007
5	230.4 ± 2.44	256.4 ± 7.62	255.4 ± 6.83	0.214 ± 0.007	0.266 ± 0.014	0.289 ± 0.02
6	350.4 ± 9.21	300.4 ± 8.84	361.2 ± 9.9	0.368 ± 0.018	0.341± 0.017	0.398 ± 0.02
7	355.8 ± 9.51	334.6 ± 10.91	408.2 ± 9.86	0.412 ± 0.021	0.367 ± 0.023	0.522 ± 0.029
8	385 ± 10.62	350.8 ± 11.65	440.4 ± 11.19	0.451 ± 0.027	0.388 ± 0.025	0.646 ± 0.04
9	481 ± 10.8	422.6 ± 13.01	570 ± 14.28	0.812 ± 0.048	0.601 ± 0.043	1.345 ± 0.089
10	431 ± 14.37	526.8 ± 12.76	489.2 ± 14.23	0.832 ± 0.084	1.063 ± 0.077	0.885 ± 0.062
11	550.9 ± 13.44	487.7 ± 12.12	537.9 ± 14.1	1.232 ± 0.088	0.855 ± 0.06.	1.162 ± 0.077
12	615 ± 16.75	515.6 ± 13.63	684.4 ± 15.42	1.707± 0.141	1.015 ± 0.085	2.255 ± 0.135
13	575.4 ± 14.25	554.4 ± 12.84	594.8 ± 12.77	1.379 ± 0.096	1.225 ± 0.081	1.491 ± 0.094
14	631.4 ± 15.17	558.5 ± 13.03	668 ± 12.43	1.82 ± 0.127	1.466 ± 0.094	2.078 ± 0.109