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Characterization of Nitrogen Dynamics in Different Recirculation Systems

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Marine Coastal Development
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Abstract

The need to reduce freshwater consumption in global food production has long been recognized. Food production in single- and multi-species recirculation systems offers advantages in terms of reduced water and energy consumption, in addition to improved opportunities for waste management and nutrient recycling. In aquaculture, land-based recirculation systems treat the water for waste products, and some is released as wastewater. The wastewater contains a great amount of nitrogen (N), and represents an unutilized resource with potential for further use as fertilizer in plants and microalgae production. Nitrogen is a fundamental element in both animal and plant cells. It is a key component in protein-rich fish feed, it is the nutrient required in largest amounts by plants, and a main element in microalgae fertilizers.

This thesis describes nitrogen dynamics in different bio-producing recirculation systems: A recirculating aquaculture system (RAS) producing Atlantic salmon (*Salmo salar*), a hydroponic system producing strawberries (*Fragaria x ananassa "Elan"*), an aquaponic system with integrated trout (*Oncorhynchus mykiss* and *Salmo trutta*) and lettuce (*Lactuca sativa*) production, and a pilot system with combined copepod (*Acartia tonsa*) and microalgae (*Rhodomonas baltica*) production (Copeponics). Possibilities for increased system performances, in terms of sustainable use of water and nitrogen, were investigated.

Implementation of integrated or end of pipe bio-producing treatments is suggested as a sustainable wastewater treatment strategy in the RAS. An ongoing and unidentified in-tank removal of ca. 24.0% NO_3^--N was discovered. Phototrophic nitrate removal by microalgae cells and other microorganisms in biofilm is expected. In the Hydroponic system, elevated NO_3^- concentrations (429-566 mg L^{-1}) to commercial hydroponic nutrient solutions (49-210 mg L^{-1}) were detected. *L. sativa* in the Aquaponic system removed dissolved nitrogen inefficiently: ca. 26% removal of TAN and only ca. 5% removal of NO_3^--N. Copeponics showed very good copepod egg production and normal microalgae production. Compared with production in standard flow through systems, internal recirculation reduced the total water consumption (10% day^{-1}), and the lowered energy and microalgae need. Microalgae was cultivated with copepod wastewater, and additional fertilizer use in the microalgae production was on average reduced with ca. 42% compared to standard production. The recirculation system allowed for storing of the microalgae cells in the system, and microalgae consumption in the copepod production in Copeponics was on reduced with ca. 15% compared with standard flow through production.

Sammendrag

Behovet for å redusere vannforbruket i globalt matproduksjon er anerkjent. Matproduksjon i resirkuleringssystemer med en eller flere arter innehar fordeler som redusert vann- og energiforbruk, i tillegg til gode muligheter for avfallshåndtering og resirkulering av næringsstoffer. Resirkuleringsanlegg i landbasert akvakultur må behandle vannet for å fjerne avfallsstoffer, og noe deponeres som avløpsvann. En stor andel av avfallsstoffene inneholder nitrogen (N), og representerer en ressurs som potensielt kan brukes som gjødsel i produksjon av planter og mikroalger. Nitrogen er et livsviktig grunnstoff for både plante- og dyreceller. Det er en hovedkomponent i proteinrikt fiskefôr, det grunnstoffet som kreves i største mengder av planter, og et hovedelement i gjødsel til mikroalger.

Denne avhandlingen beskriver nitrogendynamikk i ulike resirkuleringsanlegg med bioproduksjon: Et resirkulerende akvakultursystem (RAS) med produksjon av Atlantisk laks (*Salmo salar*), et hydroponics-system med produksjon av jordbær (*Fragaria x ananassa "Elan"*), et aquaponics-system med integrert produksjon av ørret (*Oncorhynchus mykiss* og *Salmo trutta*) og salat (*Lactuca sativa*), og et pilotsystem med kombinert produksjon av copepoder (*Acartia tonsa*) og mikroalger (*Rhodomonas baltica*) (Copeponics). Systemenes potensiale for en bærekraftig håndtering av vann og nitrogen ble utforsket.

Innstallering av integrerte eller separerte bioproduserende vannbehandlingsmetoder er foreslått som et bærekraftig tiltak i RAS. En pågående og uidentifisert fjerning av ca. 24% NO_3^--N fra tankvannet ble avdekket. Fototrofisk nitratfjerning av etablerte mikroalger og andre mikroorganismer i RAS er forventet årsak. Høye konsentrasjoner av NO_3^- (429-566 mg L^{-1}) sammenlignet med konsentrasjoner i kommersielle næringsløsninger (49-210 mg L^{-1}) ble målt i Hydroponics. I Aquaponics viste *L. Sativa* seg å være ineffektiv i fjerning av oppløst nitrogen fra vannet: ca. 26% fjerning av TAN og ca. 5% fjerning av NO_3^-. Copeponics tilrettela for god produksjon av copepodeegg og normal produksjon av mikroalger. Sammenlignet med standard produksjon i gjennomstrømningsanlegg tilrettela Copeponics som resirkuleringssystem for redusert totalt vannforbruk (10% day^{-1}), lavere behov for mikroalger og energi, og gjødselforbruket ble i gjennomsnitt ca. 42% lavere sammenlignet med normal mikroalgeproduksjon. Resirkuleringssystemet tillot også en forlenget oppholdstid for mikroalgecellene i copepodetanken. Dette reduserte forbruket av mikroalger som levendefôr til copepoder med ca. 15%, sammenlignet med standard produksjon i gjennomstrømmingsanlegg.

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1 Introduction

The aquaculture production of Atlantic salmon (*Salmo salar*) fry and smolts constitutes the greatest fraction of the total land based aquaculture production in Norway (Hess-Erga et al., 2013). Production of salmon smolts in freshwater flow-through systems is the most common practice in today (Terjesen et al., 2013). Integrated water treatment strategies that lowers water consumption in flow-through systems has been predicted necessary for an up-scaled smolt production (Kittelsen et al., 2006).

The interest towards producing smolts in a recirculation aquaculture system (RAS) is increasing in Norway (Terjesen et al., 2013). Regardless of the degree of water recirculation, an integrated wastewater treatment is essential, but this is costly (Blancheton, 2000). Phytoremediation of aquaculture wastewater in hydroponics (Adler et al., 1996, Ghaly et al., 2005) and integration of organisms that feed on fish waste (Blancheton, 2000, Wang, 2003) have been suggested as sustainable and profitable alternative to technical wastewater strategies in RAS today. Wastewater treatment by microalgae has also proven to be an efficient wastewater strategy (Arbib et al., 2012, Di Termini et al., 2011). As the interest towards RAS production is growing in Norway (Terjesen et al., 2013), and an increasing number of RAS installations observed in Europe (Blancheton, 2000), a potential for re-use of effluent wastewater from RAS in integrated production should be investigated to improve sustainability in aquaculture.

Nitrogen (N) is an important compound in all the recirculation systems studied in this thesis. Aquaculture species, such as fish and copepods, will produce nitrogenous waste products from protein degradation. Hargreaves (1998) indicated only 25% recovery of feed-N in cultivated animals in aquaculture, leading to a 75% discharge of feed-N to the water and sludge. If accumulated, nitrogenous compounds (e.g. ammonia and nitrite) will be toxic to the reared animals in aquaculture (Jensen, 1995, Jepsen et al., 2015, Fivelstad et al., 1993). To sustain low concentrations of dissolved inorganic nitrogen (DIN) and sufficient water quality, plants or algae can serve a water treatment function. N is the nutrient required in largest amounts by plants (Marschner, 2011), and is normally supplied through fertilizers as nitrate (NO_3^-) and/or ammonia (NH_4^+) (Marschner, 2011). Despite low volumes, wastewater is discharged from RAS installations every day. Instead of discharging nutrient rich wastewater

to the surrounding ecosystem with possibility of eutrophication, produced plants and algae have the potential to utilize NO_3^- and NH_4^+ from wastewater in a more sustainable way (Tyson et al., 2011). However, proper nutrient balance is a challenge in integrated production systems (Tyson et al., 2011). Increased understanding on balancing uptake by plants or algae with aquaculture output could contribute to efficient nutrient utilization in integrated multi trophic production systems such as aquaponics.

1.1 Recirculating aquaculture system (RAS)

RAS is an aquaculture system where the outlet water from fish tanks is treated and re-used (Lekang, 2008), and less than 10% of the total water volume is replaced per day (Timmons and Ebeling, 2007). The system typically includes cultivation tanks, a water treatment circuit, and daily replacement of the discharged water (Timmons and Ebeling, 2007). Major advantages of RAS are the need for low intake water volume, hence lowered freshwater consumption (Lekang, 2008). This can lower energy use due to the reduced intake volume of water that needs heating (Lekang, 2008). The system also offers a unique opportunity for control and stability, together with an ability to conserve waste products such as organic material, phosphorous (P) and N products (Timmons and Ebeling, 2007, Van Rijn, 1996). This is normally discharged in a recipient water body or transported for treatment. Further, the biofilter units in RAS offer microbial control, as dominance and selection of slow growing bacteria (K-strategists) can increase water quality and support a healthy rearing environment for the organisms (Blancheton, 2000, Attramadal et al., 2012, Skjermo et al., 1997). Hence, K-strategic bacteria are more beneficial bacterial communities for the reared organism. In order for a biofilter to mature water with K-strategic bacteria, a low and stable concentration of substrate load to the system is required. The opposite can trigger growth of opportunistic and fast growing bacteria (r-strategists) (Vadstein et al., 1993), which can become pathogenic when cultured organisms are stressed (Olafsen, 1993).

The role of nitrogen in RAS

An important challenge in RAS is accumulation of nitrogenous waste products (Lekang, 2008). Protein degradation in the reared organism will lead to nitrogenous waste metabolites, which are excreted and released through urine and excrements, the skin, cation exchange in the gills, and gill diffusion in fish (Smith, 1929, Wood, 1958, Handy and Poxton, 1993). The main quantity of the excreted nitrogen will be ammonia in ionized (NH_4^+-N) or un-ionized

(NH_3-N) forms (Timmons and Ebeling, 2007), and the sum of the two forms is noted as total ammonia nitrogen (TAN). pH, salinity and temperature determines the fraction of TAN present as NH_3-N or NH_4^+-N (Bower and Bidwell, 1978, Timmons and Ebeling, 2007, Trussell, 1972). Unionized NH_3 is toxic to aquatic animals (Brownell, 1980, Rogers and Klemetson, 1985, Wuhrmann et al., 1947, Fivelstad et al., 1993, Knoph, 1992, Kolarevic et al., 2013, Jepsen et al., 2015) and should be kept in low concentrations. The degree of ammonia toxicity differs between animals and species (Wright and Fyhn, 2001, Terjesen et al., 2008). Developmental stage, metabolic activity, and feeding rate also have its say on the poisonousness. Timmons and Ebeling (2007) suggests to keep NH_3-N concentrations in the range 0.05-0.1 mg L^{-1}.

In intensive aquaculture recirculation systems, inefficient oxidation of TAN in biological filters can cause accumulation of NO_2^- (Kroupova et al., 2005). Toxicity of NO_2^- to animals is well documented (Brownell, 1980, Kroupova et al., 2008, Siikavuopio and Sæther, 2006) and high concentrations of the compound should be avoided. NO_2^- can accumulate in animal bodies as it has affinity to Cl^- (Jensen, 1995). Cl^- uptake through the gill epithelium can be shifted towards NO_2^- (Jensen, 1995). This can have an effect on oxygen binding in the blood and further reduce growth and cause physiological disturbances in the animal (Jensen, 1995). Presence of Cl^- in the water can increase nitrite tolerance in fish (Bath and Eddy, 1980), and hence marine fish is more protected from nitrite toxicity than freshwater fish.

The biofilter in RAS plays a crucial role in microbial oxidation of TAN into less toxic nitrate (NO_3^-) (Lekang, 2008). NO_3^- is also excreted from fish, but the source is unknown (Clark et al., 1985). Autotrophic bacteria are responsible for the nitrification process, where NO_2^- is the intermediate product in the formation of NO_3^- (Timmons and Ebeling, 2007). The total nitrification reaction can be presented as followed:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad (1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (2)$$

Ammonia oxidizing bacteria (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrisolobus and Nitrosovibrio*) are responsible for (1), while nitrifying bacteria (*Nitrobacter, Nitrococcus, Nitrospira and Nitrospina*) are responsible for (2) (Haynes, 2012, Zehr and Kudela, 2011).

Feed will affect TAN production in RAS (Terjesen et al., 2013), and generation of waste and production of TAN is related to quality and quantity of feed input. The concentration of TAN will in turn influence NO_3^- production in the biofilter, as presented in equation 1 and 2. The feed that is not accumulated as fish biomass will be released into the rearing environment through metabolic processes (Wood, 1958). Given time, bacteria in RAS will break down feed waste to toxic ammonia and CO_2 (Blancheton et al., 2013). Different models have considered N-retention from feed in fish; Timmons and Ebeling (2007) suggested 42% retention, while Grisdale-Helland and Helland (1997) reported 52.8% retention and 7.7 fecal N-loss in a study with 80 g initial weighted Atlantic salmon. Thus, there is a high waste production in aquaculture and in RAS, largely due to the natural anatomy of fish, where leaves a large amount of feed undigested and excretes it (Amirkolaie, 2005).

Protein is a main component in feed, due to its importance for growth and role as energy source in fish (Hepher, 1988). As presented, the breakdown of protein will lead to ammonia-rich excrement in fish. Terjesen et al. (2013) suggests to use published species and life stage-specific N-retention data when dimensioning for TAN removal in RAS. Uneaten feed will also influence the physical water environment for the rearing organism. Solids from uneaten food, feces, and bioflocs (living and dead bacteria) can accumulate in RAS (Timmons and Ebeling, 2007, Chiam and Sarbatly, 2011), as organic load in bioreactor has proven to lower the nitrification efficiency in bioreactors (Tal et al., 2003).

Dependent on water exchange with new intake water, nitrate has the potential to accumulate in high concentrations (400-500 mg L^{-1}) in RAS (Van Rijn et al., 2006). The most common strategy for NO_3^- removal in RAS is through via dilution (Freitag et al., 2015). A denitrification process, where NO_3^- is converted to nitrogen gas (N_2), can also be used as a NO_3^- removal strategy (Van Rijn et al., 2006). Nitrite or nitrate is reduced to N_2 gas, conducted by facultative anaerobic microorganisms (Van Rijn et al., 2006). Recirculation systems often use heterotrophic de-nitrification using external electron and carbon donors (e.g. CO_2) (Van Rijn et al., 2006). The use of this technology is limited due to high investment costs, required expertise and accumulation of total oxygen demand (TOD) (Martins et al., 2010). Denitrification can be considered sustainable as the make-up water volume necessary for controlling NO_3^- concentrations is reduced (Martins et al., 2010). Still, an even more sustainable approach can be met by utilizing the unwanted nitrate in bio-producing systems (Martins et al., 2010).

Martins et al. (2010) suggest that recycling of nutrients through integrated farming can contribute in improvement of environmental sustainability in RAS. Incorporation of wetlands and algal controlled systems are presented as alternative and sustainable water treatment strategies (Martins et al., 2010), as plants and algae assimilate N and P in ionic forms (Marschner, 2011). Hence, instead of de-nitrification, this can be a more sustainable NO_3^--removal strategy in RAS. The produced plant or algae biomass can in turn represent a food source for humans and feed for aquatic species (Martins et al., 2010, Wang, 2003).

1.2 Hydroponics

Hydroponics is a recirculation system where plants are cultivated in water with dissolved nutrients, instead of soil (Roberto, 2004, Jones Jr, 2004). It has its name from the Greek words *hydro* (water) and *ponor* (labor) (Jones Jr, 2004). Advantages of hydroponics presented by Jensen (1997) are the ability to grow crops where soil is contaminated or do not exist, production in controlled and stable environments, conservation of water, lowered associated land and water pollution, and lowered labor costs. The system is also easy adaptable, making it possible for amateur horticulturist to construct in private gardens and on rooftops (Jensen, 1997). Associated disadvantages of hydroponics are high construction costs per acre, high knowledge demand on optimal growth conditions and nutrition, rapid spreading of diseases to all plant beds, high research demand on adaptable plant species, and the production requires daily maintenance and observation (Jensen, 1997).

Two common hydroponic techniques are **media-based grow bed** and **deep-water culture (DWC) bed** (Lennard and Leonard, 2006). In a media-based grow bed, the roots are supported by an inert (chemically inactive) substrate (e.g. clay, pumice or gravel) that serves as microbial substrate, nitrification and filtering medium for solids (Roberto, 2004), and water is supplied sequentially in an ebb and flow pattern (Goddek et al., 2015). The DWC system contains floating rafts with plants in pots containing coco, rock wool or pumice for root support (Goddek et al., 2015). Different from the media-based grow bed, the roots in DWC are constantly submerged in water for nutrient uptake (Roberto, 2004).

Hydroponics: A strategy to reduce nitrogen losses in agriculture production

Loss of agricultural fertilizer-N to the surrounding environment through leaching and run-off (Hochmuth and Hanlon, 1995, Hochmuth, 2000), denitrification and gaseous losses (Hofman

and Van Cleemput, 1999, Cockx and Simonne, 2003) is common knowledge for conventional (soil-based) crop producers (Tyson et al., 2011). Globally, only ≈50% of fertilizer-N is recovered in crop production (Eickhout et al., 2006). Hence, farmers lose a high portion of fertilizer due to natural occurrences in soil-based production. Moreover, the industry is under high pressure to reduce pollution of natural ecosystems due to fertilizer inputs (Mitsch and Gosselink, 2000). Fedoroff et al. (2010) also calls for new and innovative agriculture systems to meet the predicted future predicted challenges in growing global populations, freshwater shortage (Watkins, 2006), soil degradation and arable land (Bindraban et al., 2012). If hydroponics is well-designed and properly managed, the system may work as a sustainable and effective alternative to field-grown agriculture production (Smither-Kopperl and Cantliffe, 2004).

1.3 Aquaponics

Aquaponics is integrated production of fish and plants in a recirculating system (Rakocy et al., 2006). The system combines aquaculture (RAS) with hydroponics, and offers integrated production of aquaculture species and plants (Tyson et al., 2011). Aquaponics is known as a sustainable system where metabolic by-products from RAS are incorporated into plant biomass instead of being discharged (Rakocy et al., 2006, Adler et al., 2003). Soil-free plant production occurs naturally in ponds and lakes, and the use of animal waste as fertilizer for plants has been recorded to take place already in the gardens of Mexican Aztecs (14^{th} –16^{th} century) (Roberto, 2004). The development of RAS technology in the 1970's influenced the work on aquaponics (Love et al., 2014). Removal of accumulated nitrogen in RAS by plants in soilless systems was tested as a possible water treatment strategy before more technical water treatment strategies were developed (Naegel, 1977, Lewis et al., 1978). Aquaponics has gained more attention the last years (Love et al., 2014), and is considered a sustainable and innovative production strategy for animal protein and agriculture products in the meet of global population rise, constrained freshwater supplies, climate change and soil nutrient depletion (Goddek et al., 2015, Bindraban et al., 2012, Klinger and Naylor, 2012, Tyson et al., 2011). Still, more research on aquaponics is needed in order to improve cultivation strategies and food security (Goddek et al., 2015).

Fish and plants constitute the main components of aquaponics, but mechanical filtration units for particle removal is common (e.g. particle filter, drum filter, and settling tank), together

with a biofilter for nitrification and microbial control (Attramadal et al., 2012). Typically the water flow starts in the fish tanks and continues in a loop to a mechanical filter, a biofilter, a settling tank or sump, a hydroponic bed, and flows back to the fish tanks (Goddek et al., 2015). As in an ordinary RAS, the biofilter is essential for nitrification and establishment of microbial communities in the system (Blancheton, 2000). It can control and select for slow growing bacteria (K-strategists) and establish microbially matured water (Blancheton, 2000, Attramadal et al., 2012).

The role of nitrogen in aquaponics

The N dynamic presented in Figure 1 is of particular interest in aquaponics (Tyson et al., 2011). Fish release TAN, whereas some NH_3^+ ionizes with water to form NH_4^+ (Tyson et al., 2011). In the biofilter ammonia is oxidized into NO_3^- trough nitrification, as presented in equation 1 and 2. After carbon, nitrogen is the most needed macronutrient for plants; 1-5% of dry matter in plants constitutes of N and it is vital for plant growth (Marschner, 2011). Plants can absorb NO_3^- and NH_4^+ (Marschner, 2011), which is readily available in the wastewater stream from the fish. NO_3^- is often preferred to NH_4^+ by the plants (Marschner, 2011)

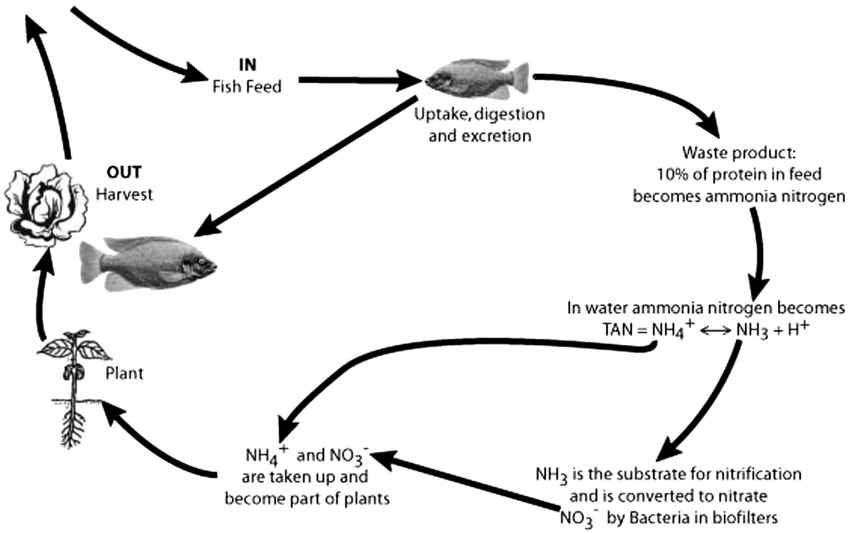

Figure 1 – The nitrogen cycle in aquaponics (Tyson et al., 2011).

System sizing aquaponics systems

A challenge in aquaponics is scaling and optimal dimensioning of fish and plant biomass (Tyson et al., 2011). Optimal nutrient balance is achieved when fish output is balanced with plant uptake (Adler et al., 1996). Fish waste production is directly related to quality and

quantity of fish feed (Fivelstad et al., 1993). The feed should be easy to digest and supplied in proper amounts. Overfeeding should be avoided in order to reduce organic load to the system, as this can enhance proliferation of fast growing and opportunistic bacteria (Vadstein et al., 1993) and in turn reduce fish and plant welfare (Vadstein et al., 2004). Fish fed on a proper diet can benefit from this through good growth, and hence secure production yield from high fish biomass production (Buzby and Lin, 2014). The nutrient uptake by plants must be balanced with nutrient production from the fish (Buzby and Lin, 2014). An inadequate plant growing area can lead to accumulation of nutrients, while too much plant biomass can improve water quality but lower plant growth rates (Buzby and Lin, 2014, Tyson et al., 2011). This will in turn have a negative effect on plant crop production (Buzby and Lin, 2014). Different models have suggested an appropriate fish feed ratio to plant growth area (Endut et al., 2010, Rakocy et al., 2006, Al-Hafedh et al., 2008), considering tilapia (*Oreochrimis* spp.) and African catfish (*Clarias gariepinus*) production. These recommendations ranged from 15 to 100 g feed m^{-2}. As these systems produce different fish and plant species and differ in hydroponic designs, water temperature, and fish waste production rates, it is questionable how transferable these studies are to other systems. More species and system specific models should be investigated.

1.4 Batch system: A culture system for microalgae

A batch system is the most common method for cultivation of microalgae (Lee and Shen, 2004), and can be used to measure growth rates of microalgae cultures (Wood et al., 2005). The system is preferred for its inexpensive cultivation approach, and it occupies advantages in terms of required volume of media, ease of manipulation, and various of manipulation possibilities (Wood et al., 2005). The system is simple: It requires an algal inoculum and a reduced amount of complete culture medium, placed in a culture vessel (often a conical flask) (Lee and Shen, 2004). In order to arrange for photosynthesis, CO_2 is added to the culture by capping the vessel and purging it with CO_2 enriched air (e. g. 5% v/v CO_2 in air), or through continuous gassing with CO_2 enriched air. The culture can also be illuminated (natural or artificial light sources) to stimulate photosynthetic growth (Lee and Shen, 2004).

The lag phase, the exponential phase, and the linear growth phase are phases that may occur in a batch culture (Lee and Shen, 2004). The lag phase characterizes the start-up of the batch culture, and in this period microalgae cells are adjusting to the environment and hence low

growth rates are normally observed (Lee and Shen, 2004). In the exponential phase, the growth increases as a fixed percentage of the total per unit time (Wang, 2003), indicating cell growth. This phase is maintained as long as nutrients and mineral substances are present (Lee and Shen, 2004). The exponential phase can be followed by a linear growth phase, where biomass accumulates at a constant rate (Lee and Shen, 2004). This phase is usually hampered by a limiting factor, such as sufficient input of light to all cells, or lack of one or more nutrients (Wang, 2003).

The kinetics of microbial growth in a batch culture can be calculated using the following equation (Guillard, 1973):

$$\mu = \frac{\ln N_t - \ln N_0}{\Delta t}, \tag{3}$$

where μ is the specific growth rate (SGR) per day. t refers to time. Δt is the length of a time interval ($t_t - t_0$), N_0 is number of cells at the beginning of a time interval, and N_t is number of cells at the end of the time interval (Wood et al., 2005). The SGR is the increase in cell mass per unit time, and is calculated in the exponential phase (Wood et al., 2005). The relationship can be explained by the following equation (Guillard, 1973):

$$N_t = N_0 \cdot e^{\mu t} \tag{4}$$

1.5 Concept for integrated copepod and microalgae production

Copepods are a group of crustaceans systematically divided into ten orders, whereas Cyclopoida, Calanoida, Harpacticoida, and Mormonilloida are represented in marine environments (Marcus, 2005). The calanoid copepod is especially abundant in the pelagic part of estuaries and coastal areas (Marcus, 2005), and creates a vital link in the food web between phytoplankton and fish (Støttrup, 2003). Production of copepods is considered a bottleneck of cultivation of marine fish in aquaculture (Marcus, 2005). The use of copepods as live feed for marine fish larvae in aquaculture, together with development of efficient cultivation strategies, has been studied since the 1980's (Støttrup et al., 1986, Schipp, 2006). Copepod production in intensive cultivation systems has been suggested to support a healthy and low-parasitic culture (Støttrup, 2003), and Drillet et al. (2011) recommended RAS as a cultivation

strategy to improve water quality conditions for the crustacean. *Acartia tonsa* Dana is a calanoid copepod species cultivated in saline environments (Mauchline et al., 1998), and production of this species in intensive systems has also proven to be beneficial for high-nutritional and viable egg-development (Drillet et al., 2006). This is beneficial, since copepod eggs forms the basis of the copepod production. Hence, intensive production of calanoid copepods in RAS may seem logic and necessary in order to meet the challenges of the bottleneck of marine aquaculture.

Wastewater effluents follow production of organisms in RAS, and the need for wastewater treatment is vital. The use of microalgae as a wastewater treatment strategy has the advantage of converting N and phosphorous (P) into biomass, and high value products can be extracted from the microalgae cells (Arbib et al., 2012). Microalgae biomass can be used as live feed for fish larvae, crustaceans, and mollusks (Meireles et al., 2003), and algal cells can be extracted for proteins (Kuhad et al., 1997, Brown et al., 1997), carbohydrates and lipids (Brown et al., 1997), hydrocarbons (Chisti, 2007), and pigments (Wiltshire et al., 2000). Moreover, microalgae can be a possible resource for biofuel production (Chauton et al., 2013, Chisti, 2007), and they host a potential for CO_2 capture from aquaculture wastewater (De Morais and Costa, 2007).

Integrated production with microalgae has the potential to reduce industrial costs and to offset carbon emission (Chauton et al., 2015, Hughes and Benemann, 1997). A multi-trophic production system with microalgae and aquaculture species has been described, where ditaoms were cultivated on wastewater form shrimps (Wang, 2003). They were supplied to the oysters as feed, from where the treated water returned back to the shrimp tank by (Wang, 2003). As microalgae can be used as feed for copepods (Brown et al., 1997), a similar system integrating microalgae and copepod production may be suggested: *Copeponics*. This strategy can benefit sustainability of aquaculture feed production, and present an innovative treatment strategy in production of copepods.

1.6 Aims of the study

The aims of this study are specific for the different recirculation systems, with a main focus on nitrogen dynamics and possibilities for a sustainable re-use of nutrients in effluent water from land based aquaculture production systems.

RAS

- Measure the concentrations of NO_2^--N, NO_3^--N and TAN (NH_3-N + NH_4^+-N) in the influent and effluent water of a fish tank by using a spectrophotometric method, and describe the nitrogen dynamics.
- Discuss possibilities for sustainable re-use of nutrients in wastewater from RAS.

Hydroponics

- Measure the concentrations of NO_2^--N, NO_3^--N and TAN (NH_3-N + NH_4^+-N) in the system with by using a spectrophotometric method, and describe the nitrogen dynamics.
- Discuss possibilities for re-use of nutrients in wastewater from land-based aquaculture in Hydroponics.

Aquaponics

- Measure the concentrations of NO_2^--N, NO_3^--N and TAN (NH_3-N + NH_4^+-N) in all main compartments of the system by the use of a spectrophotometric method, and describe the nitrogen dynamics.
- Investigate efficiency of lettuce as a wastewater strategy to remove dissolved nitrogen compounds from the aquaponic water.

Copeponics

- Cultivate microalgae with copepod wastewater in a batch system. Compare growth of microalgae cultivated in copepod wastewater with growth of microalgae cultivated in a standard fertilizer media.
- Evaluate performance of a small-scale recirculating system with integrated production of copepods and microalgae (Copeponics). Investigate the water quality conditions for the copepods, the copepod egg production compared to normal flow-trough production, and the growth performance of microalgae cultivated on copepod wastewater.
- Measure the concentrations of NO_2^--N, NO_3^--N and TAN (NH_3-N + NH_4^+-N) in all compartments of the Copeponics and in water medias of the batch system, by using a spectrophotometric method, and an online NO_3^--N sensor in Copeponics.

2 Material and methods

Nitrogen dynamics were studied in four different recirculation systems and one batch system. One experiment was conducted in each of the five systems, at different experimental facilities and production sites in Norway:

- *Recirculation aquaculture system* (RAS), Salmon smolt production at Marine Harvest (Nordheim)
- *Hydroponics*, strawberry production at Norwegian University of Science and Technology (NTNU) Social Research (Trondheim)
- *Aquaponics*, integrated trout and lettuce production at Norwegian Institute of Bioeconomy Research (NIBIO – former Bioforsk, Landvik)
- *Batch cultivation* of microalgae at NTNU and SINTEF Fisheries and Aquaculture (Trondheim)
- *Copeponics*, integrated microalgae and copepod production at NTNU and SINTEF Fisheries and Aquaculture (Trondheim)

The experiments in RAS, Hydroponics and Aquaponics were all parts of a collaborative project named BioSys founded by Regionalt Forskningsfond Midt (RFFMIDT), with an ongoing progress from 1st of March 2014 until 1st of March 2015. The experiments in batch system and copeponics were both part of a collaborative project named Copeponics founded by RFFMIDT. Fieldwork for this master project was carried out from February to December 2015.

Different bio-producing recirculation systems were assessed in this project, both integrated and single species production systems. The results are presented in two parts, seawater- and freshwater-based systems respectively. The systems named **Aquaponics**, **Hydroponics** and **Recirculation aquaculture system** (**RAS**) are included in the freshwater part, and the experiment and system named **Batch experiment** and **Copeponics** are included in the seawater part.

2.1 Physicochemical water quality

The dynamics of the nitrogenous waste products (total ammonia-nitrogen, TAN, nitrite-nitrogen, NO_2^--N, and nitrate-nitrogen, NO_3-N, concentrations) were measured in all freshwater and seawater systems with a DR/890 HACH Colorimeter/HACH, USA. NO_3-N measurements in seawater were calibrated for Cl^- interference (Appendix 6). Unionized ammonia-nitrogen (NH_3-N) concentration was calculated from TAN, temperature and pH, in saline (Bower and Bidwell, 1978) and non-saline (Trussell, 1972) systems. Each following section explains where in the systems the nitrogen analysis was performed. An inline sensor (SAC Sensor VIOMAX CAS51D, Endress+Hauser, Switzerland) performed continuous online measurements of nitrate in the copeponic systems. Particulate organic nitrogen content (PON) was obtained in all systems by analyzing water samples that were immediately vacuum filtered through ignited (480^{o}C, 2 h) 1.2 μm, 25 mm diameter GF/C glass microfiber filters (Whatman International Ltd., England). The filters were stored at -20 °C and inorganic CO_2 was removed from filters with hydrochloric acid vapor (37%, 20 min). Each filter was transferred to a tin cup (Säntis Analytical AG, Switzerland) and analyzed in a HN-S/N Elemental Analyser 1106 (Carlo Erba Instruments, Italy). This procedure was followed for both the freshwater and seawater experiments.

2.2 Freshwater systems

The **RAS experiment** was conducted in March 2015 at Marine Harvest's production site at Nordheim, the **hydroponics experiment** was conducted during February 2015 at NTNU Social Research in Trondheim, and the **aquaponics experiment** was conducted in April 2015 at NIBIO in Landvik. A description of the different systems is presented in Table 1. The recirculation ratio is based on the following equation:

$$Recirculation\ ratio\ in\ \% = \frac{Internal\ recirculation\ flow}{New\ water\ intake + Internal\ recirculation\ flow} \cdot 100 \quad (5)$$

Table 1 – A summary of technical and biological parameters of the freshwater recirculation systems in the period of study.

	Hydroponics	**RAS**	**Aquaponics**
Fish species	No	*Salmo salar*	*Oncorhynchus mykiss, Salmo trutta*
Fish biomass, kg	No	117244.3±995.8	23*
Single fish tank area, m^3	No	190	0.6
Total fish tank volume, m^3	No	1900	2.4
Plant species	*Fragaria x ananassa "Elan"*	No	*Lactuca sativa*
Plant biomass (wet weight), kg	36.7	No	26.0
Total plant bed area, m^2	12	No	20
Plant system	Media-based grow bed	No	Deep-water floating raft
Total plant tank volume, m^3	1	No	6
Recirculation ratio, %	99	99	99
Flow, $m^3\ h^{-1}$	0.03	240	0.3
kg fish feed day^{-1}	No	2193.3±133.3	0.8±0.0
L fertilizer day^{-1}	0-0.5	No	No

* Measured only on day 1 of the experiment

2.2.1 Input of feed and fertilizers in freshwater systems

Table 2 presents an overview of input of feed/fertilizer to the different freshwater recirculation systems, affecting the nitrogen dynamics of the bio-producing systems. The

RAS and Aquaponic system were added pellet feed (no fertilizer added in the hydroponic part of Aquaponics). The main nitrogen source in fish feed is protein. 16% of the protein is considered nitrogen (Siri Tømmeraas, Skretting, pers. comm.). The hydroponics was added fertilizer (standard nutrient solution Kristalon Indigo, 10%, and Calcinit, 10%) until electrical conductivity (EC) was 1.5. Kristalon Indigo contained 7.5% NO_3-N and 1% NH_4-N, while Calcinit contained 14.4% NO_3-N and 1.1% NH_4^+-N.

Table 2 – Input of feed or fertilizer to RAS, Aquaponics and Hydroponics (n=5 in RAS).

System	**Producer/ Recipe**	**Feed/Fertilizer**	**Protein content (%)**	**Nitrogen content (%)**	**kg feed m^{-3} day^{-1}/ L fertilizer m^{-3} day^{-1}**	**kg feed kg $fish^{-1}$ day^{-1}/ L fertilizer $plant^{-1}$ day^{-1}**
RAS	Skretting	Nutra Olympic (3 mm, day 1-3)	47.8	16	0.90±0.10	0.02±0.00
RAS	Skretting	Nutra Supreme (3 mm, day 4-5)	47.8	16	1.00±0.10	0.02±0.00
Aquaponics	Skretting	Nutra RC (3 mm)	47.8	16	0.08±0.00	0.03±0.00
Hydroponics	YARA	Kristalon Indigo (10%), Calcinit (10%)	-	8.5, 15.5	28.8±0.00	1.20±0.00

2.2.2 RAS

The experiment was carried out for five days in the period from 05.03.15 to 09.03.15 at Marine Harvest's RAS unit in Nordheim, Møre and Romsdal. The system is a commercial RAS where 99% of the water is re-used. It produces smolts of Atlantic salmon (*Salmo salar*) for further growth and production in semi-exposed sea-cages along the Mid Norwegian coast.

The RAS consisted of a start-feeding department (A) and on-growing department (B). This experiment took place in department B, see Figure 2 for schematic setup and flow scheme.

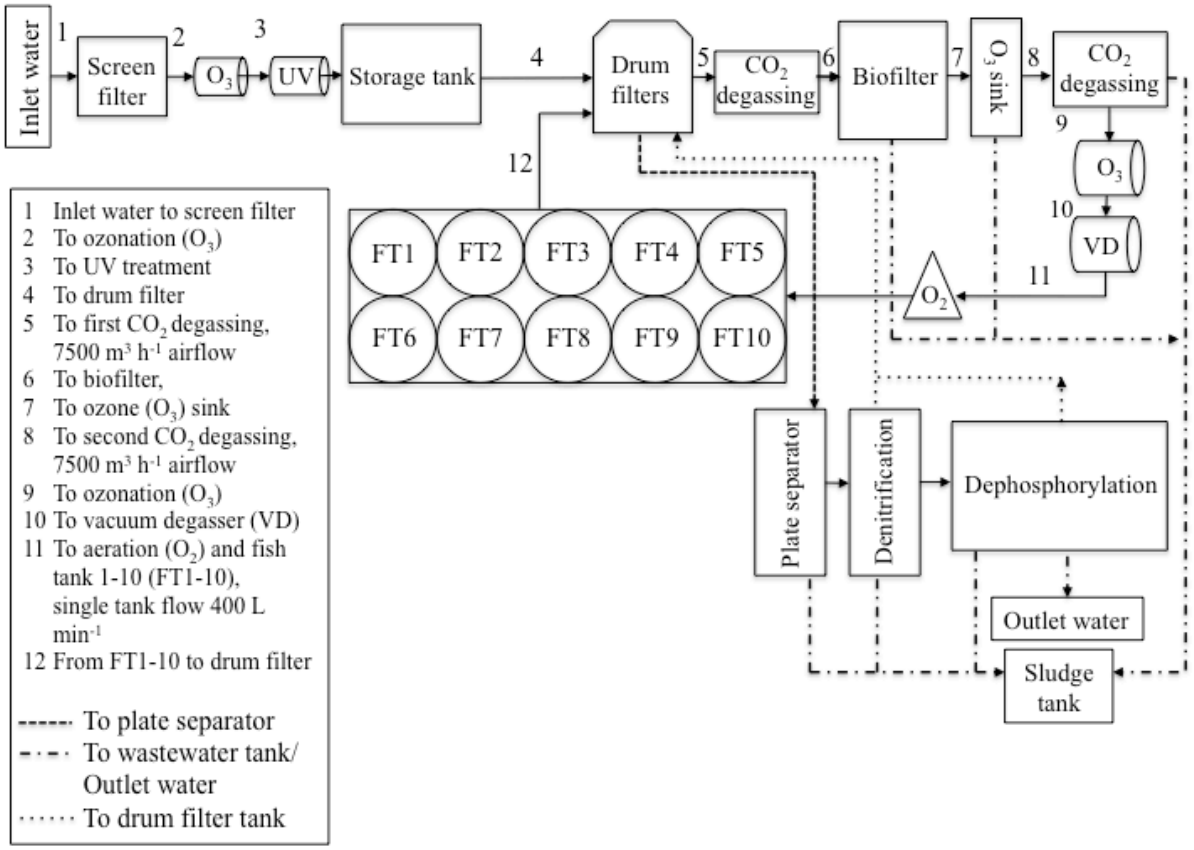

Figure 2 - Schematic drawing and flow scheme for department B in RAS (not to scale). The system is a Zero Water Change (ZWC) designed by Aquatec Solution A/S.

Department B consisted of 10 circular rearing tanks for Atlantic salmon made out of fiberglass. Wastewater from the tanks was treated in a water treatment loop consisting of three drum filters (30, 40 and 60 μm mesh size), a CO_2-degassing compartment, three biofilter units (250 m^3 filter medium in each), an ozone (O_3) sink (2 min exposing time), a second CO_2-degassing compartment, and an ozone reaction chamber. A side stream was treated for N_2 saturation in a vacuum degasser, while all water was added oxygen by an O_2 cone before it entered the fish tanks. Sludge from the biofilter and the second CO_2-degassing compartment was transported directly to the wastewater tank. Solids were filtered out from the drum filters and led through a plate separator for sedimentation of particles. Sludge was transported to the wastewater tank, while solid-free water was led to de-nitrification. From the de-nitrification treatment, sludge water was transported to the wastewater tank, while rinsed water was either led back to the drum filters or to de-phosphorylation. In the de-phosphorous system phosphorous, metals and fine particles were chemically removed from the water. Sludge was led to the wastewater tank, while rinsed water was either transported back to the drum filters

or out to the sea (outlet water). Marine Harvest could not provide names of brand and producer or volume of the water treatment units.

Approximately 0.05 m^3 of sludge day^{-1} was deposed from the RAS into the sea (wastewater tank). About 3-5 m^3 rinsed water was deposited from RAS day^{-1} (outlet water). The inlet water was collected from the Sagvikvannet just outside of the RAS facility, filtered through a screen filter, disinfected with ozone and ultraviolet (UV) radiation, and further pumped to a storage tank (freshwater supply). The treated inlet water was further pumped to the drum filters in the treatment loop. The total water volume in compartment B was 2400 m^3 added salt (2-3 ppt), and the total water volume of all the 10 fish tanks was 1900 m^3.

The fish was supplied by Marine Harvest, selected and bred for cultivation. During the experiment the fish was entering the smoltification process to develop seawater tolerance. Each tank had a volume of 190 m^3. The initial weight of the 197 788 salmonoids in fish tank 5 (**FT5**) on the start of the study was in total estimated as 14 402 kg. The average fish density in the fish tanks of RAS was estimated to be 69.5±2.3 kg m^{-3} (MEAN±SE, n=6). The fish were fed with commercial pellets feed (Nutra Olympic, 3 mm, Skretting, Norway; Appendix 1; Nutra Supreme, 3 mm, Skretting, Norway; Appendix 2), in amounts of 307.1 ± 2.6 kg day^{-1} in FT5. The amount of feed increased on all days, except for on day 6. Marine Harvest provided all rights on data.

Physiochemical water quality and sampling

Water quality measurements of temperature and oxygen, pH, salinity and CO_2 were measured at the same time as the water samplings (Handy Polaris, OxyGuard, Denmark; pH Manta, OxyGuard, Denmark; Pro fast, GLI International, USA; OxyGuard CO2 Analyser, OxyGuard, Denmark). The water quality parameters were measured in the sampling point of effluent water from FT1-10 before it entered the water treatment circuit. The Marine Harvest staff collected these measurements daily. The only measurement gathered directly from FT5 was the oxygen concentration (Oxygen Probe, OxyGuard, Denmark).

Water samplings for analysis of dissolved nitrogen compounds were performed every day at 09.00, 12.00, and 16.00 in order to detect possible daily variations. Two night measurements were also performed between day 3 and 4, at 22.00 and 02.00. Samplings were collected at two locations in the system: 1) Water returning from the biofilter and flowing in to all fish

tanks in department B (from here on named **in to FT1-10**), and 2) water in fish tank 5 (**FT5**). Samplings (30 – 40 mL) for PON analysis were collected on day 1, 3 and 5 in water in to FT 1-10 and in FT5 (during the 09.00-sampling).

2.2.3 Hydroponics

The study was carried out for three days from 28.02.15 to 02.02.15 at NTNU Social Research (Trondheim). This was a short-term investigation where no significant fluctuations in water parameters were expected, since the plants were in a fully developed stage (generative stadium; pers. comm., Irene Karoliussen, CIRiS) with a relative stable behavior regarding uptake of nutrients. Fertilizer was added once a week.

The setup with media bed hydroponics was placed in a greenhouse to perform uniform conditions throughout the experiment (20 °C air temperature). Flow and schematic setup of the system is presented in Figure 3. A hydroponic trough (6 m) with 24 Strawberry Elan F1 (*Fragaria x ananassa 'Elan'*) plants, a slow flowing biofilter (pumice), and a head tank with nutrient medium made up the system setup. Seeds were sown in pumice and covered in plastic the first week to ensure high humidity conditions. After 3 weeks, the plants were transferred to hydroponics, prefilled with pumice (12 L). Artificial light was given in a 16h/8h light/dark cycle. Pipelines made out of polyethylene connected the different components and a pump (Maxi Jet 50-100, Norway) was connected to the head tank. Loss of water trough evaporation and transpiration was replenished with water in the control tank three times a week. The transpiration and evaporation rate by the plants was approximately 10-15 L day^{-1}. No other water displacement or discharge took place in the system.

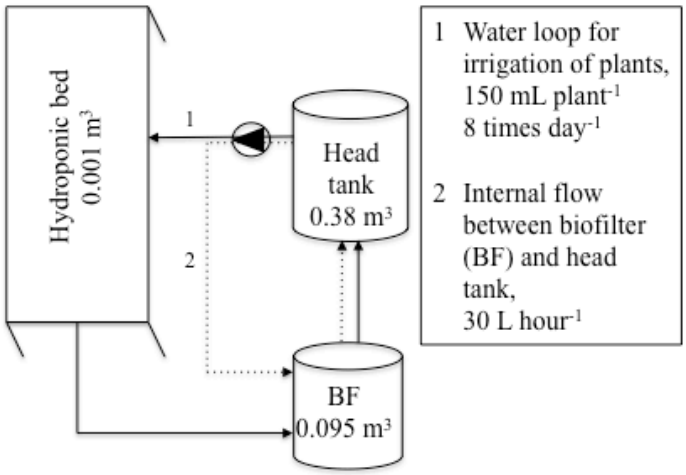

Figure 3 – Schematic drawing and flow scheme of the hydroponic system (not to scale).

The freshwater source was regular tap water regulated by the Municipality of Trondheim (Trondheim Kommune). Two water loops were connected to the system. During irrigation of plants, the water was pumped from the head tank to the hydroponic bed. The irrigation lasted for 5 min, and occurred every third hour. Eight times daily, each plant was irrigated with 150 mL of standard nutrient solution (10% Kristalon Indigo, YARA, Norway; 10% YaraLiva Calcinit, YARA, Norway; Appendix 2). The second loop was internal circulation between the biofilter and the head tank. The biofilter supported microbiological control in the system. Both flow loops passed the biofilter in order to ensure recirculation of matured water.

Physicochemical water quality and sampling

The water quality parameters temperature, oxygen, pH and conductivity were measured at the same time as the water samplings with a portable electrode, and a combined pH electrode cartridge and an EC/TDS graphite electrode respectively (ProODO Optical Dissolved Oxygen Meter, YSI Inc, USA; LH-T28, China; HI98130 pH/Conductivity/TDS Tester, HANNA instruments). Water sampling for analysis of dissolved nitrogen compounds was performed every day, three times a day from the control tank: At 09.00, 12.00 and 15.00 from the same sampling point in the head tank. Sampling for PON analysis (200-690 mL) was carried out every day.

2.2.4 Aquaponics

The study was carried out for six days in the period from 13.03.15 to 18.03.15 at NIBIO Landvik in Aust-Agder. The aquaponics system was located in a greenhouse (20-21°C). Total water volume in the system was 10 m^3 freshwater. The system included four rearing tanks for fish, two hydroponic troughs, a sump, a biofilter, an air blower/aeration tank, a particle remover/bead filter, and four swirl separators (17 L each) connected to each fish tank. For schematic setup and data on volume, flow and dimensions of all compartments of the system, see Figure 4. The rest of the volume was in the pipelines (polyethylene). The aquaponic system contained a DWC hydroponic system, modified after design described by Rakocy et al. (2006). The biofilter was a Moving Bed Bioreactor (MBBR) with K1 Kaldnes Media. The bead filter was a Polygeyser DF-6 with Enhanced Nitrification (EN) bead media. The fish tanks were shaded with curtains (86% shade effect) to reduce algae growth in the fish tanks, and oxygenated by air stones in all tanks (Diaphragm air pumps HP 60, 230V/50Hz~1 with air diffuser discs, 20 L min^{-1}, HIBLOW, USA Inc.).

Both the swirl separators and the bead filter removed particles from the water, and excess particles also settled in the sump. The system was designed as a zero discharge system with wet composting of the sludge. Loss of water trough evaporation, sludge removal and transpiration was replenished with water in the sump (17.6 L day^{-1}). No other water displacement or discharge took place in the system. Due to problems in the systems with indications of nitrite toxification, chloride was added occasionally to the system by the addition of $CaCl_2$ to a Cl^- concentration (± standard deviation, SD) of 100±11.6 mg L^{-1}. Also, $CaCO_3$ was added to the system to increase pH. Lime slurry was added using a Watson-Marlow 313 peristaltic pump. A regulation principle determined the dosage, with pH value as set point.

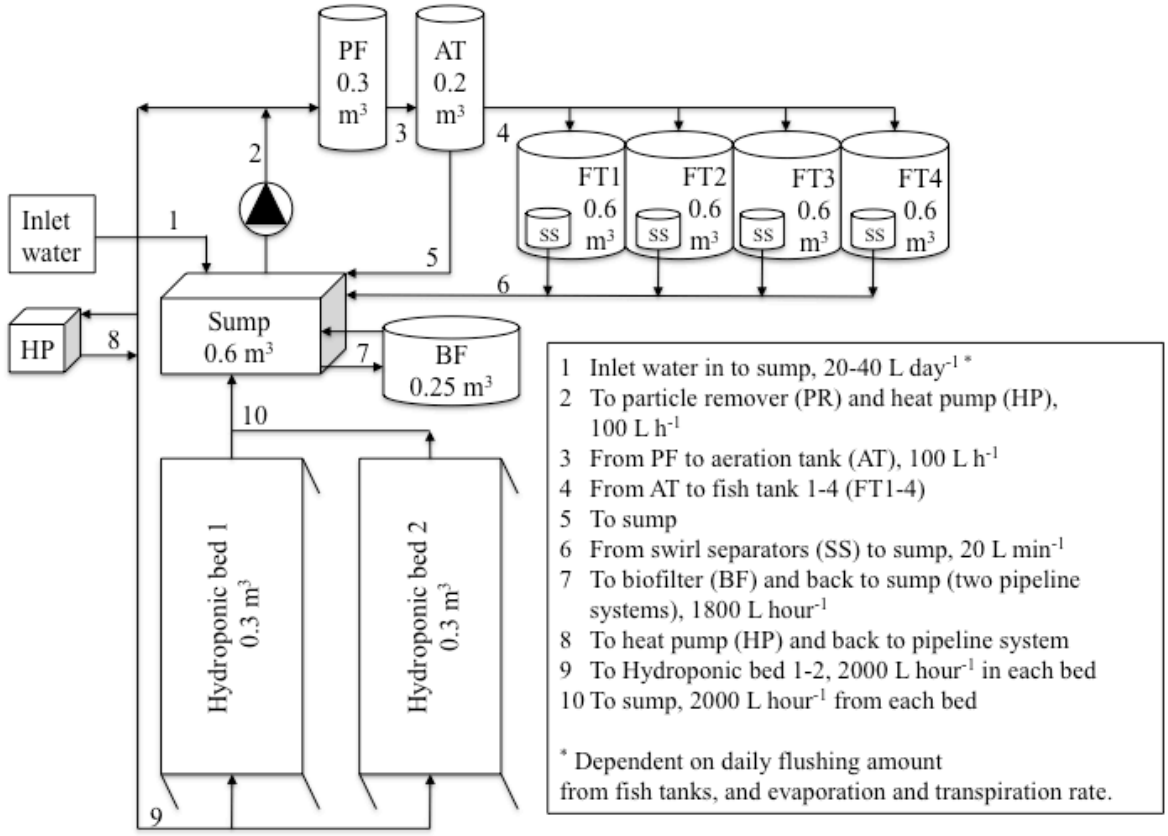

Figure 4 – Schematic drawing and flow scheme of the aquaponic system (not to scale).

Inlet water was regular tap water regulated by the Municipality of Grimstad. The pump (BADU® Eco Touch 0-29 m^3 $hour^{-1}$, SPECK Pumpen, Tyskland) ensured water circulation. Water from the sump flowed to the hydroponic beds and was sprinkled over the crispy lettuce (*Lactuca sativa*), and outflow from the hydroponic beds flowed back to the sump. The water was further pumped to in to the biofilter for nitrification, and the outflow was pumped back to

the sump after aeration. The third outflow from the sump was to the bead filter, in which continued into the fish tanks. All wastewater from each fish tank was transported back to the sump.

Brown trout (*Salmo trutta*) with an initial total body weight of 11 kg in fish tank 1 (FT1) and 12 kg in fish tank 2 (FT2), and rainbow trout (*Oncorhynchus mykiss*) with an initial total body weight of 6.6 kg in fish tank 3 (FT3) and 5.4 kg in fish tank 4 (FT4) were used in the study. The rainbow trout was supplied by Lerøy AS, selected and bred for cultivation. The brown trout was of a 1st generation of wild caught fish (from the river Otra, Aust-Agder), delivered by Syrtveit Fiskeanlegg AS. Feed dispensers (Clockwork Feeders, Sterner AquaTech UK Ltd, Scotland) ensured continuous feeding of the fish. FT1 and FT2 were daily fed 150 g, and FT3 and FT4 were daily fed 250 g of the same feed (Nutra RC, 3 mm, Skretting, Norway; Appendix 3).

Seeds were first planted into rock wool for germination and growth in a separate nursing system (3 weeks), using aquaponic water, before being transferred to the hydroponic beds. Two plant beds for DWC constituted the hydroponic system. The tanks were made out of steel and wood, with a pond cover in plastic, deep water (30 cm), and a total surface are of 20 m^2. Lettuce 'Crispy' (*Lactuca sativa*) was grown in 28 floating boards made out of polystyrene (Dry Hydroponics, The Netherlands). Each board contained 24 plants that were fertilized with aquaponics water exclusively from nursery stage to product.

Physicochemical water quality and sampling

Water temperature and oxygen, and CO_2 concentration were measured manually every day in each compartment of the system with portable electrodes (Handy Polaris 2, OxyGuard, Denmark; Portable Dissolved CO2, OxyGuard, Denmark). The pH was measured continuously in the sump with a pH electrode (Polilyte Plus 120, Hamilton Co., Switzerland) which was connected to an automatic monitoring system for pH, temperature, oxygen, system flow through and signal controlled dosing pump for additional buffer solution ($CaCO_3$ powder). The parameters were logged by an analog AAC 3100 data logger equipped with a Siemens GSM-modem for communication with an external host at The Norwegian Institute for Water Research (NIVA), and powered by UPS 24 VDC.

Water samplings for analysis of dissolved nitrogen compounds were performed at 09.00 every day at the same sample points. Samplings (30 – 40 mL) for PON analysis were collected on

day 1, 3 and 5 in the sump at 09.00. One night sampling was also performed at midnight between day 4 and 5. Samplings were collected from the common outlet of all four fish tanks, inlet to fish tanks, outlet from hydroponic beds, outlet from biofilter, and from the sump. Water quality measurements of temperature, oxygen, pH and conductivity was measured in all main compartments of the system concurrent with timing of the water samplings (Handy Polaris 2, OxyGuard, Denmark; Polilyte Plus 120, Hamilton Co., Switzerland; Portable Dissolved CO2, OxyGuard, Denmark).

2.3 Seawater system

All of the following experiments took place at NTNU and SINTEF Fisheries and Aquaculture. A **batch cultivation experiment** with the microalgae *Rhodomonas baltica* was conducted in June 2015. The final **Copeponics system** was conducted during November and December 2015. A description of the different systems is presented in Table 3. The recirculation ratio is based on Equation 5.

Table 3 - A summary of technical and biological parameters of the seawater recirculation system Copeponics during the days of study.

	Copeponics
Copepod species	*Acartia tonsa*
Copepod biomass, million individuals	3-10
Copepod tank volume, m^3	1.3
Algae species	*Rhodomonas baltica*
Algae density, cells mL^{-1}	1.3-5.0 x 10^6
Single algae bag volume, m^3	0.3
Total algae bag volume, m^3	0.9
Recirculation ratio, %	89
Flow	7100 L day^{-1}
L microalgae as feed to copepod tank day^{-1}	203±15
Biofilter, m^3	Moving bed
CO_2-stripping	In-tank aeration
Other supplements	Conwy
In-line measurements	Nitrate-nitrogen, NO_3^--N

2.3.1 Input of feed and fertilizers in the seawater system

Table 4 presents an overview of input of feed/fertilizer to the Copeponics, affecting the nitrogen dynamics of the bio-producing system. The microalgae in Copeponics were fertilized with a reduced dose of Conwy medium, compared with normal production (1.2 mL). The Conwy medium contains 16.5% NO_3^--N and 6.8% NH_4^+-N.

Table 4 – Input of fertilizer (Conwy medium) to Copeponics during the days of study.

System	Recipe	Fertilizer	Nitrogen content (%)	mL fertilizer m^{-3} of copepod tank	mL fertilizer L^{-1} seawater
Copeponics	Walne (1979)	Conwy	23.3	0.08±0.01	0.7±0.1

2.3.2 Batch experiment

A batch experiment was conducted in May 2015. The aim was to analyze the growth response of *R. baltica* to four different water mediums originating from:

1) The copepod tank, normal production, flow-through system (**CT**)
2) The biofilter receiving water from CT (**BF**)
3) Standard Conwy medium (1.2 mL L^{-1} seawater; modified from (Walne, 1979), Appendix 5) (**CON**)
4) Seawater (**SW**)

12 bottles (1.2 L each) were filled with the four different waters; three replicates per water medium. To assure normal conditions in water from BF and CT, water quality parameters were measured in the compartments before it was filtered and transferred to the bottles of the experiment. Water from the CT tank and BF were filtered with a double layer of a straining cloth with a mesh size of 10 µm, to remove copepods and particles >10 µm from the water. This allowed algae cells from the copepod tank to pass to the experimental bottles for these two treatments (CT and BF). All 12 bottles were added 20 mL inoculation from a *R. baltica* stem culture. Cell density was not measured in the stem culture before addition in the 12 bottles, but it was made sure that the culture was sufficiently mixed before the initial samples were taken from the culture. Cell counts in the bottles started on day 1 of the experiment, 24 hours after the experiment started.

The 12 bottles were placed in a room that held an air temperature of 20 ^{o}C and continuous light exposure. Air with 1-2% CO_2 was supplied to the bottles, and the pH was kept between 7.5 and 8.5 by regulating the CO_2 supply to ensure optimal photosynthesis for the algae cells, growth and stirring.

Physicochemical water quality and sampling

Water pH was measured daily (pH/mV-meter, WTW pH 315i, Germany). Cell density was also measured daily using a Beckman MultisizerTM3 Coulter Counter, based on particle concentration (cells mL^{-1}) with the diameter range from 5-10 µm. Dissolved nitrogenous compounds in the waters were measured on day 1 and the final day of the cultures, and sampling and filtering (2 mL) for PON measurement in all bottles was carried out at the same time. When the cell growth of *R. baltica* was less than 5% the bottles were removed from the experiment and the final analyses were performed.

2.3.3 Copeponics

The study was carried out for 32 days in the period 06.11.15 – 07.12.15. The study included 2 periods. In the first stage, the copepod tank had a biomass of approximately 3 million adult copepods of *A. tonsa*, while in the second stage 7 million extra (adult) copepods were added (2 weeks younger than the first 3 million copepods). The copepods were supplied from C-feed AS. The aim was to test production of the copepod *A. tonsa* and the cryptomonade *R. baltica* in an integrated recirculation system. The same copepod tank and biofilter as described in the batch experiment were used in Copeponics, but now the two compartments were coupled together, which ensured internal circulation (not a flow-through system).

Figure 5 presents schematic setup and data on volume, flow and dimensions of all compartments of copeponics. The system included one polyethylene rearing tank for copepods, two MBBR containing K1 Kaldnes Media, one extra tank made of polyethylene, three plastic bags for algae cultivation, an extra tank, and a polyethylene feeding tank, connected with polyethylene pipelines. Loss of water through evaporation and waste removal was replenished with new intake seawater (sand filtered, 1 µm; exchange rate 5.5 times day^{-1}) every day: 10% of copepod tank volume day^{-1}.

Water flowed passively from the copepod tank (CT) through a filter (64 µm) to the biofilters for nitrification. Water from biofilter 1 (BF1) flowed passively on to biofilter 2 (BF2). The water was collected in an extra tank (ET), where one flow of nitrified water was pumped back to CT and the remaining water was pumped to a 5 µm cross flow filter. From there, one flow of infiltrated water was pumped back to the CT, while the second flow was pumped through the filter and on to a second 0.5 µm cross flow filter. The filtrated water was pumped to algae

bag 1, 2 and 3 (AB1, AB2 and AB3 respectively) and supplied them with water. See Figure 3 for flow scheme and schematic setup. Any overflow from AB1-3 was pumped back to BF2.

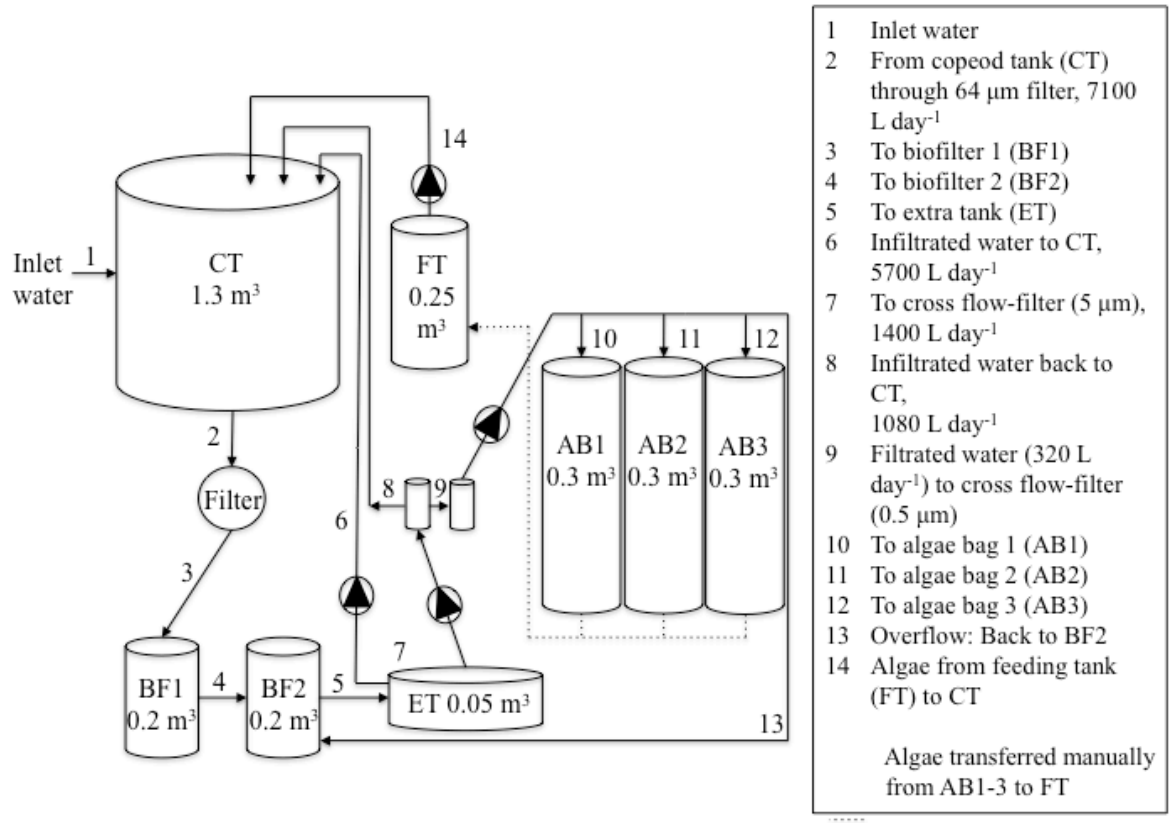

Figure 5 – Schematic drawing and flow scheme of the Copeponics (not to scale).

Algae were harvested every morning and transferred to a separate feeding tank (FT) connected to the copepod tank. From the FT *R. baltica* was provided continuously to the copepods by a pump during 24 h day^{-1}. The cell density of the algae culture in FT was estimated daily during weekdays using a Beckman MultisizerTM3 Coulter Counter, based on particle concentration (cells mL^{-1}) with a diameter range of 5-10 µm. Between 100 and 300 L of algae was supplied to FT daily, different in stage 1 and stage 2 of the study, since the higher copepod biomass required higher food supply. The harvested amount of algae from the different algae bags was dependent on which bag supplied the algae of best quality. Quality was determined by the culture's appearance, color and (high) cell density.

The algae used in AB1-3 were initially started from intermediate cultures (80 L in each bag, 1.3 x 10^6 cells mL^{-1}). The original algae were cultivated on seawater that was sand filtered (1 µm), heated to 20 °C, chlorinated (NaOCL (10-15 %), 0.25 ml L^{-1}, no aeration, > 5 hours) and dechlorinated (3 g $Na_2S_2O_3$ 100 L^{-1} seawater, heavy aeration, > 5 hours). Air with 1-2%

CO_2 was supplied to the bags, and the pH was kept between 7.5 and 8.5 by regulating the CO_2 supply. The same physiochemical conditions were held in the Copeponics project and AB1-3 were under continuous illumination by 2 fluoresecent tubes (GE Polylux XL 830, F58W, GE Lightning, USA). After addition of 80 L of *R. baltica* culture in each bag, 50 L seawater was added to AB1-3 respectively on the first day, and another addition of 170 L of seawater the next day (20 ^{O}C, 24-29 ppt) to achieve a bag volume of 300 L.

On to day 6 of the study, the copepod tank was supplied algae from AB1-3 only. In this period the algae received biofiltered water from the copepod tank only. Due to reduced algae growth and optimal cell quality, the algae in AB1-3 were supplied with Conwy medium to ensure optimal growth (0.7 ± 0.1 mL L^{-1} seawater, n = 34). There were some replacements of the algae bags throughout the production period, which was natural as an algae bag normally has production duration of 7-9 days. AB1 was harvested on day 7, and AB1 and AB3 was harvested on day 11 and replaced with new startup-cultures (same procedures as in the start of the production were followed). Before addition of the new cultures, the cylinders were cleaned, chlorinated and dechlorinated before new algae were started from intermediate cultures (80 L, 1.3×10^6 mL^{-1} or 30 L, 5.0×10^6 mL^{-1}), seawater (added as described over) and Conwy medium (1.2 mL L^{-1} seawater).

A. tonsa was cultivated in a 1300 L tank. The outlet filter (64 µm) was cleaned daily. Debris and eggs were siphoned daily from the bottom of the copepod tanks and filtered through two sieves of 100 and 120 µm mesh size to remove waste and dead copepods. Eggs were cleaned with seawater and transferred to NUNC EasyFlasks™ (NUNC A/S, Denmark) and stored at 2 °C (SANYO Pharmaceutical Refrigerator MPR-311D (H), Japan). As presented in Figure 5, the copepods were supplied *R. baltica* continuously from FT by a pump. The algae density in the tank was estimated daily (except for weekends).

Physicochemical water quality and sampling

Water quality measurements of temperature, oxygen and pH were measured weekly (ProODO Optical Dissolved Oxygen Meter, YSI Inc, USA; pH/mV-meter, WTW pH 315i, Germany). Water samplings from the copepod tank, biofilter, and cop algae tank was performed on day 1, 4, 8, 15, 22 and 32 for analysis of dissolved nitrogen waste products. The samples were collected at 10.00 at the same sampling points. Samples (2 mL) for PON analysis were collected on day 1 and 32.

2.4 Statistical analysis

Statistical analyses were conducted using SigmaPlot™ 12.0 (SigmaPlot, Systat Software Inc., USA). Tables were made in Microsoft Work for Mac OS X (Microsoft Coorporation, USA). Graphs were made in SigmaPlot™ 12.0.

Environmental parameters (dissolved oxygen, temperature, salinity, pH, dissolved CO_2 and conductivity), nitrogenous waste products (NO_2^--N, NO_3^--N, TAN, and NH_3-N), PON, and specific growth rate (SGR day^{-1}) were tested for significant differences. Statistical analysis was performed at the 95% confidence level ($p < 0.05$). One way ANOVA, Kruskal-Wallis one way ANOVA on ranks, and one way ANOVA Holm-Sidak, was used to compare the amount of variation and significant differences of the means of three of more samples for numerical data, non-numerical data, and pairwise comparisons respectively. A Dunn's test was used as a post-hoc test after rank-based ANOVA when the treatment groups were unequal. When dealing with only two water compartments, t-tests were used to compare the amount of variation and significant differences.

3 Results

3.1 Freshwater systems

3.1.1 RAS

Temperature, salinity, dissolved oxygen and pH showed only slight variations in water in to FT1-10 of the RAS during the days of study, as shown in Table 5. The concentration of dissolved CO_2 was considerably high. In the regulations relating to Operation of Aquaculture establishments ("*Akvakulturforskriften*") (Norwegian Food Safety Authorities, 2008) a threshold levels of 15 mg CO_2 L^{-1} is recommended. Table 5 presents the measured values of particular organic nitrogen (PON) measured from influent water to all fish tanks (FT1-10) and effluent water from a single fish tank (FT5). No significant differences in PON concentrations between the inlet and effluent water were found. Still, the mean value was higher in the effluent water from FT5. A decrease in concentration of PON was observed in both water sources during the period of study. The NH_3-N level in FT5 was significantly higher than in the influent water (t-test, $p < 0.001$).

Table 5 – Water quality parameters (MEAN±SE) in to FT1-10 and from FT1-10 in the RAS (n=5, n=6 for PON, n=15 for NH_3-N).

Parameters	In to FT1-10	From FT1-10
Temperature (°C)	-	13.9±0.1
Oxygen (%)	-	90.0±0.3*
Salinity (ppt)	-	2.3±0.09
pH	-	6.8±0.04
CO_2 (mg L^{-1})	-	23.4±0.90
NH_3-N (µg L^{-1})	0.89±0.13	2.9±0.22*
PON (mg N L^{-1})	0.3±0.03	0.4±0.02*

*Measured directly in FT5

Nitrogen dynamics

Fluctuations in concentrations of NO_2^--N, NO_3^--N and TAN in the influent water to all fish tanks (FT1-10) are presented in Figure 6.A, while water concentrations from FT5 are presented in Figure 6.B. Due to significant differences in concentration between the samplings performed at 09.00, 13.00 and 17.00 in both water compartments, all of the mean

values of the daily measurements are presented in Figure 6. The NO_2^--N and TAN concentrations in to FT1-10 showed a particular similar trend during the investigation, with fluctuations in the range of 0.52 – 1.81 mg L^{-1} NO_2^--N and 0.23 – 0.90 mg L^{-1} TAN. The highest measured concentrations of the two compounds were discovered in the first measurement taken at 0 hours. The concentration of NO_3^--N fluctuated between 54.0 mg L^{-1} and 63.3 mg L^{-1}.

Figure 6.B presents the dynamics of the measured concentrations of NO_2^--N, NO_3-N and total TAN in FT5. As seen in the influent water of FT1-10, similar trends of NO_2^--N and TAN concentrations were also found in this compartment. The concentrations fluctuated between 0.63 – 1.74 mg L^{-1} and 1.43 – 2.40 mg L^{-1} respectively, whilst NO_3^--N concentrations ranged between 39.7 mg L^{-1} and 48.7 mg L^{-1}. This means that lower NO^-_3-N concentrations were observed in FT5 compared with in to FT1-10.

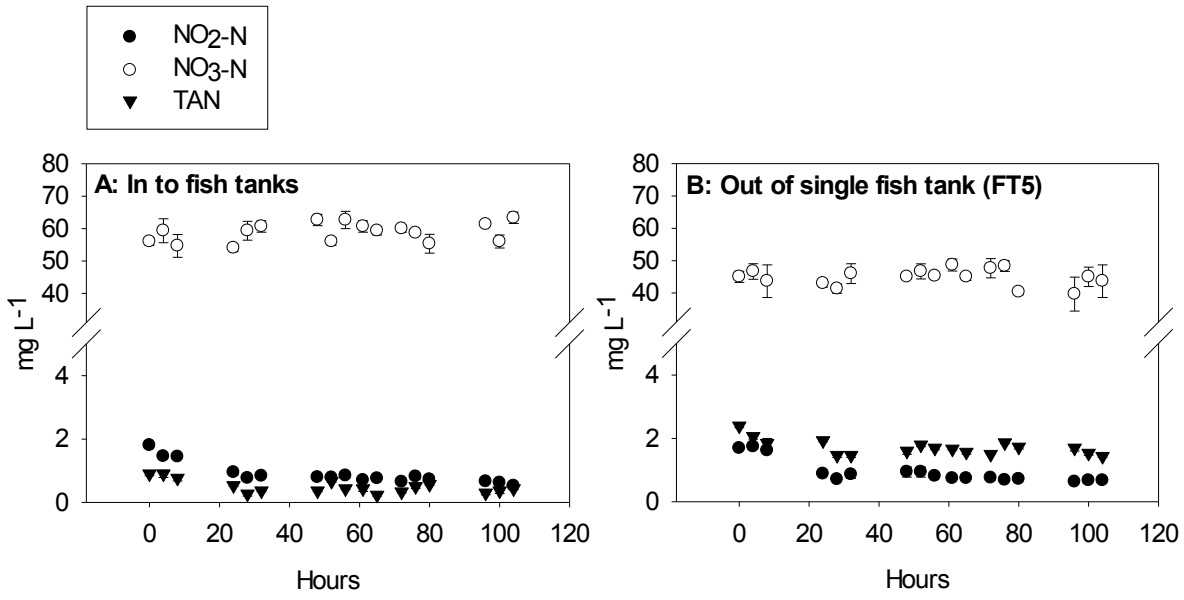

Figure 6 – Mean (±SE) measured values of nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and total ammonia-nitrogen (TAN) in influent water to FT1-10 (A) and effluent water from FT5 (B) in RAS.

To compare the dynamics of the two different water compartments, the concentrations of the different nitrogen compounds were tested for significant differences in variation on a day-to-day basis (t-test). On day 5 the NO_2^--N concentration in FT5 was significantly higher than in water flowing in to FT1-10 ($p = 0.035$). The NO_3^--N concentration in to FT1-10 was significantly higher than in FT5 on all days ($p < 0.001$), and at both night measurements

(p = 0.009 for 22.00, p = 0.001 for 02.00). TAN concentrations were found to be significantly higher in FT5 on all day and night measurements ($p < 0.001$). These results indicate higher waste production as TAN in the fish tank, compared to the influent water. NO_3-N was also dissipated in or on its way to FT5.

The night measurements were compared with the NO_2^--N, NO_3^--N, and TAN concentrations measured during day 3 (One way ANOVA and Kruskal-Wallis one way ANOVA on ranks). For NO_2^--N the compound was of a significantly higher concentration at 09.00 than at 22.00 ($p = 0.015$), measured in to FT1-10. Further, the same waste product was of a significantly higher concentration at 17.00 than at 22.00 and 02.00 in FT5 ($p = 0.003$, $p = 0.009$ respectively). No significant differences were found for any NO_3^--N measurements in any compartments. Meanwhile, significantly higher concentrations of TAN were detected in water in to FT1-10 for the measurements from 09.00 and 13.00 compared with the levels at 02.00 ($p = 0.047$, $p < 0.001$ respectively). No significant diurnal differences were found for TAN in FT5.

3.1.2 Hydroponics

Temperature, oxygen, pH and conductivity showed only slight variations in the collective tank (sump) of the hydroponic system, as shown in Table 6. Temperature was on average 24.6 °C and oxygen concentrations never fell below 6.23 mg L^{-1} saturation. The pH declined by 0.14 during the period of study. Conductivity increased by 0.055 on day 3 as a response to addition of fertilizer to the system.

Table 6 – Water quality parameters (MEAN±SE) in the sump of the hydroponic system (n=9).

Parameters	**Hydroponic sump**
Temperature (°C)	24.6±0.2
Oxygen (mg O_2 L^{-1})	6.2±0.04
pH	6.4±0.03
Conductivity	1.43±0.01
NH_3-N (µL^{-1})	> 0.3±0.2 *
PON (mg N L^{-1})	0.02±0.005

* Percentage of un-ionized ammonia for pH values < 6.5 not found. Presented values are for pH = 6.5.

Nitrogen dynamics

The concentrations of nitrogenous compounds are shown in Figure 7. The results indicate well-balanced addition of fertilizer with uptake and growth of *F. ananassa,* as no nitrogen compound seemed to accumulate in the system. NO_2^--N and TAN seemed to decrease in concentration during the first 30 hours, but increased with 0.007 mg L^{-1} and 0.39 mg L^{-1} respectively, from 30 to 51 hours after the investigation started. This rise happened in accordance with addition of fertilizer (0.4 L) after 47 hours. NO_2^--N and TAN increased with 0.007 mg L^{-1} and 0.39 mg L^{-1} respectively, from 30 to 51 hours after the start of the study. The NO_3^--N concentration showed a rise in concentrations after 27 hours and was the compound showing most fluctuations, ranging from 98.4 mg L^{-1} at 0 hours, 128.0 mg L^{-1} after 27 hours, and declined to 106.6 mg L^{-1} after 54 hours.

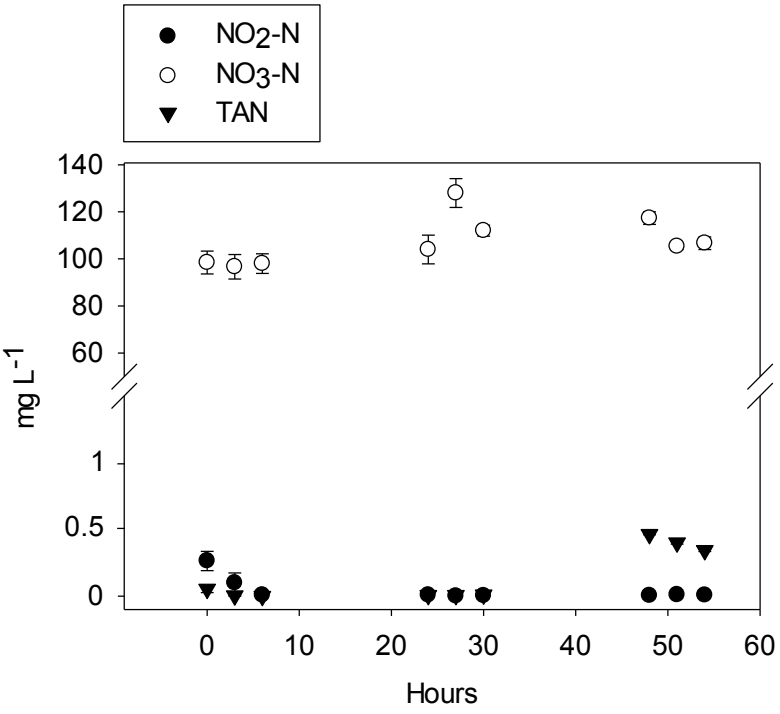

Figure 7 – Mean (±SE) measured values of nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and total ammonia-nitrogen (TAN) in sump of the hydroponic system.

It was tested whether significant differences in similarity were found on a daily basis and in the samplings performed at 09.00, 12.00 and 15.00 on the different days (one way ANOVA). Significant differences in NO_2^--N measurements were not found. However, for NO_3^--N the measurements on day 2 differed significantly from each other ($p = 0.008$). Also, the concentration of the same compound after 27 hours was significantly higher than all measurements from day 1, after 24 hours and 52 hours ($p \leq 0.004$, $p = 0.029$, $p = 0.045$

respectively). Measurements of TAN on day 3 differed significantly ($P \leq 0.003$), with highest measured values after 57 hours.

3.1.3 Aquaponics

Table 7 shows a summary of the physiochemical water quality of the aquaponics during the period of study. The temperature was similar and stable in all the compartments. There were differences in the variation of oxygen over time between the different compartments of the aquaponics system, with significantly lower oxygen concentrations in the water from FT1-4 compared with water from the hydroponics, sump, biofilter and in to fish (particle remover) ($p < 0.001$ for all comparisons). The pH was stable, and the CO_2 concentration was low and stable. No significant difference was detected for PON in the different compartments.

Table 7 – Water quality parameters (MEAN±SE) in the water from FT 1-4, hydroponic system, sump, biofilter and particle remover (in to FT 1-4) in the Aquaponic system (n=9).

Parameter	FT1-4	Hydroponics	Sump	Biofilter	In to fish
Temperature (ºC)	17.0±0.2	17.0±0.6	17.1±0.6	17.1±0.2	17.1±0.2
Oxygen (mg O_2 L^{-1})	9.2±0.10	9.7±0.05	9.7±0.05	9.7±0.06	9.7±0.04
pH	-	-	6.9±0.02	-	-
CO_2 (mg L^{-1})	1.4±0.3	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
NH_3-N (µL^{-1})	0.4±0.03	0.2±0.02	0.3±0.03	0.2±0.02	0.2±0.01
PON (mg N L^{-1})	1.2±0.2	0.9±0.2	1.5±0.3	1.1±0.2	1.1±0.3

Nitrogen dynamics

The levels of NO_2^--N, NO_3^--N and TAN showed stable values throughout the period of study, as presented in Figure 8. To compare the dynamics in the five different water compartments, the concentrations of the different nitrogen compounds were tested for significant differences in variation on a day-to-day basis (one way ANOVA, Holm-Sidak). The mean value of NO_2^--N was found to be significantly lower in the biofilter compared to all the other compartments (0.13 mg L^{-1}, $p < 0.001$), while the highest mean value was detected in water from the hydroponics (0.175 mg L^{-1}). The highest measured mean value of the NO_3^--N was found in the water from the particle remover (91.8 mg L^{-1}), while the lowest measured mean value was found in the water from the hydroponics (86.9 mg L^{-1}). The mean TAN concentration in the water from FT1-4 (0.197 mg L^{-1}) was significantly higher than the mean values in water from

the hydroponics, the sump, the biofilter, and the particle remover ($p < 0.001$ for all respectively), meaning all other compartments. The test also indicated that the mean TAN concentration in the sump (0.12 mg L^{-1}) was significantly higher than water from the biofilter, the hydroponics, and the particle remover ($p < 0.001$ for all respectively). The lowest measured mean value of TAN was found in the water from the biofilter (0.0761 mg L^{-1}).

The night measurements from each compartment were compared with the NO_2^--N, NO_3^--N and TAN concentrations measured in the same compartment during day 4 to assess whether diurnal variation could be found (t-test). It was found higher NO_2^--N values at night for water from FT1-4, the hydroponics and biofilter ($p = 0.001$, $p = 0.088$, $p = 0.009$ respectively), while significantly lower NO_2^--N values were measured in the water from the particle remover during night ($p = 0.006$). No significant difference was detected for NO_3^--N in any compartment. Significantly higher TAN values during night were measured in the water from the hydroponics and in the sump ($p = 0.035$, $p < 0.001$ respectively).

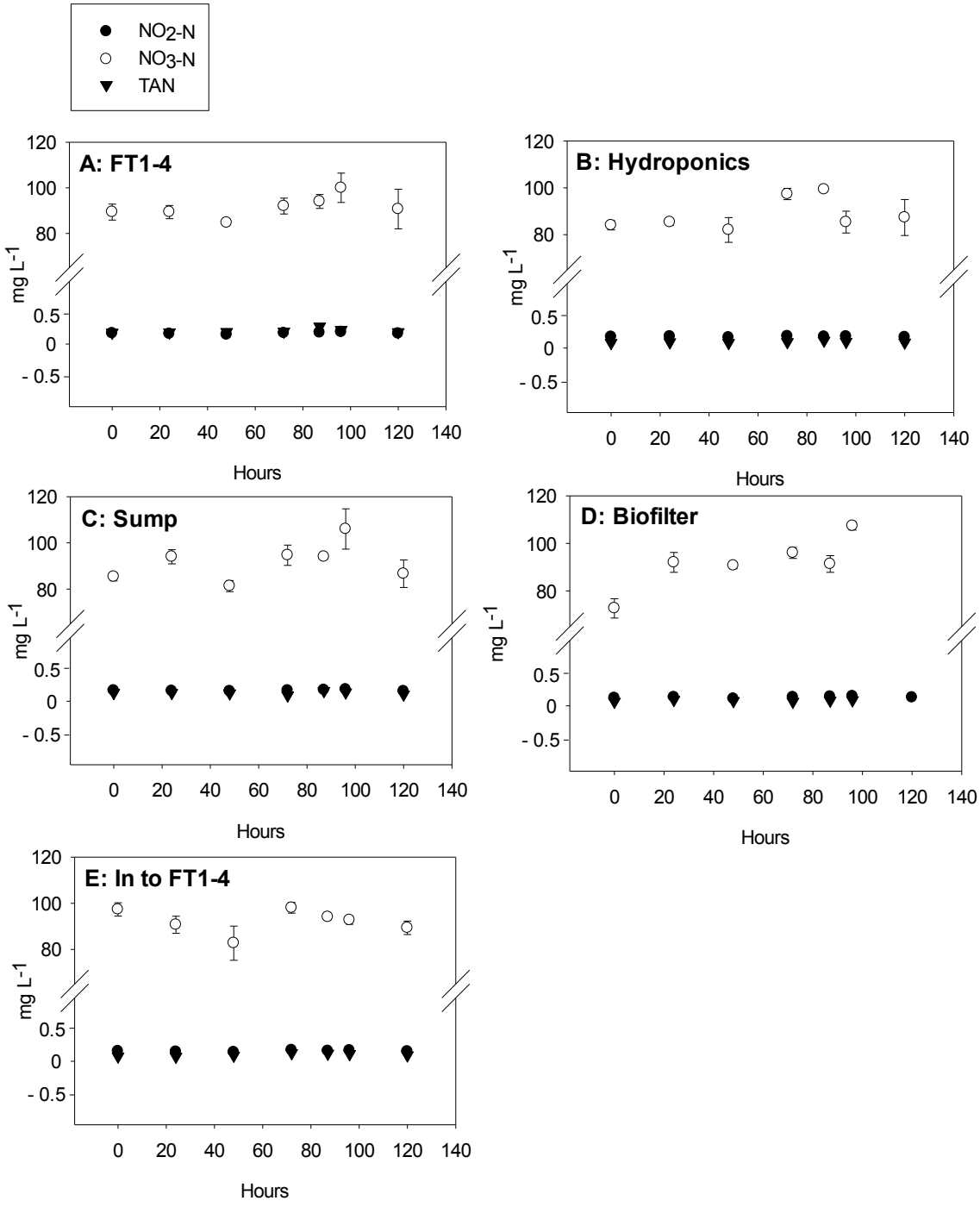

Figure 8 – Mean (±SE) measured values of nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and total ammonia-nitrogen (TAN) in water from FT1-4 (A), from hydroponics (B), the sump (C), the biofilter (D), and influent water to fish tank (particle remover) (E) in aquaponics.

3.2 Water quality in seawater systems

3.2.1 Batch experiment

Table 8 presents the measured water quality parameters in the copepod tank and biofilter ahead of the experiment, and PON concentration on the final day of cultivation in all water media.

Table 8 – Water quality parameters in the water in copepod tank (CT) and biofilter (BF) concurrent with sampling for the batch experiment (n=1). PON sampling was performed in all media (n=3).

Parameter	CT	BF	CON	SW
Temperature (ºC)	22.3	21.6	Not measured (N. m)	N. m.
Oxygen (mg L^{-1})	5.02	5.86	N. m.	N. m.
pH	7.7	7.8	N. m.	N. m.
CO_2 (mg CO_2 L^{-1})	3.0	2.0	N. m.	N. m.
Salinity (ppt)	25.0	25.0	N. m.	N. m.
NH_3-N (µg L^{-1})	15.0	2.0	N. m.	N. m.
PON (mg N L^{-1})	9.24±1.1	12.2±2.5	8.62±1.1	1.27±0.3

The pH was measured in each bottle throughout the experiment, to assure optimal input of CO_2 and pH conditions. As presented in Table 8, the pH was stable and variation between the cultures was small.

Table 9 – pH (MEAN ± SE) in the bottles containing water from CT, BF, CON, and SW in the batch experiment (n=15 for CT, BF and CON, n=9 for SW).

Parameter	CT	BF	CON	SW
pH	8.4±0.10	8.3±0.03	8.3±0.10	8.1±0.02

Cell quality

The red color of the *R. baltica* cultures weakened throughout the experiment, changing first from red to light brown, and then to and yellow. This change in color was reported for the cultures in CT (day 3), BF (day 3) and CON (day 4). The bottles containing seawater (SW) never expressed a red color from the very beginning, likely due to low cell density.

Specific growth rate

The growth of *R. baltica* (cells mL^{-1}) in CT, BF, CON and SW is presented in Figure 9. Since a filter of 10µm mesh size was used to remove particles and biological matter from the water, algae cells from CT and BF remained in the water. As presented in Figure 5, a significantly higher amount of cells mL^{-1} was therefore measured in CT and BF after 24 hours, compared with CON and SW ($p < 0.001$, one way ANOVA). Specific growth rate (SGR) was calculated for the exponential phase of microalgae growth between 48 and 72 hours. SGR of *R. baltica* varied in the different cultivation media. With a SGR of 0.13 the microalgae in CON exhibited the highest growth rate. Further, the BF supported a slightly higher SGR compared with growth in CT (0.11 and 0.10 respectively). A negative SGR was found for SW (-0.01). After 72 hours the SW cultures were terminated. After 96 hours BF exhibited a significantly higher density (cells mL^{-1}) compared with CT and CON ($p < 0.001$). No significant difference in density was detected for measurements after 72 and 120 hours.

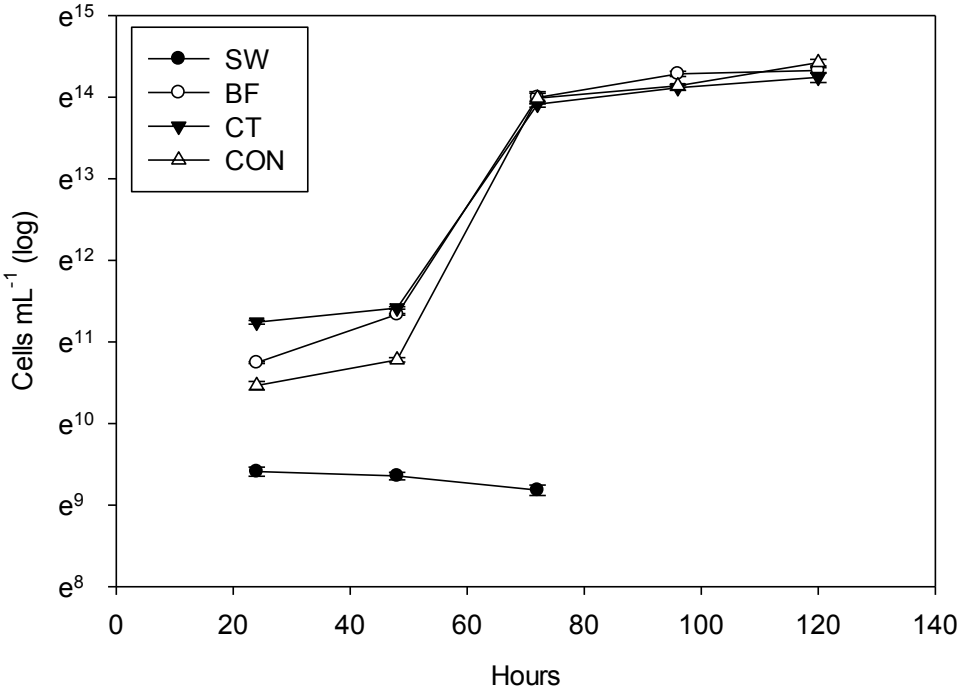

Figure 9 – Growth performance of *R. baltica* in seawater (SW), water from biofilter (BF), copepod tank (CT), and Conwy medium (CON) (n=3).

Nitrogen dynamics

To compare the basis of the different media, the nitrogen compounds were tested for significant difference in concentration (one way ANOVA). As presented in Figure 10.A, the

CT culture contained highest mean NO_2^--N concentration in the start of the experiment (0.65 mg L^{-1}) and was significantly higher than all other water media ($p < 0.001$). SW contained the lowest mean NO_2^--N concentration, and was significantly lower than BF and CON as well ($p = 0.005$, $p = 0.015$ respectively), as illustrated in Figure 10.D. Regarding NO_3^--N, the highest mean value was found in CON (8.3 mg L^{-1}), see Figure 10.C. This concentration was significantly higher than in COP, BF and SW ($p < 0.001$, $p = 0.001$, $p < 0.001$ respectively). BF supported the second highest NO_3^--N-concentration (mean value 6.3 mg L^{-1}, Figure 10.B). Meanwhile, SW had the significantly lowest mean NO_3-N concentration (0.4 mg L^{-1}, $p < 0.001$).

The concentration of TAN differed significantly between the groups ($p = 0.017$) with zero detection in CON and a mean start value of 0.8 mg L^{-1} in CT. The total nitrogen content (Total N, the sum of NO_2^--N, NO_3^--N and TAN) was highest in CON on day 1 (8.4 mg L^{-1}), and 6.7 mg L^{-1}, 6.5 mg L^{-1} and 0.49 mg L^{-1} in CT, BF and SW respectively, on the same day.

The degree of removal of NO_2^--N, NO_3^--N, TAN and total N was calculated as percent removal from the cultures. Table 10 indicates greatest decrease of NO_2^--N and TAN in CT, while NO_3^--N was removed in a highest degree in CON. The SW cultures had a negative removal percentage, indicating a release of nutrients instead of uptake. Both CT and CON experienced a 95% decline of total N during the cultivation period.

Table 10 – Mean values (±SE) of decrease (%) of NO_2^--N, NO_3^--N, TAN and total N (mg L^{-1}) in *R. baltica* cultures containing water from CT, BF, CON and SW in the batch experiment (n=3).

	NO_2-N	**NO_3-N**	**TAN**	**Total N**
Media	% removal	% removal	% removal	% removal
CT	99.6±0.1	91.4±0.3	96.8±3.2	95.9±1.2
BF	95.4±0.1	88.1±2.7	63.1±12.1	82.2±4.1
CON	96.9 ± 0.2	94.4±0.2	0	95.6±0.1
SW	-122.2±72.2	-18.5 ±26.7	-5.6±33.8	-48.8±32.4

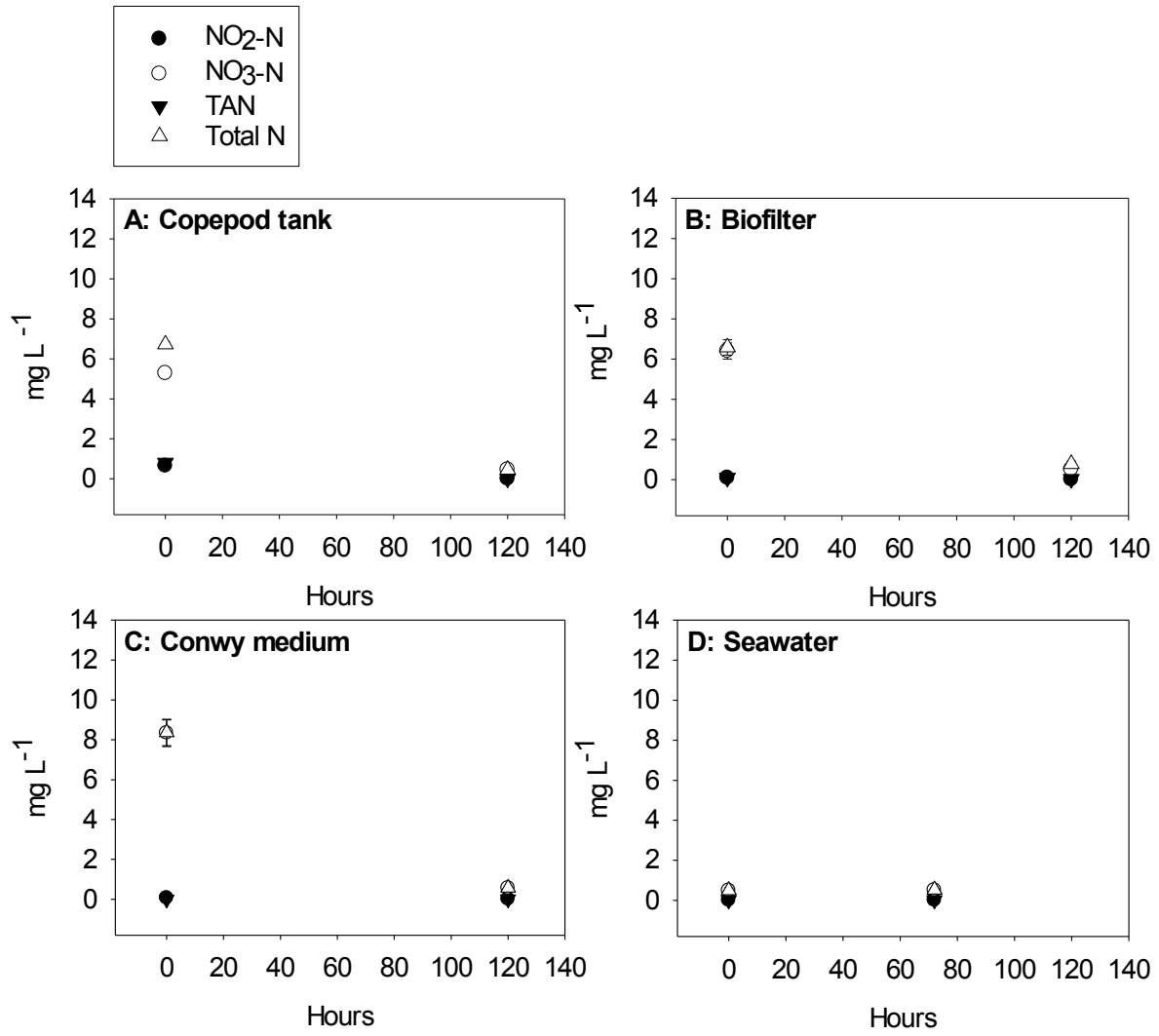

Figure 10 – Mean (±SE) measured values of nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), total ammonia-nitrogen (TAN), and total nitrogen (Total N) in water from CT (A), from BF (B), CON (C), and SW (D) in the batch experiment. Total nitrogen is a summary of NO_2^--N, NO_3^--N and TAN on the different days, with denoted SE.

3.2.2 Copeponics

A summary of the physicochemical water quality parameters in the three compartments of Copeponics is presented in Table 11. Water quality parameters measured from January to December 2015 in a copepod tank (Control CT) with flow through system are added to Table 11, in order to compare water quality in the normal production tank to the recirculation system. In Copeponics, the salinity rose throughout the period of study, from 24.0 ppt to an average level of 28.3 ppt for all compartments. A significant difference in temperature was observed between AT and both CT and BT respectively ($p < 0.001$), with highest measured values in the AT. The oxygen concentration was similar and high (> 5.2 mg L^{-1} saturation) in all compartments, but decreased in CT and BF as more biomass was added to the system. The

pH was similar and stable, whereas a small reduction was observed in CT and BF during the last 10 days (from 8.2 to 7.6, and 8.3 to 7.8 respectively). The water quality parameters in copeponics were similar to the conditions in the normal production system.

Table 11 – Water quality parameters (MEAN±SE) in the water from copepod tank (CT), biofilter (BF), algae tank (AT) and a flow-through copepod tank (Control CT) (n=6 for all water parameters in CT, BF and AT, n=3 for PON in AT and Control CT, and n=13, n=18, n=17, n=16 for O_2, temperature, pH and salinity in Control CT respectively).

Parameter	CT	BF	AT	Control CT
Temperature (ºC)	22.2±0.2	21.7±0.2	24.2±0.5	22.8±0.2
Oxygen (mg O_2 L^{-1})	6.4±0.30	7.0±0.01	6.3±0.20	6.7±0.4
pH	8.0±0.2	8.1±0.1	8.2±0.1	7.9±0.1
Salinity (ppt)	25.7±0.8	26.2±0.9	26.7±1.2	27.8±0.6
NH_3-N (µg L^{-1})	22.0±13.0	1.0±0.50	0.28±0.20	-
PON (mg N L^{-1})	0.39±0.069	0.31±0.082	3.2±0.15	0.40±0.039

Particulate organic nitrogen (PON) increased significantly in the CT during the investigated production period ($p = 0.002$). Further, the BF showed an opposite development, with a significantly lower PON concentration in the end of the study ($p = 0.038$). To study differences between nitrogen dynamics in the RAS system of copeponics, the PON concentration from CT was compared with PON levels of an extra external copepod tank (named **control CT**) with water flow through system. The level of PON in CT was higher than compared with the control CT, but no significant difference between the groups was found ($p = 0.071$).

Cultivation of R. baltica

The Copeponic system was run as a fully integrated system for the first two days, where AB1-3 received nutrients from the BF2 only (Figure 5). On day 3, AB1-3 continued being supported with water from BF, but 0.7±0.1 mL Conwy media L^{-1} seawater (MEAN±SE, n=34) was additionally supplied in order to secure optimal quality of the *R. baltica* cells. Until day 7, the copepods received algae from AT1-3 only. From this day, additional algae were supplied occasionally (day 7-10, 13-19, 22-32) from external *R. baltica* cultures due to insufficient production in AB1-3, in the amount of 145.3±18.8 L (MEAN ±

SE, n=17). AB1 was emptied on day 7, as for AB3 on day 11 and AB2 on day 15. AB1 and AB3 were started up again on day 15, while AB2 was starter up again on day 16.

Cell density

The cell density of *R. baltica* (MEAN±SE) was $3.1\times10^4 \pm 4.4\times10^3$ cells mL^{-1} in CT, $3.5\times10^4 \pm 5.2\times10^3$ cells mL^{-1} in BF, and $1.1\times10^6 \pm 1.5\times10^5$ cells mL^{-1} in AT throughout the production of Copeponics. The feed/algae volume (MEAN±SE) added to CT during low biomass (ca. 3 million copepods) cultivation was 134.0 ± 11.2 L, and 292.0 ± 13.7 L during high biomass (ca. 10 mill copepods) cultivation.

Nitrogen dynamics

Dynamics of NO_2^--N, NO_3^--N and TAN in Copeponics is presented in Figure 11: A, B and C respectively. The concentration of NO_2^--N was significantly different between the compartments of the copeponics system ($p < 0.01$, Dunn's test) with the highest mean value in AT (0.18 mg L^{-1}). Both CT and BF had stable and low levels of NO_2^--N, but a rise was measured on day 32 in CT (0.4 mg $L^{-1)}$. Except for that, low NO_2^--N values were experienced ($\leq$ 0.09 mg L^{-1}, $\leq$ 0.04 mg L^{-1} respectively).

Likewise, the mean value of the NO_3^--N concentration was significantly higher in AT (13.2 mg L^{-1}) and differed from CT and BF ($p < 0.01$, Dunn's test). A gradual accumulation of NO_3-N was measured in both CT and BF. The sensor data presents a detailed picture of increasing NO_3-N concentration in BF until day 20 (around 10 mg NO_3^--N L^{-1}), followed by an immediate decrease to 5.4 mg L^{-1} on the last day of sensor measurements (day 25). No significant difference between sensor and manual measurements was detected (t-test). Highest degree of fluctuations of NO_3-N was found in AT, with a peak on day 15. The NO_3-N levels were significantly higher in the BF than in the CT ($p = 0.001$, Dunn's test).

Regarding TAN, the mean value was significantly higher in CT (0.25 mg L^{-1}) compared with BF and AT ($p < 0.001$, Dunn's test). There was a peak in the concentration of TAN on day 15 (2.3 mg L^{-1} TAN), probably reflecting the addition of 7 million more copepods to the system the day before. Otherwise, the concentration of TAN was stable and low in all compartments throughout the period of study (0.7 mg L^{-1}).

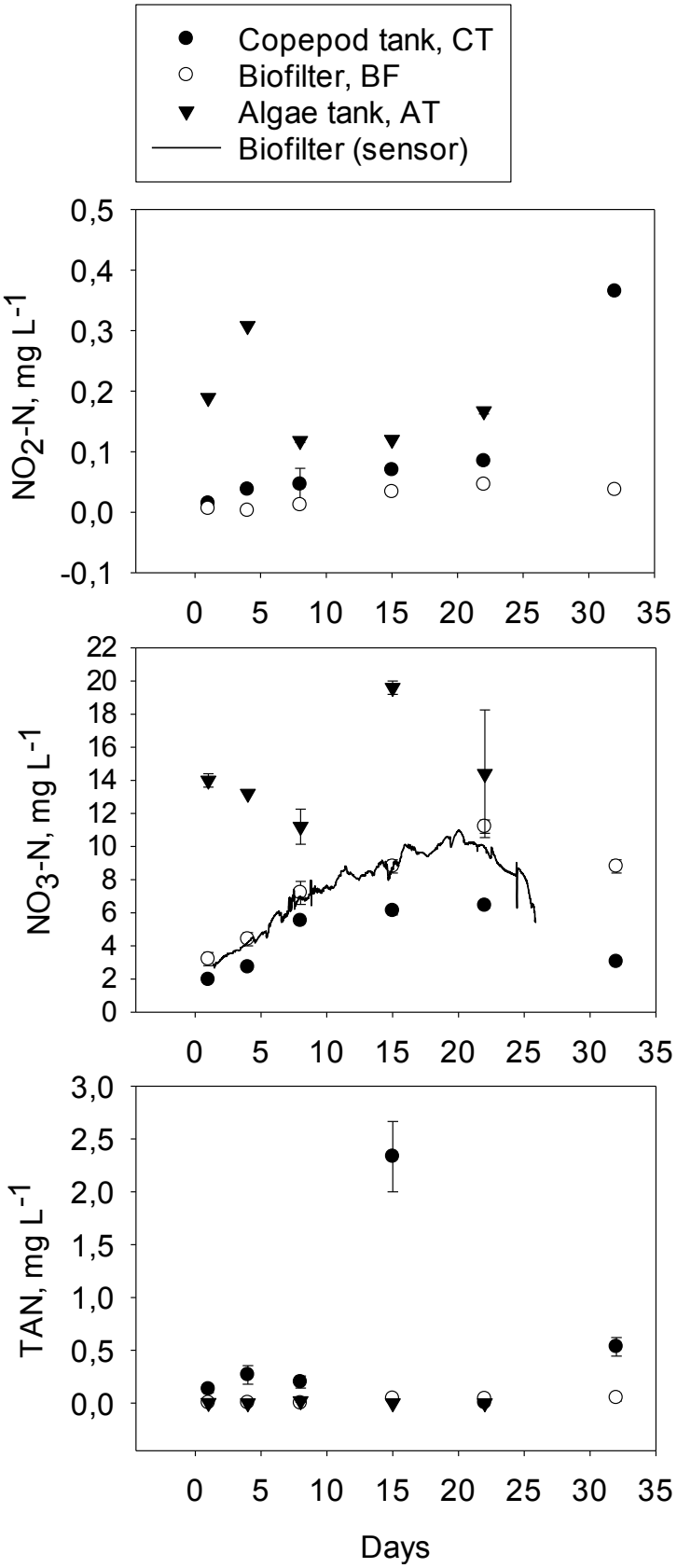

Figure 11 – Mean (±SE) measured values of nitrite-nitrogen, NO_2^--N (A), nitrate-nitrogen, NO_3^--N (B), and total ammonia-nitrogen, TAN (C), in water from CT, BF, and AT in the copeponics (n=3 for manual measurements).

4 Discussion

4.1 Freshwater systems

4.1.1 RAS

Phytoremediation as a wastewater treatment strategy in RAS

The results indicate stable and high dissolved nitrogen levels in the wastewater of RAS during intense smolt production. Nitrate constituted the greatest part of the total dissolved nitrogen fraction. As this is the preferred nitrogen source of plants (Marschner, 2011) and a main component in algae fertilizers (Walne, 1979, Andersen, 2005), the nitrate content of the aquaculture wastewater indicates its present potential for further use as fertilizer in plant and algae production. Removal of NO_3^- by denitrifying biofilters is common for intensive RAS with high biomass production (Van Rijn et al., 2006). The denitrification unit of the RAS in this respective study, converted dissolved NO_3^- to N_2 gas from a side stream (180 L min^{-1} in department B). The removal efficiency was not measured, but it has a potential to remove 200-300 mg NO_3^- L^{-1} (ca. 45-68 mg NO_3^--N L^{-1}) during full production of smolts (pers. comm. Nils Ole Klevjer, Marine Harvest). However, incorporation of a hydroponic system and removal of NO_3^- through a second biomass production is reported to comply better with the requirements of sustainable aquaculture (Waller et al., 2015).

The RAS in this study discharges 3-5 m^3 of rinsed water every day. Measurements of nitrogen in the outlet water were not performed. However, since the discharged water goes through a denitrification process ahead of deposit, the nitrate concentration in the outlet water should be close to zero (pers. comm. Jesper Lund, Aquatec Solutions). TAN and NO_2^- was detected in the water from the biofilter, and hence some is predictably present in the outlet water. Despite low concentrations, this might still have an effect on the natural cycle in the local coastal environment and stimulate excessive phytoplankton production (Martins et al., 2010). Phytoremediation of the aquaculture wastewater can assist in lowering adverse environmental impacts (Ghaly et al., 2005).

Besides ecological impacts, the discussion brings along an aspect on use of global resources. In denitrification, NO_3^- is converted to N_2 gas (Van Rijn et al., 2006). The fertilizer industry converts atmospheric nitrogen (N_2 gas) into NH_4^+ (the Haber-Bosch process) (Kitano et al., 2012), a practice consuming >1% of the global power production (Kitano et al., 2012). As

both NH_4^+ and NO_3^- is readily present in the wastewater of RAS systems as an unwanted waste product, re-use of nitrogen from waste-streams for bio-producing purposes appears to be logical for a sustainable resource management in aquaculture. The nitrogen flux in the RAS investigated in this study complies with nitrogen requirements of plant production in hydroponics. The concentration of NO_3^- in the RAS was ca. 200 mg L^{-1} or more (different between the measurements of inlet and outlet water of the fish tank), whereas hydroponic solutions contains 49-210 mg NO_3^- L^{-1} (Jones Jr, 2004). The ammonium concentration in hydroponic fertilizers generally ranges 0-154 mg NH_4^+ L^{-1}. The amount of TAN available as NH_4^+ in RAS depends on salinity, pH and temperature of the water (Bower and Bidwell, 1978, Trussell, 1972), and TAN will be converted to nitrate through nitrification. The TAN levels of the treated (nitrified) water in the RAS ranged 0.2-2.3 mg L^{-1}, and the concentration was highly dependent on the metabolic activity of the fish. If all the TAN in the RAS were to be available as NH_4^+, the same range could be expressed as 0.25-3.0 mg L^{-1}, indicating low but adequate NH_4^+ concentrations for hydroponic use. In general, extra micro- and macronutrients vital for horticultural survival must normally be supplied with the recirculated process water to endure proper growth of the plant or algae used in phytoremediation (Waller et al., 2015).

Implementation of a plant or algae production area instead of a denitrification unit has been reported in previous studies (Buhmann et al., 2015, Schneider et al., 2005, Deviller et al., 2004), and pushes aquaculture production strategies towards management approaches of aquaponic systems. Moreover, a study on integrated production containing similar fish density (ca. 70 kg m^{-3}) and feed load (0.9-1.0 kg m^{-3}, Table 2) as the RAS in this respective study has not been obtained. The Aquaponics studied in this thesis reports a fish:plant ratio of 1:4.3 (Skar et al., 2015). In order to fully replace a denitrification unit, sufficient biomass of plants or algae is required for efficient nutrient removal and wastewater treatment. If taking into consideration the production ratio of the Aquaponics, the hypothetically associated phytoremediation area of RAS would be tremendous. It would undoubtedly require a larger area in comparison to a denitrification unit.

Unidentified removal of NO_3^- in RAS

Significantly higher levels of NO_3-N were measured in the influent water of the fish tank (Figure 6.A) than in the fish tank (Figure 6.B). As much as 24.0±1.45% mg NO_3-N L^{-1} (MEAN±SE, n=15) was on average removed from the water on its way from the biofilter to

the fish tank (Figure 2, 7-11). The removal of NO_3-N is noticeably high and strongly indicates established microbial or eukaryote consumers in the system, as none of the compartments between the biofilter and fish tank aim to remove NO_3^-. A biofilm is a multispecies microbial community, with associated bacteria and microalgae (Flemming and Wingender, 2010). Biofilm is present in the biofilter of RAS, but may also be present on surfaces (e.g. pipes and fish tanks) in systems with low hydraulic retention time, and are normally found on all components of an aquaculture system (Blancheton et al., 2013). The microbes found in biofilms are sorted in layers, with different bacterial community members with associated nutritional requirements (Schreier et al., 2010). Further, eukaryotic organisms such as algae, fungi and rotifers can also be found in the water of RAS and have complex filtration strategies and feed sources (Schreier et al., 2010). As NO_3^- is a an important source for nitrogen in microalgae (Andersen, 2005), ongoing NO_3^- assimilation by settled microalga species is a likely explanation to the high NO_3^- removal.

Bacteria may also consume NO_3^- as a source for nitrogen (Lananan et al., 2014). Microalgae will consume CO_2 and produce O_2 through photosynthesis (Lananan et al., 2014), while bacteria will compete with both fish and nitrifying bacteria for O_2 (Blancheton et al., 2013). Fast growing heterotrophic bacteria (r-strategists), often found associated with the outer layer of the biofilm, are superior O_2 consumers (Elenter et al., 2007). Reduced O_2 concentration in the water may in turn reduce the nitrification efficiency and hence lower water quality (Elenter et al., 2007). Moreover, established biofilm has the potential to host pathogenic and opportunistic bacteria and in turn cause outbreaks of diseases if released to the water (King et al., 2004). For biosecurity reasons, the NO_3^- removal in the RAS should be further investigated in order to clarify whether this is beneficial or potentially detrimental for the fish. Measurements should also be performed in each fish tank or in the common outlet (Figure 2) in order to clarify whether the removal yields for all fish tanks or only for fish tank 5.

General physicochemical water quality conditions

Of all the studied recirculation systems, the RAS exhibited the largest production volume (Table 1 and Table 3). This regards water volume, biomass production, and feed load to the system. Compared to the other investigated systems in this study, the RAS is a commercial production system producing millions of smolts yearly. Water renewal rates of ca. 1% of the total system volume day^{-1} induces production stability (Orellana et al., 2014), but demands frequent control of water quality parameters. Dissolved oxygen and pH were within the

national recommendations for land based salmon production (Norwegian Food Safety Authority, 2008). The measured dissolved carbon dioxide concentrations were high (24-25 mg CO_2 L^{-1}) and exceed the national recommended criterion for smolt production (CO_2 concentration <15 mg L^{-1}) (Norwegian Food Safety Authority, 2008). The carbon dioxide concentration in smolt farms can be within the range 10-25 mg L^{-1} during spring time, simultaneous with the smoltification of the salmon (Fivelstad, 1999). This corresponds to the situation in the RAS, which also held high production densities. A lower CO_2 concentrations is highly recommended for *S. Salar* to avoid chronic sub-lethal concentrations and to lower the risk of nephrocalcinosis (Fivelstad et al., 2003).

Elevated NO_2^--N and TAN levels on day 1 of the investigation (Figure 6) might be associated with handling, sorting and holding during vaccination of the fish that day. These practices may have led to stress and increased metabolic activity in the fish (Portz et al., 2006), and increased release of metabolic waste products to the water. Except from this, the fish were fed on a continuous light and feeding regime and the daily and annual variations in metabolite production rates were small. The recommended upper ammonia level in Norwegian regulations is 2 mg L^{-1}. It is not specified if this regards TAN or toxic NH_3. The TAN levels in RAS were > 2.0 mg L^{-1} on day 1, but < 2.0 mg L^{-1} the following days. The NH_3-N levels (Table 5) in both influent and effluent water of the fish tank were low and not toxic to *S. salar*, according to recommendations from previous studies on NH_3 toxicity on *S. salar* (Fivelstad et al., 1993, Kolarevic et al., 2013). The long-term effect of ammonia on Atlantic salmon in freshwater is not well studied and should be further investigated.

Treated and in-tank water contained similar NO_2^--N concentrations (Figure 6). A concentration of 0.1 mg nitrite L^{-1} is the recommended threshold value in water of land based aquaculture systems by Norwegian regulations (Norwegian Food Safety Authority, 2008). The regulation does not specify is this value applies to NO_2^- or NO_2^--N, and examples using both 0.1 mg L^{-1} NO_2^--N (Terjesen et al., 2013) and 0.1 mg L^{-1} NO_2^- as threshold values has been obtained (Wedemeyer, 1996, Timmons and Ebeling, 2007). NO_2^--N measurements considers only the nitrogen fraction of the NO_2^- molecule, and hence a lower number will follow. Salmonids are among the fishes most sensitive to NO_2^- and show little species variation (Lewis Jr and Morris, 1986). Literature discussing concrete toxic NO_2^- levels for adult *S. salar* has not been obtained, but presence of Cl^- in the water is documented to counteract toxicity for the species (Eddy et al., 1983). NO_2^--N measurements were <0.1 mg

L^{-1} in both influent and effluent water of the fish tank during the period of study. A Cl^-:NO_2^--N ratio of 17 has been recommended for salmon in order to avoid NO_2^- toxicity (Kroupova et al., 2005). A mean salinity of 2.3 ppt was measured in RAS (ratio>17). Water pH can also influence nitrite toxicity (Wedemeyer, 1996), but the pH obtained in the RAS encounters the possible production of nitrous acid (HNO_2) (Wedemeyer, 1996). Low mortality numbers during the days of the investigation was also reported for the intensive production system (9 individuals, >99% survival). Hence, the NO_2^--N level might not have been harmful to the salmon. Maintaining a proper water quality for the fish in recirculation systems with production of high fish biomass and low water dilution rates is a challenge for fish producers. However, lower concentrations of NO_2^--N are recommended, as they exceed recommended threshold levels.

National recommendations on optimal NO_3^- concentrations in land based aquaculture systems are not given. Suggested guidelines on safe NO_3^- concentrations are gaping, with a range from 1 mg L^{-1} (Wedemeyer, 1996) to 400 mg L^{-1} (Timmons and Ebeling, 2007). Measured NO_3^--N values in the fish tank of the RAS ranged from 39.7 to 48.7 mg L^{-1} and were only within the recommended level recommended by Timmons and Ebeling, 2007. Moreover, juveniles of *S. salar* have been suggested to be insensitive to nitrate, in terms of growth, in NO_3^- -N concentrations up to 101.8 mg L^{-1} (Freitag et al., 2015). This indicates measured NO_3^- -N concentration in the RAS to be within the tolerable level for Atlantic salmon.

4.1.2 Hydroponics

Cultivation in closed recirculation systems occupies many advantages: Reduced use of pesticides, lowered run-off of nutrients, and reduced soil fungi and bacteriosis compared with conventional soil-based cultivation (Cecatto et al., 2013). It also allows for year-round production (Cecatto et al., 2013). Horticultural production in hydroponics is environmentally friendly, but requires species-specific management and adaptation of cultural management to the conditions of the system (Gruda, 2009). Water quality parameters in the Hydroponics were stable and satisfactory, according to recommendation found in literature (Sardare and Admane, 2013, Roberto, 2004, Jones Jr, 2004), and arranged for proper growth of the strawberry plants. Hence, the management of the Hydroponics was likely to be sufficient. pH normally changes in hydroponic systems in response to plant growth (Sardare and Admane, 2013), and may reason for the observed pH-decline of the Hydroponics in this respective

study. As this was a short-term investigation, growth and fruit production was not monitored. Meanwhile, high performance was reported in the end of the long experiment, indicating adequate environmental conditions for the plants (pers. comm., Irene Karoliussen, CIRiS).

Nitrogen dynamics

As mentioned, NO_3^- is considered the most important N-source in plants, and is normally found in the range 100-200 mg L^{-1} in most hydroponic nutrient solutions (Jones Jr, 2004). In this study NO_3^--N was found in the range 97-128 mg L^{-1}, equivalent to 429-566 mg L^{-1} NO_3^-. Hence, the hydroponics contained particularly high levels of NO_3^-. The plants absorbed NO_3^- by some extent, but high amounts of unutilized NO_3^- were present. Nitrate is the main component of the fertilizer recipe used in the Hydroponics (Appendix 4). Reduced consumption might be economically favorable. The stable levels of all dissolved nitrogen compounds indicates a proper irrigation strategy, where fertilizer addition is well balanced to plant uptake and nitrogen demands.

Re-use of aquaculture wastewater in hydroponics

The waste stream from fish production has the potential to become a crop production asset (Tyson et al., 2011). Furthermore, fertilizer cost can range from 5 to 10% of the total crop production expenses due to the high amount of fossil fuels needed in fertilizer production (Hochmuth and Hanlon, 2000). A further increase in fertilizer expenditures is expected (Hochmuth and Hanlon, 2000), and hence use of excess nutrients from wastewater streams can become profitable for the horticultural producer. An important question is if production of large quantities of plants will be in the interest of the fish producer. Therefore, a so-called decoupled aquaponics strategy, where filtrated nutrient rich wastewater from RAS is transported to hydroponic facilities, should be investigated. Studies where aquaculture wastewater is combined in separate small-scale hydroponic systems have been reported with various plant species (Takeda, 1997, Hess-Erga et al., 2013, Snow and Ghaly, 2008, Gjesteland, 2013). Direct discharge of fish wastewater from RAS to the surrounding environment of a RAS facility is an easy and cheap removal strategy for the fish producer. Still, more sustainable approaches should be investigated. Re-use of wastewater in production of crops with potential for further use as fish feed (Ghaly et al., 2005) has been suggested a economical advantageous strategy for fish producers.

Moreover, this study focused on nitrogen dynamics only. It is possible to grow crops in wastewater from RAS, but presence of other micro- and macronutrients in the effluent water should be investigated, e.g. presence of phosphorous (P), potassium (K), calcium (Ca^{2+}), manganese (Mn), sulphur (S), and iron (Fe) (Jones Jr, 2004, Rakocy et al., 1997, Adler et al., 1996), together with presence of pathogens and diseases. The salinity of 2-3 ppt in the RAS wastewater in this respective study could potentially cause salinity stress in production of *F. ananassa*, followed by reduced plant growth and dehydration (Pessarakli et al., 1989). This has been reported in a study on re-use of wastewater from a salmon fish farm in hydroponic lettuce (*L. sativa*) production (Gjesteland, 2013). Waller et al. (2015) integrated production of European seabass and salt-tolerant halophytes. In comparison to halophytes, barley (*Hordeum vulgare*) is used in both human food and animal feed (Katerji et al., 2006), and has proven to tolerate cultivation in saline environments (Maas and Hoffman, 1977). Hence, barley can be pinpointed as a potential candidate in integrated production in RAS effluents.

4.1.3 Aquaponics

The water quality parameters were stable throughout the period of study and within the recommendations by Norwegian regulations for salmonid production (Norwegian Food Safety Authority, 2008), with O_2 saturation >80% in outgoing water, pH > 6, CO_2 < 15 mg L^{-1}, and temperature within the range of 6-20°C. pH was 6.9 (±0.2), indicating low risks regarding accumulation of NH_3-N to toxic levels. The lower CO_2 concentration in the Aquaponics, compared with in the RAS, reflects the difference in fish biomass production and fish density in the two systems. One general challenge for aquaponic systems is accumulation of solids. A sampling campaign performed by NIBIO, Feedback Aquaculture, AqVisor AS and NIVA (16.01.2015-13.03.2015) reported adequate levels of suspended solids in the water (Skar et al., 2015) throughout the long-term experiment.

Performance of fish and plants

No mortalities of fish or plant and no occurrence of pests and diseases were registered in the period of study (and production throughout spring 2015). Meanwhile, Skar et al. (2015) monitored fish frowth in the aquaponics system in spring 2015 and reported better growth of *O. mykiss* (rainbow trout) compared with *S. trutta* (brown trout). *O. mykiss* were selected and bred for cultivation, while *S. trutta* were wild caught and bred for strengthening of wild stocks. If the aim in of the aquaponic production is to produce fish for sale, the price for

product can decide what species to cultivate. Good growth of lettuce was reported. The crispy salad was a sough after product in the local sales marked. A production sale volume of 89.8 kg lettuce (plant roots and bad quality plants not included) was reported (after 8 weeks of production) by (Skar et al., 2015). This indicates good performance of both fish and plants in aquaponics. Input of fish feed only supported healthy growth of the crispy salad, and the Aquaponics provided adequate water for integrated fish and plant production.

Nitrogen dynamics

The concentration of NO_2^--N remained stable in the Aquaponics at <0.18 mg L^{-1}. The lowest nitrite concentration was detected in the biofilter. This indicates that the biofilter is able to convert nitrite to nitrate, but not to fully avoid accumulation of NO_2^--N. Microorganisms in the system may also be present in the water, biofilm, particles or surface areas and hence produce NO_2^--N and explain presence of the compound in the system (Blancheton et al., 2013). This is normal in recirculation systems with low dilution rates (Blancheton et al., 2013). The plants could benefit from higher conversion of NO_2^--N to NO_3^--N, as NO_3^--N has potential to be utilized for growth and enhanced biomass production. The highest NO_2^--N concentration was detected in outflowing water from the plants (0.175 mg L^{-1}), suggesting zero or minimal uptake of NO_2^--N by the plants, in addition to presence of microorganisms generating NO_2^--N.

Studies report high expressed NO_2^- sensitivity in trout species (Williams and Eddy, 1986, Kroupova et al., 2005, Thurston et al., 1978). The mean NO_2^--N in Aquaponics concentration exceeds the already mentioned recommended levels for fish production in freshwater systems (Wedemeyer, 1996, Timmons and Ebeling, 2007). Species-specific data on nitrate-tolerance for *O. mykiss* (Kroupova et al., 2008, Russo et al., 1974, Russo et al., 1981) and *S. trutta* (Bartlett and Neumann, 1998) has suggested increased chloride (Cl^{-1}) concentrations to counteract nitrite toxicity. Prior to this investigation (in 2014), the Aquaponics facility experienced fish mortality due to nitrite toxification. This was managed by addition of chloride to the system (100 mg L^{-1}) through CaCl. A chloride concentration of 100 mg L^{-1} is recommended to avoid nitrite poisoning in recirculation systems (Svobodova et al., 2005), and had a good effect on the fish in the Aquaponic system.

The highest mean NO_3^--N value was measured in the particle remover, indicating a second nitrification process in this compartment, together with the biofilter. This extra nitrification

process may have been important in order to maintain a low level of NO_2^--N. Not surprising, the plants in hydroponics seemed to remove NO_3^--N as the lowest mean value was detected from the hydroponic outlet. Meanwhile, when comparing mean influent and effluent concentration of NO_3^--N in hydroponics, the results indicate ca. 5% removal of NO_3^--N in hydroponics. This suggests that approximately of 95% of the dissolved NO_3^--N was left unutilized for biomass production. Removal of NO_3^--N by lettuce has been reported in previous studies (Rakocy et al., 1993, Lennard and Leonard, 2005). On the other hand, Buzby and Lin (2014) reported ineffective (zero) removal of NO_3^- by *L. sativa* cultivated in waste water from *O. mykiss*. The plants in this study were supplied a mean NO_3^--N concentration of 91.3 mg L^{-1} (from sump), which equals > 400 NO_3^- mg L^{-1}. This is a higher concentration than reported by Lennard and Leonard (2005) and Rakocy et al. (1993), which was >11 and >22 mg NO_3^- L^{-1} respectively.

When calculating removal of TAN by *L. sativa* in this study (same way as for NO_3^--N), ca. 26% was removed in hydroponics. Most TAN in aquaponics was present as NH_4^+-N, which can be utilized for plant growth. Nitrification activity in hydroponics may also have impact on TAN removal. Nevertheless, higher removal of TAN than NO_3^--N by *L. sativa* seemed to take place in aquaponics. Xu et al. (1992) predicted NH_4^+-N to be preferred to NO_3^--N as nitrogen source for maize in case of low N concentrations. As NO_3^--N concentrations in this study were higher than the other presented studies, other underlying factors may be considered. Jones Jr (2004) reported that sizable concentrations of Cl^- can reduce NO_3^--N uptake in plants. As high Cl^- was found in this system, this scenario should be considered likely for aquaponics.

The nitrate level of in the Aquaponics was high (390-440 mg L^{-1} NO_3^-), and exceeds the levels recommended by Timmons and Ebeling (2007) (maximum 400 mg L^{-1} NO_3^-). Westin (1974) reported a 96 h LC_{50}[1] of 1355 mg L^{-1} NO_3^--N for *O. mykiss*. Hence, the nitrate levels were probably within the tolerable range for the rainbow trout. Similar studies on *S. trutta* have not been obtained. To avoid accumulation of nitrate to toxic levels for the fish, increased focus nitrate removal is recommended. Implementation of plant species more effective in nitrate removal is a suggested action.

[1] The lethal concentration of the chemical that kills 50% of test animal, after 96 hours exposure

Suggestions to increase nitrogen utilization in aquaponics

Approximately 40% of the biomass of *L. sativa* in the Aquaponics consisted of young plants just transported from the separate nursing system (3 weeks old). The remaining part consisted of 7 weeks old salad (weeks from seeding). Growth rate and nutritional needs partly regulate rate of nutrient uptake in plants (Clarkson 1985). Young plants are found to have higher growth rate than elder (Buzby and Lin, 2014), and as only ~40% of the plant biomass in this system consisted of younger plants this may have influence on nitrogen removal. The Conveyor system presented by Adler et al. (2003) suggests to place youngest plants closest to the hydroponics inlet and elder plants at the outlet, as inlet water is highest in nutrients. Furthermore, elevated hydraulic loading rate (HLR) may diminish the contact time of nitrogen to plant roots, thus decrease removal of nutrients (Endut et al., 2010). The HLR was has not calculated for this system, but lowed flow in/out of the hydroponics might enhance nutrient removal by the plants.

When considering dissolved nitrogen only, there are undoubtedly unused resources present in the water. NO_3^--N is considered the most important nitrogen source for plant growth (Marschner, 2011), and it is therefore reason to consider a more efficient production strategy and system design to improve nutrient use efficiency. NH_4^+-N was also a highly present source in this system and has the potential to support production of more plant biomass. A 1:4.3 ratio of fish and reported for the Aquaponic by Skar et al. (2015). Increased plant biomass and an extended hydroponic area can potentially increase removal of dissolved nutrients, but it is important to find the right balance of plant biomass in the Aquaponics. High plant numbers can decrease nutrient concentration in the water and increase water quality for the fish, but the problem arise when the nutrient concentration is too lot to sustain plant growth (Tyson et al., 2011). System sizing and optimal dimensioning of fish and plant biomass is a challenge in aquaponics (Tyson et al., 2011), but optimization can increase the sustainability of the system.

4.2 Seawater systems

4.2.1 Batch experiment

Considering nitrogen removal and growth response, *R. baltica* has a high potential for removing nitrogen from copepod wastewater. Specific growth rate (SGR) can indicate if a microalgae culture is productive (Chaloub et al., 2015). Cultures to which added Conwy medium was added exhibited highest SGR (0.13), but the cultures added water from biofilter and copepod tank were also productive (SGR 0.11 and 0.10 respectively). As expected, the culture containing seawater did not grow, indicating *R. baltica* being nutrient limited.

Nitrogen dynamics

The observed color change in the *R. baltica* cultures might be associated with NO_3^- content, as cultures added highest amount of NO_3^- changed color later to cultures containing less NO_3^-. The literature on *R. baltica* growth and development is limited to only a few publications. A study describing a change in cellular concentration of the red-colored phycoerythrin (PE) pigment in *Rhodomonas* sp. when the microalgae was exposed to different NO_3^- concentrations (Chaloub et al., 2015) reported increased PE content in cultures containing high NO_3^- concentrations. PE is a phycobiliprotein that may function as a nitrogen reserve in cryptomads (da Silva et al., 2009). Hence, if *Rhodomonas* sp. exhibits lower PE content as a response to lower surrounding NO_3^- concentrations, it can in turn explain the fading of red color in the cells of *R. baltica* over time.

All dissolved N compounds were assimilated by *R. baltica*, but since measurements were only monitored at the end of the experiment, nitrogen dynamics on which nutrient is more favored by *R. baltica* is difficult to discuss. Nevertheless, it is important to highlight that a great amount of NO_3^- in the copepod wastewater take its origin from the excess fertilizer associated with microalgae feed with water added to the copepod tank. As presence of other macro- and micronutrients in the wastewater was not investigated, the results can only suggest that the biofiltered water has potential to replace nitrogen in Conwy. A full investigation on nutrient composition in copepod wastewater should be performed in order to declare its full potential as fertilizer in microalgae production.

4.2.2 Copeponics

The objective of this study was not to increase production of copepods, but rather to test a new and innovative production system where wastewater was re-used while keeping the total production stable and high. Copeponics supported egg production similar to production in a flow through system (20-25 mL egg day^{-1}, data provided by C-feed), and water consumption was reduced to only 10% new intake water day^{-1}. This lowered energy use on heating of new water, and established a stable rearing environment for the copepods. Moreover, the use of fertilizer in microalgae production was reduced, compared to normal consumption. The nitrate sensor also provided detailed real-time data on nitrate dynamics in the system.

Performance of Copeponics

As already presented, the algae cultures in Copeponics were supplied with wastewater only for the two first days. Conwy medium was added from day 3, because of reduced growth and performance of the algae cultures. Since the algae in the algae bags were to be used as feed for the copepods, this was considered necessary. This means that the Copeponics did not function as a system with zero input of fertilizers, as for Aquaponics. Still, in Copeponics the use of Conwy medium was reduced by ~42% compared to normal consumption (1.2 mL L^{-1} seawater). Microalgae production is associated with high expenditures on algae fertilizers, and Chauton et al. (2015) suggests the use of waste streams containing nitrogen, phosphorous and trace nutrients as a replacement, in order to reduce production costs. The expense on fertilizer will increase as the algae production up scales, indicating that re-use of waste water components from copepods may support a more profitable microalgae production as consumption of expensive algae medium may be lowered.

In the beginning, Copeponics faced challenges with dysfunctional filters between the biofilter compartment and the algae bags, followed by supplementation of unfiltered water to the microalgae production. To avoid algae culture crash, presence of pathogenic bacteria and unwanted microalgae should be avoided. A membrane filter with a proper mesh size would be the right solution, but since this would be a costly action it was not prioritized in this project.

Water quality and performance of copepods and microalgae

Most of the water quality conditions (temperature, dissolved oxygen and pH) in Copeponics were relatively stable throughout the production period (Table 11), but an increase in salinity was experienced in all compartments of copeponics (24-30 ppt). Despite the increment,

salinity values were still within tolerable levels suggested for *A. tonsa* (Holste and Peck, 2006, Hansen et al., 2012). Dissolved oxygen concentrations, temperature and pH also held adequate concentrations for sufficient hatchability and growth of *A. Tonsa* (Mauchline et al., 1998).

The initial density of copepods was 2 300 ind L^{-1} at day 0 and increased to approximately 7 700 ind L^{-1} on day 15, in connection with the addition of 7 million copepods to the copepod tank. These densities resemble reported densities (both flow through and recirculation systems), with 50-2000 ind L^{-1} in studies by Støttrup et al. (1986) and (2003), and 7 000 ind L^{-1} in Drillet et al. (2006). *R. baltica* were fed continuously to the copepods and maintained a density around 30 000 cells mL^{-1} in CT (Berggreen et al., 1988, Skogstad, 2010). The algae cells survived in the biofilters, explaining the high cell densities in the biofilter compartment. The average consumption of microalgae added as live-feed to the copepods was ca. 200 L day^{-1} (Table 3). This is ca. 15% lower than normal consumption during production of copepods in a flow-through system. A conventional flow-through production system is continuously diluted in order to remove metabolic waste products and to restore oxygen concentrations (Lekang, 2008). The water exchange rate is a key factor in order to maintain optimal water quality in flow through systems (Lekang, 2008, Timmons and Ebeling, 2007), but for copepod production this is followed by a loss of valuable algae cells. In Copeponics, the water exchange rate was reduced to 10% of the normal dilution rate, and hence algae cells remained in the system.

Combining of fish and plant production in freshwater systems dates back to the 1970's and 1980's (Lewis et al., 1978, Watten and Busch, 1984), but examples of co-production in marine (seawater) systems is restricted (Waller et al., 2015). But reported studies on efficiency of algae biofilters in removing nitrogen from fish effluents (Deviller et al., 2004, Metaxa et al., 2006, Cohen and Neori, 1991, del Rio et al., 1996) suggests integrated production to be advantageous for an adequate water environment. Deviller et al. (2004) and Metaxa et al. (2006) tested a high-rate algae pond (HRAP) as a second loop of water treatment in a marine RAS, in order to reduce water requirements and nutrient discharge levels. In total, 25% of nitrogen was removed over a year by the macroalgae in the HRAP. It had a positive effect on survival of sea bass and did not reduce the nitrification in the biological filter. The same was true for copeponics, with high nitrogen removal rates, stable egg production numbers, and efficient nitrification.

After termination of the Copeponics project, the production of copepods continued with internal recirculation without integrated microalgae production. During Copeponics and the following two months, the RAS unit experienced a cleaner cultivation tank compared to flow-through production tanks. The copepod production in flow-through tanks required cleaning of tanks every 7-8th week. The tank with RAS production, did not need cleaning until the total shutdown of the system after 12 weeks, which was due to termination and relocation of the cultivation site. Production of copepods in RAS has been reported to provide improved water quality conditions to flow-through systems (Drillet et al., 2011). Water quality control in RAS can be achieved by bio-process technology (Orellana et al., 2014), where a high hydraulic retention time (HRT) and a low dilution rate can facilitate establishment of grazers eating off biofilm in the tanks, and further improve water quality conditions.

Nitrogen dynamics

Since Copeponics is a recirculation system with a low dilution rate, accumulation of nitrogenous waste products to toxic levels is a known concern (Timmons and Ebeling, 2007). Even low levels of ammonia can be toxic to copepods (Sullivan and Ritacco, 1985). According to Jepsen et al. (2015) the No Observed Effect Concentrations (NOEC) of dissolved NH_3 is 477 µg L^{-1} for *A. tonsa*. Thus, the un-ionized concentrations in the copepod tank were seemingly within the safe range for cultivation of *A. tonsa* (Table 11). Ammonia removal is required in RAS, and in addition to nitrification removal can be handled by exchange or removal of water, or through assimilation by algae. The ammonia removal in the biofilters was efficient, as the TAN concentration in the copepod tank remained low (<0.1 mg L^{-1}), except for day 15 (Figure 11.C). Occasionally there was observed lower TAN concentrations in the algae cultures than in the biofilter, probably indicating photoautotrophic removal by the microalgae and assimilation of TAN into new biomass (Ebeling et al., 2006, Brune et al., 2004). This indicates a double process of photoautotrophic removal of TAN from the system, in addition to the ongoing autotrophic bacterial conversion of TAN to NO_3^--N in the biofilters. Copeponics is hence provided with an extra nitrogen removal pathway supported by the algae culture. This might have a positive effect water quality control of the system.

To date, there has have been performed studies reporting exact toxic levels of NO_2^--N and NO_3^--N for *A. tonsa* as Jepsen et al. (2015) described with ammonia. Hence, the discussion on tolerable production levels is inconclusive. More research upon NO_2^- and NO_3^- tolerance on

A. tonsa is needed. The slight accumulation of NO_2^--N in CT and BF (Figure 11.A) may be reasoned by aggregation of finer solids that might have led to a less efficient nitrification process, by leaving some of the biological conversion of TAN as NO_2^--N (Holan et al., 2013). Meanwhile, NO_3^--N also accumulated in Copeponics, with highest reported levels in the biofilter (Figure 11.B). The drop in concentration on day 20 is pronounced. Decreased nitrate concentrations are often associated with increased dilution rates in RAS. In Copeponics, the dilution rate stayed unchanged. The input of microalgae to the copepod tank, with associated nitrate-rich fertilizer, increased when more copepod biomass was added to the system. Hence, a further accumulation of nitrate would be the expected outcome. The reason behind this stays unclear, but since nitrate is important for the microalgae to grow, the underlying reason should be investigated in order for Copeponics to function as a suitable system for microalgae production.

Performance of real-time monitoring of nitrate

The use of the continuous nitrate sensor in Copeponics allowed for a detailed report on NO_3^--N dynamics in the system. Use of in-situ monitoring sensors in aquaculture production systems is not common. Continuous measurements of NO_3^--N in recirculate bio-producing systems can convey accurate and time-specific data, making it a valuable tool in understanding the complex interactions of water parameters in a multi-trophic system as Copeponics. In aquaculture, monitoring nitrate-behavior can be considered valuable, as it is the end product of nitrification: A key process in aquaculture recirculation systems (Timmons and Ebeling, 2007). Manual sampling for water quality evaluation is time demanding and labor intensive (Glasgow et al., 2004), and it only presents a state of the art picture from the given time the sampling was performed. The NO_3^--N data presented in Copeponics (Figure 11.C) shows that manual samplings are in accordance with the concentrations measured by the sensor, but the manual sampling strategy carries a risk to lose valuable data in the time period between each measurement is performed. In general, manual monitoring efforts can be intensive, but they are limited to address factors influencing developments of events, such as sudden production kill, oxygen depletion, or contamination (Glasgow et al., 2004). The advances in real-time technologies allow for important early warning information to the producers (Glasgow et al., 2004). Hence, up-scaled aquaculture production systems can benefit from implementing continuous surveillance technology in their production strategies, as it gives producers time to respond to observed changes, and provides them with increased control and understanding of intricate and composed water dynamics.

5 Conclusions and recommendations

Freshwater systems

The levels of NO_2^--N, NO_3^--N and TAN were stable in influent and effluent water of a single fish tank in the RAS, but the levels of NO_2^--N were high (>0.1 mg L^{-1}). TAN was within national threshold levels on 4 out of 5 days, but levels of toxic NH_3 were low. Unidentified removal of ca. 24% NO_3^- in the system was detected, and accumulation by algae cells or other microorganisms in biofilm in the system is predicted. High NO_3^--N content of the wastewater in the RAS indicate a present potential for further use as fertilizer in plant or algae production. The level is predicted to support nitrogen requirements for plant or algae production. Replacing the denitrification unit with bio-producing systems is recommended to support a more sustainable utilization of the nitrate rich wastewater from the fish production, but has a drawback with a need for a high production area compared to the denitrification unit. The study also discovered elevated CO_2 levels (24-25 mg L^{-1}), and production with a lower level is recommended to avoid nephrocalcinosis in the fish.

In the Hydroponics, water quality parameters were stable and satisfactory for cultivation of *F. ananassa*. The levels of NO_2^--N, NO_3^--N and TAN were stable, but the NO_3^- concentration was particularly high when comparing with standard hydroponic nutrient solutions. Lowered fertilizer consumption is recommended as associated costs on expensive nitrogen can be reduced. Considering nitrogen input only, wastewater from RAS could support growth of *F. ananassa* in a decoupled hydroponic system, but volume of total daily wastewater from RAS would require a larger hydroponic production unit. The composition of micro- and macronutrients for plants in the RAS wastewater should be investigated. Salt in the water is predicted to induce salinity stress in *F. ananassa*, and hydroponic cultivation of a more salt-tolerant plant is recommended.

In the Aquaponics, water quality parameters were stable and satisfactory for cultivation of *O. mykiss*, *S. trutta* and *L. sativa*. The levels of NO_2^--N, NO_3^--N and TAN were stable, but elevated NO_2^- concentrations were measured. Nitrite toxicity to *O. mykiss* and *S. trutta* was probably counteracted with presence of Cl^- (ca. 100 mg L^{-1}). The results indicate ineffective removal of NO_3^- (ca. 5%) and TAN (ca. 26%) by *L. sativa*. Reduced uptake of NO_3^--N could be influenced by a high Cl^- concentration in the water. Organizing the plants in a Conveyor system, lowered flow in the hydroponic compartment (increased retention time of nutrient

rich water), and increased plant biomass in the system is recommended to increase nitrogen utilization in the Aquaponics.

Seawater systems

In the batch experiment, the Conwy medium supported more nitrogen and higher SGR to water from copepod wastewater. Still, high growth was observed in the microalgae cultivated in copepod wastewater, which is promising for improved nutrient utilization of aquaculture wastewater.

In the Copeponics, TAN levels were within recommended levels for *A. tonsa*, but tolerance of NO_2^--N and NO_3^--N in *A. tonsa* should be further investigated. NO_2^--N and NO_3^--N accumulated in the system, but an unexplainable drop in NO_3^--N was experienced after 20 days. Manual and online measurements were similar, and the sensor provided valuable continuous data on NO_3^--N dynamics in the system. Growth of *R. baltic*a was supported by copepod wastewater for only two days, but Copeponics allowed reducing the fertilizer consumption with on average ca. 42 % compared to normal production, and still maintain adequate microalgae cell density. Egg production was stable and high, and copepod production in Copeponics was followed by a reduced consumption of water, energy (for heating) and microalgae as live-feed. Longer production time between wash-down compared with a normal flow-through production was also experienced. Optimal water quality and growth conditions for *R. baltic*a was not studied in this thesis, but should be further investigated.

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Appendix 1

Godkjent: 03.02.2016

SKRETTING
a Nutreco company

Nutra Olympic 1,2 - 1,5 - 2 - 3 og 4

Bruksområde: Settefiskfôr til laks og ørret
Produksjonssted: Averøy
Produksjonsmetode: Ekstrudert
Sekkestørrelse: 25 og 500 kg

Beskrivelse

Nutra Olympic er et settefiskfôr til laks og ørret i ferskvannsfasen.

Produktegenskaper

- Gir rask vekst
- Gir godt karmiljø
- Svevefôr definert med synketid fra 15-20 sek/m og 5-20 % flyt

		Nutra Olympic 1,2	Nutra Olympic 1,5	Nutra Olympic 2	Nutra Olympic 3	Nutra Olympic 4
Fiskestørrelse	g	2 - 7	5 - 20	15 - 60	40 - 200	150 - 350
Protein	%	50 - 53	49 - 52	48 - 51	47 - 50	45 - 48
Fett	%	21	21	22	23	25
NFE*	%	10 - 12	11 - 13	11 - 13	11 - 13	11 - 13
Fiber	%	0,6 - 4,5	0,6 - 4,5	0,6 - 4,5	0,6 - 4,5	0,6 - 4,5
Aske	%	10 - 11	10 - 11	10 - 11	10 - 11	9 - 10
Brutto energi	MJ/kg	22 - 23	22 - 23	22 - 23	22 - 23	22,5 - 23,5
Vitamin-D	IU/kg	2200	2200	2200	2200	2200
Vitamin-E	mg/kg	300	200	200	200	200
Vitamin-C	mg/kg	200	200	200	200	200
Pigment**	mg	5	5	5	5, 70	5, 70
Pelletstørrelse	mm	1,2	1,5	2	3	4
Pellet pr.kg		1 mill.	440 000	175 000	70 000	25 000

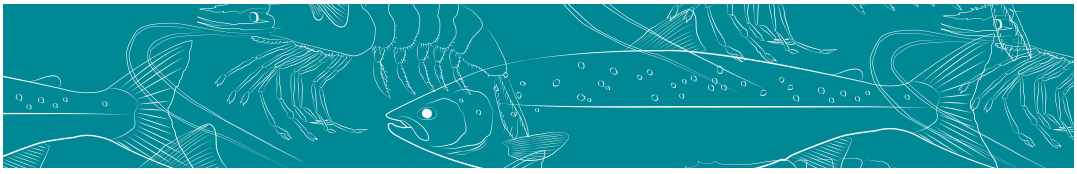
*Nitrogenfrie ekstrakter
** Pigmentkilde: Astaxanthin. Flere pigmentnivå kan være opprettet.

Råvarer

Fiskemel, fiskeolje, soya protein konsentrat, hvetegluten, rapsolje, hvete, solsikkemel.
Innholdet i dette produktdatabladet viser veiledende verdier. Produksjonssertifikatet for det enkelte parti angir nøyaktige verdier.

SEA™ Sustainable Economic Aquafeeds
nutrace
MicroBalance™

Skretting AS certifications:
NS-EN ISO 9001:2008
NS-EN ISO 14001:2004
NS-EN ISO 2200:2005
GlobalGAP CFM



Appendix 2

Settefiskfôr
Nutra Supreme 2, 3 og 4

Godkjent dato: 18.07.2014
Produksjonsmetode: Ekstrudert
Produksjonssted: Averøy
Sekkestørrelse: 25 kg, 500 kg
Bruksområde: Settefiskfôr til laks
Informasjon: Tabell under viser veiledende verdier. Produksjonssertifikatet for det enkelte parti angir nøyaktige verdier.

Beskrivelse

Overgangsfôr den siste perioden i ferskvann og som aktivt stimulerer fisken til en jevnere smoltifisering. Nutra Supreme er et fôr som skal brukes sammen med Spirt Supreme (startfôr i sjø). Alle Nutra Supreme produkter er sveveför definert slik; synketid fra 30-40 sek/m og 5-20% flyt. Alle råvarer som brukes i Skretting's fiskefôr gjennomgår en nøye kvalitetskontroll. AminoBalance i fôret gjør at fisken nyttiggjør en større andel av proteinet til muskelbygging. Vitaminer og pigment er oppgitt som total innhold i fôret.

Innhold:	Nutra Supreme 2	Nutra Supreme 3	Nutra Supreme 4
Pellet st.	2 mm	3 mm	4.0 mm
Pigment	5 mg	5 mg	5 mg
Protein	49-50 %	48-49 %	45-46 %
Fett	21 %	22 %	24 %
NFE	10-13 %	10-13 %	10-13 %
Trevler	0,4-3 %	0,4-3 %	0,4-3 %
Aske	10-13 %	10-13 %	10-13 %
Brutto energi	22-23 MJ/kg	22-23 MJ/kg	23-24 MJ/kg
Pigmenttype	Astaxanthin	Astaxanthin	Astaxanthin

Råvarer
Fiskemel
Fiskeolje
Karbohydratråvarer
Soyaprotein
Rapsolje
Vitaminer og mineraler
Nukleotidrikt gjærekstrakt
Gjærglukan
Astaxanthin (Carophyll Pink)

Tilsetningsstoffer
Vit D: 2200 IU/kg
Vit E: 300 mg/kg
Vit C: 500 mg/kg

Appendix 3

Godkjent: 03.02.2016

SKRETTING
a Nutreco company

Nutra RC 1,2 - 1,5 - 2 - 3 - 4 - 7

Bruksområde: Settefiskfôr til laks og ørret
Produksjonssted: Averøy
Produksjonsmetode: Ekstrudert
Sekkestørrelse: 25 og 500 kg

Beskrivelse
Nutra RC er et settefiskfôr til laks og ørret i ferskvannsfasen tilpasset resirkulerings- og gjennomstrømingsanlegg med utslippsbegrensning for nitrogen og fosfor.

Produktegenskaper
- Tilpasset resirkulerings- og gjennomstrømningsanlegg
- Rask vekst og godt karmiljø
- Svevefôr definert med synketid fra 15-20 sek/m og 5-20 % flyt.

		Nutra RC 1,2	Nutra RC 1,5	Nutra RC 2	Nutra RC 3	Nutra RC 4	Nutra RC 7
Fiskestørrelse	g	2 - 7	5 - 20	15 - 60	40 - 200	150-350	350 -
Protein	%	50 - 54	49 - 52	48 - 51	47 - 50	45-48	39 - 42
Fett	%	21	21	22	23	25	28
NFE*	%	10 - 12	11 - 13	11- 13	11 - 13	11-13	16 - 18
Fiber	%	0,6 - 4,5	0,6 - 4,5	0,6 - 4,5	0,6 - 4,5	0,6-4,5	0,6 - 4,5
Aske	%	9 - 11	9 - 11	9 - 11	9 - 11	9 - 11	3 -5
Brutto energi	MJ/kg	22 - 23	22 - 23	22 - 23	22 - 23	23 - 24	24 - 25
Vitamin-D	IU/kg	2200	2200	2200	2200	2200	1400
Vitamin-E	mg/kg	300	200	200	200	200	200
Vitamin-C	mg/kg	200	200	200	200	200	100
Pigment**	mg	5	5	5	5, 70	5, 70	5, 70
Pelletstørrelse	mm	1,2	1,5	2	3	4	7
Pellet pr. kg		1 mill.	440 000	175 000	70 000	25 000	

*Nitrogenfrie ekstrakter ** Pigmentkilde: Astaxanthin. Flere pigmentnivå kan være opprettet.

Råvarer
Fiskemel, fiskeolje, soya protein konsentrat, hvetgluten, rapsolje, solsikkemel.
Innholdet i dette produktdatabladet viser veiledende verdier. Produksjonssertifikatet for det enkelte parti angir nøyaktige verdier.

SEA™ Sustainable Economic Aquafeeds
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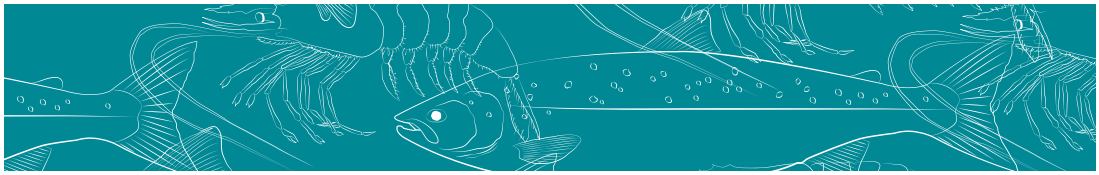
NS-EN ISO 2200:2005
GlobalGAP CFM



Appendix 4

Kristalon Indigo

The feed was produced at Glomfjord, Norway.

Grain shape: Prill

Nutrients	**%**
N (nitrogen)	8.5
NO_3^- (nitrate)	7.5
NH_4^+-N (ammonia-nitrogen)	1
P (phosphorous)	4.9
K (potassium)	24.7
Mg (magnesium)	4.2
S (sulfur)	5.7
B (boron)	0.027
Cu (copper)	0.004
Fe (iron)	0.2
Mn (manganese)	0.06
Mo (molybdenum)	0.004
Zn (zink)	0.027

YaraLiva Calcinit

The feed was produced at Glomfjord, Norway.

Grain shape: Prill

Nutrients	**%**
N (nitrogen)	15.5
NO_3^- (nitrate)	14.4
NH_4^+-N (ammonia-nitrogen)	1.1
Ca (calcium)	19

Appendix 5

Conwy medium

The algae medium is modified from Walne (1979), with a smaller amount manganese chloride than the original recipe.

Algal nutrient stock solution – contents per 1000 mL:

	grams (g)
$FeCl_3 \bullet 6H_2O$ (Ferric Chloride, 6-hydrate)	1.30
$MnCl_2 \bullet 4H_2O$ (Manganous Chloride, 4-hydrate)	0.36
H_3BO_3 (Boric Acid)	33.6
Na-EDTA (EDTA Disodium Salt)	45.0
$NaH_2PO_4 \bullet H_2O$ (Sodium Phosphate, monobasic)	20.0
$NaNO_3$ (Sodium Nitrate)	100.0
$ZnCl_2$ (Zink Chloride)	0.0211
$CoCl_2 \bullet 6H_2O$ (Cobalt Chloride, 6-hydrate)	0.0200
$(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ (Ammonium Molybdate, 4-hydrate)	0.0900
$CuSO_4 \bullet 5H_2O$ (Copper Sulphate)	0.0200
Thiamin HCl (Vitamin B_1)	0.10
Cyanocobalamin (Vitamin B_{12})	0.005
Deionized water to 1000 mL	

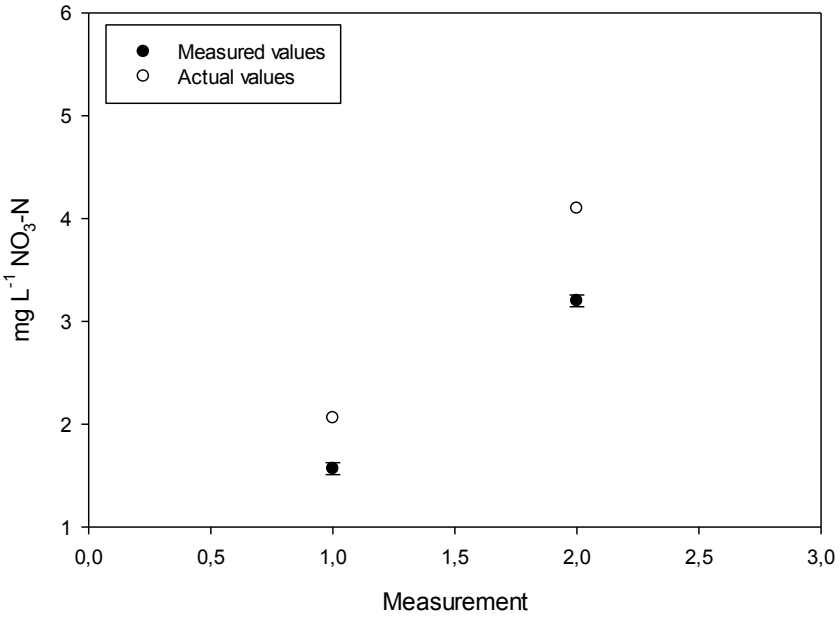
Appendix 6

Chloride concentrations above 100 mg L^{-1} will cause low NO_3-N results when measuring with the DR/890 HACH colorimeter. To use the method in high chloride concentrations (seawater) a calibration was performed using standards spiked to the same chloride concentration (33 ppt), as specified in the DR/890 HACH Colorimeter manual.

Table 12 – Overview of measured NO_3-N values with HACH DR/890 to actual NO_3-N values in a known solution.

Measured values (mg NO_3-N L^{-1})	Actual value (mg L^{-1})	Deviation (mg L^{-1})	Average deviation (mg L^{-1})	Average deviation (%)
1.5	2.06	0.27	0.23	22.9
1.5	2.06	0.27		
1.7	2.06	0.17		
3.2	4.10	0.22		
3.1	4.10	0.24		
3.3	4.10	0.20		

In solutions containing 4.10 mg NO_3-N L^{-1} and 2.06 mg NO_3-N L^{-1} the DR/890 HACH colorimeter measured 3.20±0.057 mg L^{-1} and 1.57±0.067 mg L^{-1} respectively (MEAN±SE, n=3), see Figure 12.



Figur 12 – Measured NO_3-N concentrations of solutions containing 4.10 mg NO_3-N L^{-1} and 2.06 mg NO_3-N L^{-1}.