Overcoming major bottlenecks in aquaponics - A practical approach

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Hendrik Monsees
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It was the right time, the right place and the best topic for me to undergo the adventurous journey of preparing a dissertation.

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Contents

Summary

Aquaponics is the combination of fish production in aquaculture and hydroponic (soilless) production of crop plants. Despite of representing already a sustainable, innovative approach for future food production systems, aquaponics are still missing economic success and up to date major bottlenecks were not scientifically addressed. Therefore the main aims of this thesis were (I) to identify safe nitrate concentrations under which best growth and health status of tilapia can be guaranteed in aquaponics and recirculating aquaculture systems (RAS), (II) to evaluate the best design concept for an optimal combined production of fish and plants concerning professional aquaponic applications and (III) to increase the overall system efficiency by recycling waste water and nutrients deposited in the sludge of the mechanical filtration unit.

The growth and health status of Nile tilapia (*Oreochromis niloticus*) is negatively affected by high nitrate concentrations (> 500 mgL^{-1} NO_3^--N) commonly reported for RAS. Specific growth rate (SGR) of Nile tilapia decreased significantly to up to 1.1 % per day (± 0.1) and feed conversion ratio (FCR) increased significantly to 1.1 g g^{-1} (± 0.2) at the highest nitrate concentration of 1000 mgL^{-1} NO_3^--N, confirming possible negative effects on fish production within a realistic concentration range for RAS. Nevertheless, optimal nitrate concentrations for plant production in aquaponic systems (~ 200 mgL^{-1} NO_3^--N) are not affecting fish welfare and allow for an efficient production of Nile tilapia. With increasing concentrations, uptake of nitrate and conversion to nitrite in the stomach have been identified here as alternative pathway mediating nitrate toxicity in fish.

A study on the optimization of aquaponics under a realistic, medium scale production revealed that the choice of system design has a considerable influence on the overall system performance. Decoupled aquaponics proved to be favorable for professional aquaponic production, whereas coupled systems were suboptimal for a combined production of fish and plants. There were no differences in fish production, whereas tomato production within the decoupled system was considerably increased by 36 %. The advantages of decoupled aquaponic systems were mainly attributed to the possibility of an independent regulation (separately for fish and plants) of different productions parameters, e.g. the pH (important for nitrification and nutrient availability) and the increased effectiveness of the supplementation / fertilization of limited minerals, most importantly K, P.

A closer look was also taken at the improvement of the recycling efficiency in terms of nutrient and water management. Therefore, mineralization under aerobic and anaerobic conditions were experimentally compared. Aerobic mineralization of phosphate revealed best

phosphate recovery with only minor losses of nitrate. Within only 14 days the phosphate concentration increased from 9.4 mgL^{-1} (±0.7) to 29.7 mgL^{-1} (±2.1) and simultaneously the nitrate concentration was reduced by only 16 %. In contrast, anaerobic mineralization did not result in an increase in phosphate, but nitrate concentration was up to 97 % lower. Due to a complete loss of nitrate, the main nitrogen source in aquaponic systems and because of the potential formation of toxic byproducts, anaerobic mineralization is more problematic for aquaponic applications. Recycling of water sludge mixture from clarifiers resulted in a substantial phosphor recovery, an increase in potassium and additional water savings.

Conclusively, the results of this holistic thesis clearly revealed the bottlenecks in aquaponic technology and provided guidance in overcoming mayor obstacles in terms of optimized nutrient and resource management to increase the overall sustainability of these systems and improve production efficiency and profitability.

Zusammenfassung

Der Begriff Aquaponik beschreibt die kombinierte Produktion von Fisch in Aquakultur mit der hydroponischen (erdlosen) Produktion von Pflanzen. Obwohl dies an sich einen sehr nachhaltigen, innovativen Ansatz für die zukünftige Lebensmittelproduktion darstellt, hat sich bis heute noch kein flächendeckender, ökonomischer Erfolg eingestellt und wesentliche systemische Engpässe wurden wissenschaftlich nicht untersucht. Daher waren die Hauptziele dieser Dissertation, (I) sichere Nitratkonzentrationen in geschlossenen Kreislaufanlagen (RAS) zu ermitteln, unter denen optimales Wachstum und Tierwohl produzierter Tilapien gewährleistet ist, (II) die Evaluierung des besten Designkonzeptes für die optimale, kombinierte Produktion von Fisch und Pflanzen in professionellen aquaponischen Systemen und (III) die allgemeine Effizienz bei der Wiederverwertung des Abwassers und der Nährstoffe aus dem Schlamm der mechanischen Filtrationseinheiten in aquaponischen Systemen zu erhöhen.

Das Wachstum und die Gesundheit von Niltilapien (*Oreochromis niloticus*) wird durch hohe Nitratkonzentrationen (> 500 mgL^{-1} NO_3^--N), die in RAS erreicht werden können, negativ beeinflusst. In der höchsten Behandlungsgruppe des durchgeführten Expositionsversuches (1000 mgL^{-1} NO_3^--N) wurde die spezifische Wachstumsrate (SGR) signifikant auf bis zu 1.1 % pro Tag (± 0.1) reduziert und der Futterquotient (FCR) gleichzeitig auf 1.1 g g^{-1} (± 0.2) erhöht, was die vermuteten negativen Effekte auf die Fischproduktion innerhalb realistischer Konzentrationsbereiche in Kreislaufanlagen bestätigte. Dementsprechend haben Nitratkonzentrationen, die für die Produktion von Pflanzen in aquaponischen Systemen (~ 200 mgL^{-1} NO_3^--N) optimal sind, keinen negativen Einfluss auf das Tierwohl, weshalb eine sichere Produktion von Niltilapien in aquaponischen Systemen gewährleistet ist. Zusätzlich wurde eine alternative Möglichkeit der Nitrataufnahme bzw. der toxischen Wirkung des Nitrats vorgeschlagen und überprüft. Es stellte sich heraus, dass die toxische Wirkung des Nitrats nicht durch einen direkten Einfluss auf die Fische zu Stande kommt, sondern durch eine vorherige Reduktion zu Nitrit im Magen der Tilapien hervorgerufen wird, welches dann ins Blutgefäßsystem aufgenommen wird und zur Bildung von Methämoglobin führt.

Eine weitere Studie zur Optimierung aquaponischer Systeme ergab, dass die Wahl des Systemdesigns einen erheblichen Einfluss auf die Gesamtproduktivität hat. Entkoppelte Kreislaufsysteme sind bei einer professionellen aquaponischen Produktion von Fisch und Pflanzen zu bevorzugen, da klassische, gekoppelte Systeme nur suboptimale Produktionsbedingungen bieten können. Bei der Produktion von Fisch ergab sich keinerlei Unterschied, jedoch wurde eine deutlich gesteigerte Tomatenproduktion von 36 % in

entkoppelten Kreislaufsystemen erreicht. Die Vorteile der entkoppelten Systeme sind hauptsächlich darauf zurückzuführen, dass die verschiedenen Produktionsparameter, z.B. der pH-Wert (wichtig für die Nitrifikation und Nährstoffverfügbarkeit) individuell reguliert werden können und das eine zusätzliche Düngung deutlich effektiver appliziert werden kann.
Die Effektivität des Nährstoff- und Wassermanagements ist ausschlaggebend für die Nachhaltigkeit und sollte optimiert werden. Dafür wurde die aerobe und anaerobe Mineralisation zur verstärkten Nährstofffreisetzung miteinander verglichen. Die aerobe Mineralisation zeigte das beste Rückgewinnungpotential von Phosphat und nur geringe Nitratverluste. Innerhalb von 14 Tagen stieg die Phosphatkonzentration von 9.4 mgL^{-1} (±0.7) auf 29.7 mgL^{-1} (±2.1) und gleichzeitig wurde die Nitratkonzentration nur um 16 % reduziert. Im Gegensatz dazu ergab die anaerobe Mineralisierung keinerlei Anstieg in der Phosphatkonzentration, jedoch wurde die Nitratkonzentration um bis zu 97 % reduziert. Gleichzeitig ist die anaerobe Mineralisation problematischer für den Einsatz in aquaponischen Systemen, da sich toxische Nebenprodukte bilden können und die fachgerechte Steuerung anaerober Prozesse mehr Wissen und Arbeitskraft erfordert. Die Wiederverwendung des Wasser-Schlamm-Gemisches in aeroben Mineralisations-Einheiten führt wiederum zu einer substantiellen Phosphat-Rückgewinnung, einer Steigerung der Kaliumkonzentration und einer zusätzlichen Wasserersparnis, die in der Gesamtheit eine deutliche Effizienzsteigerung aquaponischer Systeme zur Folge hat.
Die Ergebnisse dieser Dissertation zeigen die Engpässe in der Aquaponik klar auf und liefern gleichzeitig Lösungsansätze, wie diese Hindernisse in Bezug auf das Nährstoff- und Ressourcenmanagement überwunden werden können. Dadurch kann die Nachhaltigkeit dieser Anlagen gesteigert und die Wahrscheinlichkeit des wirtschaftlichen Erfolges erhöht werden.

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General introduction

1 Aquaculture: Current status and the need for system advancement

Aquaculture is the fasted growing sector in animal food production with average growth rates of 5.4 % per year within the last decade (FAO 2016, Bostock et al. 2010). A milestone was reached, when aquaculture production overtook fisheries landings in terms of seafood supply for human consumption for the first time in 2014 (FAO 2016). Additionally, by taking into account that the world population will probably increase to 9.7 billion by 2050 (United Nations 2015), protein supply derived from seafood will be even more important. However, at present, fish supply can only be increased by an increase in aquaculture production. Here lays the chance for the aquaculture industry, but although the obligation for politics to foster a more sustainable growth of the aquaculture sector. Common aquaculture production, like other food production sectors, often lacks sustainability. Some examples are the pollution of ground and surface waters by effluent discharge, the destruction of natural sites such as mangroves and wet lands and the spread of diseases (Boyd 2003, van Rijn 2013)

Until now, aquaculture is mainly restricted to production in ponds or net cages (FAO 2016). Characteristically, these production forms are in direct contact with surface waters like rivers, lakes or coastal waters. Thus, soluble nutrients, faeces and feed leftovers are often released into the environment without prior treatment or filtration (Verdegem 2013). In Europe, according to the legislation, environmental impact of agricultural production has to be reduced to meet the goals of the EU-Water Directives and recently, even a charge on excess-nitrogen in agriculture has been suggested by the Federal Environment Agency of Germany (UBA 2017). Therefore, a shift towards sustainable aquaculture production is favorable and necessary to meet future thresholds.

Within the last decades constant technical progress has provided several solutions for a more controlled and sustainable production of fish. Herewith, recirculating aquaculture systems (RAS) are the most efficient ones in terms of water use, nutrient recycling and post-treatment of waste products derived from aquaculture production.

2 Recirculating Aquaculture Systems (RAS)

Recirculating aquaculture systems (Fig. 0.1) were developed to produce aquatic animals under controlled conditions. This includes a minimal use of water, improved hygiene measures, facilitation of disease management (Summerfelt et al. 2009, Tal et al. 2009) and a reduction of the risk of escapees (Martins et al. 2010, Zohar et al. 2005). Due to the minimal use of water

in RAS (~2-10 % of the system volume per day), waste management and water quality control are of great importance in these systems. Waste management generally implies the use of mechanical filters, like clarifiers or drum filters, to remove suspended organic waste from the water flow. This is crucial since the removal of suspended solids is directly linked to available oxygen concentration, biological oxygen demand (BOD) und CO_2-concentration in the water (Eding et al. 2006). Thus the effectiveness of solid removal determines the performance of the whole system, especially the effectiveness of nitrification in the biofilter, representing the second major treatment step in RAS (van Rijn 2013).

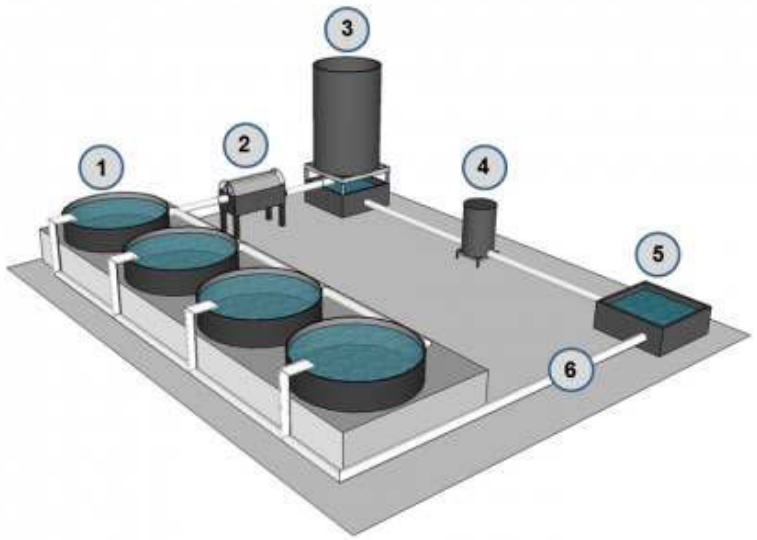

Fig. 0.1: Illustration of a recirculating aquaculture system (RAS) with major components: 1- rearing tanks, 2- mechanical filter unit (drum filter), 3- biofilter (trickling filter), 4- denitrification unit, 5- degassing tank, 6- return flow

When nitrogen-containing chemical compounds, like proteins and nucleic acids, are metabolized by fish after feeding, ammonium (NH_4^+) is released via the gills as a metabolic end product (Evans et al. 2005). Depending on the pH, a part of the ammonium is present as ammonia (NH_3). Furthermore, ammonium and ammonia can result from degradation of uneaten feed and faeces and fish toxic thresholds can be rapidly achieved (Kamstra et al. 1998, Meade 1985, Thurston et al. 1981). Consequently, different biological treatment units, like trickling filters or moving bed filters, are used in RAS to promote an efficient microbial oxidation of ammonium to nitrate (nitrification) (Brazil 2006, Hovanec und DeLong 1996, Timmons et al. 2006, van Rijn et al. 2006). In conjunction with an ongoing development of RAS and a reduction of fresh water usage in aquaculture systems in recent years, the accumulation of nitrate has been recognized as an emerging problem (van Bussel et al. 2012).

3 Nitrate in RAS

Nitrate is often discussed with regard to eutrophication of water bodies that are contaminated by agricultural production of crop plants (UBA 2017). Especially in Germany, thresholds for groundwater bodies of 50 mgL^{-1} NO_3^- are regularly exceeded, since excess nitrogen, mainly in the form of manure, liquid manure and nutrient rich sludge derived from biogas plants is spread on agricultural areas as cheap fertilizer (UBA 2017). Plants are not able to absorb all the nutrients, thus excess nutrients can contaminate surface and ground water bodies. This problem is also known from conventional aquaculture, especially in terms of production in ponds and net cages. Here, the production unit is in direct contact with adjacent water bodies and waste solids and nutrients, mainly in the form of nitrogen, are released to the environment without prior treatment (Herbeck et al. 2013, Chislock et al. 2013). To reduce the impact on the environment, the development of modern recirculating aquaculture systems (RAS) was promoted.

In closed RAS, only a small amount of process water is required compared to ponds or net cages. In modern RAS, water consumption of 2-10 % of the system volume per day is sufficient to run the system (Ebeling et al. 2006). Based on the reduced water replacement and the effective conversion of ammonium to nitrate in the biofilter of RAS, concentrations of 100 - 1000 mgL^{-1} NO_3^--N can accumulate in the process water (van Rijn 2013). However, high nitrate concentrations can negatively affect the growth and health status of fish as it was already shown e.g. for turbot (*Scophthalmus maximus*), pikeperch (*Sander lucioperca*) and African catfish (*Clarias gariepinus*) (van Bussel et al. 2012, Schramm et al 2014 a, Schramm et al. 2014 b). For Nile tilapia (*Oreochromis niloticus*), one of the most important species in aquaculture produced worldwide (FAO 2014), comprehensive studies and data are still missing.

To prevent adverse effects on the growth and health status of fish, but simultaneously avoiding increasing the water exchange rate in RAS, different techniques were developed to reduce the concentration of nitrate in the process water. One possibility to reduce nitrate in RAS is the so-called denitrification. Under anaerobic conditions, nitrate is microbiologically reduced to elemental nitrogen gas (N_2), which is then released to the atmosphere (Saliling et al. 2007, van Rijn et al. 2006). The large-scale application of denitrification reactors is still limited in RAS, since the process is technically sophisticated. A high level of knowledge is required by the technical staff, additional carbon sources like methanol or ethanol are often required and, if handled inappropriately, toxic by-products like H_2S can arise (Saliling et al. 2007; van Rijn et al. 2006).

Aquaponics is another possibility to reduce nitrate from RAS by recycling excess nutrients.

4 Aquaponics

4.1 General principle

Aquaponics is the combination of fish production in aquaculture and hydroponic (soilless) production of crop plants (Fig. 0.2). Hereby nutrients, mainly nitrate, phosphate and potassium, derived from the RAS, are recycled within the hydroponic unit. By using nutrient rich waste water from the aquaculture unit for hydroponics, water and fertilizer consumption are effectively reduced (Kloas et al. 2015, Rennert et al. 2011). Potential double usage of heating and building control systems in one building complex can reduce the overall costs of these systems and increase the overall management effectiveness compared to single systems. Additionally, in an intensive, integrated production of fish and plants, less space is needed compared to conventional production systems (Rakocy et al. 2006). Nevertheless, the combination of two non-related disciplines (aquaculture and hydroponics) in one large system is difficult. Species specific requirements of fish and plants differ, e.g. in terms of water temperature, pH, salinity (or soluble nutrients), and optimal production of both at the same time is impossible. Therefore current efforts focused on a new design concept for aquaponics, were species-specific requirements are met (Kloas et al. 2015).

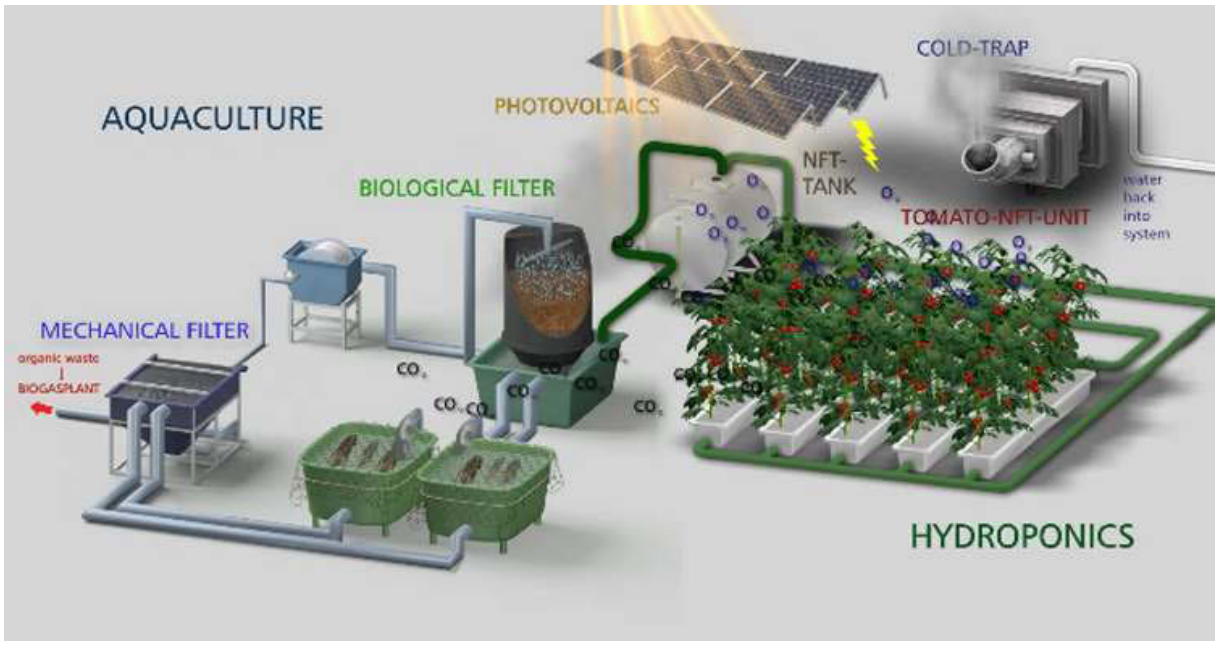

Fig. 0.2: Illustration of an aquaponic system compromising an aquaculture unit with mechanical (clarifier and drum filter) and biological (trickling filter) filters connected to a hydroponic unit (NFT-trays) for tomato production. Optional recycling of solid waste in a biogas plant, potential energy supply via photovoltaic and water recycling via cold trap are indicated.

4.2 Differences in system design

Classical aquaponic systems, also referred to as coupled or 1-loop aquaponic systems (Fig. 0.3) are arranged in a single loop. These systems are known and investigated for more than 30 years (Naegel 1977, Watten and Busch 1984). Process water from the RAS is directed to the hydroponic unit and back to the RAS; however, rearing conditions for either fish or plants are not met. The water quality for fish and plants is equal (i.e. same temperature, pH, nutrients) representing a compromise in terms of rearing conditions for both. Commercial applications are scarce and the majority of aquaponic systems are small-scale units, e.g. in schools for education purposes or in research facilities (Love et al. 2015). The reason for this is presumably the lack of control on each production unit and the need to compromise on key factors like the pH (Chapter II).

Current research has provided a new idea of decoupling the different system compartments (Fig. 0.3), i.e. RAS and hydroponic, to allow better control of species-specific requirements (Rennert et al. 2011, Kloas 2015).

In decoupled systems, the RAS and the hydroponic unit are operated in separate cycles and are connected through a one-way valve, allowing on-demand supply of process water from the RAS to the hydroponic unit. Evaporated water from plants and the aquaculture units is collected via cold trap integrated in the air condition system and returned to the RAS. The possibility of an individual management of RAS and hydroponic unit is a potential advantage compared to coupled systems (Kloas et al. 2015). Microbial nitrification in the biofilter (e.g. moving bed filter, trickling filter) requires a pH of ≥ 7 to effectively convert toxic ammonia, derived from fish metabolism, into nitrate (Chen et al. 2006). Since nitrification (the oxidation of ammonia) releases protons and thereby decreases the pH, RAS operators frequently have to artificially increase the pH by addition of e.g. limestone (Eding et al. 2006, Kloas et al. 2015). In contrast to RAS, hydroponic plant production generally requires a lower pH of 5.5 - 6.5, as most nutrients are available within this range (Hochmuth 2001). Especially in commercial production, the pH is therefore often lowered by the addition of acids, e.g. nitric acid (Wheeler et al. 1997). This example illustrates the dilemma of coupled aquaponics, especially in the context of a targeted professional production, since compromises have to be made with regard to several production parameters (Rakocy 2006). Obviously, this is not ideal for neither fish nor plants and species-specific adjustment by decoupling of both units is desirable (chapter II).

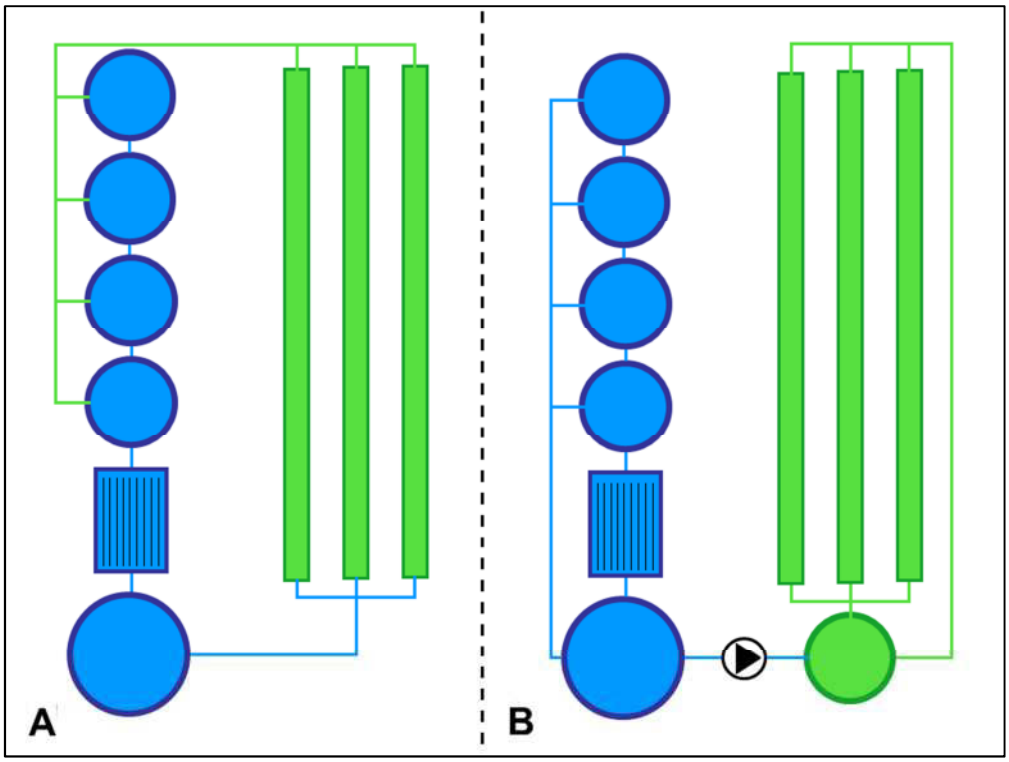

Fig. 0.3: Schematic illustration of classical (coupled) and decoupled aquaponics. A: Classical aquaponic system consisting of a recirculating aquaculture system (in blue: RAS; with rearing tanks, clarifier and biofilter) directly connected to the hydroponic unit (in green: NFT-trays). Water is constantly circulated from RAS to hydroponic and back to RAS. B: Decoupled aquaponic system consisting of a RAS connected to the hydroponic unit via one-way-valve. Water is separately recirculated in each system and water is just supplied on-demand from RAS to the hydroponic unit, but not back.

4.3 Fish and plants in aquaponics

In principle, a huge variety of fish and plants species can be used in aquaponics. The fish production unit generally represents the core of the facility due to the fact that water and nutrients have its source there. The RAS is basically the source of nutrients that are recycled in the hydroponic unit, which is, in turn, designated as sink.

In principle all species that are produced in conventional RAS are potential candidates for aquaponics. Most commonly different species of tilapia, mainly Nile-tilapia (*Oreochromis niloticus*) are produced in such systems (Rakocy et al. 2006). These fresh water fish, initially originating from Egypt, Africa, are robust against variations in temperature and water quality and have high growth rates (El-Sayed 2006, Popma und Masser 1999). Especially for intensive fish production in RAS located in a greenhouse this is a great advantage, since water temperatures can increase above 30 °C.

In aquaponic systems designed for commercial production, robust species like Nile tilapia, carp (*Cyprinus carpio*) or catfish (*Clarias gariepinus*) are generally produced (Rakocy et al.

2006, Endut et al. 2010, Naegel 1977), but there are also systems with e.g. perch (*Perca fluviatilis*) (Graber and Junge 2010).

As in RAS, all plant species produced in conventional hydroponic systems are suitable for aquaponics. Tomatoes (*Solanum lycopersicum*), basil (*Ocimum basilicum*) and lettuce (*Lactuca sativa*) are very popular in different aquaponic applications, but even ornamental plants like roses can be produced (Kloas et al. 2015, Rakocy et al. 2006, Wenger 2003). Among them tomatoes are considered as more difficult to grow, since nutrients, especially potassium, are required in high quantities (Lattauschke 2004). There are even some marine aquaponics, working with e.g. steelhead trout (*Oncorhynchus mykiss*), mussels (*Mytilus edulis*), sea-cucumbers (*Holothuria forskali*), marsh samphire (*Salicornia europaea*) and seaweeds (*Ulva spp*.) in recirculation systems (Gunning et al. 2014). Marine aquaponics are not in the scope of this thesis and are more difficult to manage e.g. in terms of salinity, corrosion and nutrition, but it should be mentioned that there is an increasing trend of marine finfish production in land based RAS and a comparable nutrient recycling here is recommended.

5 Mayor bottlenecks in aquaponics

In RAS, high nitrate concentrations (up to 1000 mgL^{-1} NO_3^--N) are accumulating during fish production (van Rijn 2013). This is beneficial, especially in aquaponic systems, because artificial nitrogen application in the form of inorganic fertilizer can be drastically reduced, ideally up to 100 %. Nevertheless, as mentioned earlier, high nitrate concentrations can negatively influence the health and growth status of fish (van Bussel et al.2012). For tilapia, one of the most frequently produced fish in RAS and aquaponic systems worldwide (FAO 2014), scientific data on potential negative effects due to chronic nitrate exposure is still missing. Considering aquaponics as a sustainable, future food production technology, animal welfare issues as well as optimal growth conditions have to be guaranteed in advance of a large-scale market implementation.

Additionally, optimal system design is of major importance for the success of a commercial production and compromises on optimal production parameters are inacceptable. In the past decades, such compromises were common practice in aquaponic applications and probably responsible for the low economic success. Lately, due to ongoing research, a new, innovative aquaponic approach was presented (Kloas et al. 2015). Up to date, this was not scientifically evaluated in comparison to the classical system design. As a consequence, the scientific basis

for a new debate on the value of aquaponic systems compared to other food productions systems was lacking.

Last but not least, aquaponic systems are resource friendly sustainable production systems, but still a big fraction of the water and nutrients are not efficiently used. A great potential for a further improvement of the overall system efficiency lies in the recycling of the discharged water, solid waste and soluble nutrients derived from the cleaning of the mechanical filters (e.g. clarifier, drum filter). To date, an efficient and easy to handle treatment unit for the recycling of these resources is lacking and research on this topic is very limited, particularly with respect to the question whether aerobic or anaerobic sludge treatment should be favoured for aquaponic application.

6 Aims and objectives

Aquaponics is a promising technology to solve multiple, complex problems commonly occurring in agriculture production systems (high water consumption, eutrophication, land use, high CO_2-footprint etc.). Objectives such as water scarcity, sustainable food production and the depletion of cheap fertilizers (especially phosphate) are addressed on several levels within aquaponic systems and these systems are likely to play a more pronounced role in future food production as natural resources will be of higher value. The aim of this dissertation was to build the scientific basis for the assessment of different aquaponic approaches for a better integration of hydroponics into RAS. The investigations were conducted to provide necessary lacking data on key aspects for the improvement of aquaponic systems and the overall system efficiency by

- identifying threshold nitrate concentrations under which best growth and health status of tilapia can be guaranteed in aquaponics and RAS
- evaluating the best design concept for optimal combined production of fish and plants in professional aquaponic applications
- increasing the overall system efficiency by recycling waste water and nutrients derived from the mechanical filtration unit in aquaponics

7 Main chapters

The dissertation is a cumulative work based on three research papers (chapters I, II and III), each including an introduction, material and methods, results, discussion, conclusion and a reference section. Chapters I and III are published, peer-reviewed research papers, reprinted with the permission of the publisher. The text was partially reformatted, figures and tables were renumbered. This thesis was funded by the Elsa-Neumann Scholarship of the Federal Country of Berlin, Germany.

Chapter I: Monsees H, Klatt L, Kloas W, Wuertz S. (2017) Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus L.*) in recirculating aquaculture systems. Aquac Res. 48, 3482–3492, doi:10.1111/are.13174

Chapter II: Monsees H, Kloas W, Wuertz S. (submitted manuscript to PLOS ONE) Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes

Chapter III: Monsees H, Keitel J, Paul M, Kloas W, Wuertz S. (2017). Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions. Aquacult Environ Interact. 9:9-18, doi: 10.3354/aei00205

Chapter I

Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus* L.) in recirculating aquaculture systems

Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus* L.) in recirculating aquaculture systems

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Abstract

Studies on chronic or acute toxicity of nitrogen species on fish in recirculating aquaculture systems (RAS) usually focused on adverse effects of total ammonia nitrogen (TAN: sum of NH_3 + NH_4^+) and nitrite (NO_2^-), while underestimating the potential effects of high nitrate accumulation on growth and health status of fish. In our study, Nile tilapia (*Oreochromis niloticus*) were exposed to five different nitrate concentrations (0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N) over 30 days. Growth parameters (feed conversion ratio: FCR, specific growth rate: SGR, hepatosomatic index: HSI), blood samples (concentrations of hemoglobin, methemoglobin, plasma NO_2^-/NO_3^-) and the histology of the gills were studied to evaluate growth and health status of the fish. At the highest nitrate concentration, the fish showed significantly reduced growth and impaired health status (SGR, FCR, plasma NO_2^-/NO_3^-, hemoglobin- and methemoglobin concentration), demonstrating that too high nitrate concentrations can negatively influence tilapia production in RAS. Here, we recommend not exceeding concentrations of 500 mg L^{-1} NO_3^--N in juvenile tilapia culture to ensure an optimal health and growth status of the fish, since below that concentration no effects on the tilapia have been observed.

1 Introduction

Recirculating aquaculture systems (RAS) have been rapidly evolving over the last two decades and are envisioned a great potential with regard to a sustainable aquaculture development due to the efficient use of water and space as well as minor environmental impact (Gutierrez-Wing & Malone 2006). However, a major drawback of RAS is the accumulation of waste products such as nitrate after biofiltration. As a consequence of improved recirculation technology and subsequently decreasing water exchange, waste products such as nutrients are accumulating in the process water (van Rijn 2013). Compared to open aquaculture systems like ponds, net cages or semi-closed systems where these products are of minor relevance to the cultured species due to high water exchange, concentrations may exceed critical levels impacting welfare as well as performance of the fish. This is particularly relevant for aquaponics, where high nitrate concentrations originating from a RAS-based fish production are desirable to fertilize the plants in the hydroponic unit. Here, nitrate concentrations in the range of 150 - 230 mg L^{-1} NO_3^--N are recommended e.g. for the hydroponic production of tomatoes, cucumbers and peppers (Lattauschke 2004)

Biofiltration in RAS is necessary to convert toxic total ammonia nitrogen (TAN) via nitrite to nitrate (Timmons, Holder & Ebeling 2006). Based on the experience in open systems and the respective concentrations, nitrate has been considered harmless to the fish (Rakocy, Masser & Losordo 2006) and only limited attention was directed to the adverse effects of nitrate in the past. However, in contrast to ponds and other open systems, nitrate can accumulate to concentrations of up to 1000 mg L^{-1} NO_3^--N in RAS (van Rijn 2010). Therefore, potential chronic effects on growth and health of fish become more likely. Furthermore, problems interfering with the production efficiency may emerge due to reduced growth performance caused by high nitrate concentrations.

The conversion of hemoglobin to methemoglobin has been reported as the main mechanism of nitrate toxicity on aquatic animals (Jensen 1996; Scott & Crunkilton 2000; Cheng & Chen 2002), but alternative modes of action (MOA) have been discussed including pathological impairment of the gills, immune suppression and endocrine effects on the thyroid system as well as on androgens and estrogens (Camargo, Alonso & Salamanca 2006; Davidson, Good, Welsh & Summerfelt 2014; Hamlin, Moore, Edwards, Larkin, Boggs, High, Main & Guillette 2008, Freitag, Thayer, Leonetti, Stapleton & Hamlin 2015). In a 30 day trial, nitrate modulated the conversion of steroids at 57 mg L^{-1} NO_3^--N, affecting key players – testosterone, 11-ketotestosterone and estradiol - in the endocrine regulation of growth and reproduction (Hamlin et al. 2008) and concentrations as low as 10 mg L^{-1} NO_3^--N raised

testosterone in Atlantic salmon (Freitag et al. 2015). In mosquitofish, embryonal dry weight was reduced and reproductive behavior of mature females was affected at minimal concentrations of 5 mg L^{-1} NO_3^--N (Edwards, Miller & Guillette 2006). Moreover, elevated nitrate concentrations up to 110 mg L^{-1} NO_3^--N lead to a decrease in the thyroid hormones T3 and T4 in rats (Eskiocak, Dundar, Basoglu & Altaner 2005). Impact on swimming performance and survival in juvenile rainbow trout has already been reported at 91 mg L^{-1} NO_3^--N (Davidson et al. 2014). Still, substantially reduced growth performance might be the most relevant for the farmer in terms of economic impact. At increasing nitrate concentrations, linear decrease in specific growth rate (SGR) was observed in turbot (*Scophthalmus maximus*) resulting in a dramatically reduced SGR (30 %) at 500 mg L^{-1} NO_3^--N (van Bussel, Schroeder, Wuertz & Schulz 2012). Similarly, Schram, Roques, Abbink, Yokohama, Spanings, de Vries, Bierman, van de Vis & Flik (2014, a) observed reduced growth performance in African catfish (*Clarias gariepinus*) at nitrate concentrations >140 mg L^{-1} NO_3^--N. Consequently, adverse effects need to be evaluated for one of the most important species in intensive aquaculture, where concentrations above 100 mg L^{-1} NO_3^--N are regularly observed and thus may be relevant upon chronic exposure.

In contrast, acute toxicity of nitrate in fish is often observed at extreme concentrations, where 96 h LC50 were observed between 1,250 mg L^{-1} NO_3^--N and 1,400 mg L^{-1} NO_3^--N e.g. in rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and Chinook salmon (*Oncorhynchus tshawytscha*) in separate studies (Tomasso & Carmichael 1986; Colt & Tchobanoglous 1976; Westin 1974). Despite the importance of tilapia aquaculture globally (FAO 2012), no data on chronic effects of nitrate exposure and safe threshold concentrations have been published so far. In addition, the uptake of nitrate in fish is not yet comprehensively described, but essential to understand nitrate toxicity in fish. Compared to NH_3 or NO_2^- nitrate uptake is presumably low as a result of low branchial permeability towards nitrate (Stormer, Jensen & Rankin 1996). Still, relatively high plasma concentrations of NO_x (sum of NO_2^- and NO_3^-) have been reported upon nitrate exposure (Schram et al, 2014 a,b; Stormer et al., 1996). Consequently, alternative uptake routes and sites may be involved.

The objective of the present study was to identify potential effects of high nitrate concentrations on growth and health status of juvenile Nile tilapia. Therefore an exposure experiment was conducted with juvenile Nile tilapia to assess the impact of nitrate in intensive aquaculture. Based on the results we give a recommendation for safe levels of nitrate in the production of juvenile Nile tilapia. In a second experiment, the reduction of nitrate to nitrite in the stomach juice was studied *in vitro* over time to clarify if nitrate

conversion and subsequent nitrite uptake is an alternative uptake route to direct uptake of nitrate, considering the plasma concentrations of nitrite and nitrate observed *in vivo*.

2 Material and Methods

2.1 Experimental setup

We conducted an experimental NO_3^- exposure of juvenile tilapia (total length 8.8 ± 0.48 cm, wet weight 13.5 ± 2.5 g) at concentrations of 0, 10, 100, 500 and 1000 mg L^{-1} NO_3-N (0, 0.7, 7, 36, 70 mM) over a 30 d period in a continuous flow-through system. Tilapia were individually stocked to forty 9 L glass aquaria (30×20×14.5 cm) with an overflow providing 7 L of rearing volume (flow rate 50 L/d). All aquaria were placed in a water bath and aerated, assuring a constant temperature of 27.3°± 0.3°C (min 26.0 °C, max 28.9°C) and 7.8 ± 0.3 mg/L O_2 (100 % O_2). Fish were fed a commercial food (Aller Futura Ex, Emsland-Aller Aqua, Germany) at 1.5 % of their body weight per day.

After acclimatization for one week, respective concentrations were established by flow controlled assembly consisting of a peristaltic pump, a rotameter flow gauge, a needle valve and a mixing chamber, diluting a 100fold stock solution with prefiltered, temperature conditioned tap water (Lutz, Kloas, Springer, Holden, Wolf, Krueger, & Hosmer 2008). The stock solution was formulated with $NaNO_3$ and KNO_3 at Na^+/K^+ weight ratio of 6.2 : 1 considering the mean ratio in the Nile (Zimmermann-Timm 2011; Dekov, Komy, Araujo, Van Put & Van Grieken 1997; Komy & El-Samahy 1995) to avoid disturbances in cellular homeostasis (van Bussel et al. 2012). $NaNO_3$ and KNO_3 were food quality grade (CHEM-DIS, Eisenberg, Germany). Each mixing chamber supplied four aquaria, referred to as cluster. For each treatment, there where two clusters assessing eight fish in total. Flow rates of nitrate stock solutions were controlled and adjusted twice a day, flow rates of tap water were controlled on a weekly basis. Temperature, pH and oxygen concentration were determined daily with a portable multimeter (HQ40d multi, Hach Lange GmbH, Germany). Salinity was measured three times over the experimental period with a portable meter (WTW LF92, WTW GmbH, Weilheim, Germany). The experiment was conducted in compliance with the local animal welfare committee (LAGESO G0367/12).

Concentrations (mg L^{-1}-N) of TAN, NO_2^- and NO_3^- in the water were determined every second day by the cadmium reduction method, the diazotization method and the ammonia salicylate method using a spectrophotometer DR3900 (Hach Lange GmbH, Germany).

2.2 Sampling

After 30 days, fish were killed and blood samples were taken from the caudal vein with heparinized syringes. Samples for the determination of hemoglobin were kept on ice and analyzed within 3 h. For methemoglobin, whole blood samples were shock frozen and stored at –80°C. Blood plasma was obtained by centrifugation (5000 *g*, 2 min), shock frozen and stored at –80°C. Fish were weighed to the nearest 0.1 g and length was recorded to the nearest of 1 mm, liver to the nearest of 1 mg. The HSI was calculated as HSI = (liver weight / final weight of fish) *100. For histology, the fourth right gill arch was dissected and fixed in 10 % phosphate buffered formaldehyde solution (Histofix, Carl Roth, Germany).

2.3 Plasma concentrations of NO_2^- and NO_3^-

We measured the sum of nitrite and nitrate (NO_x) as well as nitrite in the plasma using the nitrate/nitrite colorimetric assay kit (Cayman, USA) according to the user's manual. Briefly, for NO_x and NO_2^- determination, plasma was diluted 1:20 prior measurement. Absorbtion was determined at 530 nm with an Infinite M200 microplate reader (Tecan Trading AG, Switzerland). All samples were analyzed in duplicate. The NO_3^- concentration was then calculated as $NO_x - NO_2$.

2.4 Hemoglobin and methemoglobin determination

Total hemoglobin was determined within 3 h upon sampling with a diagnostic hemoglobin kit (DiaSys Diagnostic Systems, Germany) and calculated from a standard dilution series (12 g/dL hemoglobin standard, HEM QS, Diaglobal, Germany) as described in Wuertz, Schulze, Eberhardt, Schulz & Schroeder (2013). For the methemoglobin concentration the ratio of Meth-Hb and total-Hb was determined using the cyan ferrocyancomplex method according to Hegesh, Gruener, Cohen, Bochkovsky & Shuval (1970). Briefly, 20 µL blood was incubated (15 min) in 1 mL pure water. After addition of 600 µL saponin solution (1% saponin, 14 mM Na_2HPO_4, 42 mM KH_2PO_4, pH 6.6) and vortexing, cell debris were separated by centrifugation (10 min, 3000 *g*). Samples were analyzed in duplicates, measuring the absorption at 633 nm in (A1) 250 µL supernatant, (A2) after the addition of 5 µL 1% KCN and incubation for 10 min, in (A3) 250 µL supernatant after addition of 5 µL $K_4[Fe(CN)_6]$, followed by an addition of 5 µL 1% KCN and incubation for 10 min (A4). Total Hb:MetHb was calculated as (A1–A2)/(A3–A4).

2.5 Gill histology

After fixation in phosphate-buffered formalin for approximately 24 h at 4°C, samples were transferred to embedding cassettes and washed three times with 0.1 M phosphate buffer [0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, pH 7.3]. The last washing step was carried out overnight. Samples were dehydrated with successive washes of EtOH (70 %, 96 %, 100 %, 100 %) for 1 h each. Preinfiltratation was carried out with a 1:1 ethanol Technovit 7100 solution for 1 h, followed by infiltration in 100 mL Technovit 7100 with 1 g hardener (dissolved within 5 min) on a shaker overnight (approx. 12 h). Samples were then transferred to Histoform S, orientated and the polymerization was initiated with 1 ml hardener 2 in 15 mL solution and embedded within five minutes. After the polymerization, blocking of the embedded specimen was carried out with Technovit 3040. Samples were cut to 2 µm slices with a rotary microtome (Jung RM 2065; Leica, Germany) transferred to microscope slides, and hematoxylin-eosin (HE) stained.

Gills were analysed at 400 x magnification with the PALM Robo Imaging Software and a Zeiss AxioObserver microscope attached to a CCD camera (Carl Zeiss MicroImaging GmbH, Germany). Within 5 primary filaments per sample a total of 100 secondary lamellae were considered for each fish and histopathological changes were recorded. Dorsal and ventral secondary lamellae were considered in same amounts. Histopathological changes of the secondary lamellae and interlamellar spaces of the primary filament in-between were recorded according to Monteiro, Rocha, Fontainhas-Fernandes & Sousa (2008).

2.6 Conversion of nitrate in stomach content of tilapia

To examine the potential conversion of nitrate *in vitro*, the stomach content (1.5 ml per fish) of adult tilapia (550-650 g, n=20) was collected after sacrifice. After centrifugation (16000 *g* for 2 min), nitrate stock solution (3.035 g $NaNO_3$ in 10 mL) was added to the supernatant (gastric juice) to reach a target concentration of 1000 mg L^{-1} NO_3^--N. Samples (gastric juice and solids) were mixed gently with the tip of the pipette and incubated at room temperature for 5, 45, 90 and 150 min respectively. After incubation, samples were centrifuged (16000 g for 5 min) and supernatant was analyzed for NO_2^- and NO_3^- (mg L^{-1}-N) as described earlier.

2.7 Statistical analysis

Data are presented as means ± standard deviation (SD) of n samples. Statistical analysis was performed using Graphpad Prism (GraphPad Software Inc., La Jolla, USA). Data were tested for normality (Shapiro-Wilk) and equal variance (Kruskal-Wallis). Multiple comparisons were carried out by non-parametric Dunn's test ($p<0.05$). Results for gill histology were

expressed in percent and, prior to statistics, transformed with an arcsine-square root transformation.

3 Results

3.1 Survival and growth performance

During the experiment, mortality was only observed in the highest treatment group (1000 mg L^{-1} NO_3^--N), where three fish died. No further analyses were carried out on these fish. There was a general decrease in the specific growth rate (SGR) observed with increasing NO_3^- concentration (Fig.1.1).

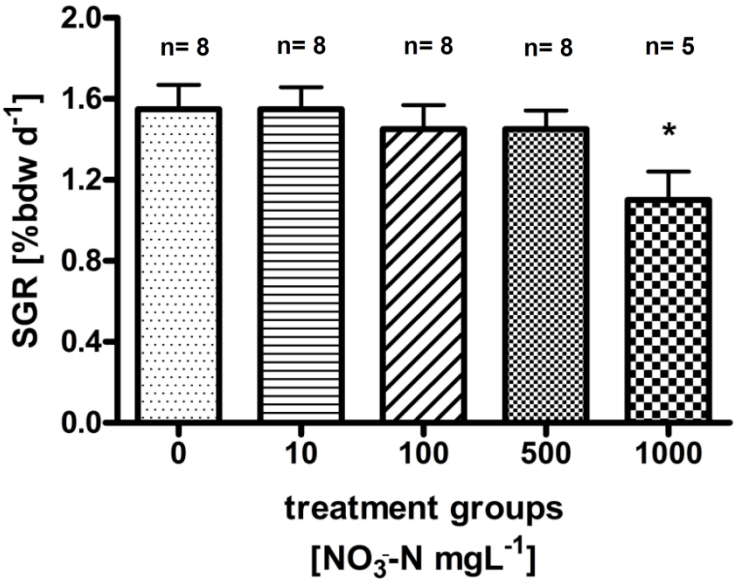

Fig. 1.1: Specific growth rate (SGR, mean ± SD) in juvenile Nile tilapia *Oreochromis niloticus* after 30 d of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N. Significant differences to the control are indicated by an asterisk (p<0.01, non-parametric Dunn's). The number of samples is indicated on top of each column. SGR= (ln final weight−ln start weight)/days*100

Lowest SGR (1.1 % d^{-1} ± 0.1) was recorded at 1000 mg L^{-1} NO_3^--N, which was significantly lower compared to the control group (P<0.01, non-parametric Dunn`s). The SGR already decreased at 100 mg L^{-1} NO_3^--N group, though not significantly different from control fish.

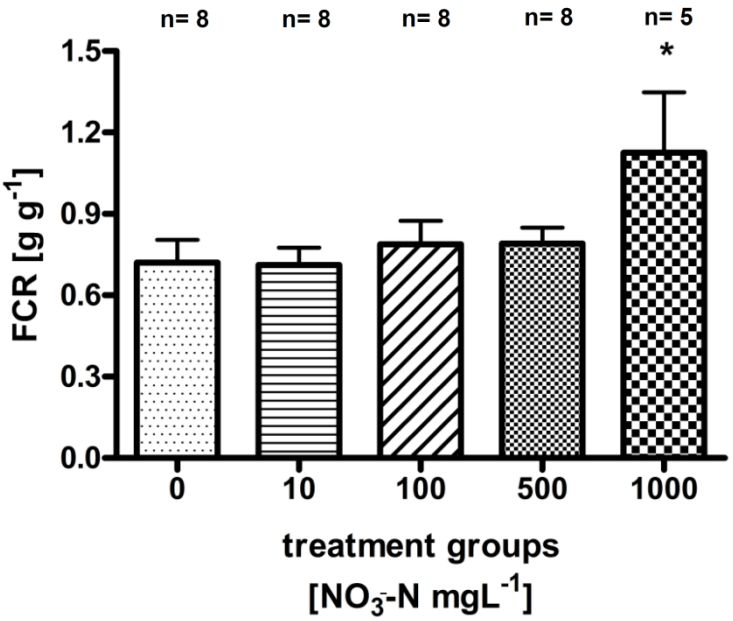

Fig. 1.2: Feed conversion ratio (FCR, mean ± SD) in juvenile Nile tilapia *Oreochromis niloticus* after 30 d of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N. Significant differences to the control are indicated by an asterisk (p<0.01, non-parametric Dunn's). The number of samples is indicated on top of each column. FCR= dry weight feed/ (final wet weight – initial wet weight)

The feed conversion ratio (FCR) increased with increasing nitrate concentration (Fig.1.2). Again, only the FCR at 1000 mg L^{-1} NO_3^--N was significantly increased at 1.1 g g^{-1} ± 0.2 compared to the control (P<0.01, non-parametric Dunn`s).

3.2 Blood parameters

There was an increase in the NO_2^-- and NO_3^-- plasma concentrations with increasing nitrate concentration (Fig.1.3). The maximum increase in plasma concentration of NO_2^- (516 µM NO_2^- ± 284) and NO_3^- (22 µM ± 2.8) was found at an exposure of 1000 mg L^{-1} NO_3^--N (P<0.01, non-parametric Dunn`s), but no statistical analysis was carried out due to low n in the highest treatment group.

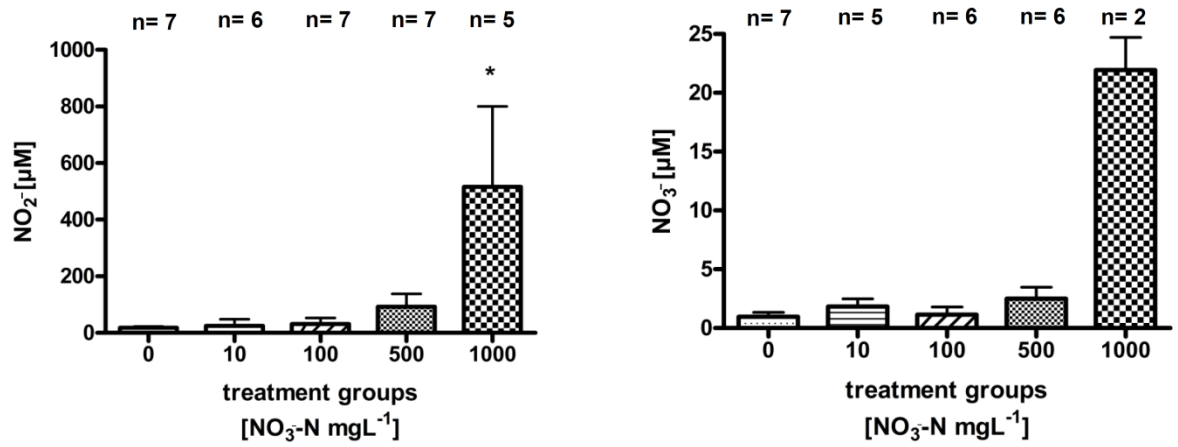

Fig. 1.3: Plasma NO_2^- and NO_3^- (mean ± SD) in juvenile Nile tilapia *Oreochromis niloticus* after 30 d of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N. Significant differences to the control are indicated by asterisk (p<0.01, non-parametric Dunn's). The number of samples is indicated on top of each column. No statistical analysis was conducted in the highest treatment group for plasma NO_3^- due to a low number of replicates.

Total hemoglobin concentration decreased with increasing NO_3^- concentration (Fig.1.4), lowest (3.5 g/dL ± 0.8) in the 1000 mgL^{-1} NO_3^--N group (P<0.05, non-parametric Dunn`s). Congruently, an increase of methemoglobin with increasing $NO_3^-$ concentration (Fig.1.4) was observed. The highest methemoglobin concentration (44 % ± 9) was recorded in the treatment group exposed to 1000 $mgL^{-1}$ $NO_3^-$-N (P<0.05, non-parametric Dunn`s).

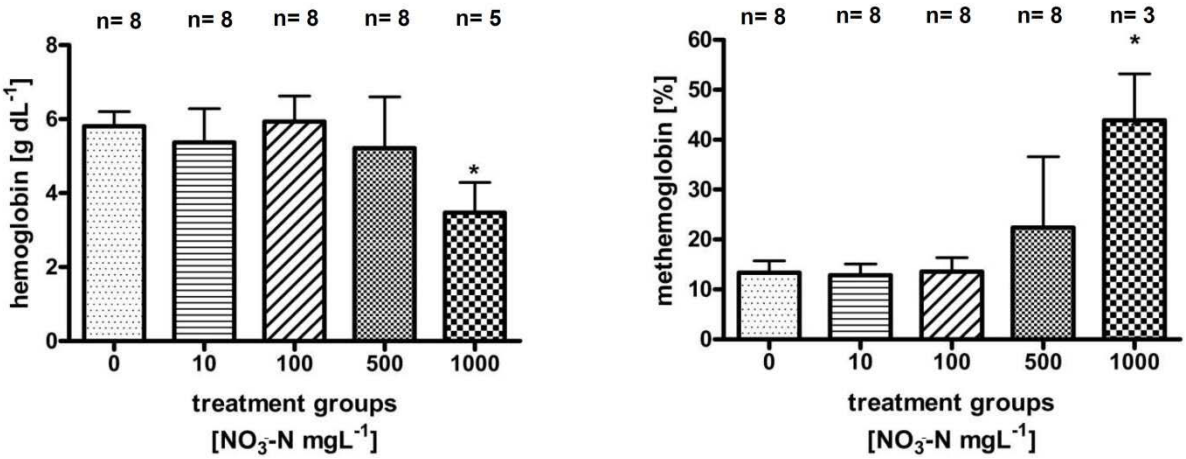

Fig. 1.4: Hemoglobin and methemoglobin concentrations (mean ± SD) in the blood of juvenile Nile tilapia *Oreochromis niloticus* after 30 d of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N. Significant differences to the control are indicated by asterisk (p<0.05, non-parametric Dunn's). The number of samples is indicated on top of each column.

3.3 Hepatosomatic index (HSI)

We observed an increase in HSI with increasing NO_3^- concentrations (Fig.1.5). The highest HSI (1.5 ± 0.5) was recorded at 1000 mgL^{-1} NO_3^--N, but no significant differences were detected (p< 0.05, nonparametric Dunn`s).

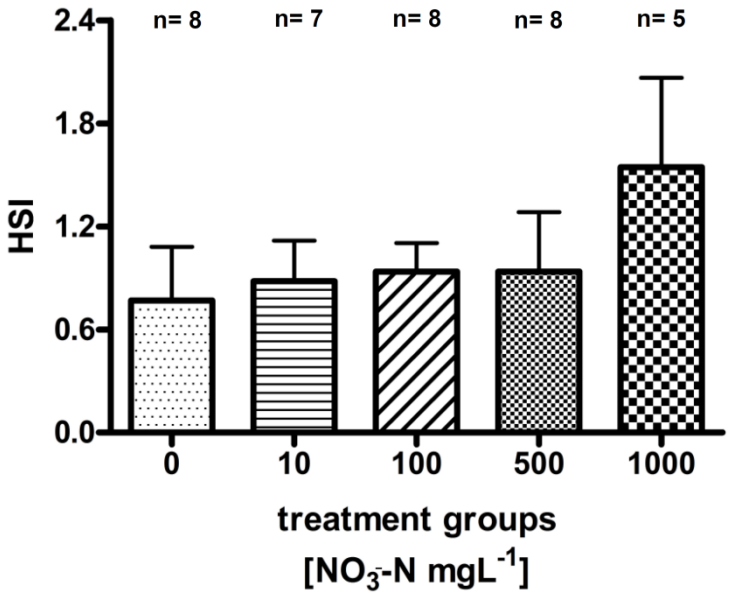

Fig. 1.5: Hepatosomatic index (HSI, mean ± SD) in juvenile Nile tilapia *Oreochromis niloticus* after 30 d of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N. No significant differences were detected ($p < 0.05$, nonparametric Dunn`s). The number of samples is indicated on top of each column. HSI = (liver weight / final weight of fish) *100.

3.4 Gill histology

Major abnormalities observed here were hyperplasia of epithelial cells, hyperplasia in cells between the lamellae, hypertrophy of pillar cells, clubbing, hypertrophy of epithelial cells, hypertrophy of mucus cells, fusion of secondary lamella and epithelial lifting (Table 1). No significant differences were analyzed between treatments, but, as a trend, most abnormalities increased with increasing NO_3^- concentrations (Table 1). Congruently, occurrence of undamaged secondary filaments decreased with increasing nitrate concentrations. Above 100 mgL^{-1} NO_3^--N less than 50% of the lamellae were undamaged compared to 62 % in the control. A strong increase of hyperplasia in epithelial cells as well as secondary lamella was recorded, particularly in the treatment group exposed to 1000 mgL^{-1} NO_3^--N. Hypertrophy of pillar cells was frequently observed (between 20 % at 1000 mg L^{-1} NO_3^--N and 56 % at 500 mg L^{-1} NO_3^--N), but revealed high individual variability. In contrast, hypertrophy of mucus and epithelial cell was very low (<5 %), again irrespective of treatment.

Table 1 Histopathological changes (relative occurrence, mean ± SD, n = 400–700) of secondary gill lamellae (100% corresponds to all examined secondary lamellae in one treatment group) in juvenile Nile tilapia *Oreochromis niloticus* after 30 days of exposure to 0, 10, 100, 500 and 1000 mg L^{-1} NO_3^--N

Treatment (mgL^{-1} NO_3^-N)	Number of secondary lamella (n)	Undamaged (%)	Hyperplasia of epithelial cells (%)	Hyperplasia between secondary lamella (%)	Hypertrophy of pillar cells (%)	Hypertrophy with clubbing (%)	Hypertrophy of epithelial cells (%)	Hypertrophy of mucus cells (%)	Fusion of lamella (%)	Epithelial lifting (%)
0	700	62 ± 17	5 ± 6	4 ± 5	29 ± 16	4 ± 5	1 ± 3	0	0	1 ± 1
10	700	53 ± 12	3 ± 5	4 ± 4	37 ± 8	10 ± 6	1 ± 3	0	0	2 ± 3
100	600	44 ± 18	3 ± 3	5 ± 5	48 ± 16	9 ± 10	0	0	1 ± 1	2 ± 1
500	600	38 ± 13	3 ± 4	4 ± 3	56 ± 13	6 ± 5	0	0	0	0
1000	400	42 ± 11	23 ± 17	19 ± 12	20 ± 20	1 ± 1	0	2 ± 2	0	2 ± 1

The major histopathological categories analysed were as follows: (a) undamaged, (b) hyperplasia of epithelial cells, (c) hyperplasia in cells between the lamellae, (d) hypertrophy of pillar cells, (e) clubbing, (f) hypertrophy of epithelial cells, (g) hypertrophy of mucus cells, (h) fusion of secondary lamella and (i) epithelial lifting. No significant differences were detected between treatment groups ($P < 0.05$, nonparametric Dunn's test).

Clubbing was equally low (<10 %) irrespective of treatment. Other abnormalities encompassed less then 5 % of the total damages.

3.5 Conversion of nitrate in the stomach of tilapia

We observed a significant conversion of nitrate in the stomach content of Nile tilapia ($p<0.01$, nonparametric Dunn`s, n=5). Nitrite already increased after 45 min, but not significantly different compared to 14 µM $NO_2^-$ (± 2) after 5 min. After 90 min, a significant increase up to 74 µM $NO_2^-$ (±14) was observed ($p<0.01$, nonparametric Dunn`s, n=5). No further increase of nitrite was observed after 150 min (Fig.1.6)

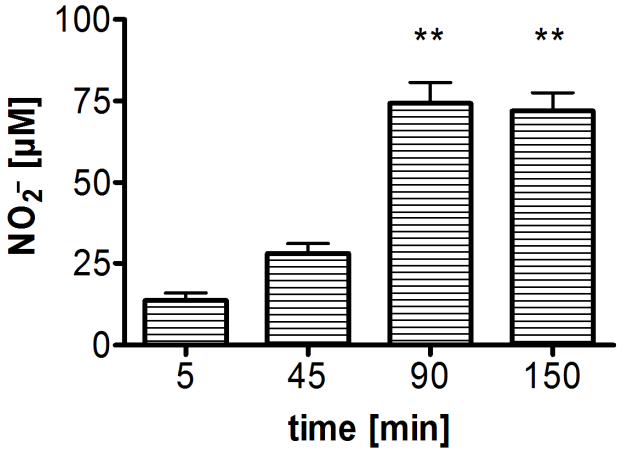

Fig. 1.6: Conversion of nitrate (nominal concentration: 1000 mg L^{-1} NO_3^--N) to nitrite in the gastric juice of Nile tilapia *Oreochromis niloticus* after incubation at room temperature. Presented are the means (± SD, n= 5). Significant differences to the start of the incubation (after 5 min) are indicated by asterisks ($p<0.01$, non-parametric Dunn's)

4 Discussion

The aim of this study was to investigate if chronic exposure to realistic nitrate concentrations observed in RAS (10-1000 mg L^{-1} NO_3^--N) induces adverse effects on growth performance, feed conversion or health status in juvenile Nile tilapia and to provide data on safe nitrate concentrations in intensive RAS-based tilapia culture. Mortalities only occurred in the highest treatment group, confirming that the range of concentrations chosen was adequate. Due to coagulation, we did not consider these fish for blood analysis. Directly after sampling, brown colored blood was recorded in fish of the highest treatment group confirming methemoglobinemia in these fish.

Both, decreasing SGR and increasing FCR were observed with increasing ambient nitrate concentrations. Still, significant differences to the control were only observed at 1000 mg L^{-1} NO_3^--N. In several studies, reduced growth performance was indicative of inadequate water quality in tilapia. For example, Shaw & Handy (2006) evaluated chronic copper toxicity in Nile tilapia, reporting depression of SGR from 1.58 (control) to 1.2. More pronounced, El-Sherif & El-Feky (2009) observed a drastic decrease of SGR from 1.16 (control) to 0.53 in tilapia fingerlings during an experiment at pH 6. Although there are no data on chronic nitrate toxicity in tilapia, reduced growth as well as increased feed conversion has been observed in other species. For example, van Bussel et al. (2012) reported a significant decrease of SGR from 1.6 to 0.45 with increasing nitrate concentration, as well as a significant increase of FCR from 1.07 to 3.80 in juvenile turbot (*Scophthalmus maximus*). In comparison to turbot (van Bussel et al., 2012), pikeperch (Schram, Roques, van Kuijk, Abbunk, van de Heul, de Vries, Bierman, van de Vis & Flik (2014, b) and catfish (Schram et al. 2014, a), results of our study suggest that tilapia is less sensitive, not surprisingly with regard to the habitat of the respective species. Here, a low feeding rate was chosen to assure an optimal water quality. Still, the decrease in SGR observed here is moderate and thus unexpectedly good with regard to the control. Congruently, feed conversion was significantly reduced at 1000 mg L^{-1} NO_3^--N with an FCR of 1.13 compared to 0.72 in the control. In a study on deleterious sub-lethal ammonia exposure (0.4 mg L^{-1} NH_3-N) to juvenile Nile tilapia, FCR increased from 1.5 (control) to 8 (El-Shafai, El-Gohary, Nasr, van der Steen & Gijzen 2004). Here, at an exposure of up to 500 mg L^{-1} NO_3^--N, neither SGR nor FCR were affected. Congruently, no effects on FCR and SGR were reported in pikeperch (*Sander lucioperca*) at nitrate concentrations up to 358 mg L^{-1} NO_3^--N (Schram et al., 2014 b).

As a conclusion, reduced growth performance and feed conversion could be a consequence of increased energy expenditure required to counteract adverse effects, for example conversion

of methemoglobin as later on discussed. Alternatively, growth depression could also arise from nitrate-mediated modulation of the thyroid axis, since nitrate competes with the uptake of iodide in the thyroid (Ward, Kilfoy, Weyer, Anderson, Folsom & Cerhan 2010). Thereby, formation of thyroid hormones T3 and T4 would be reduced which in turn leads to reduced growth. Still, plasma nitrate observed was low and nitrite much higher, supporting the conclusion that the formation of MetHb and the subsequent energy expenditure is the primary cause of reduced growth and feed conversion observed here.

The concentration of nitrate in the plasma samples was well below concentrations in ambient water. Nitrite and nitrate concentrations increased with ambient nitrate concentrations of the rearing water, but, in contrast to Schram et al. (2014, a, b), nitrite exceeded the nitrate concentrations in the plasma about 27 fold. Therefore, it seems that there was an uptake of nitrate, whether active or passive, followed by a reduction of nitrate to nitrite within the body of tilapia.

Until today, the uptake of nitrate is still poorly understood, mainly due to the fact that most tissues represent a barrier preventing the passage of the large hydrated nitrate ion. In their study on nitrate toxicity to African catfish (*Clarias gariepinus*) Schram et al. (2014, a) concluded that the integument of the fish forms a significant barrier to waterborne nitrate. As a consequence, alternative routes for nitrate uptake are limited and uptake via the gills seems most plausible with regard to the direct contact with the ambient water as well as the importance in osmoregulation and ion uptake (Hwang 2009). However, a low permeability for nitrate through the gills was discussed in trout (Stormer et al. 1996) and has been reported in freshwater crayfish (Jensen 1996). In contrast, nitrite uptake has been described for the gills as well as the intestinal wall. For example, Grosell & Jensen (2000) documented nitrite passage over the intestinal/stomach wall of the European flounder and nitrite uptake in the stomach is very fast in rats (Bryan, Fernandez, Bauer, Garcia-Saura, Milsom, Rassaf, Maloney, Bharti, Rodriguez & Feelisch 2005). Additionally, nitrite and chloride compete for the active branchial chloride uptake mechanism in freshwater fish (Williams & Eddy, 1986), and since the chloride concentration in freshwater is low, the presence of nitrite can lead to massive nitrite accumulation in the plasma (Grosell & Jensen, 2000). Furthermore, low stability of nitrite suggests rather acetic conditions to prevent fast oxidation.

Consequently we hypothesized that uptake involves a reduction of nitrate to nitrite in the stomach, prior to the actual passage of the intestinal wall. Such route would result in high plasma nitrite, similar to those observed here. Therefore, we assessed the reduction of nitrate to nitrite in stomach juice in an *in vitro* experiment. We demonstrate that nitrate is rapidly

converted into nitrite reaching a maximum of 74 µM NO_2^- after 90 min. Our findings strongly indicate that conversion of nitrate to nitrite in the gastro-intestinal system of tilapia represents the most probable uptake route. As a consequence, nitrate toxicity in tilapia is mainly a result of nitrate reduction to nitrite and irreversible oxidation of hemoglobin to methemoglobin. Nevertheless, nitrate is quite stable (~ 8 h, Webb, Patel, Loukogeorgakis, Okorie, About, Misra, Rashid, Miall, Deanfield, Benjamin, MacAllister, Hobbs & Ahluwalia 2008) and anaerobic conversion of nitrate to nitrite in the gut needs to be considered (Webb et al. 2008; Speijers & van den Brandt 2003; Fanning 2000).

In this experiment, observations, which are typically attributed to nitrite toxicity, furthermore confirm nitrite mediated intoxication. At 500 and 1000 mg L^{-1} NO_3^--N, formation of methemoglobin was 22.5 % (± 14.1) and 43.9 % (± 9.3), respecitvely. At lower concentrations, methemoglobin was low, ranging between 8.9 % and 16.5 %. Considering the actual nitrite concentrations from 23.9 µM (0 mg L^{-1} NO_3^--N) to 65.3 µM (100 mg L^{-1} NO_3^--N) in the plasma, counteracting mechanisms seem to restore homeostasis until an ambient concentration of at least 100 mg L^{-1} NO_3^--N. Here, methemoglobin reductase converts methemoglobin to hemoglobin and restores functionality of red blood cells, but also represents a substantial energy expenditure (Choury, Leroux & Kaplan, 1981). Therefore, a decrease in SGR is most likely a result of increasing methemoglobin formation and its energy demanding recycling. The presence of around 10% methemoglobin in the blood as observed between 0 mg L^{-1} NO_3^--N and 100 mg L^{-1} NO_3^--N are within the range reported as basic level in other species (Kroupova, Machova & Svobodova 2005; Wuertz et al. 2013). A visible indicator for severe methemoglobinemia is the formation of brown colored blood, which in Nile tilapia is first observed at approximately 20 % of methemoglobin with no other symptoms of toxcicity (Svobodova, Machova, Poleszczuk, Huda, Hamackova & Kroupova 2005). Here, brown color was observed during sampling of the highest treatment group at 33.9 % - 52.2 % methemoglobin. Levels above 50% methemoglobin are considered threatening to fish (Bowser, Falls, Vanzandt, Collier, & Phillips 1983), which clearly identifies NO_3^--N ≥ 1000 mg L^{-1} as intolerable for the rearing of juvenile Nile tilapia. We further recorded a significantly elevated HSI (Fig.1.5) at 1000 mg L^{-1} NO_3^--N which indicates other adverse effects on the liver. Since nitrite is an oxidizing agent this finding may indicate increased oxidative stress, but further studies are needed. Still, detoxification mechanisms to cope with oxidative stress as well as elevated nitrite include enhanced turnover by catalase and cytochrome c oxidase (summarized by Kroupova et al. 2005), which often lead to

increased liver metabolism and, subsequently, liver size. These processes are energy demanding and will hence further reduce growth performance and increase FCR.

As gills comprise the largest surface in direct contact with the surrounding water (Evans, Piermarini & Choe 2005) and subsequently represent the organ most heavily exposed, abnormalities such as fusion of the secondary lamellae have been regarded as defense mechanism limiting the uptake of toxins (Reiser, Schroeder, Wuertz, Kloas & Hanel 2010). Although some histopathological changes have been recorded in the gills, high individual variation was observed here. With regard to the low brachial permeability of nitrate, such lower incidence of gill abnormalities seems plausible. Nevertheless, a decreasing trend of undamaged secondary filaments from the control group to the highest treatment group was recorded (Table 1). We also observed increased hyperplasia of the epithelial cells as well as cells of the secondary lamella in the highest treatment group, which are typically regarded as mild responses to increase the diffusion barrier towards toxins in the water, compared to strong ones such as fusion of the lamella.

To our knowledge this investigation is the first one demonstrating that high nitrate concentrations, realistic for commercial RAS, impact juvenile tilapia at high concentrations of 500 mgL^{-1} NO_3^--N and 1000 mgL^{-1} NO_3^--N. Thus, tilapia is relatively robust towards nitrate and subsequent nitrite toxification. Here, no significant impacts on growth performance, feed conversion and health status were observed between 10 mgL^{-1} NO_3^--N and 500 mgL^{-1} NO_3^--N. Once more, it has been shown, that tilapia is well suited for intensive RAS-based aquaculture, but nutrient management such as decoupled aquaponics can improve animal health and welfare and production effectiveness.

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5 References

Bowser, P. R., Falls, W. W., Vanzandt, J., Collier, N. & Phillips, J. D. (1983) Methemoglobinemia in Channel Catfish - Methods of prevention. *Progressive Fish-Culturist* 45, 154-158.

Bryan, N. S., Fernandez, B. O., Bauer, S. M., Garcia-Saura, M. F., Milsom, A. B., Rassaf, T., Maloney, R. E., Bharti, A., Rodriguez, J. & Feelisch, M. (2005) Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nature Chemical Biology* 1, 290-7.

Camargo, J. A., Alonso, A. & Salamanca, A. (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* 58, 1255-1267.

Cheng, S.-Y. & Chen, J.-C. (2002) Study on the oxyhemocyanin, deoxyhemocyanin, oxygen affinity and acid–base balance of *Marsupenaeus japonicus* following exposure to combined elevated nitrite and nitrate. *Aquatic Toxicology* 61, 181-193.

Choury, D., Leroux, A. & Kaplan, J. C. (1981) Membrane-bound cytochrome b5 reductase (methemoglobin reductase) in human erythrocytes. Study in normal and methemoglobinemic subjects. *Journal of Clinical Investigation* 67 149-155.

Colt, J. & Tchobanoglous, G. (1976) Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. *Aquaculture* 8, 209-224.

Davidson, J., Good, C., Welsh, C. & Summerfelt, S. T. (2014) Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout *Oncorhynchus mykiss* within water recirculating aquaculture systems. *Aquacultural Engineering* 59, 30-40.

Dekov, V. M., Komy, Z., Araujo, F., Van Put, A. & Van Grieken, R. (1997) Chemical composition of sediments, suspended matter, river water and ground water of the Nile (Aswan-Sohag traverse). *Science of the Total Environment* 201, 195-210.

Edwards, T. M. Miller, H. D.; Guillette, L. J. (2006) Water quality influences reproduction in female mosquitofish *(Gambusia holbrooki)* from eight Florida springs *Environmental Health Perspectives* 114*,* 69-75.

El-Shafai, S. A., El-Gohary, F. A., Nasr, F. A., Van Der Steen, N. P. & Gijzen, H. J. (2004) Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). *Aquaculture* 232, 117-127.

El-Sherif, M. S. & El-Feky, A. M. I. (2009) Performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. I. Effect of pH. *International. Journal of. Agriculture and Biology* 11, 297-300.

Eskiocak, S., Dundar, C., Basoglu, T. & Altaner, S. (2005) The effects of taking chronic nitrate by drinking water on thyroid functions and morphology. *Clinical and Experimental Medicine* 5, 66-71.

Evans, D. H., Piermarini, P. M. & Choe, K. P. (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological reviews* 85, 97-177.

Fanning, J. C. (2000) The chemical reduction of nitrate in aqueous solution. *Coordination Chemistry Reviews* 199, 159-179.

FAO (2014) *Oreochromis niloticus* - Cultured Aquatic Species Information Programme. http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en.

Freitag, A. R., Thayer, L. R., Leonetti, C., Stapleton, H. M. & Hamlin, H. J. (2015) Effects of elevated nitrate on endocrine function in Atlantic salmon, *Salmo salar*. *Aquaculture* 436, 8-12.

Grosell, M. & Jensen, F. B. (2000) Uptake and effects of nitrite in the marine teleost fish *Platichthys flesus*. *Aquatic Toxicology* 50, 97-107.

Gutierrez-Wing, M. T. & Malone, R. F. (2006) Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquacultural Engineering* 34, 163-171.

Hamlin, H. J., Moore, B. C., Edwards, T. M., Larkin, I. L. V., Boggs, A., High, W. J., Main, K. L. & Guillette Jr, L. J. (2008) Nitrate-induced elevations in circulating sex steroid concentrations in female Siberian sturgeon (*Acipenser baeri*) in commercial aquaculture. *Aquaculture* 281, 118-125.

Hegesh, E., Gruener, N., Cohen, S., Bochkovsky, R. & Shuval, H. I. (1970) A sensitive micromethod for the determination of methemoglobin in blood. *Clinica Chimica Acta* 30, 679-682.

Hwang, P.-P. (2009) Ion uptake and acid secretion in zebrafish (*Danio rerio*). *Journal of Experimental Biology* 212, 1745-1752.

Jensen, F. B. (1996) Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). *Aquatic Toxicology* 34, 95-104.

Komy, Z. R. & El-Samahy, A. A. (1995) Dissolved Ions of Trace and Major Elements and in Suspended Sediments in the Nile, Egypt. *Chemistry and Ecology* 11, 25-37.

Kroupova, H., Machova, J. & Svobodova, Z. (2005) Nitrite influence on fish: a review. *Veterinarni Medicina* 50, 461-471.

Lattauschke, G. (2004) Anbau von Gewächshausgemüse: Hinweise zum umweltgerechten Anbau - Managementunterlage. Sächsische Landesanstalt für Landwirtschaft 2nd edition, Dresden, Germany. 220 p.

Lutz, I., Kloas, W., Springer, T., Holden, L., Wolf, J., Krueger, H. & Hosmer, A. (2008) Development, standardization and refinement of procedures for evaluating effects of endocrine active compounds on development and sexual differentiation of *Xenopus laevis*. *Analytical and Bioanalytical Chemistry* 390, 2031-2048.

Monteiro, S. M., Rocha, E., Fontainhas-Fernandes, A. & Sousa, M. (2008) Quantitative histopathology of *Oreochromis niloticus* gills after copper exposure. *Journal of Fish Biology* 73, 1376-1392.

Rakocy, J. E., Masser, M. P. & Losordo, T. M. (2006) Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture. *Southern Regional Aquaculture Center* 1-16.

Reiser, S., Schroeder, J., Wuertz, S., Kloas, W. & Hanel, R. (2010) Histological and physiological alterations in juvenile turbot (*Psetta maxima, L.*) exposed to sublethal concentrations of ozone-produced oxidants in ozonated seawater. *Aquaculture* 307, 157-164.

Schram, E., Roques, J. a. C., Abbink, W., Yokohama, Y., Spanings, T., De Vries, P., Bierman, S., Van De Vis, H. & Flik, G. (2014, a) The impact of elevated water nitrate concentration on physiology, growth and feed intake of African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture Research* 45, 1499-1511.

Schram, E., Roques, J. a. C., Van Kuijk, T., Abbink, W., Van De Heul, J., De Vries, P., Bierman, S., Van De Vis, H. & Flik, G. (2014, b) The impact of elevated water ammonia and nitrate concentrations on physiology, growth and feed intake of pikeperch (*Sander lucioperca*). *Aquaculture* 420–421, 95-104.

Scott, G. & Crunkilton, R. L. (2000) Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), C*eriodaphnia dubia*, and *Daphnia magna*. *Environmental Toxicology and Chemistry* 19, 2918-2922.

Shaw, B. J. & Handy, R. D. (2006) Dietary copper exposure and recovery in Nile tilapia, *Oreochromis niloticus*. *Aquatic Toxicology* 76 111-121.

Speijers, G. J. A. & Van Den Brandt, P. A. 2003. Nitrite and Potential Endogenous Formation of N-Nitroso Compounds. WHO Food Additives Series: 50 Geneva: World Health Organization.

Stormer, J., Jensen, F. B. & Rankin, J. C. (1996) Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: Effects on ionic balance. *Canadian Journal of Fisheries and Aquatic Sciences* 53, 1943-1950.

Svobodova, Z., Machova, J., Poleszczuk, G., Huda, J., Hamackova, J. & Kroupova, H. (2005) Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. *Acta Veterinaria Brno* 74, 129-137.

Timmons, M. B., Holder, J. L. & Ebeling, J. M. (2006) Application of microbead biological filters. *Aquacultural Engineering* 34, 332-343.

Tomasso, J. R. & Carmichael, G. J. (1986) Acute toxicity of ammonia, nitrite, and nitrate to the guadalupe bass,Micropterus treculi. *Bulletin of Environmental Contamination and Toxicology* 36, 866-870.

Van Bussel, C. G. J., Schroeder, J. P., Wuertz, S. & Schulz, C. (2012) The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). *Aquaculture* 326–329, 163-167.

Van Rijn, J. 2010. Denitrification. *In:* Timmons, M. B., Ebeling, J.M. (ed.) *Recirculating Aquaculture, second ed.* NewYork: 769 p. Cayuga Aqua Ventures .

Van Rijn, J. (2013) Waste treatment in recirculating aquaculture systems. *Aquacultural Engineering* 53, 49-56.

Ward, M. H., Kilfoy, B. A., Weyer, P. J., Anderson, K. E., Folsom, A. R. & Cerhan, J. R. (2010) Nitrate Intake and the Risk of Thyroid Cancer and Thyroid Disease. *Epidemiology (Cambridge, Mass.)* 21, 389-395.

Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J., Benjamin, N., Macallister, R., Hobbs, A. J. & Ahluwalia, A. (2008) Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. *Hypertension* 51, 784-790.

Westin, D. T. (1974) Nitrate and Nitrite Toxicity to Salmonoid Fishes. *The Progressive Fish-Culturis,* 36, 86-89.

Williams, E. M. & Eddy, F. B. Chloride uptake in freshwater teleosts and its relationship to nitrite uptake and toxicity. *Journal of Comparative Physiology B* 156, 867-872.

Wuertz, S., Schulze, S. G. E., Eberhardt, U., Schulz, C. & Schroeder, J. P. (2013) Acute and chronic nitrite toxicity in juvenile pike-perch (*Sander lucioperca*) and its compensation by chloride. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* 157 352-360.

Zimmermann-Timm, H. 2011. *Versalzung von Gewässern*. In: Warnsignal Klima: Wasser für alle? Vol. 3 Ed. Lozán, J.L.; Graßl, H.; Karbe, L.; Hupfer, P.; Schönwiese, C.-D. p 580. *Climate Service Center*, Germany

Chapter II

Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes

Decoupled systems on trial:
Eliminating bottlenecks to improve aquaponic processes

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Abstract

In classical aquaponics (coupled aquaponic systems, 1-loop systems) the production of fish in recirculating aquaculture systems (RAS) and plants in hydroponics are combined in a single loop, entailing systemic compromises on the optimal production parameters (e.g. pH). Recently presented decoupled aquaponics (2-loop systems) have been awarded for eliminating major bottlenecks. In a pilot study, production in an innovative decoupled aquaponic system was compared with a coupled system and, as a control, a conventional RAS, assessing growth parameters of fish (FCR, SGR) and plants over an experimental period of 5 months. Soluble nutrients (NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^{2-} and Fe^{2+}), elemental composition of plants, fish and sludge (N, P, K, Ca, Mg, Na, C), abiotic factors (temperature, pH, oxygen, and conductivity), fertilizer and water consumption were determined. Fruit yield was 36 % higher in decoupled aquaponics and pH and fertilizer management was more effective, whereas fish production was comparable in both systems. The results of this pilot study clearly illustrate the main advantages of decoupled, two-loop aquaponics and demonstrate how bottlenecks commonly encountered in coupled aquaponics can be managed to promote application in aquaculture.

1 Introduction

Aquaponic systems have been presented as a sustainable and resource friendly development of common recirculating aquaculture systems (RAS). Here, accumulated nutrients and water of RAS are recycled by an integrated hydroponic (soilless) plant production unit [1]. Nevertheless major drawbacks became obvious in comparison to both, professional aquaculture as well as hydroponic plant production.

Classical aquaponic systems, commonly referred to as coupled or 1-loop aquaponic systems, were described already more than 30 years ago [2, 3]. Here, the aquaculture unit and the hydroponic unit are arranged in a single loop where process water is directed from the aquaculture to the hydroponic unit and back. Inevitably, such systems provide the same water quality for both, fish and plants, which necessarily represent a compromise in the rearing conditions for each production line. Probably, the need to compromise and the lack of control on the production are the key obstacles why commercial applications are scarce and the majority of aquaponic systems are small-scale units, patronizingly called "backyard aquaponics", in schools for education purposes or in research facilities [4].

Current efforts aim at decoupled systems arranged in separate loops where process water is mainly recirculated within the respective unit, thereby allowing a better control of the species-specific requirements [5, 6]. Here, water is recirculated within the respective unit (RAS or hydroponics) and water loss due to evapotranspiration of the plants is compensated on-demand, directing process water from the fish tanks via a one-way valve into the hydroponic reservoir. Thus, water from the hydroponic unit is not redirected into the fish tanks and conditions within the hydroponic unit can be managed separately, if necessary. To improve water efficiency further, [5] described a greenhouse production equipped with an additional air conditioning system with an integrated cold trap to condensate water that is evapotranspirated by plants as well as from the RAS, redirecting the condensate (pure water) to the RAS unit.

A high diversity of fish species has been produced in aquaponics, among them catfish, carp perch and, most prominently, tilapia [2, 7-9]. The number of established crop plants may even be higher, including strawberries, tomatoes, basil and [5, 10, 11]. Here, tomatoes are considered as more difficult to grow, since nutrients, especially potassium, are required in big quantities [12].

In principle, the most important nutrients derived from the fish rearing and subsequently utilized by the growing plant crops are nitrogen (N), phosphorus (P) and potassium (K). Among them, dissolved nitrogen is primarily considered for balancing fish and plant

production during system design. Ideally, fish provide the nitrogen to sustain the plant crop growth without the need for additional nitrogen fertilization. Most of this nitrogen originates from the protein metabolism of the fish and is excreted via the gills as ammonia. Due to the high toxicity of ammonia, biofilters (moving bed, trickling filter) are integrated in the fish unit to support microbial nitrification, converting ammonia to nitrate. For optimal operation this reaction requires a pH $\geq$ 7 [13]. Since the process of nitrification results in the release of protons during ammonia oxidation[14], RAS operators have to counteract the decrease in pH by the addition of e.g. limestone [5]. On the other hand, during plant production, most nutrients become available at a pH of 5.5 - 6.5 [15]. Thus, in commercial hydroponic production, pH is controlled by the addition of acids, e.g. nitric acid [16]. Consequently, in coupled aquaponics, compromises have to be taken with regard to the production parameters including a commonly reported pH 7 [9]. Obviously, this is not ideal for neither fish or plants and species-specific adjustment by a decoupling of both units is desirable. Also, from an animal welfare perspective, addition of fertilizers in situations of nutrient imbalances is controversial due to the fact that fish are intentionally confronted with suboptimal or even negative rearing conditions. Recently, concepts for decoupled systems have been presented [1, 5]. Still, direct comparison of decoupled and coupled systems is lacking.

To our knowledge this is the first study comparing coupled and decoupled aquaponics under realistic production conditions. The results of this pilot study demonstrate the main advantages of decoupled aquaponics and highlight the bottlenecks of classical aquaponic systems. Furthermore, practical and theoretical recommendations should serve as guidance for future system design and best practices.

2 Material and methods

2.1 Aquaponic system

Experiments were conducted at the aquaponic research facility of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Berlin, Germany). Briefly, three identical RAS with a total volume of 16.5 m^3 each (culture volume 6.8 m^3, four separate rearing tanks of 1.7 m^3 each) were stocked with Nile tilapia (*Oreochromis niloticus,* weight: Ø 68 g) according to Table 2.2 and purchased at a commercial supplier (Kirschauer Aquakulturen, Germany). For biofiltration (nitrification) each RAS was equipped with a moving bed filter (2 m^3) providing a substrate surface of approximately 1350 m^2. In the first RAS (A) a drumfilter (mesh size: 100µm) was used to remove suspended solids, representing the most frequently used technology used in commercial RAS.

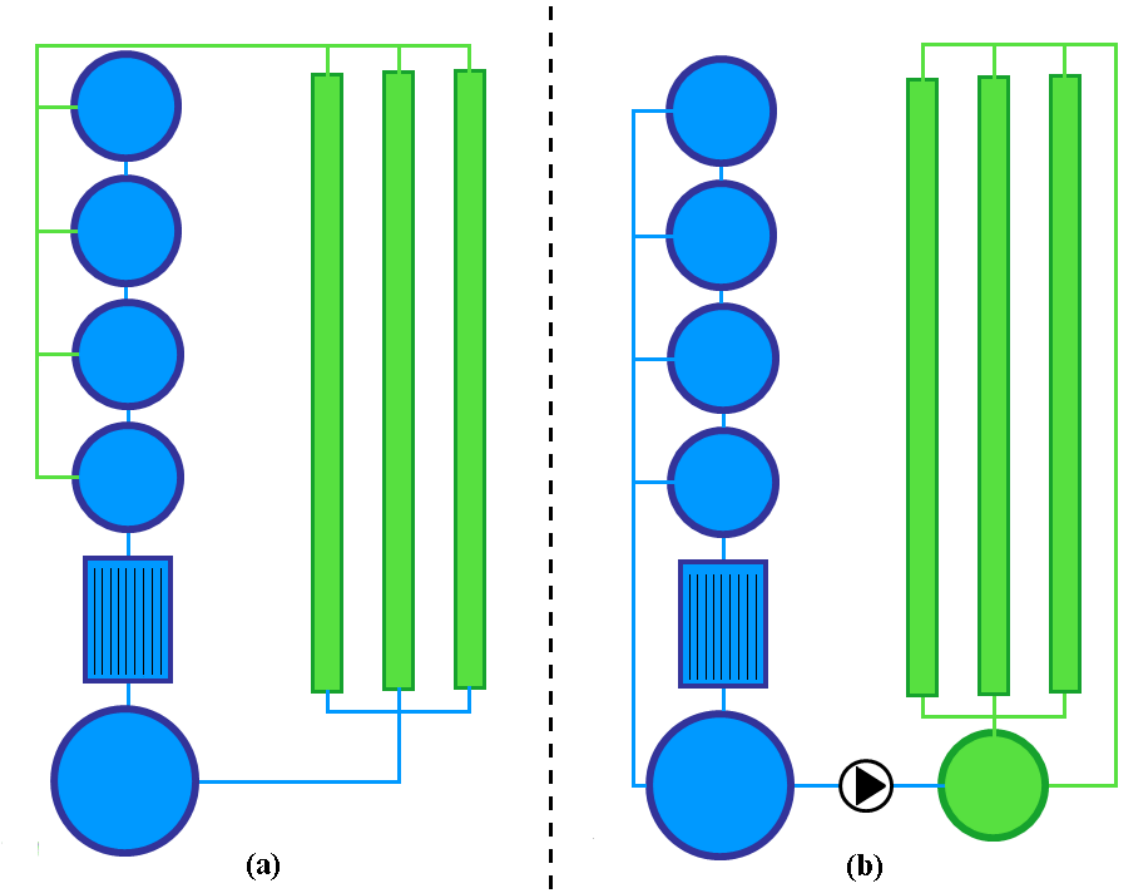

Fig. 2.1: Schematic illustration of classical (coupled) and decoupled aquaponics. (a): Classical aquaponic system consisting of a RAS (blue: rearing tanks, clarifier and biofilter) directly connected to the hydroponic unit (green: NFT-trays). Water is constantly circulated from RAS to hydroponic and back to RAS. (b): Decoupled aquaponic system consisting of a RAS connected to the hydroponic unit (with additional reservoir) via one-way-valve. Water is separately recirculated in each system and water is just supplied on-demand from RAS to the hydroponic unit, but not back.

Here, no hydroponic unit was integrated and this system was used as control (conventional aquaculture reference). In the two remaining, coupled (RAS C) and decoupled (RAS D) systems (Fig. 2.1), suspended solid removal was achieved with a clarifier (1.5 m^3), which is often used in aquaponic applications due to the energy and water efficiency. Here, five NFT-trays (l45 cm * 30 cm, h: 28 cm each) were arranged as hydroponic unit, integrated to the RAS (C, D). RAS D was connected to the hydroponic units via one-way-valve, providing a decoupled, two-loop aquaponic system [5]. As a consequence, water from RAS D was only directed on demand to the respective hydroponic unit, but not redirected to the RAS. RAS C was operated as a single-loop aquaponic system (coupled, classical approach) where five hydroponic units were connected to the RAS with a by-pass using a pump (10L/min) installed in the pump sump. To prevent clogging and fouling of the plant roots by suspended solids originating from the RAS, a small filter (Eheim, Germany) was interposed and cleaned on a regular basis. Over the experimental period, fish were fed a commercial food (Aller Float 37/10 2 mm and 3 mm, Emsland-Aller Aqua, Germany) according to Table 2.2. Temperature, pH and oxygen were determined daily (HQ40d multi, Hach Lange GmbH, Germany); pH was regulated with $Ca(OH)_2$ according to Table 2.2. Selected nutrients (NO_3^--N, NO_2^--N, TAN, PO_4^{3-}, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, Cl^- and Fe^{2+}) in the water were determined spectrophotometrically (DR3900 Hach Lange, Berlin, Germany) with the respective kit.

2.2 Tomato plants

Tomato plants (*Solanum lycopersicum*, variety: Pannovy) originated from a company specialized on hydroponic vegetables (Schwanteland GmbH, Germany). They were grown in rock wool cubes (10 cm * 10 cm) and had a mean height of 42.1 cm (± 4.3 cm). Per RAS, 15 tomato plants were randomly distributed to the trays of the respective hydroponic unit. Water consumption and fertilizer supply was according to Table 2.1. The fertilizers had the following composition: Krista K Plus (Yara, Germany): 13.7 % total N (13.7 % NO_3-N) and 46.3 % K_2O; CalciNit (Yara, Germany): 15.5% total N (14.4% NO_3-N and 1.1 % NH_4-N) and 26.3 % calcium oxide (CaO). Manna Lin M Spezial is a NPK fertilizer with 18 % total N (11 % NO_3-N and 7 % NH_4-N), 12 % P_2O_5, 18 % K_2O, 2 % MgO and trace elements including Fe, Mn, Zn, B, Cu, and Mo. Partly $KHCO_3$ was also used to increase the potassium concentration.

2.3 Elemental analysis

Over the five month experimental period, samples of leaves and fruits were taken according to Table 2.5. Plants were chosen randomly, per sampling point and system five replicates of two

leaves were taken (always the fifth fully developed leave) as well as five replicates of two fully ripe tomatoes. Samples were freeze dried prior to elemental analysis. Total phosphorus (TP), magnesium (Mg), calcium (Ca), potassium (K), sodium (Na) were determined by ICP-OES (inductively coupled plasma optical emission spectrometry; iCAB 6000, Thermo Fisher Scientific Inc., USA) after wet digestion (HCl 37%, HNO_3 65%, volumetric ratio 1:3) in a high pressure microwave oven (Gigatherm, Switzerland). C/N analysis of plants and fish were performed using freeze dried (to a constant weight), weighed samples and analyzed in a Vario EL© system (Elementar Analysensysteme GmbH, Germany). Composition of sludge (n= 4) and fish (n=3) was determined accordingly.

2.4 Determination of total solids (TS) and total suspended solids (TSS) in the RAS

For the evaluation of the weekly loss of TS due to cleaning of the clarifier (RAS C and D), water-sludge mixture from the clarifier (1.5 m^3) was collected three times within the experimental period in a 2 m^3 tank and homogenized with a pump. Per sampling five subsamples were taken in 10 L containers each. Aliquots of fresh sludge (n=15) were freeze dried to determine the dry weight: wet weight ratio.

For TSS, water samples (100 ml) were taken in triplicate at the inflow of a fish tank at the beginning of the experiment, after 3 months and at the end of the experimental period. Briefly, samples were filtered through pre-weighted 0.45 µm CA membrane filters (GE Healthcare, United Kingdom), freeze dried to a constant weight and weighed.

2.5 Estimated fate of nitrogen

The schematic illustration of the fate of nitrogen (Fig. 2.3) was developed according to the results of the present study and literature values. Literature values considered were those for % N of proteins [17, 18], the excretion of N [19-21], nitrification [13], uncontrolled denitrification [22] and nitrate uptake of tomatoes [23, 24].

3 Results

3.1 Plant growth, fertilizer supplementation and water consumption

Plant growth, fertilizer supplemented and water consumption in the hydroponic units of the coupled and decoupled aquaponic system (Hydro C, Hydro D) are presented in Table 2.1. Over the entire experimental period of 154 d, more tomatoes were harvested from Hydro D (123.5 kg) than from Hydro C (90.9 kg), corresponding to a 36 % higher tomato yield in the decoupled system. In contrast, 31 % more leaves (63.7 kg), 60 % more roots (5.8 kg) and 50 % more stem biomass (5.8 kg) were harvested from the coupled system. At the same time, fertilizer supplementation was identical in both systems (Table 2.1). Water consumption was lowest in the beginning and at the end of the experiment with 1.4 L per plant per day in Hydro D. Between the 07.05 and the 06.08.2015, water consumption was highest and ranged between 2.0 and 2.4 L per plant per day.

Table 2.1: Plant growth (fresh weight of fruit, leave, root, stem), fertilizer supplementation and water consumption in the hydroponic unit of the coupled (Hydro C) and decoupled (Hydro D) aquaponic system after 30, 63, 94, 122 and 154 d. Water consumption is only indicated for Hydro D, since Hydro C is coupled to the RAS C and is only given for the entire system (Table 2.2). Roots and stems were only sampled at the end of the experiments and fresh weight therefore not determined (n.d.) earlier.

Hydroponic	sampling intervals	days [d]	harvest [kg]				fertilizer [g]				water consumption [L]
			fruit	leave	root	stem	Krista K +	Calcinit	Manna Lin M Spezial	$KHCO_3$	
C	07.04.-06.05.15	30	0.24	11.1	n.d.	n.d.	325	130	60	0	bypass
	07.05.-08.06.15	63	25.90	12.4	n.d.	n.d.	179	140	65	0	bypass
	09.06.-09.07.15	94	13.67	12.7	n.d.	n.d.	160	0	50	300	bypass
	10.07.-06.08.15	122	11.41	6.4	n.d.	n.d.	30	0	0	0	bypass
	07.08.-07.09.15	154	39.66	21.1	5.8	25.7	0	0	0	0	bypass
	total	154	**90.9**	**63.7**	**5.8**	**25.7**	**694**	**270**	**175**	**300**	bypass
D	07.04.-06.05.15	30	1.6	11.7	n.d.	n.d.	325	130	60	0	634
	07.05.-08.06.15	63	41.2	11.2	n.d.	n.d.	179	140	65	0	990
	09.06.-09.07.15	94	27.2	7.4	n.d.	n.d.	160	0	50	300	964
	10.07.-06.08.15	122	18.6	6.0	n.d.	n.d.	30	0	0	0	983
	07.08.-07.09.15	154	34.9	11.7	2.3	17.1	0	0	0	0	670
	total	**154**	**123.5**	**48.0**	**2.3**	**17.1**	**694**	**270**	**175**	**300**	**4961**

3.2 Fish growth and RAS performance

Fish growth, feed conversion ratios (FCR) and specific growth rates (SGR) are presented in Table 2.2 and were in the same range among all three RAS (A, C, D) over the entire experimental period. The average FCR in each system ranged between 1.2 and 1.3, increasing over time from 1.0 to 1.6, identifying an increased feed conversion in larger fish. In each system, the average SGR was 1.0 whereas a continuous decrease down to 0.5 (A and D) and 0.6 (C) was observed towards the end of the experiment. Water consumption was also

comparable between the aquaponic systems. Still, in the aquaculture control RAS (A) the water consumption was higher at 5-6 % RAS d^{-1}. Also, in both aquaponic systems, addition of limestone was similar and increased from 0.7 g to 6.1 kg within the experimental period. Approximately 22 % less limestone was used in the aquaculture control RAS A to regulate the pH to comparable levels. Initial, final weight and subsequently overall weight gain revealed no difference (<2 %) between fish units. Over the entire period mortalities (< 1.5 %) were very low in all systems.

Table 2.2: Details on the stocking, amount of feed fed, specific growth rate (SGR), food conversion ratio (FCR), mortalities, water consumption and limestone added to control pH in the fish units of the coupled (RAS C) and decoupled aquaponic system (RAS D) compared to the control (RAS A) after 30, 63, 94, 122 and 154 d .

RAS	sampling intervals	days [d]	stocked tanks [n]	tank volume [m^3]	RAS stocking start [kg]	RAS stocking end [kg]	fish growth [kg $month^{-1}$]	feed [kg]	FCR	SGR	mortalities [kg]	water consumption [m^3]	water consumption [%RAS d^{-1}]	limestone addition [kg]
A	07.04.-06.05.15	30	1	1.7	66.9	104.3	37.4	37.2	1.0	1.5	0.11	14.92	3.0	0.9
	07.05.-08.06.15	63	2	1.7	104.3	153.1	48.8	58.2	1.2	1.2	0.33	29.33	5.4	1.3
	09.06.-09.07.15	94	2	1.7	153.1	220.5	67.4	75.6	1.1	1.2	0.56	29.33	5.7	2.4
	10.07.-06.08.15	122	3	1.7	220.5	273.4	52.9	71.0	1.3	0.8	0.34	28.51	6.2	2.4
	07.08.-07.09.15	154	3	1.7	273.4	324.6	51.3	83.7	1.6	0.5	1.40	32.02	6.1	5.1
	total / *average*	**154**					**257.7**	**325.6**	***1.3***	***1.0***	**2.75**	**134.12**	***5.3***	**12.1**
C	07.04.-06.05.15	30	1	1.7	66.8	101.0	34.2	37.2	1.1	1.4	0.00	15.24	3.1	0.7
	07.05.-08.06.15	63	2	1.7	101.0	147.6	46.6	58.2	1.2	1.1	0.60	13.05	2.4	1.7
	09.06.-09.07.15	94	2	1.7	147.6	218.9	71.3	75.6	1.1	1.3	0.00	13.86	2.7	4.1
	10.07.-06.08.15	122	3	1.7	218.9	275.1	56.2	71.0	1.3	0.8	0.72	16.47	3.6	3.1
	07.08.-07.09.15	154	3	1.7	275.1	330.2	55.1	83.7	1.5	0.6	1.16	15.25	2.9	6.0
	total / *average*	**154**					**263.4**	**325.6**	***1.2***	***1.0***	**2.48**	**73.87**	***2.9***	**15.6**
D	07.04.-06.05.15	30	1	1.7	66.8	102.4	35.6	37.2	1.0	1.4	0.17	15.91	3.2	0.5
	07.05.-08.06.15	63	2	1.7	102.4	145.5	43.2	58.2	1.3	1.1	0.19	12.52	2.3	1.4
	09.06.-09.07.15	94	2	1.7	145.5	217.9	72.3	75.6	1.0	1.3	0.15	13.86	2.7	4.0
	10.07.-06.08.15	122	3	1.7	217.9	271.5	53.7	71.0	1.3	0.8	0.44	16.07	3.5	3.3
	07.08.-07.09.15	154	3	1.7	271.5	323.7	52.2	83.7	1.6	0.5	0.48	13.59	2.6	6.1
	total / *average*	**154**					**256.9**	**325.6**	***1.3***	***1.0***	**1.42**	**71.95**	***2.83***	**15.3**

3.3 Rearing conditions in the fish and the hydroponic units

Rearing conditions are presented in Table 2. 3. The dissolved oxygen concentration was high (6.3-6.5 mg L^{-1}) and within the same range between RAS A, C and D. Over the experimental period a higher average oxygen concentration was recorded in Hydro D (8.2 mg L^{-1}) compared to Hydro C (6.5 mg L^{-1}) and all fish units. Similarly, the pH was in the same range between fish units RAS A, RAS C / Hydro C and RAS D (pH 7.1-7.4), but substantially lower in the decoupled Hydro D (pH 6.4). The average temperature in all three RAS and Hydro C oscillated around 27 °C. In Hydro D a lower average temperature (24.3°C ± 1.7) was observed. The conductivity ranged between 1.1 mS cm^{-1} and 1.5 mS cm^{-1} in the three RAS and Hydro C, but was nearly two fold increased at 2.8 mS cm^{-1} in Hydro D compared to Hydro C (1.5 mS cm^{-1}).

Table 2.3: Rearing conditions in the fish unit and the hydroponic unit, including dissolved oxygen (O_2), pH, temperature and conductivity in the fish (RAS) and hydroponic (Hydro) units, assessed over 154 days (07.04 - 07.09.2015).

experimental system	experimental period	days [d]	O_2 [mgL^{-1}]	pH	temperature [°C]	conductivity [$mScm^{-1}$]
RAS A	07.04.-07.09.15	154	6.4 (± 1.0)	7.4 (± 0.4)	26.8 (± 1.5)	1.1 (± 0.1)
RAS C / Hydro C	07.04.-07.09.15	154	6.5 (± 1.1)	7.1 (± 0.3)	26.8 (± 1.0)	1.5 (± 0.3)
RAS D	07.04.-07.09.15	154	6.3 (± 1.1)	7.2 (± 0.3)	27.2 (± 1.2)	1.5 (± 0.3)
Hydro D	07.04.-07.09.15	154	8.2 (± 0.4)	6.4 (± 0.7)	24.3 (± 1.7)	2.8 (± 0.9)

3.4 Dissolved nutrients in RAS and hydroponics

Dissolved nutrients in RAS and hydroponics were determined weekly and are presented in Table 2.4. In all three RAS, a constant accumulation of nitrate was observed over the 154 d experimental period, increasing from 15.7-19.8 mg L^{-1} during the first sampling interval up to 65.9 -100.8 mg L^{-1} at the end of the experimental period. In Hydro D, nitrate concentration increased from 98.8 mg L^{-1} NO_3^--N to more than 170 mg L^{-1} from the third month on. During the entire experimental period, nitrite in all fish and hydroponic units was very low (≤ 0.1 mgL^{-1} NO_2^--N). Ammonium revealed concentrations ≤ 0.4 mgL^{-1} NH_4^+-N in the RAS units and Hydro C. Only in Hydro D a maximum of 6.4 mg L^{-1} NH_4^+-N was observed at the beginning of the experimental period, which constantly decreased to low levels comparable the other systems. In all fish and hydroponic units, the phosphate concentration decreased to 5.6-9.6 mg L^{-1} towards the end of the experimental period. Still, during the first two months, phosphate concentrations were more than 2-fold higher in Hydro D than in Hydro C. Potassium concentrations in both aquaponic systems were generally higher than in the RAS A, but levels in all units ranged between 17 and 50 mg L^{-1}. Exceptionally low potassium concentrations < 5 mg L^{-1} were only observed during the last month in Hydro D. Also, no substantial differences were observed with respect to the chloride concentrations in the fish units and Hydro C, ranging between 29 - 46.5 mg L^{-1} Cl^-. Only in Hydro D an accumulation of chloride from 46 mg L^{-1} to 89.7 mg L^{-1} Cl^- was observed. Sulfate ranged between 157.5 and 195 mg L^{-1}, only in Hydro D substantially elevated concentrations (295-660 mg L^{-1}) were observed. Similarly, calcium was 3-fold increased in Hydro D (362.8-558.5 mg L^{-1}) compared to Hydro C (119.9-148.5 mg L^{-1}). Iron and magnesium were within the same range between all RAS and Hydro C; only Hydro D revealed higher concentrations.

Table 2.4: Dissolved nutrients in the fish (RAS A, C, D) and hydroponic units (Hydro C, Hydro D) assessed over a 154 d experimental period (07.04 - 07.09.2015). Nutrients in RAS C correspond to the nutrients in Hydro C, since both are arranged as coupled aquaponic system.

RAS / Hydro	sampling intervals	NO_3^--N [mgL^{-1}]	NO_2^--N [mgL^{-1}]	NH_4^+-N [mgL^{-1}]	PO_4^{3-} [mgL^{-1}]	K^+ [mgL^{-1}]	Ca^{2+} [mgL^{-1}]	Mg^{2+} [mgL^{-1}]	SO_4^{2-} [mgL^{-1}]	Cl^- [mgL^{-1}]	Fe^{2+} [mgL^{-1}]
A	07.04.-06.05.15	15.7 ± (4.7)	0.09 ± (0.08)	0.12 ± (0.06)	14.8 ± (0.9)	22.0 ± (1.4)	123.4 ± (0.8)	14.1 ± (0.0)	165 ± (35.4)	36.5 ± (7.1)	0.01 ± (0.00)
	07.05.-08.06.15	27.6 ± (3.6)	0.07 ± (0.03)	0.09 ± (0.04)	10.4 ± (2.2)	21.6 ± (1.8)	130.6 ± (6.0)	21 ± (7.7)	178.8 ± (2.5)	30.3 ± (5.9)	0.01 ± (0.01)
	09.06.-09.07.15	30.4 ± (10.9)	0.06 ± (0.02)	0.16 ± (0.16)	6.7 ± (1.1)	19.3 ± (4.8)	134.1 ± (5.8)	17.7 ± (3.0)	161.3 ± (6.3)	29.0 ± (2.3)	0.01 ± (0.01)
	10.07.-06.08.15	52.3 ± (4.8)	0.04 ± (0.01)	0.08 ± (0.01)	6.4 ± (0.9)	18.7 ± (2.1)	136.8 ± (5.4)	16.3 ± (0.9)	168.8 ± (2.5)	30.6 ± (1.8)	0.01 ± (0.01)
	07.08.-07.09.15	65.9 ± (6.1)	0.04 ± (0.01)	0.07 ± (0.04)	5.6 ± (0.8)	17.0 ± (1.4)	141.1 ± (10.8)	16.2 ± (1.2)	160 ± (5.0)	38.2 ± (5.8)	0.01 ± (0.01)
C Hydro C	07.04.-06.05.15	19.8 ± (6.2)	0.05 ± (0.00)	0.06 ± (0.01)	17.1 ± (0.4)	27.8 ± (3.9)	119.8 ± (0.8)	13.8 ± (0.7)	175 ± (35.4)	39.8 ± (7.4)	0.01 ± (0.01)
	07.05.-08.06.15	36.2 ± (14.9)	0.08 ± (0.03)	0.04 ± (0.01)	12.8 ± (2.0)	28.0 ± (2.6)	138.5 ± (11.6)	21.9 ± (7.6)	197.5 ± (2.9)	31.0 ± (6.1)	0.01 ± (0.01)
	09.06.-09.07.15	59.2 ± (14.0)	0.07 ± (0.02)	0.15 ± (0.12)	9.8 ± (0.7)	40.8 ± (10.6)	148.5 ± (5.3)	19.4 ± (1.8)	191.3 ± (8.5)	34.6 ± (3.6)	0.01 ± (0.01)
	10.07.-06.08.15	65.3 ± (11.5)	0.05 ± (0.01)	0.06 ± (0.01)	8.3 ± (1.6)	38.7 ± (19.4)	144.8 ± (7.3)	19.3 ± (1.8)	195 ± (7.1)	39.1 ± (2.3)	0.02 ± (0.01)
	07.08.-07.09.15	72.8 ± (19.9)	0.05 ± (0.02)	0.06 ± (0.02)	6.3 ± (0.9)	27.3 ± (4.0)	149.2 ± (2.8)	20.4 ± (1.3)	190 ± (18.0)	46.5 ± (7.5)	0.02 ± (0.02)
D	07.04.-06.05.15	17.5 ± (7.4)	0.02 ± (0.01)	0.03 ± (0.01)	16.9 ± (3.8)	22.0 ± (3.5)	125.2 ± (1.1)	14.9 ± (0.5)	157.5 ± (10.6)	38 ± (10.6)	0.01 ± (0.00)
	07.05.-08.06.15	27.1 ± (7.0)	0.06 ± (0.01)	0.05 ± (0.02)	14.2 ± (0.9)	25.4 ± (2.9)	140.2 ± (8.6)	22.9 ± (8.2)	173.8 ± (6.3)	29.7 ± (6.5)	0.01 ± (0.00)
	09.06.-09.07.15	50.4 ± (9.6)	0.06 ± (0.02)	0.12 ± (0.19)	13.4 ± (0.9)	40.0 ± (10.1)	152.0 ± (4.4)	19.7 ± (1.2)	186.3 ± (9.5)	33.8 ± (3.0)	0.01 ± (0.01)
	10.07.-06.08.15	77.6 ± (6.5)	0.05 ± (0.02)	0.06 ± (0.01)	11.9 ± (1.1)	41.7 ± (20.8)	149.0 ± (2.5)	23.4 ± (7.3)	193.8 ± (8.5)	38.5 ± (1.3)	0.01 ± (0.01)
	07.08.-07.09.15	100.8 ± (10.8)	0.05 ± (0.02)	0.05 ± (0.02)	9.6 ± (1.2)	29.8 ± (2.9)	149.3 ± (6.3)	19.7 ± (0.9)	183.3 ± (10.4)	33.7 ± (8.9)	0.01 ± (0.01)
Hydro D	07.04.-06.05.15	98.8 ± (23.7)	0.07 ± (0.08)	3.60 ± (0.28)	29.1 ± (6.8)	207.5 ± (3.5)	556.0 ± (90.5)	49.5 ± (10.6)	295 ± (49.5)	46.0 ± (9.9)	0.01 ± (0.00)
	07.05.-08.06.15	136.9 ± (58.4)	0.02 ± (0.02)	2.25 ± (3.03)	26.1 ± (8.5)	41.8 ± (30.5)	362.8 ± (61.9)	36.4 ± (9.9)	515 ± (256.8)	36.5 ± (9.3)	0.11 ± (0.14)
	09.06.-09.07.15	175.0 ± (38.7)	0.01 ± (0.01)	0.64 ± (0.67)	12.9 ± (1.9)	50.0 ± (41.4)	558.5 ± (137.4)	57.3 ± (27.2)	660 ± (468.5)	76.6 ± (43.3)	0.05 ± (0.03)
	10.07.-06.08.15	207.5 ± (70.1)	0.01 ± (0.00)	0.08 ± (0.05)	7.2 ± (2.9)	24.0 ± (25.6)	442.8 ± (43.4)	56.3 ± (21.9)	470 ± (194.9)	69.1 ± (15.1)	0.12 ± (0.08)
	07.08.-07.09.15	174.5 ± (38.5)	0.00 ± (0.02)	0.02 ± (0.01)	6.7 ± (1.7)	4.2 ± (4.4)	482.0 ± (147.8)	50.1 ± (16.9)	373.3 ± (50.3)	89.7 ± (23.8)	0.10 ± (0.04)

In Fig. 2.2 the development of key nutrients (N, P, K) is presented over the experimental period with respect to recommended concentrations for tomato production. In all RAS systems there was a general accumulation of N without reaching the recommended threshold (dashed line). A constant decrease of P and a more or less stable concentration of K with a peak in the middle of the experimental period was observed. Again recommended concentrations were not reached and in the case of K stayed far beyond the recommended threshold. In all cases RAS A showed the lowest concentrations of key nutrients and highest observed concentrations occurred in Hydro D. Here, recommended levels of N were often reached or even exceeded. The K concentration was just close to optimum conditions towards the start of experiments but lowered considerably towards the end of the experimental period. Also, during the first third of the experimental period, the P concentration was frequently higher than in all other systems but showed the same decreasing trend towards the end.

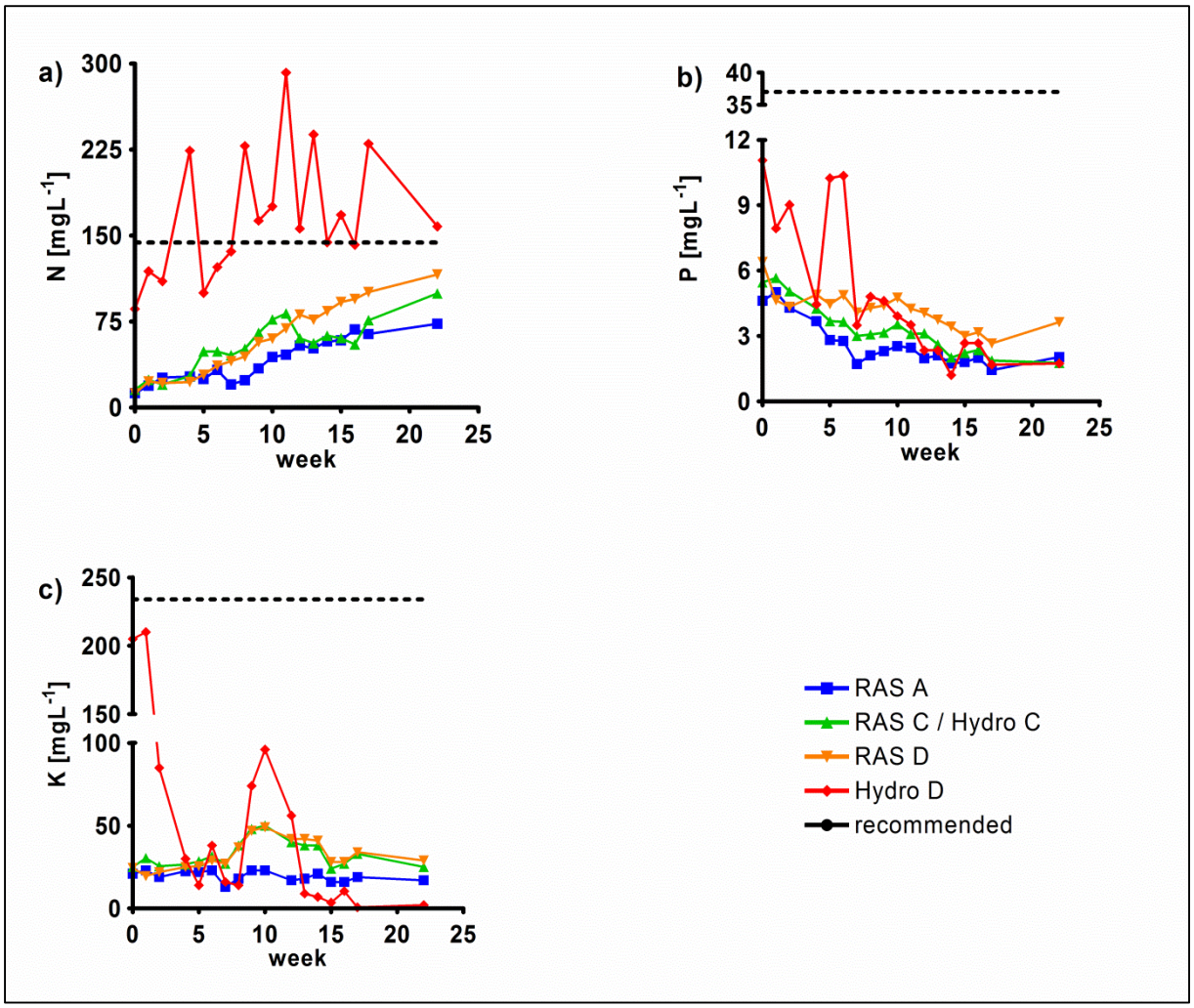

Fig. 2.2: Development of the key nutrients (N, P, K) for plant production in the fish (RAS) and hydroponic (Hydro) units of the coupled (RAS C/Hydro C) and decoupled (RAS D, Hydro D) aquaponic system compared to the control (RAS A) over 22 weeks. Nutrients in RAS C correspond to the nutrients in Hydro C since both are arranged as coupled aquaponic system. Recommended nutrient requirements for tomato production are indicated (dashed line).

3.5 Elemental composition of plants, fish and sludge

In general, composition of plant leaves and tomatoes revealed no major differences of the respective plant parts between Hydro C and Hydro D, neither in ICP-OES analysis nor C/N ratio (Table 2.5). Only the phosphate contents of tomatoes and leaves were lower in Hydro C compared to Hydro D. In addition, sodium concentrations in the fruit were slightly higher in Hydro D compared to Hydro C. Mean elemental composition of fish and sludge were also determined and are provided to complete the picture of the overall aquaponic system.

Table 2.5: Element analysis (ICP-OES and C/N) of plant leaves and tomatoes harvested from the coupled (Hydro C) and decoupled (Hydro D) aquaponic system after 30, 63, 94, 122 and 154 d within the experimental period. Additionally data for fish and sludge are presented.

system	experimental period / date	sample	Ca [g kg^{-1}]	K [g kg^{-1}]	Mg [g kg^{-1}]	Na [g kg^{-1}]	P [g kg^{-1}]	N [%]	C [%]	C/N
Hydro C	07.05.-08.06.15	leaf	30.4 ± (1.9)	45.4 ± (1.3)	4.4 ± (0.2)	0.3 ± (0.0)	5.1 ± (0.2)	3.4 (± 0.1)	36.6 (± 0.1)	10.9 (± 0.5)
	09.06.-09.07.15	leaf	32.4 ± (3.0)	40.3 ± (7.3)	4.8 ± (0.5)	0.3 ± (0.0)	4.4 ± (0.3)	3.0 (± 0.2)	37.5 (± 0.4)	12.3 (± 0.8)
	10.07.-06.08.15	leaf	26.0 ± (2.3)	35.3 ± (2.2)	3.9 ± (0.2)	0.3 ± (0.0)	4.7 ± (0.3)	3.2 (± 0.2)	38.1 (± 0.3)	11.9 (± 0.8)
	07.08.-07.09.15	leaf	34.0 ± (3.6)	33.2 ± (3.2)	3.8 ± (0.4)	0.4 ± (0.0)	4.3 ± (0.5)	2.6 (± 0.3)	37.1 (± 0.3)	14.2 (± 1.3)
	07.05.-08.06.15	tomato	2.2 ± (1.0)	47.5 ± (0.2)	1.3 ± (0.1)	0.3 ± (0.0)	4.6 ± (0.2)	2.0 (± 0.1)	38.8 (± 0.4)	19.1 (± 1.3)
	09.06.-09.07.15	tomato	2.1 ± (0.3)	41.6 ± (2.5)	1.4 ± (0.1)	0.2 ± (0.0)	4.3 ± (0.2)	1.7 (± 0.2)	39.9 (± 0.2)	24.3 (± 3.2)
	10.07.-06.08.15	tomato	1.3 ± (0.3)	41.0 ± (1.6)	1.5 ± (0.0)	0.3 ± (0.0)	4.0 ± (0.5)	2.0 (± 0.2)	39.3 (± 0.3)	19.8 (± 1.5)
	07.08.-07.09.15	tomato	1.1 ± (0.1)	42.0 ± (4.4)	1.5 ± (0.3)	0.3 ± (0.2)	4.4 ± (0.3)	2.0 (± 0.5)	39.7 (± 0.7)	20.3 (± 4.5)
Hydro D	07.05.-08.06.15	leaf	26.7 ± (4.3)	39.9 ± (2.4)	3.9 ± (0.2)	1.1 ± (0.1)	2.7 ± (0.1)	3.9 (± 0.1)	38.7 (± 0.7)	10.1 (± 0.4)
	09.06.-09.07.15	leaf	23.1 ± (3.3)	46.0 ± (0.9)	3.2 ± (0.3)	1.3 ± (0.1)	2.6 ± (0.4)	3.2 (± 0.1)	39.1 (± 0.5)	12.3 (± 0.4)
	10.07.-06.08.15	leaf	25.5 ± (2.8)	36.0 ± (1.6)	4.0 ± (0.2)	0.9 ± (0.1)	2.9 ± (0.2)	3.8 (± 0.1)	39.1 (± 0.6)	10.4 (± 0.2)
	07.08.-07.09.15	leave	26.7 ± (11.1)	32.8 ± (7.5)	3.2 ± (0.9)	0.7 ± (0.2)	2.6 ± (0.5)	3.2 (± 0.4)	38.9 (± 1.0)	12.2 (± 2.1)
	07.05.-08.06.15	tomato	1.7 ± (0.2)	45.6 ± (5.2)	1.2 ± (0.2)	0.4 ± (0.0)	3.7 ± (0.6)	2.1 (± 0.4)	39.5 (± 0.4)	19.6 (± 4.3)
	09.06.-09.07.15	tomato	1.3 ± (0.1)	36.1 ± (3.9)	1.3 ± (0.1)	0.5 ± (0.0)	3.1 ± (0.6)	2.0 (± 0.2)	39.4 (± 0.4)	20.1 (± 1.9)
	10.07.-06.08.15	tomato	1.1 ± (0.4)	40.5 ± (2.9)	1.3 ± (0.1)	0.4 ± (0.1)	3.0 ± (0.8)	2.0 (± 0.3)	39.6 (± 0.1)	20.2 (± 2.9)
	07.08.-07.09.15	tomato	1.2 ± (0.5)	41.5 ± (2.8)	1.4 ± (0.1)	0.4 ± (0.2)	3.4 ± (0.6)	2.1 (± 0.4)	39.3 (± 0.1)	19.0 (± 3.3)
RAS A-B-C	09.09.2015	fish	31.7 (± 1.0)	1.5 (± 0.1)	2.1 (± 0.1)	0.7 (± 0.0)	17.7 (± 0.5)	7.6 (± 0.2)	53.3 (± 4.2)	7.0 (± 0.3)
RAS C-D	09.09.2015	sludge	11.9 (± 5.8)	8.3 (± 0.1)	0.6 (± 0.1)	3.5 (± 0.1)	8.9 (± 2.8)	4.1 (± 0.2)	36.6 (± 1.0)	9.0 (± 0.6)

3.6 TSS and loss of solids in RAS

TSS was determined three times in triplicate (n=3) over the experimental period for each RAS (Fig. 2.3). During the first sampling interval, all three RAS had a comparable low TSS of about 0.75 - 1.15 mg L^{-1}. Thereafter, a constant increase of TSS was observed in all RAS over the experimental period, revealing highest removal in the RAS A equipped with a drum filter. Towards the last month of the experimental period TSS was highest in RAS D (6.9 (± 0.5)) and lowest in RAS A (3.6 (± 0.2)). TSS in the RAS arranged as coupled system (RAS C) was slightly lower compared to the decoupled aquaponic system (RAS D).

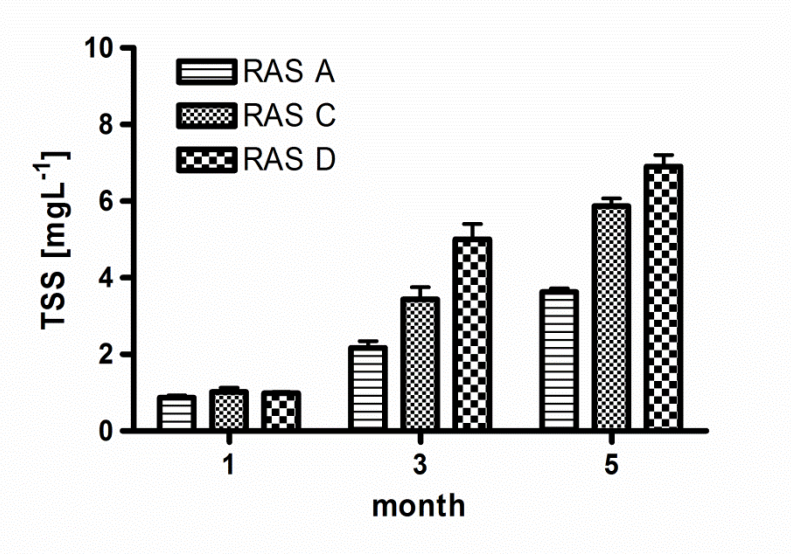

Fig. 2.3: Total suspended solids (TSS, g dry weight/L rearing water) in the fish units (RAS) the coupled (RAS C) and decoupled (RAS D) aquaponic system compared to the control (RAS A) after 30 (1 month), 94 (3 month) and 154 d (5 month) within the experimental period. Presented are the means (± SD, n=3).

The removal of solids in the clarifier (Table 2.6) due to the weekly cleaning was within the same range between the two fish units RAS C and RAS D and ranged around 1.8 - 2.0 g dry weight * L^{-1}. For the clarifiers used (V = 1.5 m^3) a weekly loss of 2.7 - 3 kg of organic matter (dry weight) was thus calculated here.

Table 2.6: Solid removal (g dry weight * L^{-1}) in the fish unit of the coupled (RAS C) and decoupled (RAS D) aquaponic system due to weekly cleaning of the clarifier (V = 1.5 m^3) after 30 (1 month), 94 (3 month) and 154 d (5 month) within the experimental period. Presented are the means (± SD, n=5).

sampling [month]	RAS C [g L^{-1}]	RAS D [g L^{-1}]
1	1.9 (± 0.18)	2.0 (± 0.17)
3	1.8 (± 0.11)	2.0 (± 0.04)
5	1.8 (± 0.06)	1.9 (± 0.04)
mean	1.8 (± 0.07)	2.0 (± 0.09)

3.7 Estimated fate of nitrogen in RAS and aquaponics

For a better estimation of nitrate accumulation in RAS and potential nitrate supply of crop plants (e.g. tomatoes) in aquaponics per kg feed fed to the fish, a simplified schematic illustration of the fate of nitrogen (mainly nitrate) was developed here (Fig. 2.4).

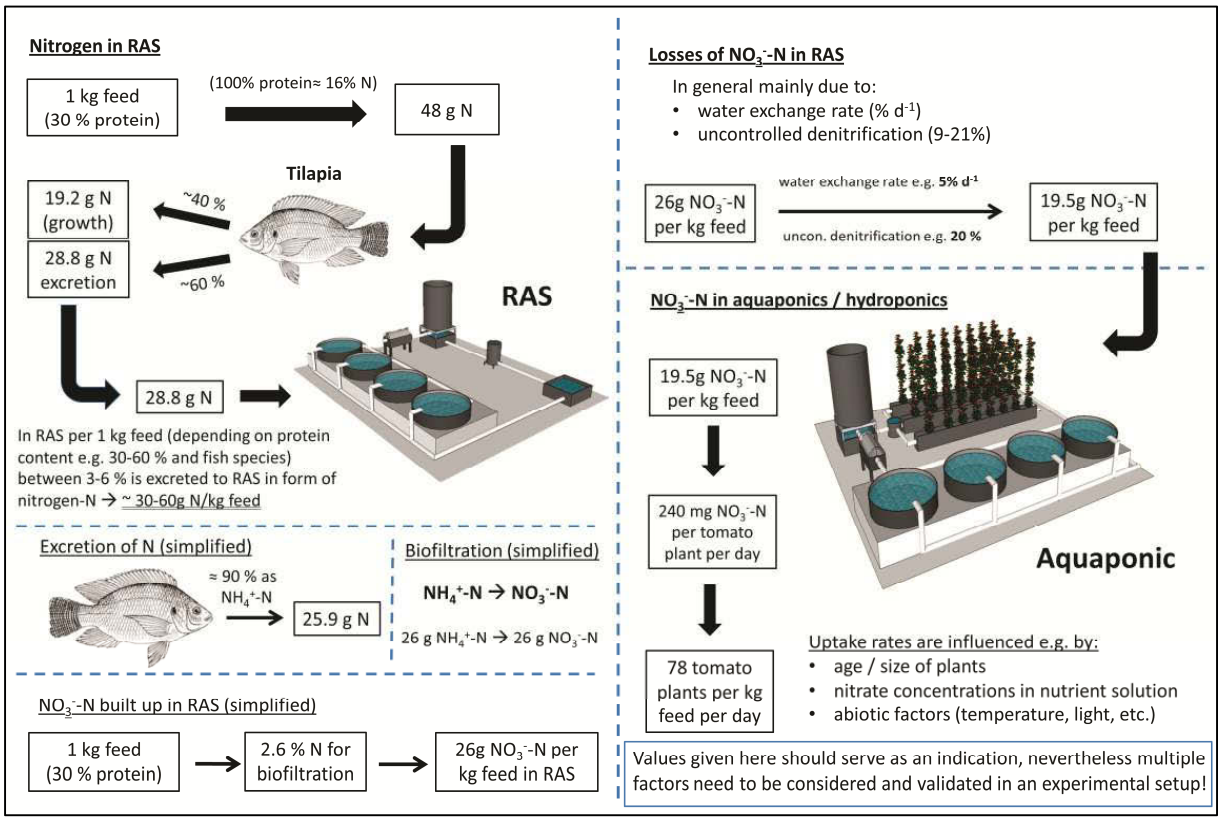

Fig. 2.4: Estimated fate of nitrogen in RAS and potential nitrate supply to the crop plants (tomatoes) in aquaponics.

4 Discussion

Here, a new approach for aquaponics is presented, comparing an innovative decoupled (2-loop system) and a coupled (1-loop system) medium scale aquaponic system experimentally in a pilot study. There are some obvious reasons why a decoupling of RAS and hydroponics in a commercial aquaponic facility is favorable compared to a classical coupled approach. The most important ones should be discussed in the following section based on the results of this pilot study and supplemented by some theoretical considerations.

In our pilot study fish were stocked at around 40 kg/m^3 providing the nutrients for plant growth in the hydroponics according to Table 2.4. The amount of fertilizer was continuously reduced with increasing biomass in the systems. Thereby, tomato harvest in the aquaponic systems differed substantially (Table 2.1). In the decoupled system 123.5 kg of tomatoes were obtained compared to 90.9 kg in the coupled system, corresponding to 36 % higher tomato yield in the decoupled system. Equal amounts of fertilizers (Table 2.2) were added in both systems, allowing a substantially improved nutrient supply in the decoupled but not in the coupled system due to the increased water volume of the coupled system. Thereby, more leave, root and stem biomass was produced in the coupled system. This has been reported before and is often related to suboptimal nutrient supply [25]. Here, the increase of root surface and the subsequent change of shoot to root ratio boost the nutrient uptake and have been frequently observed [25, 26]. Suboptimal plant growth in RAS C had probably two main reasons. In a coupled aquaponic system the pH is generally not optimal for plant growth [9] and thus not all nutrients are equally available. At the same time, fertilizers added are diluted within coupled systems due to the higher water volume encompassing the fish rearing unit, compared to decoupled systems, which allows exclusive supplementation in the hydroponic unit. Of course fertilizer applications could be increased in the coupled system, but this is neither economical nor a good solution in the context of animal welfare. Supplementation of substantial amounts of nutrients to the fish culture bares the risks of acute or chronic toxicity [27, 28]. Further, intentionally reducing water quality for the fish irrespective the degree of adverse effects is hardly acceptable with regard to the code of best practice and will also threaten the acceptance of the public as well as the envisioned potential for a sustainability label. Nevertheless, tilapia has been shown to be relatively robust in terms of nitrate and no adverse effects below 500 mg L^{-1} NO_3^--N have been observed [29]. For other ions this is mostly unclear and, if at all recommendable, optimal fertilizer formulations have to be evaluated for each fish species cultured.

In a previous study, [5] tested for the first time a prototype decoupled aquaponic systems reporting a yield of 8.89 kg plant^{-1} within 9 month. In the present study we observed a comparable tomato production of 8.2 kg plant^{-1} (Hydro D) compared to 6.1 kg plant^{-1} (Hydro C) in the coupled system within only 6 month. High greenhouse temperatures > 35°C in June and July probably contributed to a reduced development of flowers and thus fruits in that period (Table 2.1). The relationship of high temperatures and decreased flower development was already reported by [30, 31]. Here, no cooling was applied, but obviously, decoupling allows such a better temperature control, which could compromise the growth of tilapia in coupled systems. A lack of pollination could be another reason for reduced flowering [32], but this was done manually at least twice a week.

In addition to harvest yield and fruit composition, composition of the leaves was determined on a regular basis (Table 2.5) to monitor the nutrient status as suggested for fertilizer programs [15]. Results revealed that the N, P and K content of all leaves were within the normal range (N: 2-5 % of dry weight, P: 0.25-0.6 %, K: 2.8-4 %). Also, concentrations of Ca (1-5 %) and Mg (0.2-0.8 %) indicated no obvious deficiencies.

In contrast to the tomato production, harvest of fish revealed no differences in growth performance and feed conversion, neither in coupled (RAS C), decoupled (RAS D) nor classical aquaculture (RAS A) (Table 2.2). Here, the average FCR ranged between 1.2 - 1.3 and is representative for commercial aquaculture [33-35]. The SGR was moderate with an average of 1.0 and lowered with increasing fish size as described elsewhere [34, 36, 37].

A higher water consumption of 5-6 % per day was reported in the state-of-the-art aquaculture system (RAS A) compared to the aquaponic systems ranging between 2-3.6 %. This is mainly a consequence of the backwash in the automatic drum filter compared to the clarifiers in the aquaponic units. Nevertheless, an average water consumption of 5.3 % of RAS volume per day for RAS A (Table 2.2) is within the range for conventional RAS as reported elsewhere [38, 39]. Also, water quality was similar between the three RAS units and within the optimal range for tilapia. Here, both ammonia ($\leq$ 0.15 mg L^{-1} TAN) as well as nitrite ($\leq$ 0.1 mgL^{-1} NO_2^--N) were far below levels generally considered critical in fish.

In RAS, nitrification is one of the key processes, converting ammonia and providing nitrate for the plants (Fig. 2.4). For optimal conversion, pH should be kept around 7 or higher [13]. The control of pH in RAS is mainly achieved by the addition of limestone to compensate drops in pH as a consequence of nitrification itself and CO_2 accumulation from respiration. In contrast, a pH of 7 is not optimal for nutrient supply of plants since availability of most nutrients is best at pH 5.5 - 6.5 [15]. Vice versa, pH < 6.5 in the RAS affects nitrification

efficiency with subsequently accumulation of ammonium and nitrite. At pH ≤ 6 nitrification finally ceases [14] and ammonium would accumulate in the process water of RAS. High ammonium concentrations in RAS bare the risk of ammonia toxicity for fish [40] even though this is mainly problematic when the pH is high (> 8) [41]. But the processes within a classical aquaponic system are interconnected and more complex than in a single RAS. Ammonium toxicity for plants can occur already at concentrations as low as 1.8 - 9 mg L^{-1} NH_4^+ and tomatoes are among the more sensitive plants [42]. Additionally at high ammonia concentrations, ammonia uptake by the plants may further decrease the pH (<5), especially in summer, due to the excretion of protons by the roots [43].

The main advantage of decoupled systems is, that no compromises have to be made in terms of optimal production parameters for both, fish and plants. Only here, nutrient solution (e.g. addition of fertilizer in hydroponics, pH regulation, temperature adjustment) as well as temperature can be adjusted for each production unit. As discussed above, addition of fertilizers challenges animal welfare concerns. Also, economic feasibility may require discontinuous production, particularly with regard to the plant crop. As a consequence, nutrient requirements for plants can vary and nutrient supply by the fish needs to be adapted dynamically. In the coupled system, at fish densities between 39 (start) and 65 kg/m^3 (end) nitrate peaked at 99.5 mg L^{-1} NO_3^--N and was thus below the recommended nutrient requirements of tomato plants of >140 mg L^{-1} NO_3^--N [12]. Similarly, P and K did not meet minimal requirements, illustrating the need for nutrient supplementation or alternatively, compromising on the production. Still, better nutrient supply can be achieved at higher densities and tilapia can be grown up to 120 kg/m^3, if oxygenation is applied [33].

As illustrated in Fig. 2.2 and Fig. 2.4, nitrogen, mainly in the form of nitrate, is the predominant macronutrient recycled from the fish unit in aquaponics. P and K are often scarce in RAS water and need to be supplemented to support the plant crop [9]. This was also observed in the present study and, again, decoupling allowed for specific supplementation using commercial fertilizers. Nevertheless, P and K can be recycled from the fish sludge, increasing the overall sustainability of the system [27]. Here, aerobic mineralization processes may be regarded superior since significant N losses have been reported for anaerobic reactors due to denitrification.

Further, irrespective the system used, pathogen treatment or health concerns may require immediate decoupling. So far, disease transmission between fish and plant units has not been evaluated sufficiently, but needs to be addressed in the near future. Decoupling allows more

managerial flexibility, including UV or ultrasound disinfection [44] and disease therapy or specific countermeasures for fish [45] or plant treatment [46].

Overall both, decoupled and classical aquaponics, have their pro and cons. For small scale production or the production of plants with low nutrient requirements like lettuce or herbs, classical systems are probably easier to handle involving fewer factors to be monitored. For large scale professional production (as well as complex, high nutrient requirements) a decoupled system is recommended, but the complexity of the system in terms of management (e.g. automation) and labor needs to be considered.

5 Conclusions

In this pilot study, comparing the performance of decoupled aquaponic systems and coupled aquaponics, considerably higher plant production was observed in the decoupled approach, whereas fish production in all systems (including a state-of-the-art aquaculture unit) revealed comparable growth performance and feed conversion. The main reasons for better performance of decoupled systems were attributed to the independent regulation of the pH and dynamic adaptation of nutrient concentrations. At moderate densities assessed here (40-65 kg/m^3) optimal nutrient supply most probably requires supplementation and thus advocates decoupling. In terms of professionalization and improvement of production performance decoupled systems are more likely to meet the demand of producers, since optimal conditions can be controlled for both, fish and plants, separately and imbalances can be managed adequately. Based on the results a decoupling of RAS and hydroponics for an optimized production is recommended, safeguarding in particular the animal welfare in the fish unit.

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6 References

1. Goddek S, Espinal C, Delaide B, Jijakli M, Schmautz Z, Wuertz S, et al. Navigating towards Decoupled Aquaponic Systems: A System Dynamics Design Approach. Water. 2016;8(7):303.

2. Naegel LCA. Combined production of fish and plants in recirculating water. Aquaculture. 1977, 10(1):17-24.

3. Watten BJ, Busch RL. Tropical production of tilapia (*Sarotherodon aurea*) and tomatoes (Lycopersicon esculentum) in a small-scale recirculating water system. Aquaculture. 1984, 41(3):271-83.

4. Love DC, Fry JP, Li X, Hill ES, Genello L, Semmens K, et al. Commercial aquaponics production and profitability: Findings from an international survey. Aquaculture. 2015;435:67-74.

5. Kloas W, Groß R, Baganz D, Graupner J, Monsees H, Schmidt U, et al. A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. Aquacult Environ Interact. 2015;7(2):179-92.

6. Rennert B, Groß R, van Ballegooy C, Kloas W. Ein Aquaponiksystem zur kombinierten Tilapia- und Tomatenproduktion. Fischer & Teichwirt. 2011;06:209-214

7. Endut A, Jusoh A, Ali N, Wan Nik WB, Hassan A. A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresource Technol. 2010;101(5):1511-7.

8. Graber A, Junge R. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. Desalination. 2009, 246(1):147-56.

9. Rakocy J, Masser M, Losordo T. Recirculating Aquaculture Tank Production Systems: Aquaponics–Integrating Fish and Plant Culture, South Region Aquacult Cent (SRAC). 2006, 454:1-16.

10. Mangmang JS, Deaker R, Rogers G. Inoculation effect of *Azospirillum brasilense* on basil grown under aquaponics production system. Org Agr 2016;6(1):65-74.

11. Pantanella E, Cardarelli M, Colla G, Rea E, Marcucci A, editors. Aquaponics vs. hydroponics: production and quality of lettuce crop. XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Greenhouse 2010 and Soilless Cultivation. Acta Hortic 2012, 927: 887-893

12. Lattauschke G. Gewächshaustomaten: Hinweise zum umweltgerechten Anbau—Managementunterlage. Book 1. Fachbereich Gartenbau, Sächsische Landesanstalt für Landwirtschaft, Pillnitz. Landesamt für Umwelt, Landwirtschaft und Geologie. 2004(1):51.

13. Chen S, Ling J, Blancheton J-P. Nitrification kinetics of biofilm as affected by water quality factors. Aquacult Eng. 2006;34(3):179-97.

14. Eding EH, Kamstra A, Verreth JAJ, Huisman EA, Klapwijk A. Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. Aquacult Eng. 2006;34(3):234-60.

15. Hochmuth GJ. Fertilizer Management for Greenhouse Vegetables Florida Greenhouse Vegetable Production Handbook. 2001;3:1-25.

16. Wheeler R, Sager J, Berry W, Mackowiak C, Stutte G, Yorio N, et al., editors. Nutrient, acid and water budgets of hydroponically grown crops. International Symposium on Growing Media and Hydroponics 481; 1997.

17. Chibnall AC, Rees MW, Williams EF. The total nitrogen content of egg albumin and other proteins. Biochem J. 1943;37(3):354-9.

18. Jones DB. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins: US Department of Agriculture Washington, DC; 1941.

19. Kutty M. Respiratory quotient and ammonia excretion in *Tilapia mossambica*. Mar Biol. 1972;16(2):126-33.

20. Wilkie MP. Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. J Exp Zool. 2002;293(3):284-301.

21. Wood C, Evans D. Ammonia and urea metabolism and excretion in fish. CRC Marine science series. 1993, 379-426.

22. Thoman ES, Ingall ED, Davis DA, Arnold CR. A nitrogen budget for a closed, recirculating mariculture system. Aquacult Eng. 2001;24(3):195-211.

23. Andriolo JL, Le Bot J, Gary C, Sappe G, Orlando P, Brunel B, et al. An experimental set-up to study carbon, water, and nitrate uptake rates by hydroponically grown plants. J Plant Nutr. 1996, 19(10-11):1441-62.

24. Le Bot J, Adamowicz S, Robin P, Andriolo J, Gary C. Modelling nitrate uptake by greenhouse tomato crops at the short and long time scales. II Modelling Plant Growth, Environmental Control and Farm Management in Protected Cultivation 456. 1997:237-46.

25. Hermans C, Hammond JP, White PJ, Verbruggen N. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 2006;11(12):610-7.

26. López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol. 2003;6(3):280-7.

27. Monsees H, Keitel J, Paul M, Kloas W, Wuertz S. Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions. Aquac Environ Interact. 2017;9:9-18.

28. van Bussel CGJ, Schroeder JP, Wuertz S, Schulz C. The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). Aquaculture. 2012; 326:163-167.

29. Monsees H, Klatt L, Kloas W, Wuertz S. Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus L.*) in recirculating aquaculture systems. Aquacult Res. 2016: Aug 06 doi: 10.1111/are.13174

30. Peterson RH, Taber HG. Tomato flowering and early yield response to heat buildup under rowcovers. J Am Soc for Hortic Sci. 1991;116(2):206-9.

31. Smith O. Relation of temperature to anthesis and blossom drop of the tomato together with a histological study of the pistils. J Agr Res. 1932;44:183-90.

32. Picken A. A review of pollination and fruit set in the tomato (*Lycopersicon esculentum Mill*.). J Hortic Sci. 1984;59(1):1-13.

33. DeLong DP, Losordo TM, Rakocy JE. Tank culture of tilapia. South Region Aquacult Cent (SRAC). 2007, 282: 1-8.

34. Ridha MT. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus, L*. at two stocking densities. Aquacult Res. 2006;37(2):172-9.

35. Shnel N, Barak Y, Ezer T, Dafni Z, van Rijn J. Design and performance of a zero-discharge tilapia recirculating system. Aquacult Eng. 2002;26(3):191-203.

36. Abdel-Tawwab M, Ahmad MH, Khattab YAE, Shalaby AME. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 2010;298(3–4):267-74.

37. Hanley F. Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion, and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 1991, 93(4):323-34.

38. Ebeling JM, Timmons MB, Bisogni JJ. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture. 2006;257(1–4):346-58.

39. Verdegem MCJ. Nutrient discharge from aquaculture operations in function of system design and production environment. Rev Aquacult. 2013;5(3):158-71.

40. Meade JW. Allowable ammonia for fish culture. Prog Fish Cult. 1985;47(3):135-45.

41. Randall DJ, Tsui TKN. Ammonia toxicity in fish. Mar Pollut Bull. 2002;45(1–12):17-

42. Britto DT, Kronzucker HJ. NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol. 2002, 159(6):567-84.

43. von Wirén N, Gazzarrini S, Gojon A, Frommer WB. The molecular physiology of ammonium uptake and retrieval. Curr Opin Plant Biol. 2000;3(3):254-61.

44. Bazyar Lakeh AA, Kloas W, Jung R, Ariav Ra, Knopf K. Low frequency ultrasound and UV-C for elimination of pathogens in recirculating aquaculture systems. Ultrason Sonochem. 2013;20(5):1211-6.

45. Wuertz S, Schulze SGE, Eberhardt U, Schulz C, Schroeder JP. Acute and chronic nitrite toxicity in juvenile pike-perch (*Sander lucioperca*) and its compensation by chloride. Comp Biochem Physiol C. 2013;157(4):352-60.

46. Song W, Zhou L, Yang C, Cao X, Zhang L, Liu X. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Prot. 2004;23(3):243-7.

Chapter III

The potential of aquacultural sludge treatment for aquaponics – evaluation of nutrient mobilization under aerobic and anaerobic conditions

Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions

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Abstract:

In recirculating aquaculture systems (RAS), mechanical removal of suspended solids by clarifiers or drum filters provides an organic mixture rich in nutrients. Still, in most traditional RAS, this sludge is discharged directly or following dewatering. Here, the potential recycling of nutrients from sludge is assessed, comparing aerobic and anaerobic mobilization of nutrients experimentally, ultimately aiming at an application in aquaponic systems. Nutrient mobilization processes were studied, monitoring soluble nutrients photometrically in the treatment tanks (NO_3^--N, NO_2^--N, total ammonia nitrogen, soluble reactive phosphorus [SRP], K^+, Mg^{2+} and Fe^{2+}), the nutrient composition of the sludge (total phosphorus, Fe, Mn, Al, S, Mg, Ca) by inductively coupled plasma optical emission spectrometry, as well as C:N ratio, total solids (TS) and total suspended solids (TSS). Aerobic treatment (aerated, AT) resulted in a 3.2-fold increase in mean (±SD) SRP from 9.4 (± 0.7) to 29.7 (± 2.1) mg l^{-1}, most likely owing to a decrease in pH. In contrast, in the anaerobic treatment (unaerated, UT), SRP remained unchanged between 9.4 (± 0.7) and 9.3 (± 0.4) mg l^{-1}. Both treatments resulted in increased K^+ concentrations from 28.1 (± 1.5) to 36.8 (± 2.3) mg l^{-1} in AT and to 32.2 (± 2.3) mg l^{-1} in UT. AT revealed best mobilization of P and K^+ without major losses of NO_3^--N. Thus, aerobic treatment of water-sludge mixture has a high potential for significant improvements of nutrient recycling in aquaponics.

1 Introduction

Public perception of aquaculture is often critical, raising concerns about eutrophication and pollution of the aquatic environment due to direct emissions of nutrients from fish farms (Edwards 2015, Zhang et al. 2015). Often ignored, solid waste originating from faeces and uneaten feed pellets represent a substantial nutrient reservoir. Upon microbial conversion, chemical mobilization and leaching, nutrient emissions may induce algal blooms, oxygen depletion and mass mortalities among aquatic organisms (Zhang et al. 2015). Over the last 2 decades, recirculating aquaculture systems (RAS) have been rapidly evolving to reduce such impacts on the environment. Undoubtedly, RAS technology has a great potential, particularly assigned to the efficient use of water and space (Gutierrez-Wing & Malone 2006) and supports a sustainable development of the fast growing aquaculture industry. Environmental legislation and, from an economic perspective, fees for waste disposal and nutrient emissions represent main motivations to improve waste management and reduce nutrient emission supporting the development of sound environmentally friendly aquaculture production.

RAS usually comprise two main water treatment steps. First, mechanical filters such as clarifiers or drum filters are used to concentrate suspended solids, discharged either after dewatering or directly with the backwash. Subsequently in a biofilter, toxic ammonia, (NH_4^+/NH_3) excreted from the fish gills, is converted to nitrate (NO_3^-) by nitrifying bacteria (Paredes et al. 2007). Despite the large variability observed between species, 60–90% of the excreted nitrogen is dissolved (van Rijn 2013). In contrast to classical RAS, aquaponic systems make use of such soluble nutrients derived from the fish unit to grow plants in an integrated hydroponic unit (Goddek et al. 2015). Here, standing stock of the RAS sustains the growth of the crop plants hence determining the dimensions of the hydroponic production (Rakocy et al. 2006). Consequently, in a well-balanced system, additional nitrogen fertilization is not required.

In contrast, phosphorus (P) in the process water is generally limited, but is essential for plant growth (Dawson & Hilton 2011) and can only be assimilated by plants as dissolved inorganic phosphate (PO_4^{3-}; hereafter soluble reactive phosphorus, SRP). A high percentage of the dietary P is not retained in fish but excreted and dissolved P strongly adsorbs onto particles (Neori et al. 2007). Consequently, feed leftovers and fish faeces are the main sources for P, either in organic form or inorganic as PO_4^{3-} (Barak & van Rijn 2000). Thus, mechanical removal of suspended solids removes a major part of P without considering further strategies for recycling. Recent fishmeal substitution in modern diets reduces SRP (Hua & Bureau 2006) but further increases the deposition of plant-derived organic phosphorus in the sludge.

In addition to P, the supply of potassium (K^+) is often suboptimal in aquaponic systems (Rakocy et al. 2006). Consequently, it has become standard practice in aquaponics to use synthetic chemical fertilizers, mainly nitrogen, phosphate and potassium (NPK-fertilizer) to formulate aquaponic media if specific nutrient profiles are not met (Rennert et al. 2011).

To date, the management of aquacultural sludge mostly aimed for improved water recycling in RAS as well as in aquaponics. Obviously, optimization strategies in RAS and aquaponics are quite opposite. In RAS, efforts focus on higher nutrient retention in the fish or the use of sludge as a nutrient sink. In aquaponics, retention of nutrients in the fish is not necessarily prioritized. Instead, optimized mobilization of nutrients is a key factor to ensure sustainability of the system. Currently, the prevailing approach used in RAS is anaerobic sludge digestion to reduce organic matter (Mirzoyan et al. 2010, Jung & Lovitt 2011). Here, to mobilize P, manipulation of pH is often carried out, either by addition of acids or indirectly via microbial fermentation (Jung & Lovitt 2011). Only very few studies considered aerobic treatment for nutrient recycling of sludge where inorganic P is mobilised from organic P compounds by microbial dephosphorylation (Neori et al. 2007, Rakocy et al. 2007). Still, high mobilization rates of nutrients under aerobic conditions have been documented (Rakocy et al. 2007, Neori et al. 2007). Furthermore, under aerobic conditions, excessive nitrogen loss due to denitrification is prevented. Here, relevance of nitrogen recycling remains to be evaluated in a comparative approach under realistic production conditions, as a major part of nitrogen is actually soluble. More importantly, realistic data of P and K mobilization is needed to improve nutrient management in aquaponics.

In this study, we investigated the potential utilization of aquaculture sludge (i.e. solid waste collected in the mechanical treatment unit such as clarifier or drum filter) comparatively assessing nutrient mobilization under aerobic and anaerobic conditions. This study was integrated in a 6 mo trial on the optimization of a coupled and a decoupled aquaponic system (H. Monsees et al. unpubl.).

Optimizing sludge management should ultimately provide sound data to (1) improve environmental sustainability in the context of nutrient recycling and reduced emissions as well as profitability (reduced costs due to high water recovery from the sludge, decreased fertilization and, most importantly, lower waste and emission fees) and (2) increase the self-reliance of aquaponics. Finally, this will support the development of an automated or semi-automated reactor which will allow continuous, optimized nutrient mobilization to support a closed nutrient loop in aquaponics irrespective of the mechanical filter used (e.g. drum filter, clarifier).

2 Materials and Methods

2.1 Aquaponic system

Experiments were conducted at the aquaponic research facility of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB, Berlin, Germany), using a RAS with a total water volume of 16.5 m^3 (Fig. 3.1). Three separate rearing tanks (1.7 m^3 each) were stocked with a total of 316 kg tilapia *Oreochromis niloticus* L. at rearing densities of 62 kg m^{-3} per tank.

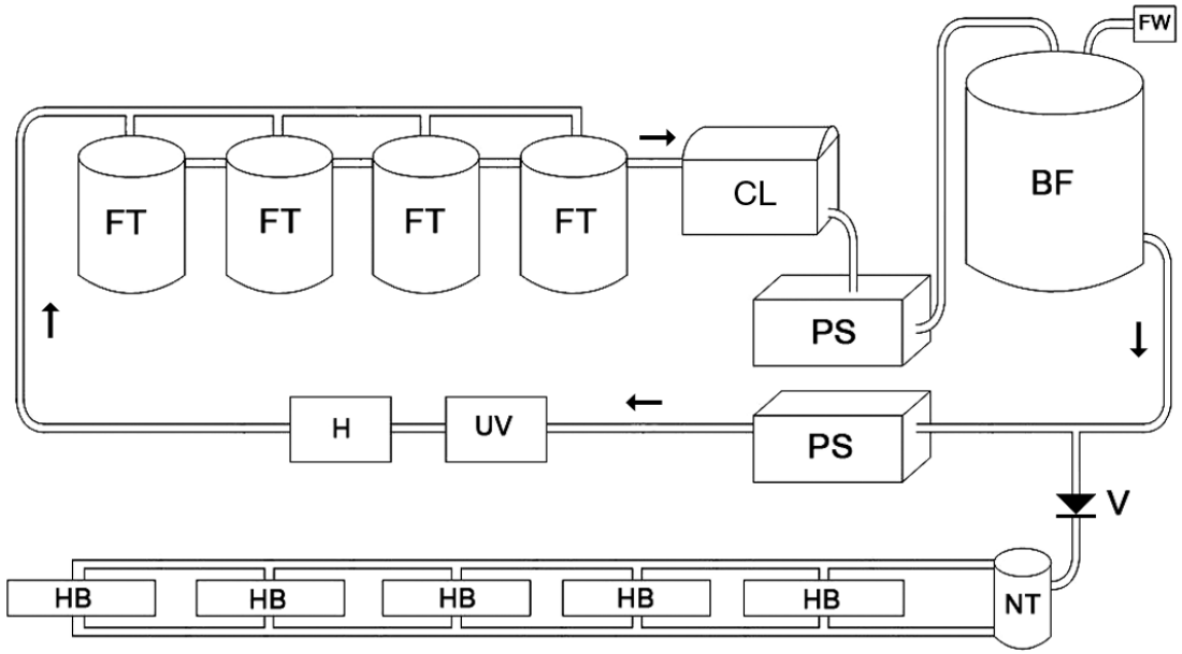

Fig. 3.1: Decoupled aquaponic system, comprising a recirculating aquaculture system and a hydroponic unit. FT: fish tank (tanks are set up in parallel, with each outflow draining to the clarifier via an open channel behind the tanks); CL: clarifier; PS: pump sump; BF: biofilter; FW: fresh water supply; UV: UV desinfection unit (optional); H: heater (optional); HB: hydroponic beds (nutrient flow technique); NT: nutrient media reservoir; V: 1-way valve

Fish originated from a brood stock established at the IGB and were not further characterized. Removal of suspended matter was carried out with a clarifier (1.5 m^3). Over the experimental period, fish were fed a commercial diet at 0.8% of their body weight per day (Aller Float 37/10 2 mm, Emsland-Aller Aqua: 37% protein, 10% fat, 38.5% nitrogen-free extract, 6% ash, 3% fibre, 1.2% P of dry weight; estimated environmental impact (feed conversion ratio = 1.0): 4.7 g N and 3 g P in faeces per kg feed, 27 g N and 2.7 g P in water per kg feed).

Temperature, pH and oxygen were determined daily (HQ40d multi, Hach Lange); pH was regulated with $Ca(OH)_2$ to maintain a target pH of 7 (±1) (Table 1). Selected nutrients (NO_3^--N, cadmium reduction method #8039; NO_2^--N, USEPA diazotization method #8507; total ammonia nitrogen [TAN], salicylate method #8155; K^+, tetraphenylborate method #8049;

Mg^{2+}, calmagite colorimetric method #8030; Fe^{2+}, 1,10-phenanthroline method #8146, all methods from the manufacturer's manual; Hach Lange) in the water were determined spectrophotometrically (DR3900, Hach Lange) at the inlet of a fish tank and the outlet of the clarifier (see Table 3.4). SRP (see Fig. 3.2a & 3.3a) was measured photometrically (Spekol® 1500, Analytik Jena) at a wavelength of 880 nm according to the molybdenum blue method (Murphy & Riley 1962). Conditions in the RAS are summarized in Table 3.1. The water-sludge mixture (1.5 m^3) from the clarifier was collected once weekly in a 2 m^3 tank, homogenized with a pump and used for the subsequent experiments.

Table 3.1: Rearing conditions for tilapia during the experimental period.

parameter	target values
Temperature [°C]	26 ± 1
Oxygen [mg l^{-1}]	> 5
pH	7 ± 1
Stocking desity [kg m^{-3}]	62 ± 2.5
Feeding rate [%]	0.8
Feed [kg d^{-1}]	2.5

2.2 Determination of total suspended solids (TSS) in the RAS

For TSS, water samples (620 ml) were taken in triplicate at the inflow of a fish tank prior to feeding at 09:00 h (0 h), and 3, 6, 9 and 24 h thereafter. Briefly, samples were filtered through pre-weighed 0.45 µm CA membrane filters (GE Healthcare), freeze-dried to constant weight and weighed.

2.3 Sludge composition

Total solids (TS) were determined in a subsample of the homogenized water-sludge mix after centrifugation and freeze-drying to constant weight. Total phosphorus (TP), iron (Fe), manganese (Mn), aluminum (Al), sulfur (S), magnesium (Mg) and calcium (Ca) were determined by inductively coupled plasma optical emission spectrometry (iCAB 6000, Thermo Fisher Scientific) after wet digestion (HCl 37%, HNO_3 65%, volumetric ratio 1:3) in a high pressure microwave oven (Gigatherm). C:N analysis was performed using freeze-dried, weighed sediment packed in tin foil and analyzed in a Vario EL© system (Elementar Analysensysteme). Dry weight: wet weight ratio was determined in freeze-dried aliquots of fresh sludge (n = 15).

2.4 Expt 1: Anaerobic lab-scale nutrient mobilization

For the verification of nutrient mobilization under anaerobic conditions in a closed container, lab-scale experiments were performed. The water-sludge mix was transferred to 18 centrifugation tubes (55 ml), ensuring that no air remained inside the tubes. To minimize temperature variation, tubes were incubated on a rotation shaker (Heidolph Reax) in a climate chamber at 25 ± 0.5°C for 4 d (with an additional 4 d for SRP sampling only). Each day, 3 tubes were sampled for nutrient determination (SRP, NO_3^--N, NO_2^--N, TAN, K^+, Mg^{2+} and Fe^{2+}). Briefly, samples were centrifuged (Multifuge 1-sr, Thermo Fisher) for 10 min at 1900 × *g*. Directly before analysis, the liquid phase was filtered through a 0.45 µm nylon syringe filter (Braun). According to O'Sullivan & Reynolds (2004), dissolved O_2 < 0.1 mg l^{-1} is considered anaerobic here. To exclude oxygenation of the small volume during measurement, oxygen was determined separately, using 500 ml glass bottles (n = 3) filled completely with water-sludge mix and continuously monitored with an oxygen probe inserted through a parafilm seal. Additionally bottles were covered with aluminium foil to prevent algal growth and placed on a magnetic stirrer (Heidolph MR 1000) for continuous movement of the liquid. Oxygen concentration was measured at 5 min intervals.

2.5 Expt 2: Aerated (aerobic) and unaerated (anaerobic) nutrient mobilization

Homogenized water-sludge mix was distributed to six 30 l polyethylene tanks providing an aerated (compressed air via airstones), aerobic (AT) and an unaerated, anaerobic treatment (UT), assessed in 3 replicates each over 14 d and repeated 3 times. All boxes were covered with a tight lid to prevent evaporation and incubated in a water bath (1.5 × 1.5 m glass fibre tank equipped with two 300 W heaters and a pump for constant circulation) at 26°C ± 0.6°C for the 14 d. The water bath was additionally insulated with foil and covered with thick, black pond foil to prevent algal growth. Samples for water analysis were collected in 50 ml centrifugation tubes and directly analyzed for dissolved ions as described for Expt 1.

2.6 Statistical analysis

Data are presented as means ± standard deviation (SD) of n samples. Statistical analysis was performed using Graphpad Prism (GraphPad Software). Data were tested for normality (Shapiro-Wilk) and equal variance (Kruskal-Wallis). Multiple comparison was carried out by non-parametric Dunn's test ($p < 0.05$), and pairwise comparisons were carried out by non-parametric Mann-Whitney *U* test ($p < 0.05$).

3 Results

3.1 Characterization of the sludge-water mixture

Sludge collected successively from a full production cycle for tilapia under realistic conditions was comparable between all 4 replicates with regard to element composition (Table 3.2).

Table 3.2: Elemental analysis by inductively coupled plasma optical emission spectrometry, C:N ratio and total solids (TS) of freeze-dried sludge collected from the clarifier (1.5 m^3) of a recirculating aquaculture system producing tilapia under realistic conditions in 4 technical replicates, illustrating the respective variation in sludge during the experimental period.

Replicate	P [mg g^{-1}]	Mg [mg g^{-1}]	Ca [mg g^{-1}]	Fe [mg g^{-1}]	Mn [mg g^{-1}]	Al [mg g^{-1}]	S [mg g^{-1}]	C [%]	N [%]	C:N	TS [g l^{-1}]
1	31.27	3.32	56.35	3.69	0.27	3.31	6.75	35.59	3.87	9.18	1.23 ± 0.05
2	25.46	3.3	47.77	2.84	0.23	2.22	6.04	37.61	4.08	9.21	1.14 ± 0.04
3	28.84	3.25	50.51	2.95	0.21	2.69	5.86	36.54	4	9.15	1.65 ± 0.03
4	35.92	3.22	70.01	3.38	0.27	3.18	7.53	33.95	4.43	7.67	---

Only slight variations (<20%) were observed, particularly P, Ca, and most prominently in TS. A mean P deposition of 59.4 g wk^{-1} was observed in the clarifier. TSS was highest in the morning (Table 3.3), but decreased within 3 h, fluctuating around 1.5 mg l^{-1} (± 0.2).

Table 3.3: Total suspended solids (TSS, g dry weight l^{-1} rearing water) in a tilapia recirculating aquaculture system over 24 h. Samples were taken at the inlet of a fish tank; sampling started at 09:00 h before feeding. Data are means ± SD (n = 3)

Time [h]	TSS [mg l^{-1}]
0	2.3 ± 0.1
3	1.5 ± 0.1
6	1.6 ± 0.3
9	1.4 ± 0.3
24	2.2 ± 0.2

The soluble nutrients measured at the outlet of the clarifier and at the inlet of the fish tanks were comparable (Table 3.4). As expected, TAN and NO_2^--N in the rearing water of the RAS were always below critical threshold. NO_3^--N concentration varied, providing different starting points for the experiments (highest concentration during the first sampling of Expt 2). Phosphate, magnesium and potassium also varied slightly, but not strictly correlated to each other.

Table 3.4: Soluble nutrients (PO_4^{3-}, K^+, total ammonia nitrogen [TAN], NO_3^--N, NO_2^--N, Mg^{2+}) measured at the inlet of a fish tank and the outlet of the clarifier of a tilapia recirculating aquaculture system. Data are the results of 3 successive samplings. nd: parameters not determined

Parameter	Sampling 1		Sampling 2		Sampling 3	
(in mg l^{-1})	tank	clarifier	tank	clarifier	tank	clarifier
TAN	0.3	0.2	0.2	0.4	0.2	0.4
NO_2^--N	0.1	0.2	0.1	0.1	nd	nd
NO_3^--N	64.0	63.0	48.5	50.0	46.5	52.5
PO_4^{3-}	7.9	8.0	8.1	7.9	9.7	9.5
Mg^{2+}	61.6	63.0	59.2	62.6	70.0	70.4
K^+	27.0	26.5	24.5	24.5	28.5	27.0

3.2 Expt 1: Anaerobic lab-scale mobilization

Within 8 d, SRP increased steadily in all 3 successively assessed sludge-water mixtures. At 0 d, SRP ranged between 7.8 and 9.2 mg l^{-1} and increased significantly ($p < 0.05$, Dunn's) to 11.2–12.6 mg l^{-1} (Fig. 3.2a). Only minor oscillations were observed in K^+, revealing concentrations of approximately 25.0 mg l^{-1} (Fig. 3.2b).

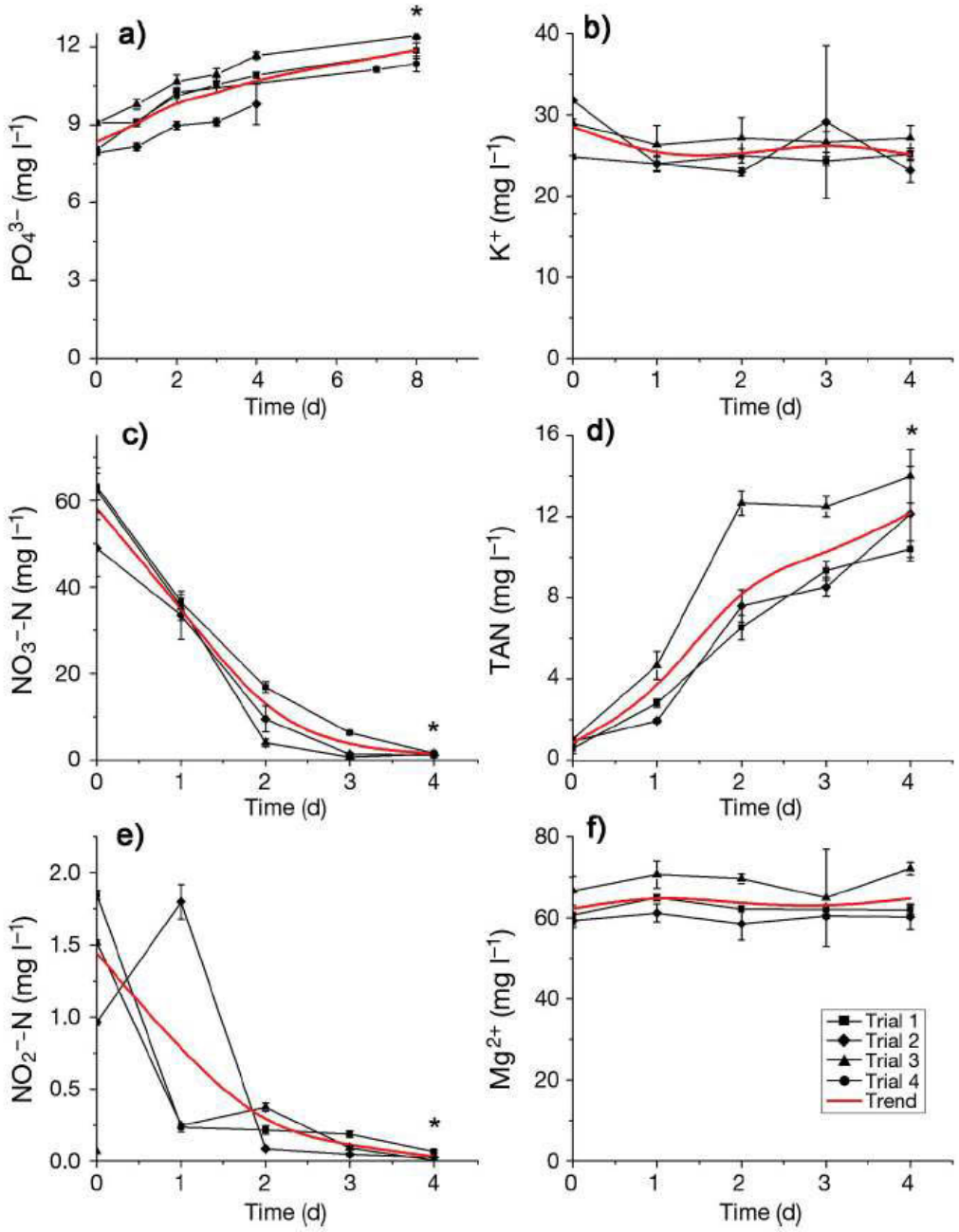

Fig. 3.2: Soluble nutrients (PO_4^{3-}, K^+, total ammonia nitrogen [TAN], NO_3^--N, NO_2^--N, Mg^{2+}) in the liquid phase of a water-sludge mixture derived from a tilapia recirculating aquaculture system over 4 d (8 d only for soluble reactive phosphorus, PO_4^{3-}) of anaerobic mobilization (Expt 1). Data from 3 successive trials (4 trials for soluble reactive phosphorus PO_4^{3-}) are presented as mean ± SD. Trend lines: means of the successive trials (technical replicates). *Significant differences compared to Day 0 are indicated by an asterisk ($p < 0.05$, Dunn's test, n = 3 or 4 trials).

The NO_3^--N concentration was reduced by 97% within 4 d from 58 (± 8) to 1.5 (± 0.2) mg l^{-1} (Fig. 3.2c). In parallel, TAN increased substantially ($p < 0.05$, Dunn's) from <1 mg l^{-1} to >10 mg l^{-1} (Fig. 3.2d). NO_2^--N concentrations decreased significantly ($p < 0.05$, Dunn's) from 1.4 (± 0.4) to 0.03 (± 0.03) mg l^{-1} (Fig. 3.2e). Mg^{2+} did not vary over the 4 d (64.1 ± 1.2 mg l^{-1}; Fig. 3.2f). Fe^{2+} concentrations were always below the detection limit (<0.01 mg l^{-1}; data not

shown). Measurement of oxygen concentration in sealed glass bottles (see 'Materials and methods') revealed a complete depletion of oxygen from 5.28 to 0 mg l^{-1} within 45 min (data not shown), confirming anaerobic conditions.

3.3 Expt 2: Aerobic and anaerobic mobilization

Within 14 d (Day 0 to Day 13), SRP increased significantly ($p < 0.05$, Mann-Whitney) in the AT from 9.4 (± 0.8) to 29.7 (± 2.1) mg l^{-1} PO_4^{3-} (Fig. 3.3a). In contrast, no changes were observed in the UT. In the AT, K^+ concentration increased by 30% from from 28.1 (± 1.5) to 36.8 (± 2.3) mg l^{-1} between 0 d and 14 d (Fig. 3.3b). Again, NO_3^--N dropped from 68.2 (± 2.8) to 9.4 (± 4.4) mg l^{-1} in the UT and was thus reduced by 86% within 14 d (Fig. 3.3c); in contrast, only a minor reduction of 16% from 68.2 (± 2.8) to 55.1 (± 11.3) mg l^{-1} was observed in the AT (Fig. 3.3c). In the UT, TAN increased from 1.0 (± 0.1) to 7.9 (± 0.8) mg l^{-1}, but decreased from 1.0 (± 0.1) to 0.1 (± 0.1) mg l^{-1} in the AT (Fig. 3.3d). Initially, NO_2^--N decreased in both treatments, but from Day 7 in the UT it then increased to 0.2 (± 0.1) mg l^{-1} (Fig 3.3e). In the AT, NO_2^--N dropped continuously from 1.3 (± 0.4) to 0.01 (± 0.005) mg l^{-1}. No changes in Mg^{2+} were observed over time, neither between treatments nor within a treatment (AT: 61–73 mg l^{-1}; UT: 53–74 mg l^{-1}; Fig. 3.3f). In both treatments, iron concentrations were always below the detection limit ($Fe^{2+} \leq 0.01$ mg l^{-1}; data not shown).

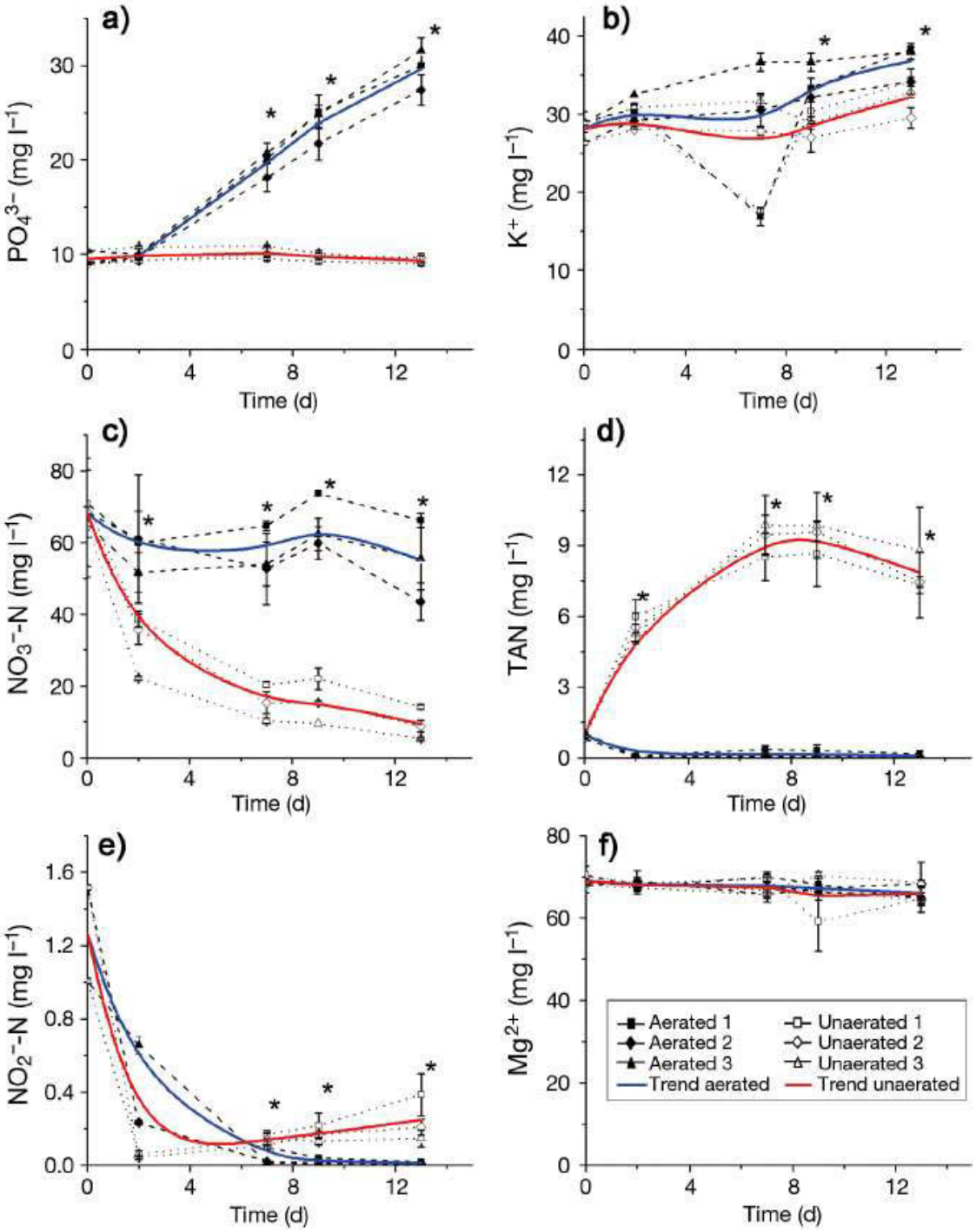

Fig. 3.3: Soluble nutrients (as in Fig. 2) in the liquid phase of a water-sludge mixture derived from a tilapia recirculating aquaculture system over 14 d (Day 0 to Day 13) of anaerobic (<0.1 mg O_2 l^{-1}, red) and aerobic (blue) treatment (Expt 2). Data from 3 successive trials are presented as means ± SD. Trend line: mean of the successive trials. *Significant differences between anaerobic and aerobic mobilization ($p < 0.05$, Mann-Whitney U, n = 3 trials per treatment)

AT and UT revealed opposite progression in pH, increasing from 6.2 (± 0.02) to 7.0 (± 0.2) in the UT and decreasing to 5.3 (± 0.01) in the AT (Fig. 3.4).

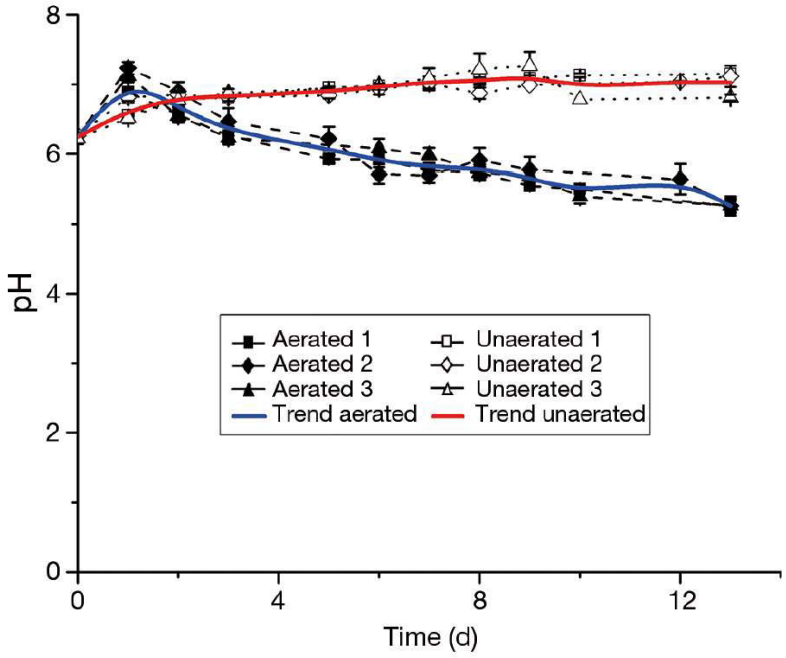

Fig. 3.4: pH in the liquid phase of a water-sludge mixture derived from a tilapia recirculating aquaculture system over 14 d (Day 0 to Day 13) of anaerobic (<0.1 mg O_2 l^{-1}, red) and aerobic (blue) mobilization. Data from 3 successive trials are presented as means ± SD. Trend line: mean of the successive trials (technical replicates)

4 Discussion

Here, sludge obtained from the clarifier of a RAS was used to demonstrate the potential of optimized nutrient mobilization for aquaponics, aiming at an easy-to-handle, inexpensive/economical incubator. Aeration treatment (AT) increased the P concentration by 215 % and the K^+ concentration by 31% within 14 d of incubation. This is highly relevant since most K^+ and P input via the feed is actually retained in the sludge. Current practice does not make use of this resource; instead, P and K concentrations in the process water are limited, requiring supplementation for aquaponics (NPK-fertilizer) (Rennert et al. 2011). Additionally, the AT reduced NO_3^--N concentrations by just 16% compared to 97% in the unaerated treatment (UT), most probably due to denitrification. Thus, AT is a good compromise considering the overall supply of the nutrients for aquaponic applications.

Following AT, the phosphate concentration of 27.7 mg l^{-1} PO_4^{3-} recorded here is still well below recommendations for industrial tomato production of around 160 mg l^{-1} PO_4^{3-} (Hochmuth & Hochmuth 2001). Our results are nonetheless very promising: a prolongation of incubation time as well as technological optimization would probably improve P mobilization further.

P is a key element for plant nutrition, essential for molecules such as ATP, nucleic acids and phospholipids (Schachtman et al. 1998). An optimal supply is thus essential to maximize plant growth. Recently, P use as fertilizer for agricultural production is subject of intense discussion in the scientific literature since estimations predict a depletion of this non-renewable resource (phosphate rock reserves, for human fertilizer utilization) in coming decades (Cooper et al. 2011, McGill 2012); price surges have already been observed (McGill 2012). Currently, P for agricultural crop fertilization is mainly produced by mining (Schmid Neset et al. 2008) and sustainable recycling on a larger scale needs to be explored. Altogether, the increase of phosphate observed in this study particularly highlights the potential for an optimized nutrient recycling in aquaponic systems.

In contrast to AT, anaerobic treatment revealed only minor increases in SRP in the lab-scale experiments and even a slight decrease in the upscaling experiments. Similarly, Jung & Lovitt (2011) reported a P-release of less than 5% within 7 d in anaerobic treatment of sludge from a trout farm. However, additional supplementation with glucose led to a final P-release of 90%. Interestingly, as suggested by those authors, glucose addition might not exhibit a direct effect on the P-release (e.g. by increase of P-solubilizing heterotrophs). Instead, lowering of the pH by glucose fermentation seemed to increase P leaching substantially (Jung & Lovitt 2011). In contrast to our study, a pH drop below 5 was observed after 24 h. Furthermore, leaching of

different nutrients including P was increased upon the addition of acids. Similarly, pH-dependent mobilization of P from fish sludge was also reported by Conroy & Couturier (2010). In our experiments, decreasing NO_3^--N indicated denitrification in all anaerobic treatments. Thus, proton consumption during denitrification (Klas et al. 2006) seemed to stabilize the pH in the anaerobic treatments, thereby reducing P (SRP) mobilization.

Accordingly, in the AT, continuous reduction in pH to 5.26 (± 0.01) over 14 d could mainly explain P leaching in the present study. Here, both, nitrification processes as well as respiratory CO_2 production contribute to acidification in the incubator (Paredes et al. 2007, Wurts & Durborow 1992). An extended retention time and/or refilling with new sludge-water mixture or concentrated sludge could consequently speed up the pH decrease required and hence improve the mobilization.

During the study, we determined the P binding fractions in the sludge according to a modified sequential P fractionation scheme according to Hupfer et al. (1995) used in aquatic and soil science (Psenner et al. 1984). P fractionation results showed that 50% of TP were Ca-associated and thus pH sensitive. In the sequential P fractionation scheme this fraction is extracted with HCl and is determined as acid-soluble P fraction. Accordingly, when the pH decreases, a major part of the P in the fish sludge can be mobilized and become available for the crop plants. The second largest P-fraction (26%) in the sludge was loosely bound P (extracted with NH_4Cl) and is thus also easily mobilized. Finally, ~5% of the extracted P were associated with organic substances (poly-phosphates and humic substances; extracted with NaOH). Here, mobilization requires complex microbial digestion. An effective microflora established in the incubator may improve mobilization in the future, compared to the static approach assessed here. In our experiment, SRP increased by 20.2 mg l^{-1} and is estimated to represent a total of 30 g P mobilized from the sludge harvested from the clarifier (1.5m^3) after 1 wk. The solid phase analyses of the fish sludge from the clarifier revealed TP values of 60 g $harvest^{-1}$. Thus, considering the fractionation analysis, this increase may only result from pH-labile P (50% of TP) in the fish sludge.

Compared to AT, anaerobic treatment is less efficient in the incubator used, but could be optimized by addition of acids, carbon sources and/or bacterial suspension. Undoubtedly, even after completion of the necessary research, an optimized anaerobic treatment process would still require further maintenance effort, resources and the reoxygenation of anaerobic water for subsequent hydroponic application. More important, nitrate, which constitutes the most important nutrient source derived from RAS, would be lost for aquaponic application.

Here, an easy-to-handle approach was evaluated particularly with regard to the requirements in aquaculture practice and the need for cost-optimization in current aquaculture operations.

Undoubtedly, the choice of fish species and feed used is utmost relevant in this respect. Particularly for tilapia *Oreochromis niloticus*, due to economic feasibility, fishmeal is often fully substituted by plant ingredients (e.g. soybean meal, rape seed press cake and meal) without adverse effects on fish performance (El-Saidy & Gaber 2002). With regard to the current trend towards sustainable aquafeeds replacing fishmeal (Samuel-Fitwi et al. 2012, Slawski et al. 2012, Tusche et al. 2012, 2013) phytate is the main storage form for P in plant ingredients. Here, phosphate bioavailability is reduced, requiring enzymatic (phytase) conversion (Kumar et al. 2012). Thus, the use of animal protein derived from sustainable resources such as blood, insect or feather meal is a worthwhile strategy to optimize diets for aquaponics in the future. Alternatively, one could increase mobilization of plant-derived, organic P by optimizing enzymatic conversion either by using phytase supplementation in the fish diet (which would also increase P availability for the fish and thus improve fish nutrition) or by increasing microbial conversion. The latter will inevitably require a more sophisticated incubator that may not be feasible under the current economic and operational conditions.

The increase of K^+ by 31% is particularly relevant in tomato production since this macronutrient is required in large amounts and is currently only covered by artificial fertilization (Lattauschke 2004). Nevertheless, K^+ is not a scarce resource like P and the increase was not as significant as the increase in P. Still, optimized nutrient management in aquaponics should ultimately aim to minimize use of artificial fertilizer. Also, to our knowledge, current legislation and fees for aquaculture emissions do not consider respective K^+ concentrations. Nevertheless, envisioning sustainable nutrient re-use, future studies should focus on an overall optimization strategy to ensure an environmentally friendly production cycle.

In this context, although not determined in our study, potential accumulation of sodium has to be considered since this is an important issue in hydroponics. Up to a point, excess NaCl in the nutrient solution can be excluded by the plants; however, in a recirculating system this will result in a steady increase in salt concentration (Blom-Zandstra et al. 1998). Therefore hydroponics nutrient solution is frequently renewed to avoid excessively high salt concentrations and thus to prevent reduction of fruit yield or increased sensitivity to diseases (Post & Klein- Buitendijk 1996a,b).

The reduction of NO_3^--N by 19% is only relevant in critical periods, when imbalances between standing stock of fish and plant production cannot be avoided, for example upon

harvest or in periods when fish growth varies unexpectedly (e.g. stress). In well-balanced aquaponic systems, the reduction could be of minor relevance since intensive RAS production supports high NO_3^--N concentrations of up to 1000 mg l^{-1} in the rearing water, and blending with water from the sludge incubator can easily be compensated (van Rijn 2013). Under anaerobic conditions, loss of nitrogen due to denitrification was substantial and has to be taken into account for the overall evaluation of AT and UT studied here.

In the present study, TS was lower than in other studies. For example, Conroy & Couturier (2010) reported 109 g l^{-1} TS before initiating anaerobic treatment, i.e. 50 to 100 times higher than in the present study. This mostly results from differently concentrated sludge. Here, we prioritized a simple, easy-to-handle harvest of sludge. Still, both studies identified a P mobilization after a drastic drop in pH below 6. Consequently, it can be concluded that mobilization is mainly observed after acidification. Furthermore, higher TS may result in acidification due to massive fermentation under anaerobic conditions, whereas at lower TS acidification due to respiration at AT is demonstrated here. Together, this emphasizes the principal role of pH in sludge treatment. With respect to handling, system safety and reducing labour costs and providing a robust sludge treatment, aerobic treatment of water-sludge mixture can easily be integrated in aquaponic systems and, compared to anaerobic treatment, does not imply a loss of nitrogen by anaerobic denitrification.

5 Conclusions

In our study we comparatively evaluated a simple, easy-to-handle sludge incubation under aerobic and anaerobic treatment to improve the mobilization of important nutrients required for plant production. Here, aeration establishes aerobic conditions, and lowers the pH (via respiration and nitrification), subsequently supporting mobilization of P and K^+ with minor losses of NO_3^--N. Thereby, the delivery of these nutrients for the crop plant production is clearly improved in the overall system, reducing nutrient emission from sludge disposal. In contrast, anaerobic conditions (e.g. as in denitrification units) revealed a complete loss of NO_3^--N, poses the risk of undesired byproducts and, in practice, is more complicated to handle under commercial conditions. Based on our results we recommend a simple aeration (aerobic treatment) for the effective nutrient mobilization for aquaponics. Still it needs to be emphasized that economic feasibility and biological safety has to be proven. Also, application might be restricted to highly technical and complex systems.

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6 References

Barak Y, Van Rijn J (2000) Biological phosphate removal ina prototype recirculating aquaculture treatment system.Aquacult Eng 22: 121−136

Blom-Zandstra M, Vogelzang SA, Veen BW (1998) Sodium fluxes in sweet pepper exposed to varying sodium concentrations. J Exp Bot 49: 1863−1868

Conroy J, Couturier M (2010) Dissolution of minerals during hydrolysis of fish waste solids. Aquaculture 298: 220−225

Cooper J, Lombardi R, Boardman D, Carliell-Marquet C (2011) The future distribution and production of global phosphate rock reserves. Resour Conserv Recycling 57:78−86

Dawson CJ, Hilton J (2011) Fertiliser availability in a resource-limited world: production and recycling of nitrogen and phosphorus. Food Pol 36 (Suppl 1): S14−S22

Edwards P (2015) Aquaculture environment interactions: past, present and likely future trends. Aquaculture 447:2−14

El-Saidy DMSD, Gaber MMA (2002) Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. J World Aquacult Soc 33: 297−306

Goddek S, Delaide B, Mankasingh U, Ragnarsdottir K, Jijakli H, Thorarinsdottir R (2015) Challenges of sustainable and commercial aquaponics. Sustainability 7:4199−4224

Gutierrez-Wing MT, Malone RF (2006) Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacult Eng 34: 163−171

Hochmuth GJ, Hochmuth RC (2001) Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. HS796. University of Florida Cooperative Extension Service, Gainesville, FL

Hua K, Bureau DP (2006) Modelling digestible phosphorus content of salmonid fish feeds. Aquaculture 254: 455−465

Hupfer M, Gächter R, Giovanoli R (1995) Transformation of phosphorus species in settling seston and during early sediment diagenesis. Aquat Sci 57: 305−324

Jung IS, Lovitt RW (2011) Leaching techniques to remove metals and potentially hazardous nutrients from trout farm sludge. Water Res 45: 5977−5986

Klas S, Mozes N, Lahav O (2006) A conceptual, stoichiometry-based model for single-sludge denitrification in recirculating aquaculture systems. Aquaculture 259: 328−341

Kumar V, Sinha AK, Makkar HPS, De Boeck G, Becker K (2012) Phytate and phytase in fish nutrition. J Anim Physiol Anim Nutr 96: 335−364

Lattauschke G (2004) Gewächshaustomaten: Hinweise zum umweltgerechten Anbau—Managementunterlage. Book 1. Fachbereich Gartenbau, Sächsische Landesanstalt für Landwirtschaft, Pillnitz

McGill SM (2012) 'Peak' phosphorus? The implications of phosphate scarcity for sustainable investors. J Sustain Finance Invest 2: 222−239

Mirzoyan N, Tal Y, Gross A (2010) Anaerobic digestion of sludge from intensive recirculating aquaculture systems. Rev Aquacult 306: 1−6

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27: 31−36

Neori A, Krom MD, van Rijn J (2007) Biogeochemical processes in intensive zero-effluent marine fish culture with recirculating aerobic and anaerobic biofilters. J Exp Mar Biol Ecol 349: 235−247

O'Sullivan P, Reynolds CS (2004) The lakes handbook: limnology and limnetic ecology. Wiley-Blackwell, Oxford

Paredes D, Kuschk P, Mbwette TSA, Stange F, Müller RA, Köser H (2007) New aspects of microbial nitrogen transformations in the context of wastewater treatment - a review. Eng Life Sci 7: 13−25

Post WHK, Klein-Buitendijk H (1996a) Zoutonderzoek bij paprika. Invloed van natrium, calcium en kalium/magnesium verhoudingen op produktie en kwaliteit. Intern Vers lag 29, Proefstation voor Bloemisterij en Glasgroente, Naaldwijk

Post WHK, Klein-Buitendijk H (1996b) Zoutonderzoek bij paprika. Invloed van natrium en kalium op produktie en kwaliteit. Intern Verslag 28, Proefstation voor Bloemisterijen Glasgroente, Naaldwijk

Psenner R, Pucsko R, Sager M (1984) Die Fraktionierung organischer und anorganischer Phosphorverbindungen von Sedimenten - Versuch einer Definition ökologisch wichtiger Fraktionen. Arch Hydrobiol (Suppl) 70:111−155

Rakocy JE, Masser MP, Losordo TM (2006) Recirculating aquaculture tank production systems: aquaponics—integrating fish and plant culture. South Region Aquacult Cent (SRAC) Publ 454: 1−16

Rakocy JE, Bailey DS, Shultz RC, Danaher JJ (2007) Preliminary evaluation of organic waste from two aquaculture systems as a source of inorganic nutrients for hydroponics. Acta Hortic 201−207

Rennert B, Groß R, van Ballegooy C, Kloas W, (2011) Ein Aquaponiksystem zur kombinierten Tilapia- und Tomatenproduktion. Fisch Teichwirt 2011(6): 209−214

Samuel-Fitwi B, Wuertz S, Schroeder JP, Schulz C (2012) Sustainability assessment tools to support aquaculture development. J Clean Prod 32: 183−192

Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus uptake by plants: from soil to cell. Plant Physiol 116: 447−453

Schmid Neset TS, Bader HP, Scheidegger R, Lohm U (2008) The flow of phosphorus in food production and consumption - Linkoping, Sweden, 1870−2000. Sci Total Environ 396: 111−120

Slawski H, Adem H, Tressel RP, Wysujack K and others (2012) Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult Int 20: 443−453

Tusche K, Arning S, Wuertz S, Susenbeth A, Schulz C (2012) Wheat gluten and potato protein concentrate - promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 344−349: 120−125

Tusche K, Nagel F, Arning S, Wuertz S, Susenbeth A, Schulz C (2013) Effect of different dietary levels of potato protein concentrate supplemented with feed attractants on growth performance of rainbow trout (*Oncorhynchus mykiss*). Anim Feed Sci Technol 183: 202−209

Van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquacult Eng 53: 49−56

Wurts WA, Durborow RM, (1992) Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. South Region Aquacult Cent (SRAC) Publ 464: 1−4

Zhang Y, Bleeker A, Liu JG (2015) Nutrient discharge from China's aquaculture industry and associated environmental impacts. Environ Res Lett 10: 04500

General discussion

1 Nutrients in aquaponic systems

Anorganic nutrients, such as nitrate, phosphate and potassium as well as organic compounds like humic substances, are accumulating in the process water of RAS during the production of fish (Hambly et al. 2015, Martins et al. 2009, Yamin et al. 2017). Fish feed is the main source providing the RAS with different chemical compounds, either directly through leaching and microbial decomposition or indirectly through metabolites excreted by the fish and subsequent conversion in the biofilters (e.g. nitrification) (van Rijn 2013). In aquaponics, many of these chemical compounds are favourable for plant growth and the recycling effectiveness can mainly be influenced by the system design, water reuse efficiency and the implementation of different treatment units. Nevertheless, some compounds like nitrate are accumulating in high quantities and critical thresholds have to be identified to prevent adverse effects and subsequently suboptimal production of fish.

This research study was conducted to illustrate the different aspects of nutrient toxicity (nitrate) and nutrient recycling to improve the overall system efficiency of aquaponics, to promote a sustainable re-usage of valuable resources and to ensure an optimal production of fish and plants in a holistic approach. The findings of this study, presented in chapter I-III, are highlighted and jointly discussed within the following section.

2 Nitrate in aquaponic systems

In aquaponics high nitrate concentrations are favorable due to the fact, that nitrate is an excellent nitrogen source for plants and it is accumulated in large quantities in RAS, as illustrated in Figure 4.1.

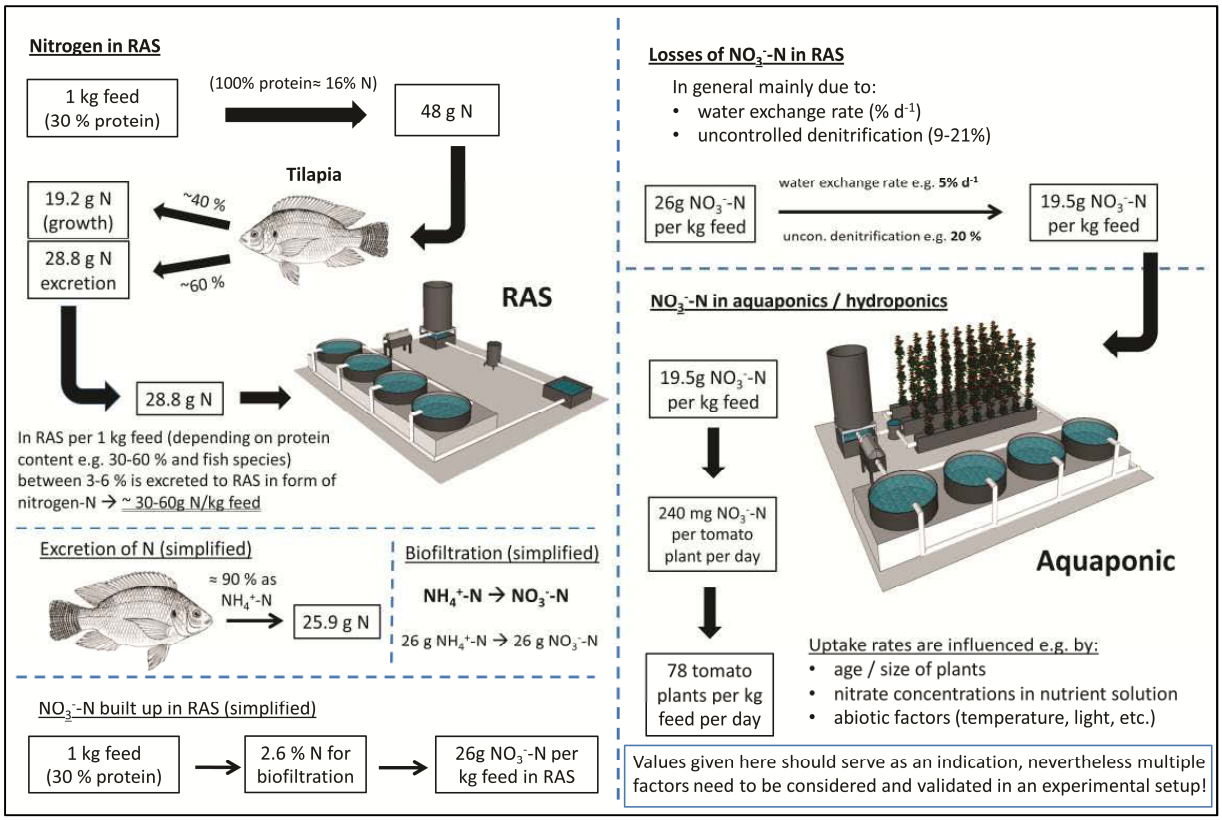

Fig. 4.1: Estimated fate of nitrogen (N) in recirculating aquaculture systems (RAS) and potential nitrate supply to the crop plants (tomatoes) in aquaponics.

In chapter I, nitrate concentrations < 500 mgL^{-1} NO_3^--N were clearly identified as not chronically affecting growth or health parameters of Nile tilapia. This is important since aquaponic systems generate (as a result of biofiltration) and require (in the hydroponic unit) large amounts of this anion for optimal fertilization of plants. In the case of tomato production at least 150 mg L^{-1} N is required and in advanced RAS even concentrations of up to 1000 mgL^{-1} NO_3^--N can accumulate (Hochmuth 2001, van Rijn 2013). In the experiment described in Chapter I, specific growth rate (SGR) of Nile tilapia decreased significantly to 1.1 % per day (± 0.1) and feed conversion ratio (FCR) increased significantly up to 1.1 g g^{-1} (± 0.2) at the highest nitrate concentration of 1000 mgL^{-1} NO_3^-N, confirming possible negative effects on fish production within a realistic concentration range in commercial RAS. Similar patterns have been observed in studies dealing with fish species like pikeperch (*Sander lucioperca*), catfish (*Clarias gariepinus*) or juvenile turbot (*Scophthalmus maximus*) (Schramm et al. 2014 a,b; van Bussel et al. 2012). Nevertheless, general underlying uptake mechanisms remained unclear. For Nile tilapia, one of the most frequently produced species in aquaculture worldwide (FAO 2014), data on chronic nitrate toxicity thresholds were, to date, lacking.

Skin and gills have been already discussed as strong barriers against nitrate uptake (Jensen 1996, Schramm et al. 2014a, Stormer et al. 1996). Accordingly, evaluation of gill histology showed only mild responses, i.e. increased hyperplasia to increase the diffusion barrier towards the toxin in the water (Reiser et al. 2010). Strong responses, such as fusion of secondary lamellae were not observed, confirming the low influence of high nitrate concentrations on the gills and suggesting different uptake pathways for this anion.

To identify alternative uptake pathways, blood was analyzed for plasma nitrite and nitrate, hemoglobin and methhemoglobin. In contrast to a study on catfisch (Schramm et al. 2014a), plasma nitrite exceeded plasma nitrate concentration by far, suggesting certain chemical alterations before or after nitrate uptake. These results were supported by the increase of methemoglobin and the decrease of hemoglobin at the highest tested nitrate concentration. This effect is often observed under nitrite intoxication as it is typically reported as "blue baby syndrome" in humans. Here, children exposed to nitrate rich diets such as spinach or baby meals prepared with nitrate rich well water, develop a blue color as a result of methemoglobin formation in the blood, leading to oxygen deficiencies (Knobeloch et al. 2000, Webb et al. 2008). But the reaction is also well described for fish (Kroupova et al. 2005, Svobodova et al. 2005, Tomasso 1986).

To confirm a potential chemical conversion of nitrate to nitrite in the gastro-intestinal tract of the fish, an additional *in vitro* experiment was conducted. Within only 90 min the nitrite concentration increased significantly to a maximum of 74 µM NO_2^- in the gastric juice of Nile tilapia, confirming the hypothesized reduction of nitrate to nitrite. Moreover, a subsequent passage of nitrite through the intestinal wall is very likely, as it was already described for European flounder (*Platichthys flesus*) (Grosell and Jensen 2000). Additionally, fast nitrite uptake from the abdominal cavity has been observed in Wistar rats (*Rattus norvegicus*) (Bryan et al. 2005). A follow-up study on the uptake of nitrite through the gastrointestinal wall could finally confirm the suggested uptake route. Nevertheless, the present results already provide a clear basis for the assumption, that the suggested pathway is likely responsible for the observed increase in plasma nitrite and subsequent methemoglobin formation.

The research presented in chapter I was the first study dealing with chronic nitrate exposure on Nile tilapia and at the same time presenting an alternative mechanism explaining indirect nitrate toxicity due to conversion of nitrate to nitrite in the stomach.

3 Aquaponics - Systems design matters

The second chapter dealt with the question, whether innovative, decoupled aquaponics are superior to coupled systems and represent a suitable alternative to the traditional approach. This is an important question, since classical, coupled systems have been developed decades ago but economic success is still missing. Extensive scientific literature was available on the combined production of fish and plants in a classical, coupled approach (Naegel 1977, Rakocy et al. 2006, Watten and Busch 1984), but recently the decoupled approach has been suggested as an alternative to the traditional concept (Kloas et al. 2015). Up to date, no information was available on the differences and missing data needs to be provided for an objective evaluation.

The results of the pilot study, presented in chapter II, clearly confirmed the advantages of decoupled systems. The most prominent advantage of decoupled systems is the possibility to run both system compartments (RAS and hydroponic) individually under optimal conditions without negatively influencing each other. It was for example possible to keep the pH of the RAS at around pH 7.2 for optimal nitrification in the biofilter and at the same time stabilizing the pH within the hydroponic around pH 6.4. For plant production this is of importance since the availability of micronutrients (e.g. copper, iron or boron) tends to decrease as pH increases and for tomatoes the recommended pH-value is between pH 5.8 - 6.2 (Hochmuth and Hochmuth 2008, Lucas and Davis 1961). Next to this, other parameters like temperature, conductivity, oxygen-concentration and many more can be controlled individually and adjusted according to specific recommendations.

Fish production was not affected by system design, but fruit production was considerably increased by 36 % in the decoupled system. Since all RAS are managed in the same manner, differences in fish growth were not expected prior to the experiments. It has to be mentioned, that fertilizer was added directly to the coupled aquaponic system (V= 16.5 m^3). The amount of fertilizer in the coupled system (16.5 m^3) corresponded to the amount that was added in the decoupled system (separate 200 L reservoir) to make both treatments comparable. However, additional fertilization in the coupled system had obviously no negative effect on fish growth. To obtain recommended nutrient concentrations in the coupled system, addition of fertilizer would need to be increased substantially, probably affecting fish growth at the same time.

Additionally, fertilization represents a manipulation within an aquaponic system. Considering that fish and plant production are combined in a coupled approach, possible negative effects of artificially added nutrients need to be evaluated and from the animal welfare point of view, addition of nutrient salts is undesired. As it was shown in chapter I, high nitrate

concentrations can negatively influence health and growth status of Nile tilapia and it is likely that other soluble nutrients will do, too. So far, no studies on animal welfare issues exist that are related to artificial fertilizer addition and fish production in aquaponics but it is likely that, with increasing economic success, the aspect will be of greater importance in the future. Also concerning this aspect, decoupled systems benefit from the advantage of independent management of both system compartments.

4 Nutrient recycling in Aquaponics

Last but not least, an improvement of the nutrient recycling was investigated, aiming at an optimized aquaponic system where as much of the waste water and excess nutrients are used for plant production. The results are presented in chapter III. A new concept of nutrient enrichment was suggested and confirmed in a technical experiment. There are controversial discussions within the aquaponic research community on whether aerobic or anaerobic nutrient enrichment should be favored for aquaponic application. This study provides a basis for a result oriented discussion.

The investigation clearly identified aerobic mineralization as an easy to handle sludge mineralization treatment, especially in terms of effective P recovery. Aerobic mineralization revealed a significant increase of phosphate of ~20 mgL^{-1} within only 14 days of incubation. In contrast, under anaerobic mineralization no increase of phosphate was observed. Additionally, the nitrate concentration was reduced by only 16 % under aerobic conditions, whereas under anaerobic conditions nitrate concentration was reduced by up to 97 %.

An additional benefit of aerobic mineralization was the increase of the potassium concentration by 31 %. Especially for tomato production in aquaponics this is of major importance since potassium is required in high quantities for optimal growth (Hochmuth 2001). The current understanding of aquaponic systems is that these systems represent already an advanced, sustainable food production, while recycling nutrients derived from RAS.

Nevertheless, a big share of nutrients in aquaponics is still unexploited and often directly discharged to the sewage system when removing solid waste from the mechanical filters (e.g. clarifier or drum filter). For instance, 60 % of the total P in aquaponics (in the RAS unit) were found to be discharged (chapter III). However, in terms of plant production requirements, phosphate is often missing in the process water of RAS due to improvements of feed formulations, a consequence of stricter environmental legislation (UBA 2017). Since phosphate fertilizer, derived from phosphate rock, is a non renewable resource (Cooper et al.

2011, McGill 2012), scientific solutions for the foreseeable deficit are needed to ensure a cost-effective production of plants.

In general, anaerobic mineralization of phosphate from organic material represents an alternative technique to recycle phosphate and earlier as well as current experiments were promising. Still, in aquaponic systems the anaerobic approach is counteracting current attempts to increase and improve the overall availability of nutrients in the hydroponic nutrient solution. This was clearly shown in chapter III. Here, nitrate (the end product of nitrification in RAS), which is seen as the dominant macronutrient in aquaponics (Rackocy 2006), was completely reduced to elemental nitrogen (N_2) as a result of anaerobic denitrification. Changes in phosphate concentrations were not observed within 14 days, but nitrate decreased simultaneously by 97 %, counteracting an overall improvement. Certainly anaerobic reactors can be efficiently used for P-recovery and RAS water can be blended with P-rich reactor water prior to hydroponic application. However, anaerobic reactors need a starting phase prior to full operation, Carbon sources and acids are often required to increase the efficiency, toxic byproducts can be produced and specialized staff is required for optimal operation (Mirzoyan et al. 2010, van Rijn et al. 2006). Additionally, the higher the complexity of an aquaponic system, the more likely it is, that mismanagement can lead to increased maintenance requirements, loss of resources and, as a result, to increasing production costs.

Finally, besides optimization of the nutrient solution, the recycling of waste water from the mechanical filtration units can result in additional water savings, increasing the overall resource efficiency of an aquaponic system. Fresh water is only required to replace the loss from the cleaning of the mechanical filter and water consumption from plants would not contribute to the overall water consumption, when formerly discarded water is re-used within the hydroponic unit. Nevertheless, depending on the size of plant production, additional water from the RAS can still be used on-demand.

5 Future directions and implications for system design

The present work presented a holistic approach to evaluate and optimize aquaponic systems. Chapter I illustrated that high nitrate concentrations, generally present in most aquaponic systems, will not negatively influence fish production. Nevertheless, the possibility of an alternative pathway of nitrate uptake via the gastrointestinal wall has to be clarified in a follow up study.

As stated in Chapter II, system design is very important to foster the professional improvement of aquaponic systems. A more detailed study on the comparison of coupled and

decoupled systems with a special focus on the improvement of plant nutrition within aquaponic systems is needed. Providing fertilizer solutions for different RAS water qualities could help future farm managers to better adapt to changing nutrient profiles.

The basis for a further improvement of nutrient recycling efficiency has been provided in Chapter III. For an implementation in aquaponic systems, more research is required, focusing on the automation of the aerobic mineralisation process. The easy applicable process is very promising and automation could increase phosphate recovery without excessive requirements for space or manpower.

Overall, for improvement of aquaponic systems and for a design of a professional production system, it is indispensable that researchers from both disciplines (aquaculture and horticulture) are working together in an interdisciplinary approach. The focus should be placed especially on the interface between aquaculture and horticulture. In this context, two research questions are of special interest: 1. What needs to be done in RAS to provide an optimal water quality for plant production (e.g. automated mineralization, post-disinfection of RAS water) and 2. How can processes be optimized in the hydroponic unit to allow a save (e.g. removal of potential pathogens) and optimal production (e.g. automation of fertilization, flexible adaptation to different nutrient profiles) of plant crops? Since two of the most efficient production systems for animal (RAS) and plant production (hydroponics) are combined in one approach, using less resources than each single one, it is likely that these systems will play a bigger role in the future of professional agriculture. The economic profitability and reliability of management is already proven for each single system and the development of decoupled systems represents a big step forward towards a more professional application. The task for future aquaponic research is to provide a feasible and technical sophisticated decoupled approach for the combination of both compartments. And representing one of the most advanced food production systems with respect to water and fertilizer utilization and CO_2 production, future aquaponic systems can contribute to adapt to the consequences of overpopulation, climate change and the depletion of natural resources.

6 Mayor findings and conclusions

Conclusively, the mayor findings of this thesis are:

- Growth and health status of Nile tilapia are negatively affected by high nitrate concentrations (> 500 mgL^{-1} NO_3^--N)
- Nitrate concentrations for plant production in aquaponic systems ~ 200 mgL^{-1} NO_3^--N are, in turn, not affecting fish welfare.
- Nitrate toxicity is rather a consequence of the conversion to nitrite in the stomach and subsequent uptake to the vascular system, than directly attributed to nitrate (e.g. effect on the gills)
- System design has a considerable influence on the overall system performance
- Decoupled aquaponics are favorable for professional aquaponic production of fish and plants
- Coupled systems are suboptimal for a combined production of fish and plants, especially in terms of plant yield
- Aerobic mineralization of phosphate revealed best phosphate recovery with only minor losses in nitrate concentration
- Anaerobic mineralization is more problematic for aquaponic applications due to a complete loss of nitrate (main nitrogen source in aquaponic systems), the potential development of toxic byproducts and an increased demand in labour
- Recycling of water sludge mixture from clarifiers results in a substantial phosphate recovery, an increase in potassium and additional water savings

7 References

Bostock, J., Mcandrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N. & Gatward, I. (2010) Aquaculture: global status and trends. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 365**,** 2897-2912.

Boyd, C. E. (2003) Guidelines for aquaculture effluent management at the farm-level. *Aquaculture*, 226, 101-112.

Brazil, B. L. (2006) Performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. *Aquacultural Engineering,* 34**,** 261-274.

Bryan, N. S., Fernandez, B. O., Bauer, S. M., Garcia-Saura, M. F., Milsom, A. B., Rassaf, T., Maloney, R. E., Bharti, A., Rodriguez, J. & Feelisch, M. (2005) Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat Chem Biol,* 1**,** 290-7.

Chen, S., Ling, J. & Blancheton, J.-P. (2006) Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering,* 34**,** 179-197.

Chislock, M. F., Doster, E., Zitomer, R. A. & Wilson, A. (2013) Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge,* 4**,** 10.

Cooper, J., Lombardi, R., Boardman, D. & Carliell-Marquet, C. (2011) The future distribution and production of global phosphate rock reserves. *Resources, Conservation and Recycling,* 57**,** 78-86.

Ebeling, J. M., Timmons, M. B. & Bisogni, J. J. (2006) Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture,* 257**,** 346-358.

Eding, E., Kamstra, A., Verreth, J., Huisman, E. & Klapwijk, A. (2006) Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. *Aquacultural Engineering,* 34**,** 234-260.

El-Sayed, A.-F. M. (2006) Tilapia culture, CABI Publ., Wallingford, UK (2006), 277pp.

Endut, A., Jusoh, A., Ali, N., Wan Nik, W. B. & Hassan, A. (2010) A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource Technology,* 101**,** 1511-1517.

Evans, D. H., Piermarini, P. M. & Choe, K. P. (2005) The Multifunctional Fish Gill: Dominant Site of Gas Exchange, Osmoregulation, Acid-Base Regulation, and Excretion of Nitrogenous Waste. *Physiol. Rev.,* 85**,** 97-177.

FAO (2014) *Oreochromis niloticus* - Cultured Aquatic Species Information Programme. http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en.

FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

Graber, A. & Junge, R. (2009) Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination,* 246**,** 147-156.

Grosell, M. & Jensen, F. B. (2000) Uptake and effects of nitrite in the marine teleost fish Platichthys flesus. *Aquatic Toxicology,* 50**,** 97-107.

Gunning, D., Harman, L., Keily, M., Nunan, R., Jones, P., Horgan, B. & Burnell, G.(2014) Designing a marine aquaponics (maraponics) system to model IMTA. *Proceedings of the Aquaculture Europe Conference,* San Sebastian, Spain

Hambly, A. C., Arvin, E., Pedersen, L. F., Pedersen, P. B., Seredyńska-Sobecka, B. & Stedmon, C. A. (2015) Characterising organic matter in recirculating aquaculture systems with fluorescence EEM spectroscopy. *Water Research,* 83**,** 112-120.

Herbeck, L. S., Unger, D., Wu, Y. & Jennerjahn, T. C. (2013) Effluent, nutrient and organic matter export from shrimp and fish ponds causing eutrophication in coastal and back-reef waters of NE Hainan, tropical China. *Continental Shelf Research,* 57**,** 92-104.

Hochmuth, G. J. (2001) Fertilizer Management for Greenhouse Vegetables *Florida Greenhouse Vegetable Production Handbook,* 3**,** 1-25.

Hochmuth, G. J. & Hochmuth, R. C. (2001) Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. *Univ. Fla. Coop. Ext. Serv. HS,* 796, Gainesville

Hovanec, T. & Delong, E. (1996) Comparative analysis of nitrifying bacteria associated with freshwater and marine aquaria. *Appl. Environ. Microbiol.,* 62**,** 2888-2896.

Jensen, F. B. (1996) Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (Astacus astacus). *Aquatic Toxicology,* 34**,** 95-104.

Kamstra, A., Van Der Heul, J. W. & Nijhof, M. (1998) Performance and optimisation of trickling filters on eel farms. *Aquacultural Engineering,* 17**,** 175-192.

Kloas, W., Groß, R., Baganz, D., Graupner, J., Monsees, H., Schmidt, U., Staaks, G., Suhl, J., Tschirner, M. & Wittstock, B. (2015) A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. *Aquaculture Environment Interactions,* 7**,** 179-192.

Knobeloch, L., Salna, B., Hogan, A., Postle, J. & Anderson, H. (2000) Blue babies and nitrate-contaminated well water. *Environmental Health Perspectives,* 108**,** 675-678.

Kroupova, H., Machova, J. & Svobodova, Z. (2005) Nitrite influence on fish: a review. *Veterinarni Medicina,* 50**,** 461-471.

Lattauschke, G. (2004) Gewächshaustomaten: Hinweise zum umweltgerechten Anbau—Managementunterlage. Book 1. Fachbereich Gartenbau, Sächsische Landesanstalt für Landwirtschaft, Pillnitz. *Landesamt für Umwelt, Landwirtschaft und Geologie***,** 51.

Love, D. C., Fry, J. P., Li, X., Hill, E. S., Genello, L., Semmens, K. & Thompson, R. E. (2015) Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture,* 435**,** 67-74.

Lucas, R. & Davis, J. F. (1961) Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Science,* 92**,** 177-182.

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., D'orbcastel, E. R. & Verreth, J. a. J. (2010) New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering,* 43**,** 83-93.

Martins, C. I. M., Pistrin, M. G., Ende, S. S. W., Eding, E. H. & Verreth, J. a. J. (2009) The accumulation of substances in Recirculating Aquaculture Systems (RAS) affects embryonic and larval development in common carp *Cyprinus carpio*. *Aquaculture,* 291**,** 65-73.

Mcgill, S. M. (2012) 'Peak' phosphorus? The implications of phosphate scarcity for sustainable investors. *Journal of Sustainable Finance & Investment,* 2**,** 222-239.

Meade, J. W. (1985) Allowable Ammonia for Fish Culture. *The Progressive Fish-Culturist,* 47**,** 135-145.

Mirzoyan, N., Tal, Y. & Gross, A. (2010) Anaerobic digestion of sludge from intensive recirculating aquaculture systems: Review. *Aquaculture,* 306**,** 1-6.

Naegel, L. C. (1977) Combined production of fish and plants in recirculating water. *Aquaculture,* 10**,** 17-24.

Popma, T. & Masser, M. (1999). Tilapia: life history and biology. *South Region Aquacult Cent* (SRAC); 282:1-8.

Rakocy, J. E., Masser, M. P. & Losordo, T. M. (2006) Recirculating Aquaculture Tank Production Systems: Aquaponics–Integrating fish and Plant Culture. *South Region Aquacult Cent* (SRAC); 454**,** 16.

Reiser, S., Schroeder, J., Wuertz, S., Kloas, W. & Hanel, R. (2010) Histological and physiological alterations in juvenile turbot (*Psetta maxima, L.*) exposed to sublethal concentrations of ozone-produced oxidants in ozonated seawater. *Aquaculture,* 307**,** 157-164.

Rennert, B., Groß, R., Van Ballegooy, C. & Kloas, W. (2011) Ein Aquaponiksystem zur kombinierten Tilapia- und Tomatenproduktion. *Fischer & Teichwirt,* 209-214.

Saliling, W. J. B., Westerman, P. W. & Losordo, T. M. (2007) Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. *Aquacultural Engineering,* 37**,** 222-233.

Schram, E., Roques, J. a. C., Abbink, W., Yokohama, Y., Spanings, T., De Vries, P., Bierman, S., Van De Vis, H. & Flik, G. (2014, a) The impact of elevated water nitrate concentration on physiology, growth and feed intake of African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture Research,* 45**,** 1499-1511.

Schram, E., Roques, J. a. C., Van Kuijk, T., Abbink, W., Van De Heul, J., De Vries, P., Bierman, S., Van De Vis, H. & Flik, G. (2014, b) The impact of elevated water ammonia and nitrate concentrations on physiology, growth and feed intake of pikeperch (*Sander lucioperca*). *Aquaculture,* 420–421**,** 95-104.

Stormer, J., Jensen, F. B. & Rankin, J. C. (1996) Uptake of nitrite, nitrate, and bromide in rainbow trout, *Oncorhynchus mykiss*: Effects on ionic balance. *Canadian Journal of Fisheries and Aquatic Sciences,* 53**,** 1943-1950.

Summerfelt, S. T., Sharrer, M. J., Tsukuda, S. M. & Gearheart, M. (2009) Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. *Aquacultural Engineering,* 40**,** 17-27.

Svobodova, Z., Machova, J., Poleszczuk, G., Huda, J., Hamackova, J. & Kroupova, H. (2005) Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. *Acta Veterinaria Brno,* 74**,** 129-137.

Tal, Y., Schreier, H. J., Sowers, K. R., Stubblefield, J. D., Place, A. R. & Zohar, Y. (2009) Environmentally sustainable land-based marine aquaculture. *Aquaculture,* 286**,** 28-35.

Thurston, R. V., Russo, R. C. & Vinogradov, G. A. (1981) Ammonia toxicity to fishes. Effect of pH on the toxicity of the unionized ammonia species. *Environmental Science & Technology,* 15**,** 837-840.

Timmons, M. B., Holder, J. L. & Ebeling, J. M. (2006) Application of microbead biological filters. *Aquacultural Engineering,* 34**,** 332-343.

Tomasso, J. R. (1986) Comparative toxicity of nitrite to freshwater fishes. *Aquatic Toxicology,* 8**,** 129-137.

Umweltbundesamt (UBA): Adler, N. E., K.; Friedrich, B.; Frische, T.; Gather, C.; Ginzky, H.; Hammerich, J.; Herrmann, T.; Hofmeier, M.; Lamfried, D.; Matthey, A.; Plambeck, N.O.; Richter, S.; Roskosch, A.; Starke, S.M.; Vogel, I.; Walter, A. (2017). Umweltschutz in der Landwirtschaft. *In:* ITTERSHAGEN, M. (ed.) *Für Mensch & Umwelt.* Dessau-Roßlau: Umweltbundesamt.

United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241

Van Bussel, C. G. J., Schroeder, J. P., Wuertz, S. & Schulz, C. (2012) The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). *Aquaculture,* 326–329**,** 163-167.

Van Rijn, J. (2013) Waste treatment in recirculating aquaculture systems. *Aquacultural Engineering,* 53**,** 49-56.

Van Rijn, J., Tal, Y. & Schreier, H. J. (2006) Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering,* 34**,** 364-376.

Verdegem, M. C. J. (2013) Nutrient discharge from aquaculture operations in function of system design and production environment. *Reviews in Aquaculture,* 5**,** 158-171.

Watten, B. J. & Busch, R. L. (1984) Tropical production of tilapia (*Sarotherodon aurea*) and tomatoes (Lycopersicon esculentum) in a small-scale recirculating water system. *Aquaculture,* 41**,** 271-283.

Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J., Benjamin, N., Macallister, R., Hobbs, A. J. & Ahluwalia, A. (2008) Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite. *Hypertension,* 51**,** 784-790.

Wenger, D. (2003) Produktion von Rosen und Tomaten in einem Polykultursystem mit Fischabwasser. *Diploma Thesis, Hochschule Waedenswil*.

Wheeler, R., Sager, J., Berry, W., Mackowiak, C., Stutte, G., Yorio, N. & Ruffe, L. (1997) Nutrient, acid and water budgets of hydroponically grown crops. *International Symposium on Growing Media and Hydroponics* 481, 655-662.

Yamin, G., Borisover, M., Cohen, E. & Van Rijn, J. (2017) Accumulation of humic-like and proteinaceous dissolved organic matter in zero-discharge aquaculture systems as revealed by fluorescence EEM spectroscopy. *Water Research,* 108**,** 412-421.

Zohar, Y., Tal, Y., Schreier, H.J., Steven, C., Stubblefield, J., Place, A. (2005) Commercially feasible urban recirculated aquaculture: addressing the marine sector. In: Costa-Pierce, B. (Ed.) Urban Aquaculture. *CABI Publishing, Cambridge***,** 159-171.

List of publications

Monsees, H., Kloas, W. & Wuertz, S. (submitted to *PLOS ONE*) Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes.

Monsees, H., Keitel, J., Paul, M., Kloas, W. & Wuertz, S. (2017) Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions. *Aquaculture Environment Interactions,* 9**,** 9-18.

Monsees, H., Klatt, L., Kloas, W. & Wuertz, S. (2017) Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (*Oreochromis niloticus* L.) in recirculating aquaculture systems. *Aquaculture Research***,** 48, 3482–3492, DOI: 10.1111/are.13174

Kloas, W., Groß, R., Baganz, D., Graupner, J., **Monsees, H.**, Schmidt, U., Staaks, G., Suhl, J., Tschirner, M. & Wittstock, B. (2015) A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. *Aquaculture Environment Interactions,* 7**,** 179-192.

Eidesstattliche Erklärung

Ich erkläre hiermit, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe. Beim Erstellen dieser Dissertation bestand keine Zusammenarbeit mit gewerblichen Promotionsberatern. Ich habe die dem angestrebten Verfahren zur Grunde liegende Promotionsordnung zur Kenntnis genommen und habe die Dissertation nicht bereits bei einer anderen wissenschaftlichen Einrichtung ganz oder in Teilen eingereicht. Die Grundsätze der Humboldt-Universität zur Sicherung guter wissenschaftlicher Praxis wurden eingehalten. Ich erkläre hiermit, dass ich zuvor noch keinen Promotionsantrag gestellt habe bzw. einen entsprechenden Doktorgrad besitze.

Berlin, 29.06.2017

Hendrik Monsees