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Aquaculture waste valorisation

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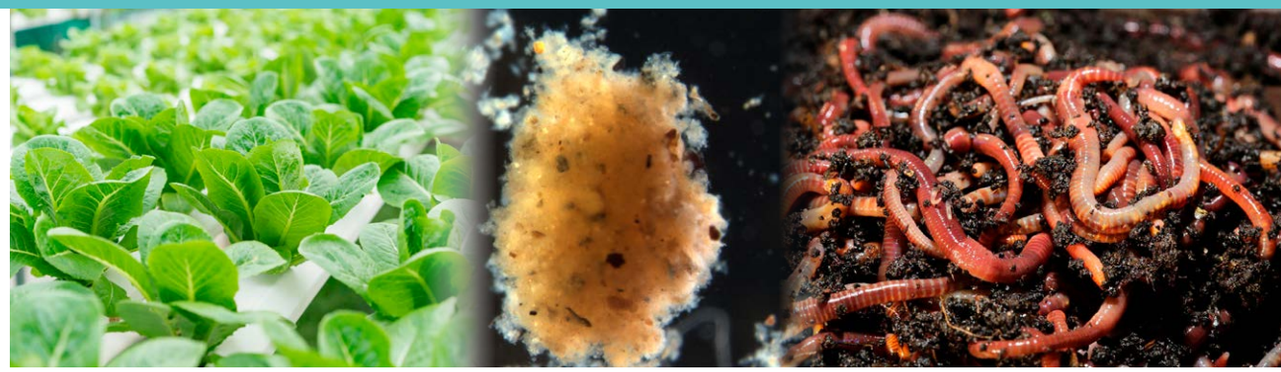
Zhodnocení odpadu z akvakultury



Doctoral thesis by
Roman Lunda

Czech Republic, Vodňany, 2021

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction

One hundred million tons of fish are consumed worldwide each year, providing 2.5 billion people with at least 20% of their average per capita animal protein intake (FAO, 2019). Fish is one of the most efficient sources of animal protein, with a great food conversion ratio (FCR) between 1 and 2 (Ebeling and Timmons, 2012). While fish demand is increasing, eighty percent of the world's oceans are fully or over exploited, depleted or in a state of collapse. Being the fastest growing sector of world food production (FAO, 2020), aquaculture seems to be the most viable answer to the growing demand.

However, rapid development of aquaculture worldwide has caused some social, economic, and environmental concerns. First, all aquaculture systems produce solids, including dissolved, colloidal, super colloidal and settleable solids (Bao et al., 2019). From this perspective, traditional aquaculture is most environmentally compatible as it uses on-farm and locally available wastes and by-products such as crop residues, animal or human manures or natural food in open water bodies as nutritional inputs for farmed aquatic organisms. Second, increasing use of pelleted feed in modern aquaculture has led to major environmental concerns (Edwards, 2015), since wastes, by-products and natural food are no longer the only sources of nutritional inputs for farmed aquatic organisms.

It seems that further restrictions on waste management are inevitable in the future. Further expansion of aquaculture depends on the development and application of waste valorisation. There is a strong hope that new technologies could intensify the fish farming by maximizing the reuse of water and nutrients in all forms (van Rijn et al., 2006; Martins et al., 2010). Many governments like China, the United States (US) and European Union (EU) have proposed restricted legislations and regulations for aquaculture activities (Zou and Huang, 2015). It looks as if there are only two directions for aquaculture, either systems that are focused only on profit regardless of environmental impact (intensive aquaculture), or the environmentally concerned option, which can never compete with intensive aquaculture in terms of profit and demand (Troell et al., 2009). However, climate change and government regulations may lead to integration of these two directions into a sustainable and profitable one.

1.2. Intensive aquaculture

Intensive aquaculture systems can be basically divided into flow-through systems (FTS) and recirculating aquaculture systems (RAS). Of course, more prototypes and hydrides of these systems exist.

1.2.1. Flow-through systems and waste footprint reduction

FTS include land-based farms, ponds, net pens, and cages. As the system name suggests, it can be assumed that all uneaten feed and feces are removed from the system constantly (Blancheton et al., 2007). Deposition or retention of waste depends on the water flow rate in the system. Basically, there is no filtration in this type of rearing system (Schumann and Brinker, 2020). The high proportion of particles in ambient water negatively affects the environment from the system outlet. There are currently a few FTS in Europe using technology for waste reduction such as solids removal or constructed wetland (CW) because of climate conditions and no-profit. Conventional wastewater treatment technologies have been applied for the treatment of aquaculture wastewater (Vymazal, 2007, 2014). Thus, CWs for the treatment of aquaculture wastewater are now on the rise (von Ahnen et al., 2020) because of the advantages in terms of cost, environmental friendliness, efficiency, and effectiveness

in wastewater treatment over the conventional treatment methods. CWs have proven their acceptability globally, which enables their implementation and usage in developing countries for waste water treatment (Omotade et al., 2019).

A huge issue has been demonstrated in net-pen farms and cages in relation to waste pollution. Initially, it was expected that all waste particles would be used by organisms living in the ocean or would be simply diluted. The enormous amount of undissolved substances that sediment to the seabed leads to vast dead zones in the oceans (Dybas, 2005). To provide environmental sustainability to aquaculture, utilization of nutrients could be attained through integrated multi-trophic systems (IMTA). The concept of IMTA was coined almost two decades ago. Chopin and Robinson (2004) presented a system that contains species from different trophic levels. Basically, it is about involving other species (crustaceans, bivalve molluscs, and aquatic weed) in the fish production system. Due to IMTA, waste particles from the intensive system are utilized and reduced (Skriptsova and Miroshnikova, 2011). All types of FTS are outdoor and directly connected to a water source. However, there are so many variations of systems that it is not possible to standardize the technology for the whole aquaculture sector. Countless factors affect the amount and concentration of waste such as type of FTS, water (saline, brackish, freshwater), fish species, location of the system, weather conditions, all water parameters, feed (type, value, composition).

1.2.2. Recirculating aquaculture systems (RAS)

The second type of intensive aquaculture system is RAS. The whole system usually consists of rearing tanks, filtration section (mechanical and biological), air/oxygen suppliers and water pumps. Together, all the parts are formed into a big loop (Losordo et al., 1999). Water from fish tanks is transported through mechanical filtration to biological filtration and back to fish tanks. Due to oxygen supply and optimal water treatment management (temperature, pH value, disinfection, filtration, carbon dioxide and nitrate removal) RAS allows high fish stocking density (30–200 $kg.m^{-3}$). RAS´s stocking density limit depends on fish species and the amount of feed (Timmons et al., 2002). This system uses 90–99% less water and land area compared to FTS (Badiola et al., 2012; Ebeling and Timmons, 2012; Dalsgaard et al., 2013). RAS management requires extra cost (maintenance of water, treatment of the tank for suspended solids removal, removal of excessive nitrogen). In addition, a daily water exchange ratio of 5–15% is still required for reducing the accumulation of dissolved nutrients in the system (Ebeling and Timmons, 2012; Topic Popovic et al., 2015). The disadvantage of RAS is the loop. Whatever is added to the system will affect all its parts (Badiola et al., 2012). Waste from RAS must be used in another way.

1.3. Aquaculture waste

In general, aquaculture waste consists primarily of total suspended solids (TSS), total dissolved solids (TDS) and waste in gas form (Thorpe and Cho, 1995; Schendel et al., 2004). Lee (2015) documented that one kilogram of eaten feed results in 35.7% of fecal solids produced by Nile tilapia (*Oreochromis niloticus*) in RAS culture. Solids in the aquaculture system may cause damage to fish because of oxygen consumption, reduce biofilter nitrification ability, lead to the accumulation of toxic materials, induce disease outbreaks, clog system components (e.g. micro screens, spray nozzle orifices and biofilters) and weaken disinfection effect (Davidson et al., 2008, 2013). Typically, a considerable amount of sludge is produced in RAS and this sludge must be treated before it can be disposed. Mechanical filtration removes particulate matter, while biological filtration removes TAN. Waste characteristics may vary widely, depending on

the fish species, feed, management, and differences in decay of organic matter within the particular culture system (van Rijn, 1996, 2013). However, formulated pelleted feed is the main food source of cultured fish currently, and feed residue is an important source of solids (Edwards, 2015; Mo et al., 2018).

1.3.1. Total suspended solids

TSS is defined as the proportion of particles (dry weight) retained by a filter with a pore size of 0.45 μm (Bao et al., 2019). Approximately 25–30% of the feed that is inputted into the system becomes suspended solids (Hambly et al., 2015). A great amount of feces is produced daily in intensive aquaculture systems. In a typical RAS treatment system, culture tank effluent generally passes through a solids removal process such as gravity clarification or micro screen sieving; rotary drum filtration, or rotary disc screening in which a wide range (from 60 to 200 μm) of screen mesh sizes is applied (Viadero and Noblet, 2002). If the mesh size is 40–100 μm, the removal efficiency of TSS can be 30–80% (Timmons et al., 2002). Screens and rotating micro screen filters have limitations in capturing solids smaller than approximately 50–60 μm (Cripps and Bergheim, 2000;Brambilla et al., 2008), and if a smaller aperture screen is used, the mechanical removal efficiency increases non linearly (efficiency increased by 24.22% from 60 to 40 μm, while efficiency increased by only 4.07% from 30 to 10 μm), increasing the filter backwash frequency, capital and operating costs (Dolan et al., 2013). In addition to the particle size, the filter performance of a micro screen is also related to the backwash frequency, screen pore size, and TSS concentration in the aquaculture water. The design and selection of mechanical filters should consider these factors to achieve better performance and benefits. In a nutshell, the final product of mechanical filtration in RAS is sludge. Typically, fish sludge is characterized by its low total solid content (1.5–3%) compared to other animal production or industrial wastewater (Mirzoyan et al., 2008). The density of sludge depends on filtration equipment and filter screen capacity, but final composition is affected by many factors as mentioned earlier.

1.3.2. Total dissolved solids

After the mechanical separation of solids, wastewater moves into another (biological) treatment. TDS refers to the dry weight of residue that passes through the filter (Bao et al., 2019). It is a dissolved substance, mostly formed by the metabolism of cultured organisms and other dissolved elements from environment. Nitrogen (N), phosphorus (P) and carbon (C) represent the bulk of elements present in aquaculture wastewater for valorisation (Neori et al., 2004; Ebeling and Timmons, 2012). These solids breaking into smaller or dissolved particles can cause solids-bound nutrients into waters and deteriorates the water quality (Chen et al., 1997). In general, most of the nitrogen waste (60–90%) is in the dissolved form-mainly ammonia (NH_3), whereas for phosphorus a larger proportion is excreted within the fecal waste (25–85%).

Nitrogen and its forms: total ammonia nitrogen (TAN), nitrite (NO_2^-) and nitrates (NO_3^-) are the most monitored and successfully treated suspended solids elements in RAS. Biological filtration is ensured by membrane biofilter (Kimbrough and Wakakuwa, 1989). The system has a required capacity of biofilter, which is appropriate to the size of the fish stock. Optimal water conditions and technology ensure TAN to NO_3 conversion (van Rijn et al., 2006).

Phosphorus effluent concentrations are high because much of the phosphorus added with the feed is unutilized by the fish (Rodehutscord and Pfeffer, 1995). In addition, appropriate methods for phosphorus removal in these systems is lacking. Enhanced biological phosphorus

removal (EBPR) from domestic wastewater inactivated sludge plants is accomplished by alternate stages in which the sludge is subjected to anaerobic and aerobic conditions. Under these conditions, phosphorus is released from the bacterial biomass in the anaerobic stage and is assimilated by these bacteria in excess as polyphosphate during the aerobic stage. Phosphorus is subsequently removed from the process stream by harvesting a fraction of the phosphorus-rich bacterial biomass (Nungesser, 1995; Barak and van Rijn, 2000; Tunçal et al., 2009). Ebeling et al. (2004) presented coagulation-flocculation aids (alum and ferric chloride) as an effective method of phosphorus removal from RAS. A whole spectrum of other organic elements like zinc (Zn), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe) and more, will always be present in the aquaculture waste, depending on the input feed (Thomas et al., 1999). Moreover, total aquaculture waste (water/sludge) composition will also be affected by additional substances for system treatment like sodium (Na) or potassium (K) which depend on water quality parameters (Lunda et al., 2019 – Chapter 2).

1.3.3. Nitrogen and carbon waste

Degassing and denitrification are topics that are often overlooked. With the water source enabled, RAS do not need these units because of economic reasons. In the present, water is cheaper than construction and maintenance of a denitrification unit. The concentration of nitrates in recirculating systems is usually as high as 400–500 $mg.L^{-1}$ (Honda et al., 1993). In RAS and traditional wastewater treatment plants, heterotrophic denitrification is often applied using external electron and carbon donors (e.g., carbohydrates, organic alcohols) or endogenous organic donors originating from the waste. Denitrifying organisms in aquaculture systems are associated with other processes relevant to water quality control. When endogenous carbon sources (originating from fish waste) are used for denitrification, in this time, the organic carbon discharge from RAS is reduced (van Rijn et al., 2006). The final production of denitrification is nitrous oxide (N_2O) and elemental form N_2.N_2O is the third most important greenhouse gas (GGS) with a global warming potential (Hu et al., 2012) – and it should be treated with caution.

In aquaculture production systems, dissolved carbon dioxide (CO_2) originates from the metabolism of organisms in the system, predominantly fish, but also bacterial populations (Skov, 2019). With the increase of the fish density to 100 $kg.m^{-3}$ or higher, to make the aquaculture system more productive, pure oxygen systems are widely used to meet the demand of the normal growth of fish. For every 10 $mg.L^{-1}$ of oxygen consumed, approximately 13–14 $mg.L^{-1}$ of CO_2 is excreted through fish gills (Summerfelt et al., 2000). CO_2 removal technology of aquaculture waters is still in the pilot study stage, and generally, a large-scale recirculating aquaculture system does not set CO_2 removal link (Colt and Bouck, 1984; Colt, 1991; Noble et al., 2012). The suggested solution includes a surface aerator with variable frequency control (Badiola et al., 2018). Currently, the mainstream CO_2 removal devices are stripping columns (Colt and Bouck, 1984). Of course, there exist techniques that utilize CO_2 with an advantageous return. It is also necessary to mention that methane (CH_4) is also one of the gases produced by aquaculture. In pond aquaculture systems, the bottom sediment is the major site for methanogenic bacteria activity as it resides at the least aerated site of the pond environment (Hu et al., 2016). In terms of large-scale CH_4 production, RAS and FTS do not represent important sources.

1.4. Innovative methods of aquaculture waste utilization and valorisation

In recent years, the EU environmental policy directives have become more stringent, bringing serious implications for aquaculture sector. These include clamping down on aquaculture input use, farm waste effluent penalties, and lowered ceilings in waste nutrient concentrations (Hoevenaars et al., 2018). RASs have been modified to respond to such increasing environmental regulations in countries with limited access to land and water (Martins et al., 2010). Aquaculture waste removal is often performed using a combination of methods that are related to several removal mechanisms – and mostly these have higher costs. The concentrated solids from aquaculture effluents can be used conditionally for composting, land application, soil amendments, conversion to bioproducts, bacterial biomass production, as fertilizer for nursery, as a feed source and as an endogenous carbon source for denitrification (Jung and Lovitt, 2011; Mirzoyan and Gross, 2013; Bao et al., 2019). Wastewater can be used in algae and crop production, the same as gases from aquaculture (Rakocy et al., 1992; Hu et al., 2013, 2015).

1.4.1. Sludge valorisation

Valorisation of organic matter solid waste can be accomplished via composting and anaerobic digestion. The advantage of producing compost is the technical simplicity of the process. To cover part of the integrated solid waste management strategies costs, it was found that valorising and recycling activities have turned into a valuable economic enterprise (Abdel-Shafy and Mansour, 2018). Aquaculture solids in the form of TSS could be suitable fertilization for the land agriculture industry, but compared to cattle manure it does not contain optimal ratio and values of nutrients (van Rijn, 2013). The simplest and most common use of sludge produced from fish farms is as fertilizer for direct land application. Fish sludge contains macro and micro nutrients, especially high levels of nitrogen and phosphorus, which potentially can be returned to the land to fertilize crops and provide much-needed organic material to certain soils. Lander et al. (2013) characterized the concentration, organic composition and size distribution of sludge released from Atlantic salmon (*Salmo salar*) farms on spatial and temporal scales and assessed their potential as a food supply for the suspension feeder, blue mussel (*Mytilus edulis)*, in IMTA. These authors found that most particles from the aquaculture net-pen system are suitable for this species. Wang et al. (2013) suggested that both Atlantic salmon feed and feces are adequate food for blue mussels and sea cucumbers co-cultured with salmon. For example, wild fish can reduce approximately 14% of the net particulate waste and convert the waste into more easily dispersible and less harmful waste (Ballester-Moltó et al., 2017). Meriac et al. (2014) revealed that denitrification on internal carbon sources using a high fiber diet (half of the cellulose and hemicellulose existing in fecal waste was used in the denitrification reactors) could remove half of the nitrogen waste produced by the rainbow trout (*Oncorhynchus mykiss*). Although nitrogen is not directly available for plants and must be decomposed by microorganisms in a stable organic product by composting to be incorporated into the soil, this represents a low-cost disposal option (del Campo et al., 2010). Geotextile method improves the drying of sludge for utilization in agriculture (Guerdat et al., 2013; Boxman et al., 2015). Composting and vermicomposting are very promising technologies for smaller farms (Chanu et al., 2017). Sludge as a source of biomass for fuel, heat, nutrient and protein production is well reported (Yusoff et al., 2003; Diener et al., 2009; Bachmann et al., 2015; Ferreira et al., 2016). An optional sludge re-utilization approach is using heterotrophic bacteria to convert solids-bound nutrients (especially nitrogen) into bacterial biomass that can potentially be used as fish feed (Lu et al., 2012). This method is called biofloc technology (BFT) and it is mostly used for shrimp culture.

The resulting aquacultural sludge is extremely susceptible to putrefaction and may contain various pathogens. This makes its direct utilisation as a fertiliser applied on the agricultural land problematic. Its dewatering and stabilisation before such application is recommended (Bergheim et al., 1998, van Rijn, 2013). Vermicomposting systems have been developed to treat high-moisture-content organic wastes from agricultural, industrial, and municipal sources, with feed stocks including manure slurries, paper mill sludge, biosolids, and food wastes (Ndegwa and Thompson, 2000; Ndegwa et al., 2000). All of these materials have used earthworms to efficiently convert wet and highly putrescible materials into earthworm protein and high-value soil amendments and biological fertilizers (Chambers, 2002)

Vermicompost is a form of organic manure, which can be produced from a variety of organic wastes (cow dung, poultry waste, piggery waste, agricultural waste, etc.) by earthworms, and is made up of worm castings (fecal excretion) and other organic material (Reinecke and Alberts, 1987). Nevertheless, vermicomposting is a technique, where vermicompost can be prepared from a variety of available plant and animal wastes (Kaur and Ansal, 2010). The application of aquaculture sludge for vermicomposting has been successfully proved in experiments (Kouba et al., 2018 – Chapter 3; Marsh et al., 2005). Among earthworm species, the ability of *Eisenia fetida* to convert waste to vermicompost has been proven in many studies (Marsh et al., 2005; Yadav et al., 2017). Other species of red worms or red wigglers such as *Lumbricus rubellus* (Bakar et al., 2014), *Perionyx sansibaricus* (Zhi-Wei et al., 2019), *Perionyx excavates* (Ananthavalli et al., 2019), *Eisenia andreii* (Kouba et al., 2018 – Chapter 3; Zainal, 2014) and some other species are successfully used in vermicompost production.

The growth performance (weight gain and survival) of earthworms are the parameters used to indicate the success of the vermicomposting process (Suthar, 2006), while the C:N ratio and contents of P, K, and Ca in vermicast determine its maturity and applicability in agronomy (Degefe et al., 2016). The major effect of C:N ratio in vermicompost is on bacterial activity. High C:N ratio decreases bacterial activity due to nitrogen shortage. This is essential for bacteria and takes part in proteins, amino acids, and other structural substances of bacteria. The worms also don't tolerate the high concentration of NH_3 and escape from such substrates. The vermicompost process progresses properly by starting the process with a C:N ratio around 25–30. In brief, the condition of vermicomposting depends on temperature, moisture content, pH value and C:N ratio (Rostami, 2011). Another less researched product of vermiculture is vermiliquer (vermicompost leachate, also known colloquially as 'worm tea'); a nutrient-enriched liquid that drains through worm-beds containing vermicomposted wastes, bedding materials and worm populations. Vermiliquer has been reported to be rich in the nutrients required for plant growth and positively influences growth and mineral uptake by plants in standard agriculture (Carlos et al., 2008) and also in hydroponics (Churilova and Midmore, 2019). According to results obtained from previous studies, it can be assumed that earthworms from vermicompost can be utilized as food for fish (Chakrabarty et al., 2009). Consequently, vermicomposting biotechnology has been integrated into aquaculture to provide nutrition, directly by supplying earthworm biomass (Zhenjun et al., 1997; Vodounnou et al., 2016) and indirectly by providing vermicast to promote ponds' natural productivity (Ghosh, 2004, 2020). Dry vermicompost from fish sludge was also used by Abdelhay et al. (2019) for the cultivation and production of algae *Spirulina platensis* (alternative protein source). Studies have shown that *E. fetida* has recommendable levels of protein, essential amino acids, and lipids, which are similar to those found in fishmeal (FM) and, are in line with the nutritional requirements of many fish species (Vodounnou et al., 2016). Therefore, there is a need for more research on simple technological advancements to promote the commercial production of *E. fetida* meal to formulate a low-cost practical and environment-friendly

nutritional feeds for sustainable production (Musyoka et al., 2019). The conclusion on this type of aquaculture sludge valorisation is that sludge can be used for the vermicomposting process and produces several beneficial products such as: vermicompost in solid or liquid form and also earthworm biomass that could be used as protein substitute in feed. Further possible applications include feeding pets, fish bait and further stocking (Kouba et al., 2018 – Chapter 3).

Sludge as a substrate for invertebrates production

Another beneficial waste valorisation is using sludge as a medium for the production of the insect *Hermetia illucens* (also known as the black soldier fly). The larvae of the fly are voracious organisms that feed on the organic matter of the waste via decomposition, excrements, etc. Its life cycle is relatively short. After 14 days an adult fly emerges. In the stage of chrysalis, the larvae reach their largest size. They are rich in proteins and lipids. In addition to the substantial reduction in organic matter volume (between 50 and 95%), the products resulting from this method are economically valuable. Similarly, the use of animal protein in fish farming as well as the use of lipids in the production of biofuels are the subject of several researchers (Diener et al., 2009). The so-called prepupa, which is the last larval stage, consists of ~40% protein and ~30% fat, this makes it a valuable alternative to fishmeal as animal feed. In addition to the yield of prepupae, the black soldier fly treatment process generates a second product: the residue or digestate. Thus, larval, and bacterial activities not only reduce the dry mass but also reduce several nutrient contents including nitrogen and phosphorus. For example, in pig manure, 80.5% of the total nitrogen and 75.7% of phosphorus were removed by black fly soldier. Treatment technology of such organic waste as aquaculture sludge, using larvae of the black soldier fly, is an important method as a feasible and sustainable treatment option.

Organisms, such as the polychaete worm *Nereis virens*, can feed on solid wastes collected from marine RAS systems and convert solids into valuable biomass that may be used as a source of food for other aquaculture species (Brown et al., 2011).

Sludge treatment plant for biogas production

The waste anaerobic digestion (AD) proved to be an efficient technology for sewage sludge treatment that allows for the generation of biogas as renewable energy. During the AD process, the anaerobic microorganisms break down the organic matter contained in the sludge and convert it into biogas, a source of energy which can be used for electricity, heat and biofuel production (Bodík et al., 2011). The produced biogas is mainly a mixture of CH_4 and CO_2. Meanwhile, the sludge is stabilized, and its dry matter content is remarkably reduced. The benefits of the AD process for sewage sludge treatment are well recognized and the technology is widely established worldwide. Nowadays, a high proportion of biogas produced by the AD plants is from several municipal wastewater treatment sites, which are used to cover the energy needed for these treatment plants in many countries. The potential of AD could be in aquaculture sludge valorisation (Bachmann et al., 2015). The AD of aquaculture sludge is a new concept because in the traditional methods of aquaculture in ponds or net-pen sludge is not collected. Consequently, information about aquaculture sludge management, in general, is scarce, and even less is known about saline aquaculture sludge from RAS (Lanari and Franci, 1998). Many of the methods of AD of aquaculture sludge result in water whose effluent quality is adequate for reuse in the RAS. In this case, lower feedwater uses because of lower water-exchange rates can be achieved. This, in turn, results in energy savings (pumping and heating) and further water savings for the farmer. As the AD of aquaculture sludge is a new concept, detailed information is still lacking, and further research is required. Aside from further optimization of the current systems, the research community should be looking at

ways to further reduce the sludge mass as well as improving on the "benefits" from the sludge treatment, such as methane production or nitrogen removal (Mirzoyan et al., 2010; Mirzoyan and Gross, 2013).

Biochar

Biochar is a high-carbon, fine-grained residue that is currently produced through modern pyrolysis processes; it is the direct thermal decomposition of biomass in the absence of oxygen (preventing combustion), which produces a mixture of solids (the biochar proper), liquid (bio-oil), and gas (syngas) products. The specific yield from pyrolysis is dependent on process conditions, such as temperature, residence time, and heating rate (Tripathi et al., 2016). These parameters can be optimized to produce either energy or biochar (Gaunt and Lehmann, 2008). Temperatures of 400–500 °C produce more char, whereas temperatures above 700 °C favour the yield of liquid and gas fuel components (Winsley, 2007). Biochar presents a stable way to store carbon in the ground for centuries, potentially reducing or stalling the growth in atmospheric GGS levels. Simultaneously, it can improve water quality, increase soil fertility, raise agricultural productivity, and reduce pressure on old-growth forests (Laird, 2008). Biochar is recognized as offering several soil health benefits. The extremely porous nature of biochar is found to be effective at retaining both water and water-soluble nutrients. Sludge from intensive aquaculture could be a biomass source for biochar production and, with a connection to hydroponic farms or yields, could present a fine fertility source for plants (Ferreira et al., 2016; Awad et al., 2017; Mopoung et al., 2020). It could also be used as a carbon filter in water treatment in RAS (Ferreira et al., 2016).

Biofloc technology

BFT has evolved from the classic *activated-sludge-based sewage bioremediation* used in wastewater treatment plants (Avnimelech, 2012). The system essentially operates on the rationale of maintaining an optimum C:N ratio (10:1 to 15:1) by daily purging with carbohydrate (carbon) source. It is done to support the formation, or rather blooming, of heterotrophic microbial biomass (flocs). These microbial flocs, otherwise known as *bioflocs*, bioremediate the nitrogenous wastes generated by fish and uneaten feeds into consumable microbial protein. The developed *bioflocs* are macro-aggregates of diatoms, macroalgae, faecal pellets, exoskeleton, dead organisms, bacteria, protists, and invertebrates that have 12–49% crude protein with low crude lipid <2% (Crab et al., 2010). Overall, BFT is an intensive aquaculture system characterized by high stocking densities (up to 90 $kg.m^{-3}$), almost zero water exchange requirements (≤1% daily), continuous aeration (24 hours), water circulation (28–32 PWA HP ha^{-1}), suspension of *bioflocs* (<60 $ml.L^{-1}$) and lesser feed input (≤70% recommended ration for RAS) (Hargreaves, 2013).

Although the biofloc biomass is partly consumed and kept in check by the fish stock, due to the constant maintenance of C:N in the system, the microbial community thrives profusely and its biomass often exceeds the recommended values of 25–50 $ml.L^{-1}$ (wet weight)for fish and 10–15 $ml.L^{-1}$ for shrimp culture (Avnimelech, 1999). Fish species cultured in BFT are mostly omnivorous filtrators. However, over several years, many aquatic species culture in BFT system were reported, such as: golden crucian carp (*Carassius auratus*), Prussian carp (*Carassius auratus gibelio*), common carp (*Cyprinus carpio*), tench (*Tinca tinca*), rohu (*Labeo rohita*), hasu (*Opsariichthys kaopingensis*), *O. niloticus*, blue tilapia (*Oreochromis aureus*), Mozambique tilapia (*Oreochromis mossambicus*), flathead gray mullet (*Mugil cephalus*), Bluegill (*Lepomis macrochirus*), Largemouth bass (*Micropterus salmonides*), African catfish (*Clarias gariepinus*), Channel catfish (*Ictalurus punctatus*), Silver catfish (*Rhamdia quelen*), darkbarbel catfish (*Tachysurus vachellii*), fathead minnows (*Pimephales*

promela) (Mahanand et al., 2013; Hastuti and Subandiyono, 2014; Abu Bakar et al., 2015; Ekasari et al., 2015; Long et al., 2015; Park et al., 2017; Verster, 2017; Vinatea et al., 2018; Battisti et al., 2020; Borges et al., 2020; da Cunha et al., 2020; Fischer et al., 2020; Green et al., 2020; Hoang et al., 2020; Chen et al., 2020; Qiao et al., 2020; Romano et al., 2020; Sousa et al., 2020; Tubin et al., 2020; Vadhel et al., 2020; Yu et al., 2020a,b). And mainly crustacean's species, e.g. Pacific white shrimp (*Penaeus vannamei*), *P. monodon*, giant river prawn (*Macrobrachium rosenbergii*), green tiger shrimp (*Penaeus semisulcatus*), Chilean river shrimp (*Cryphiops caementaurius*), Indian prawn (*Penaeus indicus*), São Paulo shrimp (*Penaeus paulensis*), Kuruma shrimp (*Penaeus japonicus*), speckled shrimp (*Metapenaeus monoceros*), narrow-clawed crayfish (*Pontastacus leptodactylus*), Australian redclaw (*Cherax quadricarinatus*) and red swamp crayfish (*Procambarus clarkii*) (Emerenciano et al., 2012; Zhao et al., 2012; Li et al., 2018, 2019; Genc et al., 2019a; Kaya et al., 2019, 2020; Azhar et al., 2020; Kavitha and Krishna, 2020; Khoa et al., 2020; Miao et al., 2020, Olier et al., 2020; Panigrahi et al., 2020; Ulloa Walker et al., 2020; Hosain et al., 2021). Excessive biofloc biomass can cause loss of appetite and chronic stress to the cultured organisms (Emerenciano et al., 2017; Kuhn et al., 2010). In BFT it is necessary to add approximately 4 kg of carbon source to produce 1 kg of microbial flocs (Anand et al., 2014). Biomass needs to be separated so often that its entire volume is not more than 10 days old. Therefore, it is advisable to drain 10% of the biomass daily from the system (Hargreaves, 2013). As a result, 'vortexing' of biofloc aquaculture systems is done at regular intervals. This step essentially removes the excessive biofloc biomass from the system. Such thinning (filtering) generates a significant amount of biofloc biomass as by-product. In turn, the bioflocs maintained in the culture possess higher bioremediation potential and nutritive quality to form a clean well–running system. However, the drained biofloc, is still of limited use. In general, excess biofloc biomass can be re-used in various ways, e.g. use of biomass in sewage treatment, as a microbial protein, fertilizer or inoculum to start a new system (van Rijn, 2013).

The structure and nutritional composition of biofloc biomass are driven by many factors. For example, indoor vs. outdoor system, seawater vs. freshwater biofloc, system C:N stoichiometry, carbohydrate (carbon) source used, fish vs. shrimp culture, stocking density, feed type, daily feed dose, etc. (Martínez-Córdova et al., 2017). Therefore, it is not trivial to determine which biomass (or meal) is suitable as an alternative source of protein for the fish or shrimp feed mixture (Kuhn et al., 2010). Long-term feeding tests are needed to assess the effects of heavy metals accumulation or anti-nutritional factors from biofloc meal (BM) feeding. Only a few studies (Bauer et al., 2012; Genc et al., 2019b; Li et al., 2019) have been concerned with the possibility of using biofloc biomass as an aquaculture waste that has the potential to be re-used; generating extra income for biofloc system farmers or saving expenditure for aquafeed manufacturers.

1.4.2. Wastewater valorisation

Aquaponics

Soilless plant growing, also known as hydroponics, is a modern agricultural method (Resh, 2012). Its basis is using nutrient balance solution on the roots of plants, mostly with controlled conditions such as light, temperature, humidity, pest management, etc. On several occasions hydroponics has been criticised due to issues with the nutrient level in crops, too much chemical use during the growing stage, and a high-energy footprint due to 24/7 system operation. However, Sir David Attenborough suggests, that horticulture by greenhouses – with its huge crop production per square meter of land, less water consumption, and possibilities of placement in the town centre, could be the only way to feed the Earth's expected 10 billion people in a sustainable way.

Aquaponics is a land-based system that combines RAS and hydroponics in a symbiotic environment (Delaide et al., 2017). Basically, they are developed from CWs basal principle with a beneficial secondary production of usable plants (Rakocy, 2012). The most well-known examples are the "stationary islands" set up in shallow lakes in central America (e.g., Aztec's *Chinampas* 1150–1350 BC), and the introduction of fish into paddy rice fields in South-East Asia about 1,500 years ago (Coche, 1967; Turcios and Papenbrock, 2014). At first sight, aquaponics presented by Rakocy (1992) may seem like an ideal technique for nutrient use and water treatment at the same time. Water from the fish tank is purged of TSS by simple mechanic filtration and subsequently transported through the plant's roots. In an ideal way, plants use and treat the water of TDS (Rakocy, 2012). This one-loop system exists in a home-build prototype with a small density of fish and plants (Graber and Junge, 2009). Another advantage of this combination lies in the fact that an excess of nutrients does not need to be removed through the periodical exchange of enriched fish water with fresh water – as practiced in aquaculture systems (Bernstein, 2011). The system results in a symbiosis between fish, microorganisms, and plants, and encourages sustainable use of water and nutrients, including their recycling (Goddek et al., 2015). Commercial and research aquaponics use RAS combined with an approved hydroponic system. There are several main aquaponics techniques widely in use worldwide: media beds, floating rafts, deep-water culture, nutrient film technique and drip irrigation. The media beds utilize various substrates in an "ebb and flow" process, while in the nutrient film technique (in a thin layer of water) and raft/deep-water culture systems (floating rafts in large water tanks) the plant roots grow directly into the water (Thorarinsdottir et al., 2015).

Within two decades of testing and innovating, it has been shown that one-loop aquaponics presents more disadvantages for both fish and plant parts (Delaide et al., 2017; Goddek and Vermeulen, 2018). The first difference we encounter in fish vs. plant requirements are water parameters (Rakocy et al., 2006; Forchino et al., 2017). Temperature, pH value, electrical conductivity (EC), and alkalinity requirements are diverse in most cases and can affect the second system. Each fish species and plant type have a preferred temperature range that should be matched for optimum fish growth, bacterial activity, and plant production (Goddek and Keesman, 2018). Generally, tropical fish thrive a 22–32 °C while cold water fish prefer 10–18 °C. Plants also have different requirements. Non-bloom crops such as salad or herbs require 15–19 °C and tropical species need higher temperature and humidity. The acceptable range for fish culture is usually between pH 6.5 to 9.0. Plants prefer pH < 6.5 and nitrifying bacteria perform optimally at pH > 7.5. Usually, pH is one of the water quality parameters in which the optimum value for fish does not match the optimum pH for plant growth (Rakocy et al., 2004).

Plants need several nutrients that are required for growth and reproduction. Hydroponics uses perfectly balanced nutrient solutions for each kind and growth stage of plant. In aquaponics, plants are reliant on wastewater from fish tanks and additional nutrients (Schmautz et al., 2016). Macro and micronutrients are presented in aquaculture wastewater in different concentrations. Some elements are represented more (N, P, Ca, Mg), some less (S, Zn, Fe, Cu, Mn) and some not at all (B, Mo, K). In the aquaponics one-loop system, the biggest disadvantage is that connection and systems interact with each other (Goddek, 2017). Additionally, the nutrient needs to be in a form that can be assimilated easily by plants (Villarroel et al., 2016; Buzby et al., 2017). The need to adjust ratios or supplement additional nutrients may result in additional costs to aquaponics (Goddek et al., 2015). Nutrient imbalances in aquaponics systems can lead to poor plant performance, nutrient deficiencies, increased disease susceptibility, and subsequently, poor economic returns (Rakocy et al., 2004). The real problem is the content of potassium in wastewater. Ideally,

when potassium is supplemented in fish feed for aquaponics, it is to benefit both fish and plants. It is therefore important to find the best dietary source of minerals as the source could affect their availability to animals. Potassium in the form of hydroxide (KOH) or chloride (KCl) can be used in RAS as a chemical addition for pH adjustment. Siqwepu et al. (2020) presented that potassium diformate (KDF) as a feed additive can improve health status via an improved haematological profile of the *C. gariepinus* in a RAS. Knaus et al. (2020) observed positive effect on aggressive behavior of *C. gariepinus* in aquponic system with KOH and KCL pH value treatment. The use of KOH or KCl in RAS is financially disadvantageous because of the price. According to Timmons et al. (2002), the most common chemical for pH adjusting in RAS is sodium bicarbonate ($NaHCO_3$), however, most plants are sodium intolerant. A higher or equal <50 $mg.L^{-1}$ concentration of sodium can destroy a plant system (Rakocy et al., 1992; Resh, 2012). Sodium concentration in RAS´s wastewater could be solved by salt-tolerant plants that are also known as halophytes (Oliveira et al., 2020; Zhu, 2001). This is just a short list of the disadvantages of one-loop aquaponics. Recently, Goddek (2017) presented a new look at aquaponics in the form of a multi-loop system. In two-loop aquaponics, the fish section must be completely standalone with TDS and TSS treatment. After mechanical filtration, a minimum volume of wastewater is transported to a separated sump tank. In this tank water is transformed to optimal nutrient solution e.g., pH, electroconductivity (EC), temperature, and needed nutrients. Thus, the created solution is used in hydroponics until the nutrient content is not optimal for plants (Goddek and Körner, 2019). After this operation, water could again be treated and used for RAS or drained out. Two-loop aquaponics have several benefits. The RAS and hydroponic part can be situated in different sectors (RAS in a hall and hydroponics in greenhouse). Although wastewater from RAS does not meet the requirements for the majority of common plants, it could be used as a base for final nutrient solution (Goddek, 2017; Goddek and Keesman, 2018, 2020; Goddek and Körner, 2019; Baganz et al., 2020). At present, aquaponics, agriculture fields and grass irrigation are well known techniques for aquaculture wastewater valorisation. More studies and techniques are focused on utilization of aquaculture sludge.

It is also important to mention waste in the form of heat in wastewater. RAS could be a very expensive system in countries with a cold climate during the winter season, especially if the focus is on thermophilic fish species such as tilapia, catfish, perch, etc. This species requires a water temperature of up to 20 °C. For example, 15.4 kWh of electricity is needed to warm 1 m^3 of 12 °C tap water to 25 °C. The average price of electricity in the year 2021 in the Czech Republic was 4.61 CZK (0.21 USD). For heating 1 m^3 water 70.99 CZK (3.25 USD) is needed. This price does not present a devastating amount, but it is important to mention that the average daily water outtake (filtration, vaporization) in RAS is approximately 5–15% (Martins et al., 2010; Ebeling and Timmons, 2012) and only a small share is evaporated. The rest of the outtake warm water could be used in greenhouses to warm air for plant cultivation or other systems requiring heat.

Wastewater for microalgae cultivation

In tropical land-based aquaculture systems, micro algae, which are a rich source of lipids and extractable fatty acids and have the potential to act as a bioresource, can occupy most of the composition of suspended solids. Microalgae are photosynthetic unicellular microorganisms that capture CO_2 from the environment or flue gases and efficiently assimilate both inorganic and organic nutrients (nitrogen, phosphorus and carbon) from the wastewater along with oxygenation of the effluent (Samorì et al., 2013). The resultant algal biomass, rich in lipids, protein and carbohydrate can be converted into biodiesel, biogas, and bioethanol, respectively. Such an integrated approach can potentially provide a solution for not only efficient wastewater

and CO_2 mitigation but can also generate additional revenues by utilization of microalgal biomass for biofuel production (Guo et al., 2013; Wuang et al., 2016; Ansari et al., 2017) or nutrient-rich ingredient for feed (Wuang et al., 2016).

1.4.3. Gas valorisation

At the current annual growth rate of 7.1%, it is estimated that aquaculture is expected to contribute 5.7% of anthropogenic N_2O emissions by 2030 (Hu et al., 2012). This estimate was based on global aquaculture production data and N_2O emission factors; however, the latter highly depends on various operating conditions of the aquaculture system. N_2O valorisation and utilization is now a much-discussed topic for the future research.

Horticulture in greenhouses is limited by several needs of plants, such as nutrients, light, humidity and also CO_2 concentration. CO_2 concentration from typical ambient values about 350–1,000 $mg.L^{-1}$ (Marchi et al., 2018). Enriching the air in an unventilated greenhouse with CO_2 has dramatically increased crop yields in northern latitudes. However, the high cost of energy to generate CO_2 has discouraged its use. An aquaponic system in a tightly enclosed greenhouse is ideal, because CO_2 and humidity are constantly vented from the aquaculture water (Rakocy et al., 2006).

1.5. Objectives of the thesis

The current study was devoted to the comprehensive investigation of aquaculture waste valorisation and utilization by means of the following objectives:

- To analyse the composition of wastewater from different types of RAS.
- To investigate the utilization of sludge from aquaculture systems by means of vermicomposting.
- To prove the possibility to use biofloc biomass as an alternative feed source for aquatic organisms.

References

Abdel-Shafy, H.I., Mansour, M.S., 2018. Solid waste issue: Sources, composition, disposal, recycling, and valorization. Egyptian Journal of Petroleum 27, 1275–1290.

Abdelhay, R., Moustafa, Y., EL-Metwaly Essa, E., 2019. The use of vermicompost in cultivation and production of *Spirulina platensis*. Egyptian Journal for Aquaculture 9, 1–11.

Abu Bakar, N.S., Mohd Nasir, N., Lananan, F., Abdul Hamid, S.H., Lam, S.S., Jusoh, A., 2015. Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing Bioflocs Technology. Int. Biodeterior. Biodegrad. 102, 100–106.

Anand, P.S., Kohli, M., Kumar, S., Sundaray, J., Roy, S.D., Venkateshwarlu, G., Sinha, A., Pailan, G., 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 418, 108–115.

Ananthavalli, R., Ramadas, V., Paul, J.A.J., Selvi, B.K., Karmegam, N., 2019. Vermistabilization of seaweeds using an indigenous earthworm species, *Perionyx excavatus* (Perrier). Ecol. Eng. 130, 23–31.

Ansari, F.A., Singh, P., Guldhe, A., Bux, F., 2017. Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. Algal Res. 21, 169–177.

Avnimelech, Y., 1999. Carbon nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227–235.

Avnimelech, Y., 2012. Biofloc technology a practical guide book. World Aquaculture Society, Baton Rouge, La, pp.1888807210.

Awad, Y.M., Lee, S.-E., Ahmed, M.B.M., Vu, N.T., Farooq, M., Kim, I.S., Kim, H.S., Vithanage, M., Usman, A.R.A., Al-Wabel, M., 2017. Biochar, a potential hydroponic growth substrate, enhances the nutritional status and growth of leafy vegetables. J. Clean Prod. 156, 581–588.

Azhar, M.H., Suciyono, S., Budi, D.S., Ulkhaq, M.F., Anugrahwati, M., Ekasari, J., 2020. Biofloc-based co-culture systems of Nile tilapia (*Oreochromis niloticus*) and redclaw crayfish (*Cherax quadricarinatus*) with different carbon-nitrogen ratios. Aquac. Int. 28, 1293–1304.

Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquac. Eng. 51, 26–35.

Badiola, M., Basurko, O., Piedrahita, R., Hundley, P., Mendiola, D., 2018. Energy use in recirculating aquaculture systems (RAS): a review. Aquac. Eng. 81, 57–70.

Baganz, G., Baganz, D., Staaks, G., Monsees, H., Kloas, W., 2020. Profitability of multi-loop aquaponics: Year-long production data, economic scenarios and a comprehensive model case. Aquac. Res. 54, 23–32.

Bachmann, N., la Cour Jansen, J., Bochmann, G., Montpart, N., 2015. Sustainable biogas production in municipal wastewater treatment plants. IEA Bioenergy Massongex, Switzerland, pp. 1910154210.

Bakar, A.A., Syed Mohd Gawi, S.N.A., Mahmood, N.Z., Abdullah, N., 2014. Vermicomposting of vegetable waste amended with different sources of agro-industrial by-product using *Lumbricus rubellus*. Pol. J. Environ. Stud. 23, 1491–1498.

Ballester-Moltó, M., Sanchez-Jerez, P., Aguado-Giménez, F., 2017. Consumption of particulate wastes derived from cage fish farming by aggregated wild fish. An experimental approach. Mar. Environ. Res. 130, 166–173.

Bao, W., Zhu, S., Jin, G., Ye, Z., 2019. Generation, characterization, perniciousness, removal and reutilization of solids in aquaculture water: a review from the whole process perspective. Rev. Aquac. 11, 1342–1366.

Barak, Y., van Rijn, J., 2000. Biological phosphate removal in a prototype recirculating aquaculture treatment system. Aquac. Eng. 22, 121–136.

Battisti, E.K., Rabaioli, A., Uczay, J., Sutili, F.J., Lazzari, R., 2020. Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. Aquaculture 524, 735213.

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, Jr W., Poersch, L.H., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342, 112–116.

Bergheim, A., Cripps, S.J., Liltved, H., 1998. A system for the treatment of sludge from land-based fish-farms. Aquat. Living Resour. 11, 279–287.

Bernstein, S., 2011. Aquaponic gardening a step-by-step guide to raising vegetables and fish together. New Society Pub., Gabriola, B.C., 288 pp.

Blancheton, J.-P., Piedrahita, R., Eding, E.H., Lemarie, G., Bergheim, A., Fivelstad, S., Roque, D'Orbcastel, E., 2007. Intensification of landbased aquaculture production in single pass and reuse systems. Aquac. Eng. Environ., 21–47.

Bodík, I., Sedláček, S., Kubaská, M., Hutňan, M., 2011. Biogas production in municipal wastewater treatment plants-current status in EU with a focus on the Slovak Republic. Chem. Biochem. Eng. Q. 25, 335–340.

Borges, B.A., Rocha, J.L., Pinto, P.H.O., Zacheu, T., Chede, A.C., Magnotti, C.C.F., Cerqueira, V.R., Arana, L.A.V., 2020. Integrated culture of white shrimp *Litopenaeus vannamei* and mullet *Mugil liza* on biofloc technology: Zootechnical performance, sludge generation, and *Vibrio* spp. reduction. Aquaculture 524, 735234.

Boxman, S.E., Kruglick, A., McCarthy, B., Brennan, N.P., Nystrom, M., Ergas, S.J., Hanson, T., Main, K.L., Trotz, M.A., 2015. Performance evaluation of a commercial land-based integrated multi-trophic aquaculture system using constructed wetlands and geotextile bags for solids treatment. Aquac. Eng. 69, 23–36.

Brambilla, F., Antonini, M., Ceccuzzi, P., Terova, G., Saroglia, M., 2008. Foam fractionation efficiency in particulate matter and heterotrophic bacteria removal from a recirculating seabass (*Dicentrarchus labrax*) system. Aquac. Eng. 39, 37–42.

Brown, N., Eddy, S., Plaud, S., 2011. Utilization of waste from a marine recirculating fish culture system as a feed source for the polychaete worm, *Nereis virens*. Aquaculture 322, 177–183.

Buzby, K.M., West, T.P., Waterland, N.L., Lin, L.-S., 2017. Remediation of flow-through trout raceway effluent via aquaponics. N. Am. J. Aqualcult. Taylor & Francis, pp. 53–60.

Carlos, G.-G.R., Dendooven, L., Antonio, G.-M.F., 2008. Vermicomposting leachate (worm tea) as liquid fertilizer for maize (*Zea mays* L.) forage production. Asian Journal of Plant Sciences 7, 360–367.

Coche, A., 1967. Fish culture in rice fields a world-wide synthesis. Hydrobiologia 30, 1–44.

Colt, J., 1991. Aquacultural production systems. J. Anim. Sci. 69, 4183–4192.

Colt, J., Bouck G., 1984. Design of packed columns for degassing. Aquac. Eng. 3, 251–273.

Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W., 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac. Res. 41, 559–567.

Cripps, S.J., Bergheim, A., 2000. Solids management and removal for intensive land–based aquaculture production systems. Aquac. Eng. 22, 33–56.

da Cunha, L., Besen, K.P., Ha, N., Uczay, J., Skoronski, E., Fabregat, T.E.H.P., 2020. Biofloc technology (BFT) improves skin pigmentation of goldfish (*Carassius auratus*). Aquaculture 522, 735132.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquac. Eng. 53, 2–13.

Davidson, J., Helwig, N., Summerfelt, S.T., 2008. Fluidized sand biofilters used to remove ammonia, biochemical oxygen demand, total coliform bacteria, and suspended solids from an intensive aquaculture effluent. Aquac. Eng. 39, 6–15.

Davidson, J., Good, C., Barrows, F.T., Welsh, C., Kenney, P.B., Summerfelt, S.T., 2013. Comparing the effects of feeding a grain-or a fish meal-based diet on water quality, waste production, and rainbow trout *Oncorhynchus mykiss* performance within low exchange water recirculating aquaculture systems. Aquac. Eng. 52, 45–57.

Degefe, G., Mengistou, S., Mohammed, S., 2016. Physico chemical evaluation of coffee husk, wastes of enset (*Enset ventricosum*), vegetable and khat (*Catha edulis*) through vermicomposting employing an epigeic earthworm *Dendrobaena veneta* (Rosa, 1886). Afr. J. Biotechnol. 15, 884–890.

del Campo, L.M., Ibarra, P., Gutiérrez, X., Takle, H.R., 2010. Utilization of sludge from recirculation aquaculture systems. Nofima Rapport Serie.

Delaide, B., Delhaye, G., Dermience, M., Gott, J., Soyeurt, H., Jijakli, M.H., 2017. Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system. Aquac. Eng. 78, 130–139.

Diener, S., Zurbrügg, C., Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manage. Res. 27, 603–610.

Dolan, E., Murphy, N., O'Hehir, M., 2013. Factors influencing optimal micro–screen drum filter selection for recirculating aquaculture systems. Aquac. Eng. 56, 42–50.

Dybas, C.L., 2005. Dead zones spreading in world oceans. BioScience 55, 552–557.

Ebeling, J.M., Timmons, M.B., 2012. Recirculating Aquaculture Systems, Aquaculture Production Systems. Wiley-Blackwell, pp. 245–277.

Ebeling, J.M., Ogden, S.R., Sibrell, P.L., Rishel, K.L., 2004. Application of chemical coagulation aids for the removal of suspended solids (TSS) and phosphorus from the microscreen effluent discharge of an intensive recirculating aquaculture system. N. Am. J. Aqualcult. 66, 198–207.

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

Ekasari, J., Rivandi, D.R., Firdausi, A.P., Surawidjaja, E.H., Zairin, Jr M., Bossier, P., De Schryver, P., 2015. Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. Aquaculture 441, 72–77.

Emerenciano, M., Ballester, E.L., Cavalli, R.O., Wasielesky, W., 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). Aquac. Res. 43, 447–457.

Emerenciano, M.G.C., Martínez-Córdova, L.R., Martínez-Porchas, M., Miranda-Baeza, A., 2017. Biofloc technology (BFT): a tool for water quality management in aquaculture. Water Qual. 5, 92–109.

FAO, 2019. The State of World Fisheries and Aquaculture: Opportunities and Challenges.

FAO, 2020. The State of World Fisheries and Aquaculture: Opportunities and Challenges.

Ferreira, C.I., Calisto, V., Otero, M., Nadais, H., Esteves, V.I., 2016. Comparative adsorption evaluation of biochars from paper mill sludge with commercial activated carbon for the removal of fish anaesthetics from water in Recirculating Aquaculture Systems. Aquac. Eng. 74, 76–83.

Fischer, H., Romano, N., Renukdas, N., Egnew, N., Sinha, A.K., Ray, A.J., 2020. The potential of rearing juveniles of bluegill, *Lepomis macrochirus*, in a biofloc system. Aquacult. Rep. 17, 100398.

Forchino, A., Lourguioui, H., Brigolin, D., Pastres, R., 2017. Aquaponics and sustainability: The comparison of two different aquaponic techniques using the Life Cycle Assessment (LCA). Aquac. Eng. 77, 80–88.

Gaunt, J.L., Lehmann, J., 2008. Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. Environ. Sci. Technol. 42, 4152–4158.

Genc, E., Kaya, D., Dinçer, S., Genc, M.A., Aktaş, M., 2019a. Biofloc application in narrow-clawed crayfish (*Astacus leptodactylus*) culture. In: 3rd International Congress on Advances in Bioscience and Biotechnology (ICABB), July 10–14, 2019, Kiev, Ukraine, pp. 71–78.

Genc, E., Kaya, D., Genc, M.A., 2019b. Biofloc as an economical and applicable production technology for the sustainability of aquaculture production. In: 3rd International Congress on Advances in Bioscience and Biotechnology (ICABB), July 10–14, 2019, Kiev, Ukraine, pp. 49–78.

Ghosh, C., 2004. Integrated vermi-pisciculture an alternative option for recycling of solid municipal waste in rural India. Bioresour. Technol. 93, 71–75.

Ghosh, S.K., 2020. Waste Management as Economic Industry Towards Circular Economy. Springer Nature, pp. 202.

Goddek, S., 2017. Opportunities and challenges of multi-loop aquaponic systems. Wageningen University.

Goddek, S., Keesman, K.J., 2018. The necessity of desalination technology for designing and sizing multi-loop aquaponics systems. Desalination 428, 76–85.

Goddek, S., Vermeulen, T., 2018. Comparison of *Lactuca sativa* growth performance in conventional and RAS-based hydroponic systems. Aquac. Int. 26, 1377–1386.

Goddek, S., Körner, O., 2019. A fully integrated simulation model of multi-loop aquaponics: a case study for system sizing in different environments. Agricultural Systems 171, 143–154.

Goddek, S., Keesman, K.J., 2020. Improving nutrient and water use efficiencies in multi-loop aquaponics systems. Aquac. Int. 28, 2481–2490.

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

Graber, A., Junge, R., 2009. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. Desalination 246, 147–156.

Green, B.W., Schrader, K.K., Rawles, S.D., Webster, C.D., McEntire, M.E., 2020. Comparison of unused water and year-old used water for production of channel catfish in the biofloc technology system. Aquaculture 519, 734739.

Guerdat, T.C., Losordo T.M., DeLong D.P., Jones R.D., 2013. An evaluation of solid waste capture from recirculating aquaculture systems using a geotextile bag system with a flocculant-aid. Aquac. Eng. 54, 1–8.

Guo, Z., Liu, Y., Guo, H., Yan, S., Mu, J., 2013. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. J. Environ. Sci. 25, S85–S88.

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

Hargreaves, J.A., 2013. Biofloc production systems for aquaculture. Southern Regional Aquaculture Center 4503, 11.

Hastuti, S., Subandiyono, S., 2014. Production Performance of african catfish (*Clarias gariepinus*, burch) were Rearing with Biofloc technology. Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology 10, 37–42.

Hoang, M.N., Nguyen, P.N., Bossier, P., 2020. Water quality, animal performance, nutrient budgets and microbial community in the biofloc-based polyculture system of white shrimp, *Litopenaeus vannamei* and gray mullet, *Mugil cephalus*. Aquaculture 515, 734610.

Hoevenaars, K., Junge, R., Bardocz, T., Leskovec, M., 2018. EU policies: New opportunities for aquaponics. Ecocycles 4, 10–15.

Honda, K., Nakamura, K., Nishiguchi, M., Yamane, K., 1993. Cloning and characterization of *Bacillus* subtilis gene encoding a homolog of the 54-kilodalton subunit of mammalian signal recognition particle and *Escherichia coli* Ffh. J. Bacteriol. 175, 4885–4894.

Hosain, M.E., Amin, S.N., Arshad, A., Kamarudin, M.S., Karim, M., 2021. Effects of carbon sources on the culture of giant river prawn in biofloc system during nursery phase. Aquacult. Rep. 19, 100607.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Khanal, S.K., 2012. Nitrous oxide (N_2O) emission from aquaculture: A Review. Environ. Sci. Technol. 46, 6470–6480.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Sharma, K., Brotto, A.C., Khanal, S.K., 2013. Nitrogen transformations in intensive aquaculture system and its implication to climate change through nitrous oxide emission. Bioresour. Technol. 130, 314–320.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C., Khanal, S.K., 2015. Effect of plant species on nitrogen recovery in aquaponics. Bioresour. Technol. 188, 92–98.

Hu, Z., Wu, S., Ji, C., Zou, J., Zhou, Q., Liu, S., 2016. A comparison of methane emissions following rice paddies conversion to crab-fish farming wetlands in southeast China. Environ. Sci. Pollut. Res. 23, 1505–1515.

Chakrabarty, D., Das S.K., Das K., Biswas P., Karmegam, N., 2009. Application of vermitechnology in aquaculture. Dyn. Soil Dyn. Plant. 3, 41–44.

Chambers, J., 2002. Continuous flow for vermicomposting. BioCycle 43, 34–34.

Chanu, T.I., Sharma, A., Ande, M.P., Prasad, J., Patnaik, R., 2017. Vermicompost production technology for organic aquaculture. Aquaculture Times, 24–28.

Chen, S., Coffin, D.E., Malone, R.F., 1997. Sludge production and management for recirculating aquacultural systems. J. World Aquacult. Soc. 28, 303–315.

Chen, X., Luo, G., Tan, J., Tan, H., Yao, M., 2020. Effects of carbohydrate supply strategies and biofloc concentrations on the growth performance of African catfish (*Clarias gariepinus*) cultured in biofloc systems. Aquaculture 517, 734–808.

Chopin, T., Robinson, S.M., 2004. Defining the appropriate regulatory and policy framework for the development of integrated multi-trophic aquaculture practices: introduction to the workshop and positioning of the issues. Bull. Aquaculture Assoc. Can. 104, 4.

Churilova, E.V., Midmore, D.J., 2019. Vermiliquer (vermicompost leachate) as a complete liquid fertilizer for hydroponically-grown pak choi (*Brassica chinensis* L.) in the tropics. Horticulturae 5, 26.

Jung, I., Lovitt, R., 2011. Leaching techniques to remove metals and potentially hazardous nutrients from trout farm sludge. Water Res. 45, 5977–5986.

Kavitha, K., Krishna, P.V., 2020. Sustainable Growth and Survival of *Litopenaeus Vannamei* Through Wastewater Recycling, Recent Trends in Waste Water Treatment and Water Resource Management. Springer, pp. 77–84.

Kaur, V.I., Ansal, M.D., 2010. Efficacy of vermicompost as fish pond manure – Effect on water quality and growth of *Cyprinus carpio* (Linn.). Bioresour. Technol. 101, 6215–6218.

Kaya, D., Genc, M.A., Aktas, M., Yavuzcan, H., Ozmen, O., Genc, E., 2019. Effect of biofloc technology on growth of speckled shrimp, *Metapenaeus monoceros* (Fabricus) in different feeding regimes. Aquac. Res. 50, 2760–2768.

Kaya, D., Genc, E., Genc, M.A., Aktas, M., Eroldogan, O.T., Guroy, D., 2020. Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: Effects of different feeding rates and stocking densities. Aquaculture, 735526.

Kimbrough, D.E., Wakakuwa, J.R., 1989. Acid digestion for sediments, sludges, soils, and solid wastes. A proposed alternative to EPA SW 846 Method 3050. Environ. Sci. Technol. 23, 898–900.

Khoa, T.N.D., Tao, C.T., Van Khanh, L., Hai, T.N., 2020. Super-intensive culture of white leg shrimp (*Litopenaeus vannamei*) in outdoor biofloc systems with different sunlight exposure levels: Emphasis on commercial applications. Aquaculture, 735277.

Knaus, U., Wenzel, L.C., Appelbaum, S., Palm, H.W., 2020. Aquaponics (sl) production of spearmint (*Mentha spicata*) with African catfish (*Clarias gariepinus*) in Northern Germany. Sustainability 12, 8717.

Körner, O., Gutzmann, E., Kledal, P., 2015. A dynamic model simulating the symbiotic effects in aquaponic systems, International Symposium on New Technologies and Management for Greenhouses – GreenSys 2015 1170, pp. 309–316.

Kouba, A., Lunda, R., Hlaváč, D., Kuklina, I., Hamáčková, J., Randák, T., Kozák, P., Koubová, A., Buřič, M., 2018. Vermicomposting of sludge from recirculating aquaculture system using *Eisenia andrei*: Technological feasibility and quality assessment of end-products. Journal of Cleaner Production 177, 665–673.

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, Jr G.J., 2010. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303, 28–33.

Laird, D.A., 2008. The charcoal vision: a win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. Agron. J. 100, 178–181.

Lanari, D., Franci, C., 1998. Biogas production from solid wastes removed from fish farm effluents. Aquat. Living Resour. 11, 289–295.

Lander, T., Robinson, S., MacDonald, B., Martin, J., 2013. Characterization of the suspended organic particles released from salmon farms and their potential as a food supply for the suspension feeder, *Mytilus edulis* in integrated multi-trophic aquaculture (IMTA) systems. Aquaculture 406, 160–171.

Lee, J., 2015. Practical applications of low-pressure hydrocyclone (LPH) for feed waste and fecal solid removal in a recirculating aquaculture system. Aquac. Eng. 69, 37–42.

Li, J., Cheng, Y., Wang, H., Wang, J., Chen, H., Li, J., 2018. Effects of different conditions on feeding biofloc of red swamp crayfish (*Procambarus clarkii*) juveniles. South China Fisheries Science 14, 58–64.

Li, J., Li, J., Li, W., Sun, Y., Liu, X., Liu, M., Cheng, Y., 2019. Juvenile *Procambarus clarkii* farmed using biofloc technology or commercial feed in zero-water exchange indoor tanks: A comparison of growth performance, enzyme activity and proximate composition. Aquac. Res. 50, 1834–1843.

Long, L., Yang, J., Li, Y., Guan, C., Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture 448, 135–141.

Losordo, T.M., Masser, M.P., Rakocy, J.E., 1999. Recirculating aquaculture tank production systems: A review of component options. Southern Regional Aquaculture Center 453.

Lu, L., Tan, H., Luo, G., Liang, W., 2012. The effects of *Bacillus subtilis* on nitrogen recycling from aquaculture solid waste using heterotrophic nitrogen assimilation in sequencing batch reactors. Bioresour. Technol. 124, 180–185.

Lunda, R., Roy, K., Másílko, J., Mráz, J., 2019. Understanding nutrient throughput of operational RAS farm effluents to support semi-commercial aquaponics: Easy upgrade possible beyond controversies. J. Environ. Manage. 245, 255–263.

Mahanand, S.S., Moulick, S., Rao, P.S., 2013. Water quality and growth of Rohu, *Labeo rohita*, in a biofloc system. J. Appl. Aquaculture 25, 121–131.

Marchi, B., Zanoni, S., Pasetti, M., 2018. Industrial symbiosis for greener horticulture practices: The CO_2 enrichment from energy intensive industrial processes. Procedia CIRP 69, 562–567.

Marsh, L., Subler, S., Mishra, S., Marini, M., 2005. Suitability of aquaculture effluent solids mixed with cardboard as a feedstock for vermicomposting. Bioresour. Technol. 96, 413–418.

Martínez-Córdova, L.R., Martínez-Porchas, M., Emerenciano, M.G.C., Miranda-Baeza, A., Gollas-Galván T., 2017. From microbes to fish the next revolution in food production. Crit. Rev. Biotechnol. 37, 287–295.

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. Aquac. Eng. 43, 83–93.

Meriac, A., Eding, E.H., Kamstra, A., Busscher, J.P., Schrama, J.W., Verreth, J.A., 2014. Denitrification on internal carbon sources in RAS is limited by fibers in fecal waste of rainbow trout. Aquaculture 434, 264–271.

Miao, S., Hu, J., Wan, W., Han, B., Zhou, Y., Xin, Z., Sun, L., 2020. Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. Aquaculture, 735280.

Mirzoyan, N., Gross, A., 2013. Use of UASB reactors for brackish aquaculture sludge digestion under different conditions. Water Res. 47, 2843–2850.

Mirzoyan, N., Parnes, S., Singer, A., Tal, Y., Sowers, K., Gross, A., 2008. Quality of brackish aquaculture sludge and its suitability for anaerobic digestion and methane production in an upflow anaerobic sludge blanket (UASB) reactor. Aquaculture 279, 35–41.

Mirzoyan, N., Tal, Y., Gross, A., 2010. Anaerobic digestion of sludge from intensive recirculating aquaculture systems. Aquaculture 306, 1–6.

Mo, W.Y., Man, Y.B., Wong, M.H., 2018. Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. Sci. Total Environ. 613, 635–643.

Mopoung, S., Udeye, V., Viruhpintu, S., Yimtragool, N., Unhong, V., 2020. Water treatment for fish aquaculture system by biochar-supplemented planting panel system. The Scientific World Journal 2020.

Musyoka, S.N., Liti, D.M., Ogello, E., Waidbacher, H., 2019. Utilization of the earthworm, *Eisenia fetida* (Savigny, 1826) as an alternative protein source in fish feeds processing: A review. Aquac. Res. 50, 2301–2315.

Ndegwa, P.M., Thompson, S., 2000. Effects of C-to-N ratio on vermicomposting of biosolids. Bioresour. Technol. 75, 7–12.

Ndegwa, P., Thompson, S., Das, K., 2000. Effects of stocking density and feeding rate on vermicomposting of biosolids. Bioresour. Technol. 71, 5–12.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.

Noble, C., Kankainen, M., Setälä, J., Berrill, I.K., Ruohonen, K., Damsgård, B., Toften, H., 2012. The bio-economic costs and benefits of improving productivity and fish welfare in aquaculture: Utilizing CO_2 stripping technology in Norwegian Atlantic salmon smolt production. Aquac. Econ. Manag. 16, 414–428.

Nungesser, P.W., 1995. Treatment of wastewater through enhanced biological phosphorus removal. Google Patents.

Olier, B.S., Tubin, J.S.B., de Mello, G.L., Martínez-Porchas, M., Emerenciano, M.G.C., 2020. Does vertical substrate could influence the dietary protein level and zootechnical performance of the Pacific white shrimp *Litopenaeus vannamei* reared in a biofloc system? Aquac. Int., 1–15.

Oliveira, V., Martins, P., Marques, B., Cleary, D.F., Lillebø, A.I., Calado, R., 2020. Aquaponics using a fish farm effluent shifts bacterial communities profile in halophytes rhizosphere and endosphere. Sci. Rep. 10, 1–11.

Omotade, I.F., Alatise, M.O., Olanrewaju, O.O., 2019. Recycling of aquaculture wastewater using charcoal based constructed wetlands. Int. J. Phytoremediat. 21, 399–404.

Panigrahi, A., Das, R., Sivakumar, M., Saravanan, A., Saranya, C., Sudheer, N., Vasagam, K.K., Mahalakshmi, P., Kannappan, S., Gopikrishna, G., 2020. Bio-augmentation of heterotrophic bacteria in biofloc system improves growth, survival, and immunity of Indian white shrimp *Penaeus indicus*. Fish Shellfish Immunol. 48, 477–487.

Park, J., Roy, L.A., Renukdas, N., Luna, T., 2017. Evaluation of a biofloc system for intensive culture of fathead minnows, *Pimephales promelas*. J. World Aquacult. Soc. 48, 592–601.

Qiao, G., Chen, P., Sun, Q., Zhang, M., Zhang, J., Li, Z., Li, Q., 2020. Poly-β-hydroxybutyrate (PHB) in bioflocs alters intestinal microbial community structure, immune-related gene expression and early Cyprinid herpesvirus 2 replication in gibel carp (*Carassius auratus gibelio*). Fish Shellfish Immunol. 97, 72–82.

Rakocy, J.E., 2012. Aquaponics – Integrating Fish and Plant Culture. In: Tidwell, J.H. (Ed.), Aquaculture Production Systems. Wiley-Blackwell, pp. 344–386. DOI: 10.1002/9781118250105

Rakocy, J.E., Losordo, T.M., Masser, M.P., 1992. Recirculating Aquaculture Tank Production Systems; Integrating Fish and Plant Culture. Southern Regional Aquaculture Center 454, 7.

Rakocy, J.E., Shultz, R.C., Bailey, D.S., Thoman, E.S., 2004. Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. in: Nichols, M.A. (Ed.), Proceedings of the South Pacific Soilless Culture Conference. International Society Horticultural Science, Leuven 1, pp. 63–69.

Rakocy, J.E., Masser, M.P., Losordo, T.M., 2006. Recirculating Aquaculture Tank Production Systems: Aquaponics – Integrating Fish and Plant Culture. Southern Regional Aquaculture 454, 1–16.

Reinecke, A., Alberts, J., 1987. The chemical and amino acid composition of the compost worm (*Eisenia foetida*) as potential source of protein for animal feed. Tydskrif Vir. Natuurwetenskap en Tengnologie 6, 1–14.

Resh, H.M., 2012. Hydroponic Food Production: A Definitive Guidebook for the Advanced Home Gardener and the Commercial Hydroponic Grower, Seventh Edition. Taylor & Francis, ISBN 9781439878675.

Rodehutscord, M., Pfeffer, E., 1995. Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). Water Sci. Technol. 31, 143–147.

Romano, N., Surratt, A., Renukdas, N., Monico, J., Egnew, N., Sinha, A.K., 2020. Assessing the feasibility of biofloc technology to largemouth bass *Micropterus salmoides* juveniles: Insights into their welfare and physiology. Aquaculture 520, 735008.

Rostami, R., 2011. Vermicomposting, Integrated Waste Management–Volume II. IntechOpen.

Samorì, G., Samorì, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: part I. Water Res. 47, 791–801.

Schendel, E.K., Nordström, S.E., Lavkulich, L.M., 2004. Floc and sediment properties and their environmental distribution from a marine fish farm. Aquac. Res. 35, 483–493.

Schmautz, Z., Loeu, F., Liebisch, F., Graber, A., Mathis, A., Bulc, T.G., Junge, R., 2016. Tomato Productivity and quality in aquaponics: Comparison of three hydroponic methods. Water 8.

Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac.12, 2109–2139.

Siqwepu, O., Salie, K., Goosen, N., 2020. Evaluation of potassium diformate and potassium chloride in the diet of the African catfish, *Clarias gariepinus* in a recirculating aquaculture system. Aquaculture, 735414.

Skov, P.V., 2019. CO_2 in aquaculture. In: Grosell, M., Munday, P.L., Farrell, A.P., Brauner, C.J. (Eds), Fish Physiology. Elsevier, pp. 287–321.

Skriptsova, A.V., Miroshnikova, N.V., 2011. Laboratory experiment to determine the potential of two macroalgae from the Russian Far-East as biofilters for integrated multi-trophic aquaculture (IMTA). Bioresour. Technol. 102, 3149–3154.

Sousa, A.A., Lopes, D.L., Emerenciano, M.G., Nora, L., Souza, C.F., Baldissera, M.D., Baldisserotto, B., Alba, D.F., Da Silva, A.S., 2020. Phosphatidylcholine in diets of juvenile Nile tilapia in a biofloc technology system: Effects on performance, energy metabolism and the antioxidant system. Aquaculture 515, 734574.

Summerfelt, S.T., Vinci, B., Piedrahita, R., 2000. Oxygenation and carbon dioxide control in water reuse systems. Aquac. Eng. 22, 87–108.

Suthar, S., 2006. Potential utilization of guar gum industrial waste in vermicompost production. Bioresour. Technol. 97, 2474–2477.

Thomas, P.M., Pankhurst, N.W., Bremner, H.A., 1999. The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. J. Fish Biol. 54, 1177–1196.

Thorarinsdottir, R.I., Kledal, P.R., Skar, S.L.G., Sustaeta, F., Ragnarsdóttir, K.V., Mankasingh, U., Pantanella, E., Ven, R.v.d., Shultz, C., 2015. Aquaponics Guidelines. DOI: 10.13140/RG.2.1.4975.6880

Thorpe, J.E., Cho, C.Y., 1995. Minimising waste through bioenergetically and behaviourally based feeding strategies. Water Sci. Technol. 31, 29–40.

Timmons, M.B., Ebeling, J.M., Wheaton, F.V., Summerfelt, S.T., Vinci, B.J., 2002. Recirculating Aquaculture Systems. Cayuga Aqua Ventures 2nd edition, 769.

Topic Popovic, N., Klobucar, R.S., Strunjak-Perovic, I., Jadan, M., Barisic, J., Coz-Rakovac, R., 2015. Piscine cytochromes P450 (CYP) and their response to antimicrobial drugs. Aquac. Res. 46, 257–271.

Tripathi, M., Sahu, J.N., Ganesan, P., 2016. Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review. Renewable and Sustainable Energy Reviews 55, 467–481.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J.-G., 2009. Ecological engineering in aquaculture – potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297, 1–9.

Tubin, J.S.B., Paiano, D., de Oliveira Hashimoto, G.S., Furtado, W.E., Martins, M.L., Durigon, E., Emerenciano, M.G.C., 2020. *Tenebrio molitor* meal in diets for Nile tilapia juveniles reared in biofloc system. Aquaculture 519, 734–763.

Tunçal, T., Pala, A., Uslu, O., 2009. Importance of particulate biodegradable organic compounds in performance of full-scale biological phosphorus removal system. Water Environ. Res. 81, 886–895.

Turcios, A.E., Papenbrock, J., 2014. Sustainable treatment of aquaculture effluents – what can we learn from the past for the future? Sustainability 6, 836–856.

Ulloa Walker, D.A., Morales Suazo, M.C., Emerenciano, M.G.C., 2020. Biofloc technology: principles focused on potential species and the case study of Chilean river shrimp *Cryphiops caementarius*. Rev. Aquac. 12, 1759–1782.

Vadhel, N., Pathan, J., Shrivastava, V., Akolkar, N., Kumar, S., Misra, C., Mahapatra, K.D., 2020. Comparative study on the performance of genetically improved rohu "Jayanti" and native rohu, *Labeo rohita* fingerlings reared in biofloc system. Aquaculture 523, 735201.

van Rijn, J., 1996. The potential for integrated biological treatment systems in recirculating fish culture – A review. Aquaculture 139, 181–201.

van Rijn, J., 2013. Waste treatment in recirculating aquaculture systems. Aquac. Eng. 53, 49–56.

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: Theory and applications. Aquac. Eng. 34, 364–376.

Verster, N., 2017. Comparison of growth rates of tilapia species (*Oreochromis mossambicus* and *Oreochromis niloticus*) raised in a biofloc and a standard recirculating. M.Sc. Thesis, Ghent University, Belgium, 67 pp.

Viadero, R.C.J., Noblet, J.A., 2002. Membrane filtration for removal of fine solids from aquaculture process water. Aquac. Eng. 26, 151–169.

Villarroel, M., Junge, R., Komives, T., Konig, B., Plaza, I., Bittsanszky, A., Joly, A., 2016. Survey of Aquaponics in Europe. Water 8.

Vinatea, L., Malpartida, J., Carbó, R., Andree, K.B., Gisbert, E., Estévez, A., 2018. A comparison of recirculation aquaculture systems versus biofloc technology culture system for on-growing of fry of *Tinca tinca* (Cyprinidae) and fry of grey *Mugil cephalus* (Mugilidae). Aquaculture 482, 155–161.

Vodounnou, D., Juste, V., Kpogue, D., Apollinaire, M., Didier, F., 2016. Culture of earthworm (*Eisenia fetida*), production, nutritive value and utilization of its meal in diet for *Parachanna obscura* fingerlings reared in captivity. International Journal of Fisheries and Aquatic Studies 4, 1–5.

von Ahnen, M., Pedersen, P.B., Dalsgaard, J., 2020. Improving denitrification in an aquaculture wetland using fish waste – a case study. Ecol. Eng. 143, 105686.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 380, 48–65.

Vymazal, J., 2014. Constructed wetlands for treatment of industrial wastewaters: A review. Ecol. Eng. 73, 724–751.

Wang, X., Andresen, K., Handå, A., Jensen, B., Reitan, K.I., Olsen, Y., 2013. Chemical composition and release rate of waste discharge from an Atlantic salmon farm with an evaluation of IMTA feasibility. Aquac. Environ. Interact. 4, 147–162.

Winsley, P., 2007. Biochar and bioenergy production for climate change mitigation. New Zealand Science Review 64, 5–10.

Wuang, S.C., Khin, M.C., Chua, P.Q.D., Luo, Y.D., 2016. Use of *Spirulina biomass* produced from treatment of aquaculture wastewater as agricultural fertilizers. Algal Res. 15, 59–64.

Yadav, J., Gupta, R., Kumar, D., 2017. Changes in C:N ratio of different substrates during vermicomposting. Ecology, Environment and Conservation 23, 368–372.

Yu, Z., Li, L., Zhu, R., Li, M., Duan, J., Wang, J.Y., Liu, Y.H., Wu, L.F., 2020a. Monitoring of growth, digestive enzyme activity, immune response and water quality parameters of Golden crucian carp (*Carassius auratus*) in zero-water exchange tanks of biofloc systems. Aquacult. Rep. 16, 100–283.

Yu, Z., Li, L., Zhu, R., Li, M., Wu, L.F., 2020b. Effects of bioflocs with different C/N ratios on growth, immunological parameters, antioxidants and culture water quality in *Opsariichthys kaopingensis* Dybowski. Aquac. Res. 51, 805–815.

Yusoff, F., Law, A., Soon, J., 2003. Effects of aeration and chemical treatments on nutrient release from the bottom sediment of tropical marine shrimp ponds. Asian Fisheries Science 16, 41–50.

Zainal, B.S., 2014. Vermicomposting of Palm Oil Mill Effluent (POME) Sludge and Effects of Vermicompost on Oil Palm Seedling Growth. M.Sc. Thesis, Universiti Sains Malaysia, 39 pp.

Zhao, P., Huang, J., Wang, X.-H., Song, X.-L., Yang, C.-H., Zhang, X.-G., Wang, G.-C., 2012. The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*. Aquaculture 354, 97–106.

Zhenjun, S., Xianchun, L., Lihui, S., Chunyang, S., 1997. Earthworm as a potential protein resource. Ecol. Food Nutr. 36, 221–236.

Zhi-Wei, S., Tao, S., Wen-Jing, D., Jing, W., 2019. Investigation of rice straw and kitchen waste degradation through vermicomposting. J. Environ. Manage. 243, 269–272.

Zhu, J.-K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.

Zou, L., Huang, S., 2015. Chinese aquaculture in light of green growth. Aquacult. Rep. 2, 46–49.

CHAPTER 2

UNDERSTANDING NUTRIENT THROUGHPUT OF OPERATIONAL RAS FARM EFFLUENTS TO SUPPORT SEMI-COMMERCIAL AQUAPONICS: EASY UPGRADE POSSIBLE BEYOND CONTROVERSIES

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Research article

Understanding nutrient throughput of operational RAS farm effluents to support semi-commercial aquaponics: Easy upgrade possible beyond controversies

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ABSTRACT

The present research attempted to address a key industry-level question amidst Recirculating Aquaculture System (RAS) waste throughput and aquaponics limitations controversies. Nutrient throughput of three operational RAS farms with progressive size proportions (16, 130, 1400 m^3), aquaculture intensity (24, 62, 86 kg stock m^{-3}) were studied. Results suggest - daily total efflux and potency of nutrients in effluents should not be generalized, extreme variability exists. Consistencies of nutrients in wastewater (except N, Ca and Na) are higher than in sludge. Asynchrony between patterns of nutrient loading and effluent nutrient concentrations exist for secondary macronutrients and micronutrients (S, Mg, Fe, Cu, Zn, B, Mo). Macronutrient output generally increases with increasing farm size and culture intensity but same cannot be said for micronutrients. Deficiency in wastewater can be completely masked using raw or mineralized sludge, usually containing 3–17 times higher nutrient concentrations. RAS effluents (wastewater and sludge combined) contain adequate N, P, Mg, Ca, S, Fe, Zn, Cu, Ni to meet most aquaponic crop needs. K is generally deficient requiring a full-fledged fertilization. Micronutrients B, Mo are partly sufficient and can be easily ameliorated by increasing sludge release. The presumption surrounding 'definite' phyto-toxic Na levels in RAS effluents should be reconsidered – practical solutions available too. No threat of heavy metal accumulation or discharge was observed. Most of the 'well-known' operational influences failed to show any significant predictable power in deciding nutrient throughput from RAS systems. Calibration of nutrient output from operational RAS farms may be primarily focused around six predictors we identified. Despite inherent complexity of effluents, the conversion of RAS farms to semi-commercial aquaponics should not be deterred by nutrient insufficiency or nutrient safety arguments. Incentivizing RAS farm wastes through semi-commercial aquaponics should be encouraged - sufficient and safe nutrients are available.

1. Introduction

The lack of space for expansion and new sites (resource competition from other users), limited fresh water availability, and concerns over pollution are considered as key obstacles for further expansion of commercial intensive aquaculture systems (e.g. cage-based and flow-through aquaculture systems). Therefore, most European countries have promoted Recirculating Aquaculture Systems (RAS) as one of the possible solutions and opportunities to further develop aquaculture (Badiola et al., 2012). In European countries, the development of RAS has been positive (Badiola et al., 2012; Eurostat, 2018; Martins et al., 2010). Aquaculture production data from freshwater RAS at the whole-EU scale is only accessible till 2010 - estimated at 20,658 tons. Denmark followed by Netherlands are the most prolific RAS producers within EU, together comprising around 90% of the total aquaculture produce from RAS. The example of the Czech Republic, a landlocked central European country, is one of its kinds. It clearly demonstrates the progressive expansion of RAS with production increasing from mere 36 tons in 2009 to 237.7 tons during 2016 *i.e.* nearly a 7-fold increase in 8 years, most intensely during 2013–2016 (Eurostat, 2018). However, there might be both good and bad sides to this prolific growth as discussed by several authors over the years (e.g. reviewed in, Badiola et al., 2012). A detailed account on the history, status and research development of RAS industry in Europe can be found in Martins et al. (2010); hence skipped from further introduction.

From the industrial point of view - fish waste management has been one of the problems having the greatest impact on the environment. Negative effects of waste from aquaculture to aquatic environment are increasingly recognized, although they are negligible to land-based pollutants (Cao et al., 2007). The varieties of wastes produced in RAS and waste recycling or disposal methods available have been well discussed in scientific literature (Badiola et al., 2012; Ebeling and Timmons, 2012; Martins et al., 2010; Rijn, 2013; Schneider et al., 2005). The overall waste treatment efficiency employing various microbial degradation techniques (the most common one in RAS) is still too low and leads to a mismatch in surface areas between fish production and microbial reactors (Schneider et al., 2002; Martins et al., 2010). Same mismatch often occurs between the mechanical filter surface area and culture water volume (Murray et al., 2014). The slow adoption of RAS technology is in part due to the high initial capital investments required by RAS (Martins et al., 2010). The average

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pay-back period under normal circumstances has been estimated at 8 years which is quite long (Badiola et al., 2012). This often compels RAS managers to employ high stocking densities in pursuit of higher system productivity to be able to cover the investment costs. This also results in an increase in both quantity and potency of 'in-system' and 'off-system' wastes. Consequently, waste management concerns concurrently arise (Martins et al., 2005, 2010). RAS investors rarely present properly researched plans and investment for farm waste utilization which quickly becomes a 'headache' as production expands (Badiola et al., 2012; Murray et al., 2014). Another ground reality being - substantial track record of RAS company failures exists in Europe and worldwide. There may be many RAS who may have ceased to exist, or production levels are quite insignificant (< 100 tons per annum) (described in Murray et al., 2014). Here the subject of integrating hydroponics (resulting into aquaponics) comes under discussion and often attains a *'prima-facie'* status among the producers. Introduction of such new 'commercially reap-able' compartments such as 'aquaponics production' is viewed as a 'by-pass' to overcome environmental or economical constraints of commercial RAS ventures. The aquaponics offer a variety of solutions- (a) decrease final environmental output, (b) valorize nutrients taking advantage of produced byproducts and, (c) generate products to supplement economical input on a regular basis (Badiola et al., 2012; Palm et al., 2018; Rijn, 2013).

Technologically speaking - RAS systems were developed for intensive fish farming, mainly where land and/or water availability is restricted: they enable up to 90–99% of the water to be recycled that too within a limited land-area. These systems allow the operator a greater control over the culture-climate, biosecurity and water quality parameters, reduced food miles (*i.e.* producing in urban set-up close to the markets) and improved product security (Badiola et al., 2012; Murray et al., 2014). Technicalities of RAS have been discussed in detail in Ebeling and Timmons (2012). Conventional RAS farms ensure > 90% water recirculation (< 10% replacement per day) or recirculation @0.1–1 $m^3 kg^{-1}$ feed (Martins et al., 2010; Murray et al., 2014). In this process they generate limited but concentrated (nutrient rich) volumes of wastewater and sludge on daily basis; providing an opportunity for improved waste management and nutrient recycling (Martins et al., 2010). Irrespective of whether a RAS farm is marine or freshwater, the wastes generated have real economic values (if re-utilized) and a wide range of recycling options is available (Badiola et al., 2012; Murray et al., 2014; Rijn, 2013). Many environmental groups support RAS over open-production systems for the same reasons (Murray et al., 2014). In recent years, the EU environmental policy directives have become more stringent bringing serious implications for aquaculture sector. These include clumping down of aquaculture input use, farm waste effluent penalties and lowered ceilings in waste nutrient concentrations (Hlavač et al., 2016; Hoevenaars et al., 2018). RASs have been modified to respond to such increasing environmental regulations in countries with limited access to land and water (Martins et al., 2010).

Aquaponics combines two technologies: recirculation aquaculture systems (RAS) and hydroponics (soil less plant production) in a closed-loop system where either complete or majority (> 50%) of nutrients sustaining the optimal plant growth is derived from RAS effluents (Forchino et al., 2017; Palm et al., 2018). Aquaponic systems range from traditional RAS and hydroponic units combined in a single loop that deems fish feed as the only plant fertilizer source (called '1-loop' or coupled aquaponics) to separated aquaculture and hydroponic units (called '2-loop' or decoupled aquaponics) with higher investment, significant nutrient addition and water control (Monsees et al., 2017a, b). Aquaponic units have also been classified as 'extensive' (with integrated RAS sludge usage) and 'intensive' (with sludge separation) (Junge et al., 2017). Aquaponics are effective at nutrient removal when sized correctly (plant surface area: fish culture volume) to balance nutrient production by fish culture and nutrient uptake by plants. It introduces vegetable crops as biofilter (phytoremediation) that reduces nutrient load from the effluents and/or improves quality of 'returning' water. The plants (vegetable crops) represent an additional 'saleable' commodity for the fish farmer; an interim income source between the periodic fish harvests that also acts as 'leverage' to accidental fish losses (Blidariu and Grozea, 2011; Buzby and Lin, 2014). Research in the field of aquaponics has been 'trending' over the last decade (Junge et al., 2017; Palm et al., 2018). Ample literature exists in terms of its history and classification (Palm et al., 2018), system variants and technicalities (Junge et al., 2017; Rakocy et al., 2006), nutrient dynamics and requirements (Bittsanszky et al., 2016; Maucieri et al., 2018), sustainability assessment (Forchino et al., 2017; Konig et al., 2016), challenges (Goddek et al., 2015, Yavuzcan Yildiz et al., 2017) and policy needs (Hoevenaars et al., 2018; Joly et al., 2015). FAO (2018) has deemed aquaponics (RAS + Hydroponics) as a major player in coping with the increased demand of a growing world population. However substantial doubts exist in this regard as many key questions about the overall feasibility of aquaponic production remain unanswered (Goddek et al., 2015; Monsees et al., 2017a, b; Short et al., 2017).

Unlike in the case of RAS, there is no dedicated database on aquaponics to probe their adoption and production successes. This leaves only few and published surveys conducted so far as the only means to gain insights on ground-level realities (e.g. Love et al., 2014, 2015; Mchunu et al., 2018; Short et al., 2017). Most of those surveys pointed out promising nature of aquaponics and tagged it as an emerging practice worldwide. However, the stigma of its scaling issues remains at large - still being a niche or 'backyard activity' performed at hobby or subsistence scale (Mchunu et al., 2018; Love et al., 2014). Owing to the scaling issues and lack of farmers' knowledge in addressing plant nutrition at larger scales, these systems have not proved commercially lucrative (Bostock et al., 2010). Nonetheless, aquaponics is indeed highly scalable to commercial systems if the basic principles and ratios of fish stocking density, feeding rates, crop growing area are maintained and coupling-decoupling needs are realized (Buzby and Lin, 2014; Monsees et al., 2017a, b; Rakocy et al., 2006). The present research addresses a key industry-level question in the middle of such contradictions: whether and, if yes, how easily European (more precisely, Czech) 'operational RAS farms' can afford to upgrade to 'semi-commercial (non-backyard) aquaponics' taking into consideration the quantity and nutrient potency of their daily discharged effluents (wastewaters, sludge) (?). By the term 'upgrade' – we imply to the primary intent of the farms in managing their waste in a eco-friendlier (vis-à-vis policy abiding) and 'commercially reap-able' way. In order to address the question, we attempted to quantify and characterize – (a) nutrient concentration in RAS effluents, (b) average system influx and effluxes of total nutrients, (c) potency of nutrient concentrations in effluents in relation to release (discharge) percentages, (d) relationships between system management protocols and nutrient discharge, (e) some empirical budgeting models based on identified relationships, and, (f) capacity of the farms to meet the nutritional needs of some common aquaponic crops.

2. Materials and methods

2.1. System selection

Two commercial RAS farms (Anapartners s.r.o., Prague http://www.ftn-aquaart.com/en/home-englisch/and Fish farm Bohemia s.r.o., Rokytno, https://www.fishfarmbohemia.cz/) and one experimental RAS facility (FROV, University of South Bohemia in Ceske Budejovice, http://www.frov.jcu.cz/en/institute-aquaculture-protection-waters/lab-nutrition) were studied during 2015–2017. Hereinafter, the farms are termed as 'FROV' (Farm A), 'ANAPARTNERS' (Farm B) and 'ROKYTNO' (Farm C); selected based on their progressive size proportions 1: 8: 80 (A: B: C). A detailed account of their operational and technical specifications (supplementary) can be found in Table 1 and Table S1, respectively. All the systems have been 'operational' for at least 5 years or more prior to the initiation of the present study; justifying our purpose of studying established systems with well laid SOPs (standard operational procedures). Furthermore, the systems were characterized by increasing intensity of aquaculture operations (e.g. no. Of species cultured, stocking density, feed rations, production) from A (lowest) to C (highest).

2.2. Sampling program

Sampling for RAS effluents were conducted intermittently at intervals of 4–5 months. By the term 'effluents', we imply 'wastewater' and 'sludge'. Sampling program were repeated 3 times for farm A (FROV), 4 times for farm B (ANAPARTNERS) and 5 times for farm C (ROKYTNO) depending on their increasing size proportions; back-stopping measure to minimize sample variability due to unknown size (scaling) influences, if any. Further details on sampling is included in supplementary text S1.

2.3. Sample analyses

Wastewaters and sludge were analyzed separately in a certified third-party laboratory (AGRO-LA, spol. s.r.o., Jindřichův Hradec) employing 'Czech standard' analytical methods (ISO verified and certified protocols in Czech Republic). Some selected 'plant-essential' elements were quantified. It includes – *primary macronutrients* (N, P, K), *secondary macronutrients* (Mg, S, Ca) and *micronutrients* (Na, Fe, Zn, Cu, B, Mo, Ni) (Resh, 2016). Additionally, some environmentally hazardous heavy metals (As, Cd, Hg, Pb, Ni, Cr) were measured from the sludge. In general, the lowest detectable limits on dry matter basis were 0.01 mg kg^{-1}, 0.01% and in wet matter 0.001 mg L^{-1}. Some elements, especially heavy metals, had element specific lower detection thresholds. All analyses were done in triplicate.

2.4. Database compilation, parameterization and descriptive statistics

Data were coded farm wise and then compiled to generate both farm-specific and pooled information. The categories of information were: (a) influx of various aquaculture inputs (b) efflux of various nutrients from the system, (c) total efflux of some 'inevitable' RAS nutrients at hypothetical exchange rates, and, (d) comparing the nutrient status in effluents with standard hydroponic solution concentrations for some common aquaponic crops. Keeping the space limitations into consideration - the parameters, their derivations (formulas) and assumptions-conditions have been provided in Table S2, category-wise.

Descriptive statistics were generated through SPSS 16.0. Mean values were

Table 1
Operational specifications of the studied RAS farms (*arranged in ascending order of size)*.

Parameters	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED*
Volume (m^3)	16	130	1400	16-1400 (630.67 ± 680.61)
Fish species cultured (no.)	2	2	4	2-4
Water exchange (% day^{-1})	5.81	1.65	0.89	0.89-5.81 (2.37 ± 2.1)
Stock density (no. m^{-3})	30	70	75	30-75 (62.08 ± 19.48)
Stock mass (kg m^{-3})	24.38	62.46	85.71	24.38-85.71 (62.63 ± 25.32)
Feeding rate (% biomass day^{-1})	2	2.5	3	2-3 (2.58 ± 0.42)
Feed input (g m^{-3} day^{-1})	490	1560	2570	490-2570 (1713.33 ± 866.19)
Feed crude protein (%)	44.2	52	32	32-52 (41.72 ± 9.11)
Feed-N Input (mg L^{-1} day^{-1})	30	130	130	30-130 (105 ± 45.23)
Feed-P (%)	1.42	1.2	1	1-1.42 (1.17 ± 0.17)
Feed-P input (mg L^{-1} day^{-1})	10	20	30	10-30 (21.67 ± 8.35)
Feed micronutrient (%)[a]	9.18	6.32	9	6.32-9.18 (8.15 ± 1.35)
Feed micronutrient input (mg L^{-1} day^{-1})	40	100	230	40-230 (139.17 ± 83.61)
Food Conversion Ratio (FCR)	1.4	1.2	1.2	1.2-1.4 (1.25 ± 0.09)
pH buffer input (mg L^{-1} day^{-1})[b]	35	20	32	20-35 (28.75 ± 6.58)
Temperature (°C)	24.7	23.1	22.4	18.5-25.5 (23.2 ± 2.3)
pH (units)	6.74	7.53	7.64	6.47-7.95 (7.38 ± 0.43)
Total Suspended Solids (mg L^{-1})	12.19	39.04	64.29	12.19-64.29 (42.85 ± 21.69)
Electrical conductivity (μS m^{-1})	1.7	2	2.3	1.7-2.3 (2.05 ± 0.25)
Dissolved oxygen (mg L^{-1})	7.38	10	7.61	5.85-10.53 (8.35 ± 1.55)
Wastewater volume (m^3 day^{-1})	0.75	1.3	5	0.75-5 (2.7 ± 2.04)
Sludge volume (m^3 day^{-1})	0.1	0.2	0.5	0.1-0.5 (0.3 ± 0.18)
Sludge Dry Matter (%)	2.7	4.9	5.84	0.5-9.3 (4.74 ± 2.63)
Wastewater: RAS-Volume ratio (%)	4.7	1	0.4	0.004-0.047 (0.017 ± 0.018)
Sludge: RAS-Volume ratio (%)	0.63	0.15	0.04	0.0004-0.0063 (0.0022 ± 0.0025)
Sludge: Wastewater volume ratio (%)	13	15	10	0.10-0.15 (0.12 ± .0.023)
Parameters significantly differing [c] ($p < 0.05$)	volume, feed input, stocking density, stocking biomass, temperature, total suspended solids, crude protein of feed, sludge dry matter content, sludge: RAS-volume ratio, sludge dry matter: RAS-volume ratio, sludge: wastewater-volume ratio			
Parameters non-significantly differing [c] ($p > 0.05$)	fish species cultured, water exchange, pH buffer input, feeding rate, dissolved oxygen, pH, electrical conductivity, sludge volume, wastewater volume, FCR, feed phosphorus, feed micronutrient, wastewater: RAS volume ratio			

* Pooled values contain range and mean ± SD (in parentheses)
[a] Total ash content of the feed (excluding P)
[b] $Ca(OH)_2$ and KOH used @1:1 in Farm A; $NaHCO_3$ used in Farms B and C.
[c] Results from Kruskal-Wallis H Test.

checked for their fitness of representation by estimating their coefficient of variation (CV = standard deviation/mean). Parameters with CV > 1 were flagged as 'extremely variable' and were considered as unfit for generalization (pooling) and comparison (Snedecor and Cochran, 1989). In view of high variability, 95% confidence intervals (C.I.) were calculated for nutrient concentrations in effluents to obtain best fitted representative data.

2.5. Mapping of inter-system operational variability and effluent nutrient consistency

Data was coded farm-wise and subjected to Kruskal-Wallis One Way-ANOVA based on Ranks (Kruskal-Wallis H Test) (McDonald, 2014). Details of the test is included in supplementary text S1.

2.6. Modeling of operational influences on nutrient output through effluents

Attempts were also made to identify the most important operational influences that play a key role in influencing nutrient generation. The data was analyzed in multiple steps, employing various statistical tools (stepwise multiple regression, log-10 transformation and non-linear LOESS smoothing). The details are included in supplementary text S1.

3. Results

3.1. System characteristics, operational variability and effluent nutrient consistency

A descriptive account of system characteristics is presented in Table 1 and Supplementary Table S1. Keeping the motto of this section in mind, we skipped presenting the trends of individual system parameters from tables to the text. Nevertheless, a generally increasing trend in system parameters from Farm A to C is easily perceptible; function of increasing size and aquaculture intensity (A < B < C). *Three farms were significantly different (p < 0.05) from each other in the following aspects*: volume (m^{-3}), feed input (g m^{-3} day^{-1}), stocking density (no. m^{-3} day^{-1}), stocking biomass (kg m^{-3} day^{-1}), temperature (°C), total suspended solids (mg L^{-1}), crude protein of chosen feed (%), sludge dry matter content (%), sludge release ratio (sludge volume: RAS volume), sludge dry matter release ratio (sludge dry matter: RAS volume), sludge: wastewater volume ratio (%) – hinting these as probable 'set of factors' responsible for significantly differing effluent nutrients if the management regimes in Czech RAS farms are normalized. *The farms did not varied significantly (p > 0.05) in terms of* fish species cultured (nos.), water exchange (%), pH buffer input (mg L^{-1} day^{-1}), feeding rate (% biomass day^{-1}), dissolved oxygen (mg L^{-1}), pH (units), electrical conductivity (μS m^{-1}), sludge volume (m^{-3} day^{-1}), wastewater volume (m^{-3} day^{-1}), FCR of the chosen feeds (units), phosphorus and micronutrient contents of chosen feed (%), wastewater release ratio (wastewater volume: RAS volume) - probably acting as the 'set of factors' behind maintaining coherence in effluent nutrients (if any) in spite of diverse management regimes in RAS farms (Table 1).

Digging deep into the daily input and loading (by fish, see Table S2 for derivations) of certain nutrients into the systems, we found out that – feed-N, P and micronutrients input (mg L^{-1} day^{-1}) varied significantly ($p < 0.05$) among the farms in conjunction with significantly different daily feed input. In terms of nutrients loading by fish, estimated N and micronutrient loadings varied significantly ($p < 0.05$) while P-loading (mg L^{-1} day^{-1}) was similar ($p > 0.05$). In terms of nutrient consistencies in wastewaters (concentrations, mg L^{-1}) among the farms, 9 out of 12 nutrients *viz.* Total-P, K, S, Mg, Fe, Cu, Zn, B, Mo were found to be consistent (non-significant differences, $p > 0.05$) irrespective of farm-specific variations. Total-N, Ca and Na were found to be significantly differing among the farms; probably due to significant differences in feed crude protein alongside fish stocking biomass (vis-à-vis nitrogen) and choice of pH buffering agents ($Ca(OH)_2$ and KOH in farm A; $NaHCO_3$ in farms B and C). If the above rationale applies true, the absence of an 'equally anticipated' K from the list despite being used in farm-A (as KOH) is questionable; although K was present in sludge at much higher concentrations (Table 4). Interestingly, a closer look in our dataset revealed that the cluster of micronutrients (Fe, Cu, Zn, B, Mo) which appeared consistent across farms might be attributed to their 'trace concentrations' (≤ 0.01 mg L^{-1}) in the wastewater (Table 3); concentrations in sludge being much higher (Table 4). *Synchrony between the patterns of nutrient loading and nutrient concentrations in wastewater was observed for N, P and Na.* In other words, N-concentration in wastewater differed significantly across farms as did the N-loading by fish. Similarly, P-concentration in wastewater followed the same pattern as P-loading *i.e.* not differing significantly among farms. Presence of Na in the 'non-consistent nutrient list' was excluded from interpretation since complete data on Na input was unavailable; only 2 out of 3 farms had measurable Na-input (using $NaHCO_3$) (Table S. *Asynchrony between the patterns of nutrient loading and nutrient concentrations were observed for the micronutrients* (represented by S, Mg, Fe, Cu, Zn, B, Mo). Despite significantly different

micronutrient loadings among the farms, their concentrations in wastewater did not reflect such trend. This also hints us a *significant partitioning of micronutrients probably from wastewater to sludge compartment of the effluents* (further elaborated below) (Table 3).

On the other hand, the significant differences observed in the sludge dry matter (%) content among farms was double-checked with another proxy parameter *i.e.* sludge-ash content (%). We found significant differences ($p < 0.05$) in sludge ash content too. After such dual confirmation, we infer that the sludge matrix is highly inconsistent and unpredictable among the farms – making any of its comparison impractical. We restrained from analyzing nutrient consistencies in sludge to avoid unknown, random interferences in our results due to variable sludge matrix consistency (also clarified under methodology section).

3.2. Nutrient output (concentration, total efflux and potency)

Keeping the space limitations into consideration, only the highlights of results have been presented in this sub-section. Detailed presentation can be found in supplementary text S2.

3.2.1. Primary macronutrients (N, P, K)

Wastewater: For total-N, nitrate was the most dominant fraction overall i.e. about 85% of the total-N concentration in wastewaters. K-concentration in wastewaters can be manipulated by using KOH as pH buffer in RAS even to the extents that it surpasses farm size influences on deciding the concentration (Farm B's K concentration < Farm A's, despite larger size). Overall in terms of primary macronutrients in wastewater – (a) the primary macronutrient efflux and potency were extremely variable in nature making it difficult to present any representative (pooled) scenario, (b) the nutrient output progressively increases with increased farm size (culture water volume) and aquaculture intensity, (c) there is a order in primary macronutrient output through wastewaters (N > K > P) and, (d) concentration of K can be manipulated beyond pre-existing 'farm size influences' by the use of .KOH as pH buffer in RAS systems (Tables 3 and S3, Fig. S1).

Sludge: All nutrient outputs through sludge are given on 'wet sludge' basis *i.e.* sludge with dry matter content of 0.5–9.3% (pooled mean 4.74 ± 2.63%). Sludge total-N concentration was over 2 times (210%) higher than in wastewater; Ammonia fractions dominating over nitrates. In the absence of nitrites and organic bound-N data we could not conclude that ammonia is the most dominant fraction. There might be a possibility that organic bound-N dominates the overall nitrogen fraction in sludge – scope for mineralization. Sludge had extremely higher concentration of total-P as compared to wastewater - 37 times higher (37873%). Sludge had almost 3 times (260%) higher K content than in wastewaters. Like in the case of wastewaters, K output through sludge can also be manipulated using KOH as a pH buffer in RAS even beyond influences of size and aquaculture intensity (Farm A's sludge K content was higher than both Farms B and C). Overall in sludge – (a) daily efflux of primary macronutrients are extremely variable making it difficult to present a generalized (pooled) picture, (b) the concentration of primary macronutrients in sludge does not necessarily increase with farm size and aquaculture intensity, (c) primary macronutrient concentrations in sludge are 2–3 times higher than in wastewaters (extremely high for P, beyond comparison with N and K), (d) the order of primary macronutrient output is N > P > K, and, (e) K output through sludge can be improved significantly by the use of KOH as pH buffer in RAS (Tables 4 and S3, Fig. S2).

Wastewater and sludge combined: All the results presented in this sub-section is estimated from a simulated release scenario where wastewater release is to the tune of 1% of total RAS volume and sludge release at 0.1% (see Table S2 for further details). Only efflux (g day^{-1} 1.1% $release^{-1}$) and potency data (mg L^{-1} day^{-1} 0.1% $release^{-1}$) were calculated. Overall in wastewater and sludge combined- (a) the order of primary macronutrient output was found to be N > K > P – matching the trend as in wastewater, (b) the macronutrient effluxes and potencies have generally extreme variability making them difficult to generalize or compare as such, (c) size and culture intensity matters, i.e. more the size and intensity, more is the nutrient output (Table S7).

3.2.2. Secondary macronutrients (Ca, S, Mg)

Wastewater: Interestingly, a peculiarity was noticed in Ca concentration among the farms. Despite not using $Ca(OH)_2$ as a pH buffer by farms B and C (as reported), they had comparable (farm B) or even higher (farm C) Ca concentration in wastewaters than farm A (used $Ca(OH)_2$ as pH buffer). We suspect an 'unreported' use of Ca $(OH)_2$ by the farms (especially farm B) as an emergency contingency measure to tackle greater drop of system pH; beyond rapid remedial capacity of the commonly used $NaHCO_3$. Especially for the revamped 'soviet-era' farm C, we suspect calcium leaching from some old calcified/cement tanks or water channels in the farm. There was some unexpected farm-level extreme variability in Mg output by farm A; unexplained. Overall in wastewater – (a) the concentration of secondary macronutrients did not generally increased as expected with increase in farm size and aquaculture intensity, (b) extreme variability exists in pooled efflux and potency of secondary macronutrients and hence cannot be generalized, (c) Ca concentration can be influenced by even emergency use of $Ca(OH)_2$ as pH buffer or leaching from old calcified structures, and, (d) the order of secondary macronutrient output is: Ca > S > Mg (Table 3, Fig. S3).

Sludge: All nutrient outputs through sludge are given on 'wet sludge' basis (also mentioned above). Due to methodological error sulfur (S) could not be measured in the sludge; although there may be significant amount locked. As presented in the case of wastewater, peculiarity in sludge Ca concentration was also observed. In fact, the lower concentration of Ca in farm A (using $Ca(OH)_2$) than both farms B and C was far from our anticipation. Moreover, higher Ca concentration in farm B than farm C reinforced our suspicion of an unreported $Ca(OH)_2$ use in farm B, probably to ameliorate high pH fluctuations (*clarified above*). The concentration Ca and Mg in sludge were almost 10 times (997%) and 4 times (388%) higher than in wastewater. Overall in sludge – (a) secondary macronutrient output unanimously increased with increasing farm size and aquaculture intensity, (b) the efflux of secondary macronutrients was extremely variable and hence cannot be generalized, (c) secondary macronutrient concentrations are over 4 times higher than in wastewater, and, (d) the order of secondary macronutrient output is: Ca > Mg, ignoring the Sulfur. Extrapolating our results from the other two secondary macronutrients, we assume that there might be approximately 3–9 times higher sludge S concentration than in wastewater (Table 4, Fig. S4).

Wastewater and sludge combined: All the results presented in this sub-section is estimated from a simulated release scenario; wastewater release (1%) and sludge release (0.1%) (clarified above). Data on sulfur could not be presented because it was not measured in sludge (mentioned above). Overall in wastewater and sludge combined – (a) secondary macronutrient output increased with increasing farm size and culture intensity, (b) extreme variability in efflux and potency exists making them difficult to generalize, and, (c) Ca is the most dominant secondary macronutrient (Table S7).

3.2.3. Micronutrients (Na, Fe, Zn, Cu, B, Mo, Ni)

Wastewater: Ni was not detected; probably absent. Unlike in other class of nutrients, the concentration of micronutrients did not show any prominent increasing trend from farm A to C. Interestingly the concentration of Na did not increased from farm B to C as anticipated due to increase in total $NaHCO_3$ input (Table 2). Cross matching this data with sludge Na concentration revealed a 'balanced' partitioning of Na from wastewater to sludge; masking the anticipated effect of increased Na concentration in wastewater with $NaHCO_3$ use (presented under sludge sub-section). Overall in wastewater – (a) the concentration of micronutrients did not exhibit any prominent increase with increasing farm size and culture intensity, (b) the output of most micronutrients except Fe are extremely variable and unfit for generalization, (c) concentration of Na did not increased with increasing $NaHCO_3$ input (pH buffer) in farms, (d) the order of micronutrient output is: Na > Fe > Zn > B > Cu > Mo, and, (e) the output of Mo was extremely low to comment upon and Ni was absent (Table 3, Fig. S5).

Sludge: Mo and B were below detection limits; could not be presented. Due to methodological error, Fe could not be measured for farms B and C. The concentrations of Cu, Zn were 15 times (1561%) and 17 times (1774%) higher than in wastewater, respectively. Interestingly, concentration of Na was 63.6% lower than in wastewater – the only nutrient showing such opposite trend. Ni was only detected in sludge and could not be compared with wastewater. Data on the concentration of Fe is only present for farm A. Comparing with Farm A's wastewater Fe concentration, we estimated a 562% (5 times) higher Fe concentration in sludge. Unlike in wastewater, almost all micronutrients in sludge showed an increasing concentration with increasing farm size and culture intensity. The increase in sludge Na concentration (farm A vs. farms B and C; Farm B to Farm C) corresponded with the increasing $NaHCO_3$ use at farm level (Table 2). Cross-matching this data with wastewater Na concentration hints a 'somewhat balanced' partitioning of Na between wastewater and sludge that on one hand masks the anticipated increasing of Na in wastewater with increased $NaHCO_3$ use and retains maximum Na in wastewater on the other hand. Overall in sludge – (a) the output of micronutrients have extreme variability, like other classes of nutrients, making them difficult to generalize or compare, (b) the concentration of micronutrients increases with increasing farm size and culture intensity (unlike in wastewater), (c) the concentration of micronutrients are usually 5–17 times higher than in wastewater, (d) Na concentration is almost 60% lower than in wastewater in spite of increasing with $NaHCO_3$ use in farms – a balanced partitioning with wastewater is apparent, (e) the order of micronutrient output is: Na > Fe (extrapolated) ≥ Zn > Cu > Ni, and, (f) Mo and B were below detectable limits (Table 4, Fig. S6).

Wastewater and sludge combined: Results on B, Mo and Ni were purposively excluded due to unavailability of concentration data in either wastewater or sludge (*explained above*). Overall in wastewater and sludge combined – (a) micronutrient output increased with increasing farm size and culture intensity, (b) extreme variability in efflux and potency exists making them difficult to generalize, (c) the order of micronutrient output (excluding B, Mo and Ni) is: Na > Fe (extrapolated) ≥ Zn > Cu (Table S7).

Table 2
Influx of various aquaculture inputs in the studied RAS farms.

Parameters*	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED#
N-loading (mg L^{-1} day^{-1})[a]	15.38	31.96	32.38	15.38-32.38 (27.99 ± 7.61)
P-loading (mg L^{-1} day^{-1})[a]	2.77	3.75	5.14	2.77-5.14 (4.08 ± 1.01)
Micronutrients loading (mg L^{-1} day^{-1})[a]	17.9	19.74	46.29	17.9-46.3 (30.34 ± 14.1)
Ca input (mg L^{-1} day^{-1})[b]	9.47	-	-	0-9.47 (2.37 ± 4.28)
K input (mg L^{-1} day^{-1})[b]	12.2	-	-	0-12.2 (3.05 ± 5.52)
Na input (mg L^{-1} day^{-1})[b]	-	5.47	8.76	0-8.76 (5.47 ± 3.62)
TSS (mg L^{-1})[a]	12.19	39.04	64.29	12.19-64.29 (42.85 ± 21.7)

[a] From selected feed
[b] From selected pH buffer
* See *Table S2* for clarification regarding calculations.
# Pooled values contain range and mean ± SD (in parentheses)

3.3. Heavy metal discharge (As, Cd, Hg, Pb, Ni, Cr)

The output of some environmentally hazardous heavy metals through sludge is given in Table S4. *The surveyed RAS farms were completely 'safe' in terms of their heavy metal discharge potential.* The concentration of all the heavy metals tested were 'far below' their respective pollution thresholds (Czech EPA limits, Table S4). Further details can be found in supplementary text S2.

3.4. Suitability of effluents in meeting nutrient requirements of common aquaponics crops

Based on our results of nutrient outputs through farm effluents, a self explanatory 'capacitogram' was generated in respect to the standard nutritional requirements of some commonly raised aquaponics crops (plants) (Table 5). Overall, considering both the capacities of wastewater and sludge, the macronutrient K is generally deficient requiring a full-fledged fertilization intervention (K fertilizers). Micronutrients like B, Mo are partly sufficient that can be easily ameliorated employing a variety of management decisions – (a) supplemental fertilization (not full-fledged), (b) by increasing wastewater exchange, or, (c) manipulating more sludge release. Nutrients like N, P, Mg, Ca, S, Fe, Zn, Cu are 'sufficiently meet-able' to plant needs using either wastewater or sludge or both 'as-it-is'. It should be noted that - *even if some nutrients are deficient in wastewater* (P, K, Ca, Mg, Ca, S, Fe, Cu) *to meet the plant needs, it can be completely masked by the use of raw or mineralized sludge* which contains almost an estimated 3–17 times (or even more, e.g. phosphorus 37 times) higher concentration of those nutrients than in wastewater. Cases on individual crops have not been elaborated here and can be easily interpreted from the capacitogram (Table 5).

The 'capacitogram' has four color blocks (green, light green, yellow, red and black) that have been defined in the legends. Counting the number of individual color blocks for each plant (Red + Black blocks; Yellow blocks; Green + Light green blocks as 'Green') and comparing the counts among plants, we prioritized the crops in terms of their 'nutritional management interventions'. By the term 'management interventions', we imply a combination of decisions on complete fertilization, supplementary fertilization or increase in wastewater exchange, sludge release manipulations. It is arranged in the descending order of 'nutritional management interventions' required: Chilli (Red + Black 10 + 2/Yellow 1/Green 9) > Cucumber (8 + 2/2/10) ≥ Tomato (7 + 2/4/9) > Lettuce and herbs (7 + 2/3/10). This order of priority should not be viewed as 'difficulty level' of culturing from plant nutrition perspective, as majority of the nutrients can be easily delivered from the effluents.

3.5. Modeling of operational influences on nutrient output

Keeping the space limitations into consideration, only the highlights of results have been presented in this sub-section. Detailed presentation can be found in supplementary text S3.

3.5.1. Wastewater

The results suggest that the concentrations of P and Mg cannot be predicted by any predictor (operational factors or variables) hinting some degree of unidentifiable, random influence on them. N, K, Ca, S and the whole cluster of micronutrients (Na, Fe, Zn, Cu, B) had some identifiable key driver influencing their concentration in wastewater. The notable factors that had key manifestation(s) on wastewater nutrient concentrations (in parentheses) were: fish species (K, Ca, S, Fe, Zn, Cu) > wastewater volume (N) > FCR (Na) > micronutrients loading (B). From practical point of view, the appearance of 'number of fish species cultured' as a key driver in determining most of nutrient concentrations in wastewater seems somewhat unrealistic. We infer it as a statistically abstract output since the data on 'fish species' had a very narrow variability (2–4 species; 2 species being the most common combination – farm A and B). Nonetheless, *it remains an interesting area to explore for future research whether increased cultured fish diversity in RAS farms generate more nutrient rich effluents (wastewater)* i.e. more the combination of fish species cultured, better the nutrient quality of wastewater (?). Appearance of FCR as a driver for Na was also partly unrealistic. Although fish feeds are known to contain 'some' amount of common salt (NaCl) in their composition, but that is far negligible in comparison to the input of Na into RAS systems through $NaHCO_3$ (as pH buffer). FCR also differed too little – by degrees of 1/10th of decimals (± 0.1) perhaps making the parameter very sensitive to predict nutrient (Na) concentrations (Table S5).

The empirical budgeting models suggest that per unit increase of wastewater volume (m^3) may lead to a corresponding change of + 602.59 mg L^{-1} (standard error, SE ± 254.81) in N content of wastewater (R = 0.599). Likewise, a unit increase in micronutrients loading (mg L^{-1} day^{-1}, see Table S2 for derivation) may result in a change of + 0.014 mg L^{-1} (SE ± 0.001) B in wastewaters (R = 0.955). A unit increase in FCR (units) corresponds to a change of − 1760.28 mg L^{-1} (SE ± 295.51) Na (R = 0.883). Such large change in Na concentration should be carefully interpreted keeping in mind that the changes in feed FCR usually occur at the scale of 1/10th (e.g. changes by ± 0.1 units); therefore, concentration of Na in wastewater changes by − 176.03 mg L^{-1} (SE ± 29.55) per 0.1 unit increase in FCR (R = 0.883). *All the above empirical estimates may presumably be considered as 'good-fit' within a range of aquaculture intensity but not universally; i.e.* the range of aquaculture intensity within which the models were generated (culture volume 16–1400 m^3, fish species 2–4, water exchange 0.89–5.81%, Stock mass 24.38–85.71 kg m^{-3}, Feeding rate 2–3% biomass day^{-1}, FCR 1.2–1.4, pH buffer input 20–35 mg L^{-1} day^{-1}). LOESS models between Total-N efflux and potency in respect to wastewater volume showed a slow but steady increase, slightly hinting a tendency of leveling-off at higher wastewater discharge (Fig. S7). The pattern of B efflux and potency in relation to increasing micronutrient loading showed an initial 'burst' followed by a 'gradual increase' at higher loading scenarios, also having an ultimate tendency to level-off like total-N (Fig. S8). LOESS models for Na could not be generated because changes in FCR were too small to generate any model.

In terms of multicollinearity between wastewater and sludge nutrient concentrations - we observed a mildly positive but non-significant partial correlation (r = 0.4, p > 0.5) between wastewater and sludge K concentrations. A mildly negative but insignificant partial correlation was observed in the case of Na (r = − 0.317, p > 0.05). No partial correlation was observed for Total-N, Total-P, Ca, Mg, Zn and Cu for concentrations between wastewater and sludge.

3.5.2. Sludge

The results suggest – concentration of macronutrients in sludge (i.e. total-N, total-P, K, Ca, Mg) cannot be predicted by any predictor (operational factors or variables) hinting some degree of unidentifiable, random influence on them. However, the concentrations of micronutrients (Na, Cu, Zn, Ni) were influenced by some key drives and can be predicted. The notable factors that had key manifestation(s) on sludge micronutrient concentrations (in parentheses) were: sludge-RAS volume ratio (Na) > feeding rate (Cu) > stock mass (Zn) > fish species (Ni). The model of Ni with 'fish species' was excluded from presentation (clarified under wastewater) (Table S6).

As per the empirical models generated – (a) per unit increase in sludge release % (sludge: RAS volume ratio) may result in a decline of sludge Na concentration by 362.23 ± 74.65 mg L^{-1} (R = 0.838); (b) per unit increase in feeding rate (%) may increase sludge Cu concentration by 4.25 ± 1.21 mg L^{-1} (R = 0.744); (c) per unit increase in stock mass (kg m^{-3}) may increase Zn by 0.75 ± 0.22 mg L^{-1} (R = 0.726). It should be noted that, in practical situations, changes in sludge release % and feeding rate % usually occur at the scale of 1/100th (i.e. ± 0.01%) and 1/10th (± 0.1%) respectively. Therefore, interpretation from the models should be made carefully. For example - sludge Na concentration will decrease by 3.62 ± 0.75 mg L^{-1} per 0.01% increase in sludge release. Likewise, Cu concentration may only increase by

Table 3
Efflux of some selected plant-essential nutrients through released wastewaters from RAS.

Parameters	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED
Primary macronutrients				
Total N (mg L^{-1})[a]	350±308.36	673.18±427.02	2907±2689.51	41.64-7272.51 (1523.1±2050.82)
N efflux (g day^{-1} % $release^{-1}$)[b]	56±49.34	875.14±555.12	40698±37653.15	6.66-102000 (17263±30719.22)
N potency (mg L^{-1} % $release^{-1}$)[c]	74.67±65.79	673.18±427.02	8139.5±7530.63	8.88-20363.04 (3634.5±6045.05)
Total P (mg L^{-1})[a]	2.29±0.84	1.79±0.39	2.94±1	1.31-4.24 (2.39±0.9)
P efflux (g day^{-1} % $release^{-1}$)[b]	0.37±0.14	2.33±0.51	41.16±14.02	0.2-59.4 (18.02±22.13)
P potency (mg L^{-1} % $release^{-1}$)[c]	0.49±0.18	1.79±0.39	8.23±2.81	0.31-11.87 (4.15±4.02)
K (mg L^{-1})[a]	43.28±35.32	18.4±5.39	109.1±32.08	7.96-155 (62.42±49.03)
K efflux (g day^{-1} % $release^{-1}$)[b]	6.92±5.65	23.92±7.01	1527.4±449.17	1.27-2170 (646.12±823.78)
K potency (mg L^{-1} % $release^{-1}$)[c]	9.23±7.54	18.4±5.39	305.48±89.83	1.7-434 (135.73±159.44)
Secondary macronutrients				
Ca (mg L^{-1})[a]	88.53±26.45	84.3±3.35	234.76±134.84	62.1–463 (148.05±112.26)
Ca efflux (g day^{-1} % $release^{-1}$)[b]	14.17±4.23	109.59±4.35	3286.6±1887.73	9-94-6482 (1409.5±2010.71)
Ca potency (mg L^{-1} % $release^{-1}$)[c]	18.89±5.64	84.3±3.35	657.33±377.55	13.25-1296.4 (306.71±385.1)
S (mg L^{-1})[a]	18.3±3	19.56±8.12	90.26±31.48	9.6-141 (48.7±41.5)
S efflux (g day^{-1} % $release^{-1}$)[b]	2.93±0.9	25.42±10.55	1263.7±440.66	2.93-1974 (535.75±695.44)
S potency (mg L^{-1} % $release^{-1}$)[c]	3.9±1.2	19.56±8.12	254.74±88.13	3.9-394.8 (112.8±134.68)
Mg (mg L^{-1})[a]	30.82±40.12	8.92±0.81	41.17±16.27	4.57-77 (27.83±24.55)
Mg efflux (g day^{-1} % $release^{-1}$)[b]	4.95±6.45	11.59±1.05	576.44±227.82	0.73-819 (245.28±323.02)
Mg potency (mg L^{-1} % $release^{-1}$)[c]	6.6±8.6	8.92±0.81	115.29±45.56	0.97-163.8 (52.66±61.85)
Micronutrients				
Na (mg L^{-1})[a]	41.75±26.65	407±54.71	383.25±129.37	15.1-549 (305.79±180.27)
Na efflux (g day^{-1} % $release^{-1}$)[b]	6.68±4.26	529.1±71.12	5365.5±1811.13	2.42-7686 (2413.7±2833.08)
Na potency (mg L^{-1} % $release^{-1}$)[c]	8.99±6.66	407±54.71	1073.1±362.23	3.22-1537.2 (585.02±508.78)
Fe (mg L^{-1})[a]	0.96±0.91	0.33±0.2	14.32±13.56	0.05-37.62 (6.32±10.82)
Fe efflux (g day^{-1} % $release^{-1}$)[b]	0.15±0.14	0.43±0.25	200.41±189.85	0.01-526.7 (83.69±154.02)
Fe potency (mg L^{-1} % $release^{-1}$)[c]	0.21±0.2	0.33±0.2	40.08±37.97	0.01-105.3 (16.86±30.73)
Zn (mg L^{-1})[a]	0.1±0.07	0.12±0.05	4±3.9	0.03-10.66 (1.73±3.09)
Zn efflux (g day^{-1} % $release^{-1}$)[b]	0.02±0.01	0.15±0.07	56.03±54.66	0.01-149.24 (23.41±43.77)
Zn potency (mg L^{-1} % $release^{-1}$)[c]	0.02±0.01	0.12±0.05	11.21±10.93	0.01-29.85 (4.71±8.74)
Cu (mg L^{-1})[a]	0.02±0.01	0.01±0.001	0.41±0.37	0.01-1.04 (0.18±0.31)
Cu efflux (g day^{-1} % $release^{-1}$)[b]	0.003±0.001	0.02±0.001	5.80±5.21	0.01-14.55 (2.42±4.33)
Cu potency (mg L^{-1} % $release^{-1}$)[c]	0.001±0.001	0.01±0.001	1.16±1.04	0.1-3 (0.49±0.87)
B (mg L^{-1})[a]	0.04±0.02	0.05±0.001	0.42±0.1	0.02-0.58 (0.21±0.2)
B efflux (g day^{-1} % $release^{-1}$)[b]	0.01±0.002	0.06±0.001	5.94±1.41	0.004-8.18 (2.5±3.16)
B potency (mg L^{-1} % $release^{-1}$)[c]	0.01±0.01	0.05±0.001	1.19±0.28	0.01-2 (0.51±0.62)
Mo (mg L^{-1})[a]	0.01±0.001	0.01±0.001	0.01±0.004	0.01-0.02 (0.01±0.001)
Mo efflux (g day^{-1} % $release^{-1}$)[b]	0.001±0.001	0.01±0.001	0.12±0.06	0.001-0.22 (0.05±0.07)
Mo potency (mg L^{-1} % $release^{-1}$)[c]	0.001±0.001	0.01±0.001	0.02±0.01	0.001-0.01 (0.01±0.01)
Consistent nutrients ($p<0.05$)[d]	Total-P, K, S, Mg, Fe, Cu, Zn, B, Mo			
Inconsistent nutrients ($p>0.05$)[d]	Total-N, Ca and Na			

[a] Observed concentration.
[b] Estimated at system scale (based on average daily wastewater output).
[c] Estimated at solution scale (based on resultant concentration of wastewater per percent culture water *i.e.* release ratio (percentage) – wastewater: RAS volume).
[d] Results from Kruskal-Wallis H Test.
Grey highlighted cells: Indicate extreme variability (CV>1) in values; rendering them unfit for generalization and comparison.

0.43 ± 0.12 per 0.1% increase in feeding rate. These models may be considered as 'good-fit' only within a range of aquaculture intensity but not universally (clarified above). LOESS models on effluxes and potencies of Na, Cu and Zn with respect to their key predictor(s) revealed some general trends. With increasing sludge release there is a steady but continuous decline in Na efflux and potency (Fig. S9). Efflux and potency of Cu and Zn seem to increase initially but gradually stagnates with increasing feeding rate and stocking biomass decisions, respectively. The effect is more pronounced in efflux rather than in potency (Figs. S10–S11). Multicollinearity results between sludge and wastewater nutrients have been presented under 'wastewater'.

4. Discussions

4.1. System characteristics, operational variability and effluent nutrient consistency

The natural feeding habit of fish species cultured, fish stocking density, total fish biomass, selection of feed, feed input rate, water quality and water management regimes are known to have decisive impact on the assimilation of nutrients in RAS and ultimate wastewater production. The main source of nutrients being – uneaten feed, fish feces, soluble excreta, pH buffer input and in-system solids or bioflocs (Ebeling and Timmons, 2012; Goddek et al., 2015). Most of the aquaponics viability studies till now have focused on the fact that waste generation by fish is directly related to the quantity and quality of feed being applied; that too predominantly from N and P perspectives (Buzby and Lin, 2014; Fornshell and Hinshaw, 2008; Schneider et al., 2005). Factors like - manipulations in wastewater-sludge release to amend nutrient concentrations, utilization of sludge as a major player in proving plant nutrition, seeing pH buffer input as a 'fertilization opportunity' have been always perceived as secondary thoughts. Under the current practices in RAS, solid wastes are only partially solubilized as they are mechanically filtered out daily (Goddek et al., 2015); soluble nutrients in RAS wastewater being the primary focus to plan aquaponics. Nonetheless, fish feed is the main nutrient input and defines, to a large extent, the sustainability of the aquaponics operation (Junge et al., 2017). We beg to differ a bit regarding the sustainability of operation by inserting 'wastewater-sludge release manipulations' and 'sludge recycling' as equally important co-factors besides the feed input. *The present study showcased that operational RAS farms are already capable of sustaining aquaponic operations with their present rate of feed input, given that they slightly increase their effluent discharge intensity.* For example - +2–3% for wastewater (by longer draining) and +0.1% for sludge (by adding more mechanical filter surface area); further discussed under nutrient output section.

Hu et al. (2015) suggested that aquaponics, with concomitant nutrient recovery, will probably become one of the widely used methods of sustainable food production soon. The contributions of such globally prevailing speculations are although 'positive vibes' for RAS farm managers or consultants to rely upon, but they are often insufficient to rationalize a decision. Especially the multitude of studies reasoning against the nutrient production from RAS being inferior for sustaining plant growth in hydroponic component – negative vibes (reviewed in Bittsanszky et al., 2016). There are already some 'established combinations' of fish and plant species that are perceived as gold-standards for venturing into aquaponics; presumably due to lower chances of failure adopting such combinations. The most common fish species are Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Onchorynchus mykiss*), common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) which can be integrated with leafy vegetables, such as lettuce (*Lactuca sativa*), basil (*Ocimum basilicum*), spinach (*Spinacia oleracea*) (Forchino et al., 2017). Entrepreneurs often plunge into 'aquaponic ventures'

Table 4
Efflux of some selected plant-essential nutrients through discharged sludge from RAS.

Parameters	FROV (Farm A)*	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED
Primary macronutrients				
Total N (mg L^{-1})[a]	2000	3850±2816.91	3400±1272.79	400-7300 (3200±1821.46)
N efflux (g day^{-1} 0.1% $release^{-1}$)[b]	32	500.5±366.2	4760±1781.91	32-7280 (2158.17±2549.58)
N potency (mg L^{-1} 0.1% $release^{-1}$)[c]	320	2502.5±1831	9520±3563.82	260-14560 (4880.83±4800.57)
Total P (mg L^{-1})[a]	354	1200±898.15	1000±353.55	100-2300 (905.17±619.68)
P efflux (g day^{-1} 0.1% $release^{-1}$)[b]	5.66	156±116.76	1400±494.97	5.66-2100 (636.74±741.8)
P potency (mg L^{-1} 0.1% $release^{-1}$)[c]	56.64	780±583.8	2800±989.95	56.64-4200 (1440.8±1403.69)
K (mg L^{-1})[a]	200	125±77.57	170±42.43	30-230 (162.5±56.71)
K efflux (g day^{-1} 0.1% $release^{-1}$)[b]	3.2	16.25±10.1	238±59.4	3.2-322 (105.38±122.64)
K potency (mg L^{-1} 0.1% $release^{-1}$)[c]	32	81.25±50.42	476±118.79	20-644 (233.42±228.16)
Secondary macronutrients				
Ca (mg L^{-1})[a]	520	2165±1596.25	1500±431.34	210-4120 (1476.67±1088.44)
Ca efflux (g day^{-1} 0.1% $release^{-1}$)[b]	8.32	281.45±207.51	2100±603.87	8.32-2954 (970.9±1072.92)
Ca potency (mg L^{-1} 0.1% $release^{-1}$)[c]	83.2	1407±1037.56	4200±1207.74	83.2-5908 (2240±2022.63)
Mg (mg L^{-1})[a]	60	110±73.49	135±67.18	20-320 (107.92±63.83)
Mg efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.96	14.3±9.55	1890±94.05	0.96-322 (83.76±109.09)
Mg potency (mg L^{-1} 0.1% $release^{-1}$)[c]	9.6	71.5±47.77	378±188.09	9.6-644 (183.73±208.55)
Micronutrients				
Total Ash (%)[a]	6.42	16.4±2.45	14.7±1.48	6.42-19.4 (13.2±4.44)
Ash-efflux (g day^{-1} 0.1% $release^{-1}$)[b]	6420	32800±4898.98	73500±7424.62	6420-84000 (43200±29191.97)
Ash potency (mg L^{-1} 0.1% $release^{-1}$)[c]	10.27	106.6±15.92	411.6±41.58	10.27-470.4 (209.6±184.22)
Na (mg L^{-1})[a]	50	215±61.24	265±81.32	50-380 (194.58±107.4)
Na efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.8	27.95±7.96	371±113.34	0.8-532 (164.1±195.45)
Na potency (mg L^{-1} 0.1% $release^{-1}$)[c]	80	139.75±39.8	742±227.69	8-1064 (357.75±370.19)
Cu (mg L^{-1})[a]	0.34	2.45±1.84	4.59±2.11	0.2-7.57 (2.81±2.34)
Cu efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.005	0.32±0.24	6.43±2.95	0.01-10.6 (2.79±3.68)
Cu potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.05	1.59±1.19	12.85±5.9	0.05-21.2 (5.9±7.15)
Fe (mg L^{-1})[a]	5.4	-	-	-
Fe efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.09	-	-	-
Fe potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.9	-	-	-
Zn (mg L^{-1})[a]	2.72	29.3±22.29	48.6±22.63	2-80.6 (30.7±26.1)
Zn efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.04	3.81±2.9	68.04±31.68	0.04-112.84 (29.63±38.97)
Zn potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.44	19.05±14.49	136.08±63.36	0.44-225.68 (63.16±75.59)
Ni (mg L^{-1})[a]	0.06	0.06±0.01	2.77±1.79	0.03-5.29 (1.22±1.74)
Ni efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.001	0.001±0.001	2.5±0.65	0.001-7.41 (1.62±2.49)
Ni potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.01	0.001±0.001	5±0.65	0.01-14.81 (3.26±4.97)

* FROV samples were pooled and sent as one sample
[a] Observed concentration.
[b] Estimated at system scale (based on average daily sludge output).
[c] Estimated at solution scale (based on resultant concentration of 'wet sludge' per percent culture water *i.e.* release ratio (percentage) – sludge: RAS volume).
Grey highlighted cells: Indicate extreme variability (CV>1) in values; rendering them unfit for generalization and comparison.

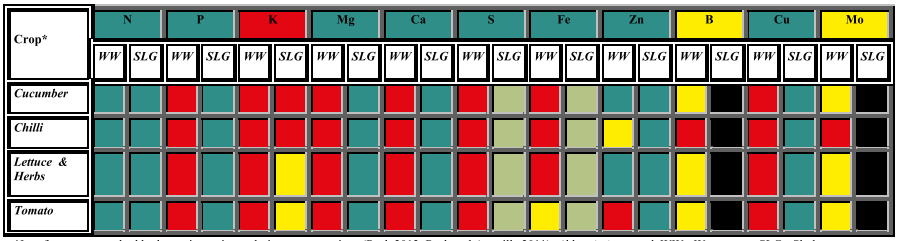
compelled by the responsibility to dispose their increasingly problematic RAS wastes to avoid legal penalties by environment regulation agencies or simply to diversify their income. Very often they are faced by lack of quantified reports or clear-cut recommendations that advocates the suitability (or unsuitability) of RAS farm effluents in upgrading to aquaponics. In such lack of confidence, some RAS farm managers take a 'leap-of-faith' while some deter their decision to upgrade to aquaponics (Anon, 2017). The present study besides commenting on the nutrient outputs by RAS farms also commented on the set of operational parameters that significantly differ or does not differ among the RAS farms (see results, Table 1). The set of operational parameters that do significantly differ among the RAS farms are the ones most likely to contribute to the success (degree of success) of the upgraded aquaponics venture, if focused upon and calibrated properly. On the other hand, the set of operational parameters which does not generally differ (significantly) among the farms can be overlooked from further calibration. This is the first kind of study which generated such type of information that too from operational RAS farms which are not yet converted to aquaponics.

In RAS systems, minerals have different solubilization rates and do not accumulate equally, which influences their concentrations in the water (Goddek et al., 2015). This was also reflected in the asynchronies we observed for some nutrients between their input and output *(see results)*. It is a well accepted notion that characteristics of RAS effluents are highly erratic and complex in nature (Goddek et al., 2015; Rijn, 2013; Seawright et al., 1998). Knowledge gap exists on identifying nutrients in RAS effluents that significantly differ or does not differ with varying scale and culture intensity of the farms. The present study gave a firsthand look on those nutrients – classified as consistent or inconsistent (see results). Future research should focus on investigating consistencies in nutrient stoichiometry and mass balance equations of effluents with varying farm conditions.

4.2. Nutrient output and meeting plant requirements

Contradictory views exist on the suitability and safety of RAS effluents to sustain plant growth under aquaponics condition. In a recent review, Bittsanszky et al. (2016) presented the diplomatic side of nutrient sustainability issues for aquaponics. Although the nutrient concentrations in fish process water (RAS) are significantly lower for most nutrients compared to hydroponic systems, plants do thrive in such sub-standard hydroponic solutions (Bittsanszky et al., 2016). They further attributed it to recent developments in the field of plant nutrition. Recently, the nearly two-century-old "Liebig's law" (briefly, plant growth is controlled by the scarcest resource) has been superseded by complex algorithms that take interactions between the individual nutrients into account (Parent et al., 2013; Baxter, 2015). These methods do not allow a simple evaluation of the effects of changes in nutrient concentrations in a hydroponic or aquaponic system (Bittsanszky et al., 2016). Generally speaking - nitrogen, mainly nitrate, is the predominant macronutrient recycled from the RAS (Bittsanszky et al., 2016); also supported by the present study. P and K are often scarce in RAS water and need to be supplemented (Bittsanszky et al., 2016; Monsees et al., 2017a, b); agreeing only with K in the present study as sludge had adequate P. Rakocy et al. (2006) opined otherwise – K, Ca, Mg are usually deficient to support plant growth; present observations contradict this view as sludge may completely mask deficiencies observed in RAS wastewater. Additional K, Ca and Mg supply can be improved by modifying the choice of pH buffers used (e.g. $Ca(OH)_2$, KOH, $CaMg(CO_3)_2$ used alternatively in combination) (Rakocy et al., 2006). Data from Bittsanszky et al. (2016) clearly show that most plant nutrients except Cu, S and Ca were at significantly lower concentrations in fish water; complying to our observations in water phase (wastewater). In terms of micronutrients - Fe, Mn, B, Mo do not accumulate significantly in RAS waters with respect to cumulative feed input (Rakocy et al., 2006); partly agreeing to our observations on B and Mo. Fe is the most commonly supplemented micronutrient supplementation in aquaponics (Rakocy et al., 2006); although we suspect Fe to be present in sufficiently high amount in sludge. Yavuzcan Yildiz et al. (2017) adds Cu and Zn to the aforementioned list of deficient micronutrients; not deficient as per our estimate if sludge taken into consideration. Promising studies have shown higher plant productivity in aquaponics comparable to hydroponics despite lower concentrations of macronutrients; attributed to 'plant beneficial micro-organisms' present in RAS effluents that can be taken up for future studies (Palm et al., 2018). Thus, a high level of

Table 5
Capacitogram of RAS farms in meeting some (prioritized) plant-essential nutrient thresholds for common aquaponics crops.



*In reference to standard hydroponic nutrient solution concentrations (Resh 2012, Resh and Anguilla 2011). *Abbreviations used:* WW= Wastewater, SLG= Sludge

Legends

Adequate= above recommended concentration

(apply wastewater or sludge 'as-it-is')

Sufficient= extrapolated based on observations (refer text)

(apply sludge 'as-it-is')

Partly sufficient= above Q3 of recommended concentration and below recommended concentration.

(supplementary fertilizer to be used or increased water exchange/sludge release necessary)

Deficient= below Q3 of recommended concentration

(Complete fertilization necessary. Beyond capacity of water exchange or sludge release manipulation to reach desired concentration)

Not detected = suspected absence.

(Complete fertilization necessary. Beyond capacity of water exchange or sludge release manipulation to reach desired concentration)

disparity in information on nutrient status of RAS effluents to sustain plant growth is evident from these examples. The present study attempted to 'clear the air' regarding these discrepancies under practical conditions (commercial RAS farm effluents) and beyond experimental systems.

The present study strongly advocates re-use of sludge as-it-is or in mineralized form. Information on available sludge digestion technologies can be found in Goddek et al. (2015), Martins et al. (2010), Palm et al. (2018), Yavuzcan Yildiz et al. (2017). According to Lennard (2015), at least 80% by weight (and often more) of the nutrients required for optimal plant growth are derived from fish waste alone. We infer this is not possible without taking sludge into consideration. Rakocy et al. (2006) estimated that in closed RAS with water exchange as low as 2%, dissolved nutrients accumulate in concentrations like those in hydroponic nutrient solutions. Nevertheless, most nutrients can be recycled from the fish sludge, to sustain an aquaponics operation without significant external fertilizer input (Monsees et al., 2017a); strongly supported by our data. Brod et al. (2017) applied dried fish sludge from RAS on 'agricultural' land and achieved a relative agronomic efficiency compared with mineral fertilizer of 50–80%. A crucial item in aquaponic systems is pH stabilization. Maximum nutrient absorption by plants occurs in mildly acidic conditions (pH 5.5–6.5 units) while pH in RAS waters are purposively kept neutral to alkaline (7–8 units) (Yavuzcan Yildiz et al., 2017). Allowing sludge digesta or raw sludge itself may likely overcome the pH conflict by dampening the pH values of resultant solution to be more skewed towards plant requirements; sludge has acidic reaction (Rijn, 2013). On the other hand, re-using sludge or its digesta to mask nutrient deficiencies in RAS wastewaters may make the process water returning to fish culture units progressively turbid; undesirable for RAS especially biofilters (Junge et al., 2017; Badiola et al., 2012). If the situation demands, decoupling of fish rearing and plant culture unit is a safer option to manipulate acidic pH conditions for plants and clearer water for fish – to address welfare and aesthetic issues in culture systems (Monsees et al., 2017a, b, Yavuzcan Yildiz et al., 2017).

Addition of sodium bicarbonate ($NaHCO_3$) to aquaponic systems for pH control is not advised; high Na^+ in the presence of Cl is phytotoxic and retards uptake of other nutrients. Rakocy et al. (2006) recommended an upper ceiling of Na^+ concentration of 50 mg L^{-1}; which was clearly breached in our findings. Contradictions occur on this aspect as well. Reviewed in Resh (2016) - the use of saline water for hydroponic growing of crops have been investigated by several workers; possibilities exist within upper ceiling as high as 1180 mg L^{-1} Na (molar mass basis from 3000 mg L^{-1} NaCl) given a few 'simple' considerations (see, Resh, 2016). Our data indicates - Na concentration (in either wastewater or sludge) seldom crossed 350 mg L^{-1} (i.e. around Q1 of the critical limit). In this light, *the prejudice of a 'definite' Na toxicity for plants should be re-visited, preferably less prioritized.* Nonetheless, with readily available RO (reverse osmosis) equipments and more complicated desalinization units these days, it is easy to remove the salts from RAS effluents (Goddek and Keesman 2018; Resh, 2016); not suggested as it may also reduce other nutrients (salts, e.g. S) in the solution. This situation can be easily avoided if the RAS farms use a combination of $Ca(OH)_2$, KOH and $CaMg(CO_3)_2$, discontinuing $NaHCO_3$ (Rakocy et al., 2006); strongly advised and backed by our data from farm A. Contrary to concerns raised from time to time regarding heavy metal accumulation and/or discharge by RAS farms (Cao et al., 2007; Martins et al., 2010), we found no such threats since the concentration of heavy metals in effluents were 'absolutely safe' (concentrations far below Q1 of pollution thresholds); also highlighted by Ebeling and Timmons (2012).

4.3. Modeling of operational influences on nutrient output

Limited information is available on modeling operational influences on nutrient output through RAS effluents. Based on our personal experience and literature search, this can be attributed to two reasons: (a) due to inherent complex nature of RAS systems itself (Monsees et al., 2017a, b), and, (b) most of the modeling attempts going un-reported due to non-realization of 'convincing' models. Limited modeling efforts, till now, have mostly concentrated on optimizing 'fish feed input (fish culture volume): plant culture area ratio' (*reviewed in*, Buzby and Lin, 2014) and recently on 'desalinization needs of aquaponics' (Goddek and Keesman 2018). Some thumb-rule models have also been listed in Ebeling and Timmons (2012) that are instrumental in planning RAS systems for emerging entrepreneurs. Interestingly, most of well-understood operational influences in RAS having implications on nutrient outputs (e.g. Ebeling and Timmons, 2012; Martins et al., 2010; Rakocy et al., 2006; Rijn, 2013) failed to make direct 'statistical appearances' as predictors in our modeling attempt. Apart from six predictors identified in the present study (viz. wastewater volume, sludge: RAS-volume ratio, feeding rate, feed micronutrient loading, FCR and stocking biomass) most of the 'well-known' operational influences failed to show any significant predictable power in deciding nutrient throughput from RAS systems. Moreover, not all the nutrients can be directly predicted or have clear cut dependencies between wastewater and sludge concentrations. Concentrations of some nutrients increase with increasing farm size and culture intensity, while in others no such tendency is apparent (see results). The limitations of our modeling approach have been clarified above. Despite that - *calibration of nutrient output from operational RAS farms may be primarily focused around the abovementioned (six) predictors.* By 'calibration' - we suggest adjusting these predictors *aka* six identified operational parameters for optimizing overall nutrient throughput from RAS farms; not merely viewing them as nutrient-specific calibration (as the models appear). The present modeling attempt generated some baseline information,

with intentions to draw-in contemplations from the global community on whether and how the predictors of nutrition output can be further precised. Nonetheless, some degree of predictability exists in RAS nutrient throughputs using limited but few available means.

5. Conclusion

Contradictory views exist on the suitability and safety of RAS effluents to sustain plant growth under aquaponics condition. The present study attempted to 'clear the air' regarding these discrepancies under practical conditions (commercial RAS farm effluents) and beyond experimental systems. Diplomatic advisories and lack of clear-cut scientific conclusion tend to retard adoption of any emerging technology. The purpose of the present study was concluded by generating applied information that can aid in future conversions, rather 'upgrades', of operational RAS farms to semi-commercial Aquaponic ventures. We emphasize - *despite inherent complexity of RAS effluents,* the *conversion of RAS farms to semi-commercial aquaponics should not be deterred by nutrient insufficiency or nutrient safety arguments.* Incentivizing RAS farm wastes (nutrients) through semi-commercial aquaponics should be encouraged - sufficient and safe nutrients are available.

Declaration

The study was a part of a national project of the Czech Republic. All necessary data have been provided in the manuscript and through supplementary files.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.05.130.

References

Anon, 2017. Information Obtained from the Farm Managers during the Study Period.
Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges. Aquacult. Eng. 51, 26–35.
Baxter, I., 2015. Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J. Exp. Bot. 66 (8), 2127–2131.
Bittsanszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B., Kőmíves, T., 2016. Nutrient supply of plants in aquaponic systems. Ecocy 2 (2), 17–20.
Blidariu, F., Grozea, A., 2011. Increasing the economical efficiency and sustainability of indoor fish farming by means of aquaponics-review. Sci. Pap. Ani. Sci. Biot. 44 (2), 1–8.
Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., Corner, R., 2010. Aquaculture: global status and trends. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365 (1554), 2897–2912.
Brod, E., Oppen, J., Kristoffersen, A.Ø., Haraldsen, T.K., Krogstad, T., 2017. Drying or anaerobic digestion of fish sludge: nitrogen fertilisation effects and logistics. Ambio 46 (8), 852–864.
Buzby, K.M., Lin, L.S., 2014. Scaling aquaponic systems: balancing plant uptake with fish output. Aquacult. Eng. 63, 39–44.
Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., Diana, J., 2007. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. Environ. Sci. Pollut. Res. 14 (7), 452–462.
Ebeling, J.M., Timmons, M.B., 2012. Recirculating Aquaculture Systems. Aquaculture Production Systems. pp. 245–277.
Eurostat, 2018. Production from Aquaculture Excluding Hatcheries and Nurseries (From 2008 Onwards) [fish_aq2a]. Available online: https://ec.europa.eu/eurostat/web/fisheries/data/database, Accessed date: 26 June 2018.
FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Food and Agriculture Organization of the United Nations, Rome.
Forchino, A.A., Lourguioui, H., Brigolin, D., Pastres, R., 2017. Aquaponics and sustainability: the comparison of two different aquaponic techniques using the life cycle assessment (LCA). Aquacult. Eng. 77, 80–88.
Fornshell, G., Hinshaw, J.M., 2008. Better management practices for flow-throughaquaculture systems. In: Tucker, C., Hargreaes, J. (Eds.), Environmental BestManagement Practises for Aquaculture. Blackwell Publishing Company, Oxford.
Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H., Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. Sustain. Times 7 (4), 4199–4224.
Goddek, S., Keesman, K.J., 2018. The necessity of desalination technology for designing and sizing multi-loop aquaponics systems. Desalination 428, 76–85. https://doi.org/10.1016/j.desal.2017.11.024.
Hlavač, D., Masílko, J., Anton-Pardo, M., Hartman, P., Regenda, J., Vejsada, P., Mraz, J., Adamek, Z., 2016. Compound feeds and cereals as potential tools for improved carp Cyprinus carpio production. Aquacul. Envir. Inter. 8, 647–657.
Hoevenaars, K., Junge, R., Bardocz, T., Leskovec, M., 2018. EU policies: new opportunities for aquaponics. Ecocy 4 (1), 10–15.
Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C., Khanal, S.K., 2015. Effect of plantspecies on nitrogen recovery in aquaponics. Bioresour. Technol. 188, 92–98.
Joly, A., Junge, R., Bardocz, T., 2015. Aquaponics business in Europe: some legal obstacles and solutions. Ecocy 1 (2), 3–5.
Junge, R., König, B., Villarroel, M., Komives, T., Jijakli, M.H., 2017. Strategic points in aquaponics. Water 9, 182.
Konig, B., Junge, R., Bittsanszky, A., Villarroel, M., Komives, T., 2016. On the sustainability of aquaponics. Ecocy 2 (1), 26–32.
Lennard, W.A., 2015. AQUAPONICS: a nutrient dynamic process and the relationship to fish feeds. World Aquacult. 20–23 September 2015.
Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X., Semmens, K., 2014. An international survey of aquaponics practitioners. PLoS One 9 (7), e102662.
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.
Martins, C.I.M., Eding, E.H., Schneider, O., Rasmussen, R., Olesen, B., Plesner, L., Verreth, J.A.J., 2005. Recirculation aquaculture systems in Europe. CONSENSUS. Oostende, Belgium, consensus working group. Eur. Aquacult. Soc 31.
Martins, C.I.M., Eding, E.H., Verdegem, M.C., Heinsbroek, L.T., 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. Aquacult. Eng. 43 (3), 83–93.
Maucieri, C., Nicoletto, C., Junge, R., Schmautz, Z., Sambo, P., Borin, M., 2018. Hydroponic systems and water management in aquaponics: a review. Ital. J. Agron. 13 (1/1012).
McDonald, J.H., 2014. Handbook of Biological Statistics, third ed. Sparky House Publishing, Baltimore, MD, USA, pp. 180–185.
Mchunu, N., Lagerwall, G., Senzanje, A., 2018. Aquaponics in South Africa: results of a national survey. Aquacul. Rep. 12, 12–19.
Monsees, H., Keitel, J., Paul, M., Kloas, W., Wuertz, S., 2017a. Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions. Aquacult. Env. Inter. 9, 9–18.
Monsees, H., Kloas, W., Wuertz, S., 2017b. Decoupled systems on trial: eliminating bottlenecks to improve aquaponic processes. PLoS One 12 (9), e0183056.
Murray, F., Bostock, J., Fletcher, D., 2014. Review of Recirculation Aquaculture System Technologies and Their Commercial Application. RAS Technologies and Their Commercial Application – Final Report. Stirling Aquaculture. https://dspace.stir.ac.uk/bitstream/1893/21109/1/HIE RAS Study Final Updated.pdf.
Palm, H.W., Knaus, U., Appelbaum, S., Goddek, S., Strauch, S.M., Vermeulen, T., Jijakli, M.H., Kotzen, B., 2018. Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. Aquacult. Int. 26 (3), 813–842.
Parent, S.É., Parent, L.E., Egozcue, J.J., Rozane, D.E., Hernandes, A., Lapointe, L., Gentile, V.H., Naess, K., Marchand, S., Lafond, J., Mattos Jr., D., 2013. The plant ionome revisited by the nutrient balance concept. Front. Plant Sci. 4, 39.
Rakocy, J.E., Masser, M.P., Losordo, T.M., 2006. Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture, vol. 454. SRAC publication, pp. 1–16.
Resh, H.M., 2016. Hydroponic Food Production: a Definitive Guidebook for the Advanced Home Gardener and the Commercial Hydroponic Grower. CRC Press.
Rijn, J.M., 2013. Waste treatment in recirculating aquaculture systems. Aquacult. Eng. 53, 49–56.
Schneider, O., Sereti, V., Eding, E.H., Verreth, J.A.J., 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacult. Eng. 32 (3–4), 379–401.
Schneider, O., Verreth, J., Eding, E.H., 2002. Framework introduction of zero nutrient discharge aquaculture by farming in integrated recirculating systems in Asia: Zafira. In: World Aquaculture 2002, Beijing. World Aquaculture Society, USA.
Seawright, D.E., Stickney, R.R., Walker, R.B., 1998. Nutrient dynamics in integrated aquaculture–hydroponics systems. Aquacult 160 (3–4), 215–237.
Short, G., Yue, C., Anderson, N., Russell, C., Phelps, N., 2017. Consumer perceptions of aquaponic systems. HortTechnology 27 (3), 358–366.
Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods, eighth ed. Iowa State Univ. Press Iowa, Ames.
Yavuzcan Yildiz, H., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D., Parisi, G., 2017. Fish welfare in aquaponic systems: its relation to water quality with an emphasis on feed and faeces—a review. Water 9 (1), 13.

CHAPTER 3

VERMICOMPOSTING OF SLUDGE FROM RECIRCULATING AQUACULTURE SYSTEM USING *EISENIA ANDREI*: TECHNOLOGICAL FEASIBILITY AND QUALITY ASSESSMENT OF END-PRODUCTS

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Vermicomposting of sludge from recirculating aquaculture system using *Eisenia andrei*: Technological feasibility and quality assessment of end-products

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ABSTRACT

Intensive aquaculture is an important and fast-growing food production industry generating significant amounts of nutrient-rich sludge, which represents a potential environmental threat. Vermicomposting aquacultural sludge has been suggested, but remained poorly understood – only survival and growth of initial earthworm stocks have been assessed so far. The present study provides a comprehensive evaluation of the production system, examining vermicomposting of three types of sludge each at four inclusion levels and the possibility of further utilising end-products (vermicomposts and earthworms). Through an 18-week experiment, high survival of initial earthworm stocks, exceeding 90% among treatments up to week 6, was documented. Higher inclusion levels and sludge types richer in nutrients positively influenced individual weight of initial stocks and their reproduction indices (cocoon and juvenile production). The most progressive treatments sustained >300 juveniles in experimental incubators containing 200 g dw of initial substrates. Original sludge and final vermicomposts were found suitable for use in agriculture, complying with limits for heavy metals given in the most usually applied regulations. In relation to the heavy metals, earthworms were found to be a generally safe feed for fish. Only arsenic concentrations may occasionally exceed given limits. Still, observed concentrations are considered safe, presuming arsenic presence primarily in organic forms having largely reduced toxicity. Vermicomposting is recommended as a clean and sustainable technology transforming aquaculture sludge into highly valuable vermicompost and earthworm biomass.

1. Introduction

Fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Since 2014, more than half of all fish for human consumption came from aquaculture. Its extent, diversification and intensification make aquaculture one of the fastest growing food-producing sectors globally. As a result of the magnitude and intensity of aquaculture production, issues related to its long-term sustainability and environmental impacts have become more pronounced (FAO, 2016).

Many flow-through and cage aquaculture systems have minimally effective, or a complete lack of, systems for treating effluent waters (van Rijn, 1996). This leads to the unacceptable rate of eutrophication of adjacent recipients. Increasingly strict regulations on discharged waters, combined with a limited number of suitable sites for conventional aquaculture systems, has led to the development of recirculating aquaculture systems (RAS). RAS have distinct advantages compared with conventional technologies, since the amount of effluent water is much lower, while the concentration of solid wastes is substantially higher. This makes treatment of effluent waters more effective, easier and cheaper (Blancheton et al., 2007). Despite improvements in digestibility of commercial feeds provided to the cultured fish, some 15% of consumed feeds is converted to faeces (Reid et al., 2009) and some 5% not consumed (Bureau et al., 2003). For RAS, an additional biomass of microorganisms is released mainly from biofilters (van Rijn, 1996). All of these resources are particularly rich in organic

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matter and nutrients.

The resulting aquacultural sludge is extremely susceptible to putrefaction and may contain various pathogens. This makes its direct utilisation as a fertiliser applied on the agricultural lands problematic. Its dewatering and stabilisation prior to such application is recommended (Bergheim et al., 1998). Further ways of RAS sludge utilisation are rarely applied and include biogas production (del Campo et al., 2010), composting or vermicomposting (Marsh et al., 2005).

Vermicomposting is a complex biological and ecological process of accelerated bio-oxidation and stabilisation of organic material. In contrast to traditional composting, it involves the joint action of earthworms and microorganisms without a thermophilic phase (Edwards, 2004), exhibiting reduced emission of greenhouse gases (Nigussie et al., 2016). The applicability of this biotechnology has been shown for a wide range of organic matrices. Vermicomposting allows transformation of potentially problematic organic solid waste into highly valuable end-products – vermicompost and biomass of earthworms (Lim et al., 2016).

Marsh et al. (2005) proposed the possibility of vermicomposting RAS sludge mixed with shredded cardboard for use as a feedstocks for the earthworm *Eisenia fetida* (Savigny, 1826). However, this study evaluated survival and growth of initial stocks only, which is insufficient for complete evaluation of the applicability of this technology. The qualitative parameters of RAS sludge vary between farms and the same is expected for different technological sections of a given RAS. Low sludge inclusion levels do not promote a suitable vermicomposting process while overdosing may led to the mortality of initial stocks. In the present study vermicomposting of three kinds of sludge obtained from a commercial RAS mixed with shredded wheat straw at four inclusion levels each was tested. Expanding on previously evaluated parameters, cocoon and juvenile production of *E. andrei* Bouché, 1972 were assessed during an 18-week experiment. Final vermicomposts were characterised and, together with earthworm biomass, contents of selected heavy metals were measured. Two of the most commonly used commercial fish diets and a dominant market-sized fish conventionally reared on the farm from which RAS sludge originated were also analysed for heavy metals. This allows qualitative evaluation of resultant earthworms as an alternative diet for feeding fish.

2. Material and methods

2.1. Substrates and earthworms

Three kinds of aquaculture sludge were obtained from a Trout farm (Mlýny, Žár, Czech Republic). Two kinds of sludge were acquired directly from the RAS. The sludge sampling sites were located either in the outlet channel from the culturing units (derived vermicomposting treatments thereafter indicated as O) or in the immersed biofilter (B). The third sludge was sourced from an adjacent pond which is used for sedimentation of effluent water (P). For a detailed description of the RAS and location of sampling sites see Supplementary Information (Fig. S1) and Buřič et al. (2016).

The O sludge was taken manually with a fine hand-held mesh screen from sedimentation zones in the RAS. The B sludge was pumped from immersed biofilters during the desludging process. For P sludge, a top layer of fine sediment was scraped manually from close to the inflow of effluent water in the sedimentation pond. Sludge of B and P origin were further sieved through a stainless steel sieve with a mesh size of 0.65 × 0.65 cm in order to eliminate large particles, mainly plastic elements (RK Plast A/S, Skive, Denmark) used for biofiltration in the RAS. Resulting sludge samples were left on polyamide meshes (mesh size of 109 μm) for 2 h for gravitational dewatering. Composition of the sampled sludge is shown in Table 1. Unless further specified, analyses were done in the accredited laboratory of the AGRO-LA, spol. s.r.o., Jindřichův Hradec, Czech Republic. Organic carbon was determined at the Institute of Soil Biology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic. The analyses followed standardised methods of Zbíral and Honsa (2010) for dry matter, pH, calcium, magnesium, phosphorus, potassium and sodium, and Zbíral et al. (2011) for organic matter, total organic carbon and total nitrogen. Heavy metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc) were determined at the accredited laboratory of the State Veterinary Institute in Prague, Czech Republic according to Zbíral (2011). The mercury concentration was determined by the AAS (AMA254, Altec, Czech Republic), chromium (Cr) by GF-AAS (SpectrAA 220Z, Varian, Australia) and the other metals by ICP-MS, Varian, Australia.

Suitability of biological material for vermicomposting is often determined by its humidity and C:N ratio. Despite species-specific differences in requirements among earthworm species typically involved in vermicomposting, these parameters are usually around 80% humidity and 25:1 C:N (Ndegwa and Thompson, 2000). The raw RAS sludge had low dry matter content and was rich in nitrogen (Table 1). In order to mitigate the impacts of both high humidity and nitrogen content, dry carbonaceous material (shredded wheat straw) was included. Wheat is one of the most widely grown cereal crops globally, being cheap and widely available. For composition of the straw see Table 1.

The stock of earthworm species, originally purchased as *E. fetida*, was obtained from a commercial supplier (Tomsovy žížaly, 2008). This species and *E. andrei* are closely related epigeic earthworms often utilised in vermicomposting (Edwards, 2004), and species may sometimes have been incorrectly assigned, particularly in older literature. Applying molecular methods (Dvořák et al., 2013) to our earthworm stock revealed the correct species assignment to be *E. andrei* (Dvořák, personal communication, July 2012).

2.2. Experimental set-up

2.2.1. Tested treatments and incubators

Experimental substrate mixtures consisting of 5, 10, 20 and 30% dry weight (dw) of respective sludges and shredded wheat straw were manually mixed. The abbreviated codes thereafter refer to the sludge content e.g. B20 for a treatment containing 20% RAS sludge sourced from the biofilter (B). Shredded wheat straw itself served as the control. Each treatment was tested in triplicate, each 200 g, with an initial humidity of 75% (i.e. 800 g wet weight, ww). The initial humidity was adjusted using distilled water.

The substrates were placed in the experimental incubators, which were made from a polypropylene pipe (inner diameter 12.5 cm, height 22.5 cm) with a fine (mesh size of 109 μm) polyamide mesh fixed on its lower part. This mesh prevented escapes of earthworms and allowed drainage of any excessive water. The upper part was closed with a tightly fitting lid with a hole in the centre (2.2 cm in diameter), which was overlain with a mesh glued on its inner side to allow ventilation. Completed incubators were placed on polypropylene plates (17 cm in diameter) which collected excess water. For more details on the construction of experimental incubators see Supplementary Information (Fig. S2).

2.2.2. Stocking of earthworms, temperature and humidity maintenance

In order to reduce the possibility of earthworm mortality in treatments with higher concentrations of RAS sludge (presumably caused by the toxicity of ammonia), all incubators, once filled, were left in a temperature-controlled room for a one-week pre-

Table 1
Composition of aquaculture sludge and shredded wheat straw used in the study, and national and international limit values for selected heavy metals in sludge and vermi/composts used in agriculture. Data are expressed on a dry weight basis.

Parameter	O sludge	B sludge	P sludge	Straw	Limit value
Dry matter (%)	9.87	15.20	30.90	86.60	
pH (H_2O)	5.41	5.70	6.31	6.35	
pH ($CaCl_2$)	5.24	5.46	6.09	6.10	
Organic matter (g kg^{-1})	547.0	358.0	179.0	932.0	
Total organic carbon (g kg^{-1})	482.8	314.1	160.0	719.9	
Total nitrogen (g kg^{-1})	28.6	22.5	11.7	9.9	
C:N ratio	16.9	14.0	13.7	72.7	
Calcium (g kg^{-1})	82.0	103.0	114.0	3.8	
Magnesium (g kg^{-1})	9.6	10.1	12.9	0.97	
Phosphorus (g kg^{-1})	14.1	11.2	11.1	1.4	
Potassium (g kg^{-1})	31.4	4.2	4.9	12.3	
Sodium (g kg^{-1})	1.4	0.1	0.9	0.07	
Arsenic (mg kg^{-1})	4.02	5.26	5.29	0.04	30[a]
Cadmium (mg kg^{-1})	0.63	0.46	0.42	0.08	5[a], 39[b], 40[c]
Chromium (mg kg^{-1})	17.0	99.5	98.7	0.8	200[a], 1,200[b]
Copper (mg kg^{-1})	14.3	22.3	12.8	3.3	500[a], 1,500[b], 1,750[c]
Lead (mg kg^{-1})	6.0	6.7	8.8	0.2	200[a], 300[b], 1,200[c]
Mercury (mg kg^{-1})	0.049	0.044	0.041	0.009	4[a], 17[b], 25[c]
Nickel (mg kg^{-1})	13.3	47.9	48.2	0.6	100[a], 400[c], 420[b]
Zinc (mg kg^{-1})	1,386.6	974.6	647.3	15.9	2,500[a], 2,800[b], 4,000[c]

[a] Decree of the Ministry of the Environment of the Czech Republic No. 437/2016 of the Code, 2016.
[b] Brinton (2000).
[c] EU (1986).

composting. The mean temperature of substrates (±SD) was 15.5 ± 0.5 °C. Temperature was recorded hourly with automatic Minikin dataloggers (Environmental Measuring Systems, Brno, Czech Republic). Ten adults of *E. andrei*, determined by the presence of a well-developed clitellum, were added to each incubator. All adults were weighed individually prior to the experiment. Animals were gently removed from the bedding by hand, and placed on a wet absorbent tissue paper which removed the majority of bedding from their surface. The remaining material was removed manually using a fine entomological tweezers. An analytical balance (Kern & Sohn GmbH, Balingen, Germany) was used for weighing the earthworms to the nearest mg. The initial individual weight of adults was identical among incubators (ANOVA, $F_{38,351} = 0.030$, $p = 1.0$), with a mean weight of 399 ± 89 mg. After stocking with earthworms, the temperature was elevated to 19.5 ± 0.6 °C for a week, followed by 27.0 ± 1.5 °C until the end of the experiment. Incubators were kept in darkness and their position was randomised throughout the experiment.

Suitable humidity was maintained through the experiment and was subjectively evaluated by pinching a sample of the substrate – humidity was considered sufficient if a few drops of water appeared between the fingers. When checking development of vermicultures (for timing see section 2.2.3), substrates were provided with additional moisture if required. Excessive water collected in the standing plates beneath was used for this purpose. If this was not available, distilled water was sprayed on the substrate surface.

2.2.3. Earthworm survival, growth and reproduction

Survival rate of adults to determine the success of the initial stocking was monitored after one week. Further checks were done every even week after stocking, when individual weights of original stock (as described above), as well as the number of cocoons and juveniles were determined. The individual weight of the original stock was evaluated up until week 16, after which it was impossible to reliably distinguish between the originally stocked earthworms and their offspring. The experiment was terminated at the end of week 18. The number of juveniles at the termination of the experiment refers to all free-living earthworm individuals, i.e. predominantly juveniles, but also adult survivors as well as low numbers of their adult offspring (exact counts were not carried out).

2.2.4. Content of heavy metals in end-products, conventional fish diets and fish from the farm

The resulting vermicomposts were separated from earthworm stocks manually (incl. cocoons) and analysed as original substrates (Table 1). Juveniles and adults were left for 24 h in plastic boxes (16 × 11.5 × 6 cm) containing wetted filter paper at 21.6 ± 0.5 °C to allow defecation. The obtained biomass of earthworms was then analysed for heavy metals.

Two of the most commonly used commercial fish diets (Efico Enviro 920 3 mm and Orbit 929 4.5 mm, BioMar A/S, Denmark, three different batches for each) and a dominant market-sized fish (a single fillet from three specimens of rainbow trout, *Oncorhynchus mykiss*, live weight of ca. 500 g) conventionally reared on the farm from which RAS sludge originated, were also analysed for selected heavy metals (as above, see section 2.1.).

2.3. Statistical analyses

Survival rates calculated as percent survival from the initial stock were arc-sine transformed. Kolmogorov–Smirnov and Cochran's C tests were performed on the data, assessing normality and homoscedasticity. When assumptions for using parametric tests were confirmed, one-way ANOVAs followed by Tukey's HSD *post hoc* tests were performed. Non-parametric Kruskal–Wallis's tests followed by multiple comparisons of mean ranks for all groups were applied on numbers of cocoons. Data were analysed using Statistica 12.0 (StatSoft, Inc.). The null hypothesis was rejected at $\alpha < 0.05$ in all tests. All data are presented as means ± SD.

3. Results and discussion

Expanding on the pilot study by Marsh et al. (2005) which utilised the earthworm *E. fetida*, the present study provides a comprehensive evaluation of the successful use of earthworms for vermicomposting sludge from RAS by means of *E. andrei*. These earthworms are widely distributed throughout temperate regions

and are the most commonly used species in vermicultures (Edwards, 2004). Literature suggests that *E. andrei* is the preferred candidate for use in temperate zones, due to its higher reproduction indices (Domínguez et al., 2005) as well as elevated innate defence mechanisms (Dvořák et al., 2013) which are beneficial when exposed to RAS sludge rich in potentially pathogenic microorganisms.

The high survival of the initial *E. andrei* stock (exceeding 90% among treatments up to week 6; Fig. 1) enabled successful reproduction and hence vermicomposting. Significant differences were seen only between treatment O30, which had absolute survival, compared with groups B30 and P5-10 at week 12. Final survival rates usually ranged from 40 to 70%. For detailed values and statistical results see Supplementary Information (Table S1). Considering there was no mortality even in the most sludge-rich treatments for lengthy periods of time (Fig. 1), the one-week precomposting stage was probably not necessary and initial sludge inclusion levels could possibly have been increased (if more efficient methods of dewatering RAS sludge are applied). Initial earthworm stocks may experience total mortality if exposed to unsuitable feedstocks e.g. fresh cattle and pig manures. Mass mortalities of initial stocks are often related to limiting conditions, including unsuitable pH, high concentrations of ammonia, high humidity – hence low aeration, elevated temperature or large quantities of inorganic salts (Edwards, 2004). The study by Marsh et al. (2005) on vermicomposting RAS sludge is not easily compared with the present study. Their earthworm stock was adapted to feedstock containing RAS sludge for an unspecified time, at least for the second experiment, and experimental sludge was frozen prior to the trials, which might mitigate its toxicity. The toxicity of high inclusion levels of sludge from RAS to earthworms is hard to predict due to widely varying qualitative characteristics. A preliminary test is recommended in order to avoid possible losses of inoculum. Such a test would consist of simply placing a small group of earthworms into a perforated box or cage containing the particular substrate to be used for vermicomposting (identically positioned directly in a vermicomposting bed), where survival of inoculum is checked over the course of at least a few days.

The control substrate and treatments with low inclusion levels (5 and 10%) of RAS sludge were not rich enough to promote growth of the original stock (Fig. 2). The opposite trends were apparent in the treatments rich in sludge, where growth peaked between weeks 4 and 6. At least one sludge-rich treatment reached higher values than the control between weeks 2 and 10. Treatments containing 30% of O sludge exhibited higher values than those of B and P origin between weeks 6 and 10. Final observed values were fairly similar among treatments. For details see Supplementary

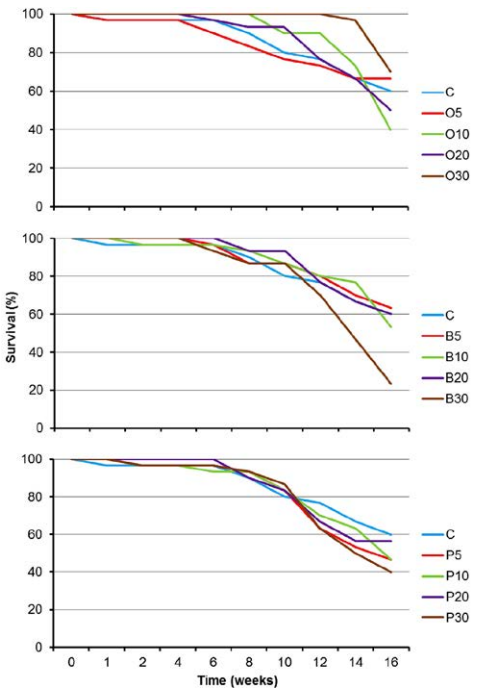

Fig. 1. Survival of the originally stocked *Eisenia andrei* adults in the experimental treatments – the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Data are presented as means.

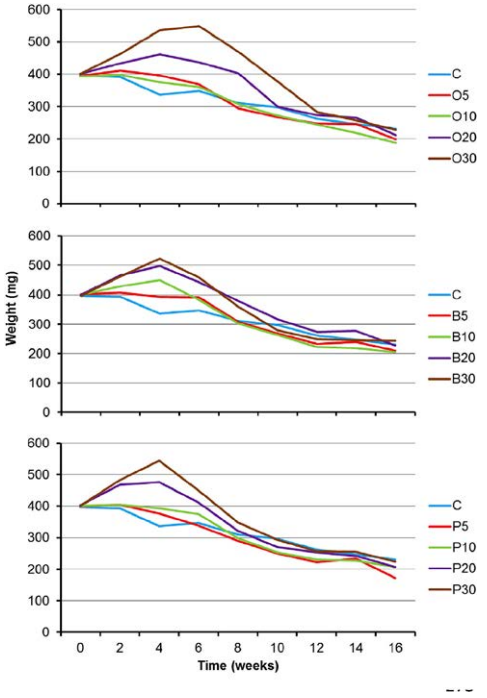

Fig. 2. The individual weight of originally stocked *Eisenia andrei* adults in the experimental treatments – the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Data are presented as means.

Information (Table S2).

Cocoons, which first occurred in week 2, sharply increased in numbers and peaked between weeks 4 and 6. These effects were mostly evident when compared with the control, while treatments containing O and B sludge attained higher absolute counts of cocoons than groups with the P sludge (Fig. 3). Later, the individual weight of the original stock, as well as counts of cocoons, converged on similar values among treatments, with very few cocoons counted after week 12, typically only a few per incubator (for details see Supplementary Information, Table S3). Juveniles first occurred in week 4, followed by a sharp increase peaking between weeks 10 and 12, even exceeding 300 juveniles per incubator on average (Fig. 4). Their counts stayed relatively stable in sludge-containing treatments, while the control tended to have lower numbers (for details see Supplementary Information, Table S4). An increase in the number of juveniles between weeks 16 and 18 can be partly attributed to the inclusion of the survivors of the initial earthworm stock, which ranged from 2 to 7 per incubator on average (Table S1) but most likely was attributed to the increase in counting thoroughness when carefully separating final vermicomposts from cocoons and free-living earthworms for further analyses. Obtained results confirm that the utilisation of RAS sludge provided suitable conditions for earthworm reproduction and was positively related to both the inclusion level and the composition of particular sludge types (Table 1). A decline in the weight of the original stock and in the production of cocoons is typical for bath cultures that do not allow progressive development of an earthworm population, unlike semi-continuous and continuous cultures. It is related to the carrying capacity of the particular environment, i.e. the depletion of resources and the accumulation of metabolites. It is likely that the individual mean weight of the original stock was affected by the energy and nutrient demanding process of cocoon production (for details see Koubová et al. (2012) and literature cited therein). It may be expected that such a decline is greater in treatments that facilitated more developed earthworm stocks in the initial phases of the experimental period.

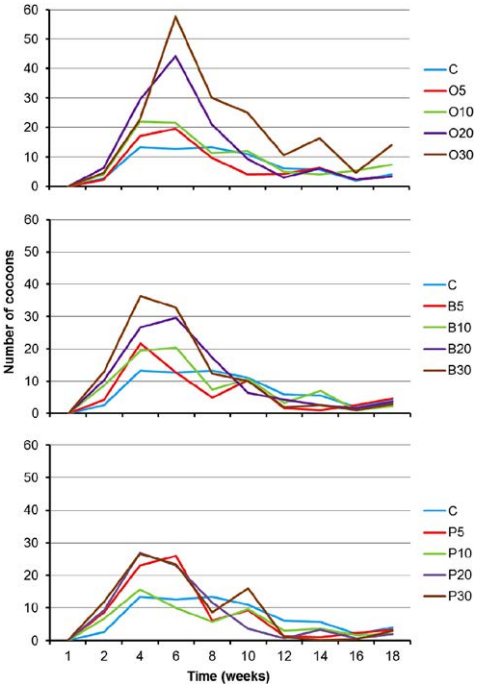

Fig. 3. Mean numbers of *Eisenia andrei* cocoons in the experimental treatments – shredded wheat straw as a control (C) or combined with aquaculture sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Data are presented as means.

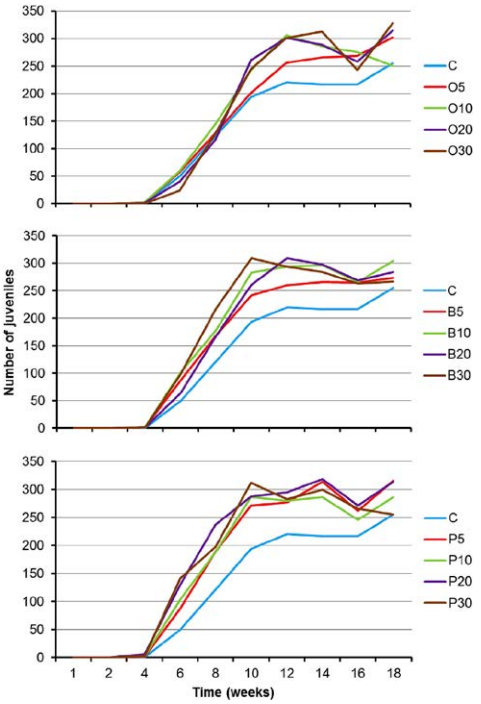

Fig. 4. The number of *Eisenia andrei* juveniles in the experimental treatments - the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Data are presented as means.

For details of changes in the appearance of substrates during vermicomposting see Supplementary Information (Fig. S3). The composition of the final vermicomposts after one-week pre-composting and 18 weeks of vermicomposting is summarised in Table 2. Although the humidity of the final vermicomposts was considered similar among treatments, the content of dry matter increased with the inclusion levels of sludge, particularly in those containing P sludge (Table 2). This suggests a lower water holding capacity of P groups, resulting from partial mineralisation prior to the experiment, as well as the presence of inorganic particles (sand). As is expected in the vermicomposting process (Albanell et al., 1988), a substantial portion of biodegradable organic matter

Table 2
The composition of final vermicomposts. Experimental treatments refer to the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Values with different lower case letters as superscripts are significantly different among treatments containing a given RAS sludge at different inclusion levels in each row. Values with differing capital letters in superscripts are significantly different among treatments containing identical inclusion levels among RAS sludge types in each row. Asterisks refer to differences between a given sludge-containing treatment and the control (one-way ANOVA, Tukey's HSD test, n = 3, $p < 0.05$). Data are presented as means ± SD and expressed on a dry weight basis.

Parameter	C	O5	O10	O20	O30	B5	B10	B20	B30	P5	P10	P20	P30
Dry matter (%)	13.6 ± 0.1	14.2 ± 0.5^{aB}	16.7 ± 1.2^{bA*}	17.2 ± 0.4^{bB*}	19.9 ± 0.2^{aB*}	14.9 ± 0.3^{cAB}	16.2 ± 0.6^{bcA}	17.8 ± 0.3^{abB*}	20.1 ± 1.6^{aB*}	15.8 ± 0.5^{cA}	18.6 ± 1.1^{cA*}	22.8 ± 0.9^{bA*}	26.0 ± 1.0^{aA*}
pH (H_2O)	7.5 ± 0.1	7.5 ± 0.1^{aA}	6.0 ± 0.4^{dB*}	6.9 ± 0.8^{cA}	6.5 ± 0.3^{dB}	7.5 ± 0.1^{aA}	7.2 ± 0.2^{abA}	7.1 ± 0.1^{abA}	6.9 ± 0.3^{cAB}	7.6 ± 0.0^{aA}	7.2 ± 0.3^{abA}	7.2 ± 0.3^{abA}	7.5 ± 0.1^{aA}
pH ($CaCl_2$)	7.3 ± 0.1	7.3 ± 0.1^{aA}	5.9 ± 0.4^{dB*}	6.7 ± 0.7^{bA}	6.3 ± 0.3^{dB}	7.3 ± 0.1^{aA}	7.1 ± 0.2^{abA}	6.9± 0.1^{abA}	6.7 ± 0.3^{bAB}	7.4 ± 0.0^{aA}	7.0 ± 0.3^{abA}	7.0 ± 0.3^{abA}	7.2 ± 0.1^{aA}
Organic matter (g kg^{-1})	81 ± 0	77 ± 1^{aA}	75 ± 3^{bbA}	62 ± 4^{bcA*}	51 ± 8^{cdB*}	73 ± 3^{abA}	68 ± 5^{bbAB}	59 ± 5^{bcA*}	52 ± 3^{cA*}	66 ± 6^{bA}	56 ± 8^{abB*}	44 ± 4^{bcB*}	37 ± 1^{cB*}
Total organic carbon (g kg^{-1})	63 ± 2	61 ± 2^{aA}	57 ± 1^{aA}	47 ± 1^{bA*}	40 ± 2^{cA*}	59 ± 1^{aA}	55 ± 0^{aA*}	48 ± 2^{bA*}	36 ± 2^{cA*}	54 ± 1^{aB*}	43 ± 2^{bB*}	32 ± 3^{cB*}	25 ± 2^{dB*}
Total nitrogen (g kg^{-1})	2.3 ± 0.1	2.2 ± 0.2^{aA}	2.5 ± 0.1^{aA}	2.6 ± 0.4^{aA}	2.7 ± 0.7^{aA}	2.2 ± 0.1^{aA}	2.2 ± 0.0^{aB}	2.1 ± 0.1^{aAB}	1.7 ± 0.1^{bAB}	2.0 ± 0.1^{aA}	1.8 ± 0.0^{aC}	1.5 ± 0.1^{bB}	1.2 ± 0.1^{cB*}
C:N ratio	27.2 ± 1.4	27.1 ± 1.4^{aA}	22.7 ± 1.1^{abA}	18.2 ± 2.9^{bbA*}	15.8 ± 3.6^{cbA*}	26.7 ± 1.1^{aA}	25.2 ± 0.6^{abA}	22.9 ± 1.5^{abA}	20.7 ± 2.4^{bA}	27.3 ± 1.2^{aA}	24.0 ± 1.2^{aA}	21.5 ± 2.7^{abA}	20.8 ± 3.0^{abA}
Calcium (g kg^{-1})	1.4 ± 0.1	2.3 ± 0.1^{dB}	3.2 ± 0.4^{cB}	5.2 ± 0.1^{abbA*}	8.0 ± 1.8^{aaA*}	2.5 ± 0.0^{dB}	3.5 ± 0.1^{cdB*}	6.0 ± 0.2^{bbA*}	7.4 ± 0.6^{aaA*}	3.0 ± 0.1^{dA}	4.6 ± 0.2^{cdA*}	6.2 ± 0.6^{bbA*}	7.9 ± 0.2^{aaA*}
Magnesium (g kg^{-1})	0.3 ± 0.0	0.4 ± 0.0^{bA}	0.4 ± 0.1^{bA}	0.6 ± 0.1^{bA}	0.9 ± 0.1^{aaA*}	0.4 ± 0.1^{cA}	0.5 ± 0.1^{bcA}	0.6 ± 0.1^{abA}	0.7 ± 0.1^{aA}	0.4 ± 0.1^{aA}	0.5 ± 0.1^{aA}	0.6 ± 0.3^{aA}	0.7 ± 0.3^{aA}
Phosphorus (g kg^{-1})	0.5 ± 0.0	0.6 ± 0.0^{bA}	0.8 ± 0.0^{abA*}	1.1 ± 0.0^{abbA*}	1.6 ± 0.3^{aaA*}	0.6 ± 0.0^{dA}	0.7 ± 0.0^{cB}	0.9 ± 0.0^{bbB*}	1.0 ± 0.0^{aAB*}	0.6 ± 0.0^{cA}	0.7 ± 0.0^{bB}	0.8 ± 0.0^{aB*}	0.9 ± 0.0^{aB*}
Potassium (g kg^{-1})	4.1 ± 0.2	3.5 ± 0.2^{aA}	3.1 ± 0.1^{aA*}	2.6 ± 0.0^{aA*}	2.7 ± 0.6^{aA*}	3.5 ± 0.1^{aA}	3.3 ± 0.1^{aA}	2.7 ± 0.0^{bA*}	2.2 ± 0.1^{cA*}	3.4 ± 0.1^{aA}	2.9 ± 0.2^{bA*}	2.3 ± 0.2^{cB*}	1.8 ± 0.0^{dA*}
Sodium (g kg^{-1})	0.02 ± 0.0	0.04 ± 0.0^{cA}	0.06 ± 0.0^{bcA*}	0.09 ± 0.0^{bbA*}	0.15 ± 0.0^{aaA*}	0.03 ± 0.0^{dA}	0.04 ± 0.0^{cA}	0.06 ± 0.0^{bB*}	0.07 ± 0.0^{aB*}	0.04 ± 0.0^{cA}	0.05 ± 0.0^{cA}	0.06 ± 0.0^{bbB*}	0.08 ± 0.0^{aB*}

was reduced and pH showed a tendency towards neutrality. For brevity compare the original composition of shredded wheat straw and final control vermicompost (Tables 1 and 2). Relatively broad C:N ratios of final vermicomposts, ranging from 15.8 to 27.3 (Table 2) can be explained by the limited inclusion of nitrogen-rich RAS sludge at the beginning of the experiment. C:N ratio is traditionally used to determine the degree of vermicompost maturity. It is believed that a C:N ratio below 20 is indicative of acceptable maturity, while a ratio below 15 is preferable (Morais and Queda, 2003). Repeated inclusion of sludge, as occurs in semi-continuous and continuous cultures, may be beneficial under real conditions. Low final values of total nitrogen (Table 2) might suggest its volatilisation in the form of ammonia, which probably occurred mainly at the beginning of the experiment as is typical for freshly obtained nitrogen-rich substrates. Regular handling with substrates during controls, leading to their aeration, might also contribute to this process. Denitrification and dissimilatory reduction of nitrate can also not be overlooked since anaerobic processes occur in vermicomposts (Taylor et al., 2003). Despite regular handling of substrates, some localised anaerobic microhabitats may still persist. Earthworm guts sustain anaerobic conditions. This specific microenvironment favours the reduction of nitrate via the availability of high quality electron donors, such as sugars, organic and amino acids (Horn et al., 2006). Nitrogen is also incorporated in the biomass of earthworms. Zhenjun et al. (1997) refer to 54.6% of protein in *E. fetida* meal. Edwards (1985) reported 60–70% of protein in dry matter. Taking various nitrogen forms and typical nitrogen:protein conversion factors into account (Mariotti et al., 2008), this is well above the content of the original substrates (cf. Table 2). Other elements such as phosphorus, calcium and magnesium were as expected also incorporated into the earthworm biomass. Phosphorus is a key component of phospholipids, energy-bounding nucleotides and nuclear acids. Calcium is essential for the functioning of calciferous glands (Edwards, 2004). Zhenjun et al. (1997) refer to 27.5 g kg^{-1} of phosphorus and 15.5 g kg^{-1} of calcium in *E. fetida* meal.

Selected heavy metal concentrations of final vermicomposts is shown in Table 3. They reflect inclusion levels and the composition of the original substrates (Table 1) and, as a result of the decomposition process, are more concentrated in the final vermicomposts. For brevity compare the original composition of shredded wheat straw and final control vermicompost (Tables 1 and 3). Specific biotransformation pathways of selected heavy metals were also influenced by the presence of earthworms. Lead and nickel occurred in earthworms to a limited extent, while their absolute values were often greater than an order of magnitude in particular vermicomposts. Chromium was always below the detection limit in earthworms (Tables 3 and 4). As expected, heavy metal concentrations in the fish diets were lower compared to RAS sludge (Tables 1 and 5). Despite the presence of chromium, lead and nickel in these diets, they, together with cadmium, were not detected in the fish muscle (Table 5). Rather than completely lacking such an accumulation, there is a tendency for this to occur in the liver or hepatopancreas of aquatic animals (Kouba et al., 2010). Copper reached similar values among final vermicomposts with absolute concentrations reduced 2 to 3 times in earthworms (Tables 3 and 4). Fish muscle also contained reduced copper concentrations as compared with the diets provided (Table 5). It can be expected that tissue specific bioaccumulation at least partly contributed to this finding (Fallah et al., 2011). Mercury concentrations increased 2 to 5 times in earthworms compared to vermicomposts (Tables 3 and 4), which is in agreement with observations on fish muscle and fish diets (Table 5). Bioaccumulation of arsenic was apparent mainly when looking at the concentrations of final vermicomposts and respective earthworm stocks (Tables 3 and 4). RAS sludge (Table 1)

Table 3
Concentrations of heavy metals in final vermicomposts. Experimental treatments refer to the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Values with differing lower case letters in superscripts are significantly different among treatments containing given RAS sludge at different inclusion levels in each row. Values with differing capital letters in superscripts are significantly different among treatments containing identical inclusion levels among RAS sludge types in each row. Asterisks refer to differences between a given sludge-containing treatment and the control (one-way ANOVA, Tukey's HSD test, $n = 3$, $p < 0.05$). Data are presented as means ± SD and expressed on a dry weight basis in mg kg^{-1}.

Parameter	C	O5	O10	O20	O30	B5	B10	B20	B30	P5	P10	P20	P30
Arsenic	<0.05	$1.0 \pm 0.3^{cB*}$	$1.3 \pm 0.1^{bcA*}$	$2.7 \pm 0.8^{abA*}$	$3.4 \pm 0.2^{aA*}$	$0.9 \pm 0.1^{bB*}$	$2.4 \pm 0.9^{abA*}$	$2.8 \pm 0.3^{aA*}$	$3.7 \pm 0.5^{aA*}$	$2.0 \pm 0.5^{bA*}$	$2.4 \pm 0.1^{abA*}$	$3.8 \pm 0.5^{aA*}$	$3.7 \pm 0.6^{aA*}$
Cadmium	0.3 ± 0.0	0.4 ± 0.0^{bA}	0.5 ± 0.0^{abA}	$0.6 \pm 0.2^{abA*}$	$0.7 \pm 0.1^{aA*}$	0.3 ± 0.0^{bB}	0.4 ± 0.0^{abAB}	0.4 ± 0.1^{abA}	$0.5 \pm 0.0^{aAB*}$	0.3 ± 0.0^{bB}	0.4 ± 0.0^{abC}	0.4 ± 0.0^{abA}	0.4 ± 0.1^{aB}
Chromium	1.4 ± 0.3	2.4 ± 0.3^{aA}	7.3 ± 5.3^{aA}	5.9 ± 1.0^{aA}	$10.1 \pm 1.3^{aB*}$	3.3 ± 1.6^{bA}	3.3 ± 0.3^{bA}	5.3 ± 0.2^{abA}	7.5 ± 0.4^{aB}	6.0 ± 1.8^{aA}	7.2 ± 0.9^{aA}	$12.4 \pm 4.4^{aA*}$	$13.3 \pm 1.5^{aA*}$
Copper	13.1 ± 1.1	12.6 ± 1.2^{aA}	14.7 ± 2.7^{aA}	16.6 ± 5.4^{aA}	18.9 ± 4.3^{aA}	12.6 ± 1.7^{aA}	13.5 ± 1.4^{aA}	13.3 ± 2.7^{aA}	15.1 ± 0.5^{aA}	11.2 ± 1.1^{aA}	13.3 ± 1.1^{aA}	12.8 ± 0.2^{aA}	12.9 ± 0.6^{aA}
Lead	0.9 ± 0.2	1.5 ± 0.2^{bA}	2.6 ± 0.3^{abA}	$5.2 \pm 1.5^{aA*}$	$5.6 \pm 1.2^{aA*}$	1.6 ± 0.2^{cA}	3.1 ± 0.5^{bA}	$3.7 \pm 0.5^{bA*}$	$5.3 \pm 0.4^{aA*}$	2.0 ± 0.2^{bA}	$3.5 \pm 0.2^{bA*}$	$5.4 \pm 0.2^{aA*}$	$6.2 \pm 1.1^{aA*}$
Mercury	0.03 ± 0.0	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.04 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.04 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aAB}	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aA}	0.03 ± 0.0^{aB}
Nickel	1.9 ± 0.4	2.9 ± 0.54^{aA}	6.6 ± 2.6^{aA}	6.2 ± 1.7^{aA}	$9.2 \pm 4.0^{aA*}$	3.1 ± 1.0^{bA}	4.1 ± 0.8^{bA}	5.3 ± 0.7^{abA}	$7.8 \pm 0.8^{aA*}$	3.5 ± 0.4^{bA}	5.9 ± 0.2^{abA}	$8.4 \pm 1.5^{aA*}$	$8.5 \pm 0.5^{aA*}$
Zinc	99 ± 17	335 ± 18^{cA}	505 ± 37^{bcA}	901 ± 243^{abA}	$1{,}076 \pm 91^{aA}$	220 ± 11^{cB}	422 ± 53^{bcA}	595 ± 114^{bAB}	841 ± 25^{aB}	175 ± 7^{cB}	289 ± 17^{bB}	396 ± 21^{aB}	455 ± 40^{aC}

Table 4
Concentrations of heavy metals in earthworm (*Eisenia andrei*) biomass at the end of the experiment. Experimental treatments refer to the shredded wheat straw control treatment (C) or the treatments with aquacultural sludge from RAS – taken either in the outlet channel (O), biofilter (B), or adjacent sedimentation pond (P) at 5, 10, 20 and 30% dw, respectively. Values with differing lower case letters in superscripts are significantly different among treatments containing given RAS sludge at different inclusion levels in each row. Values with differing capital letters in superscripts are significantly different among treatments containing identical inclusion levels among RAS sludge types in each row. Asterisks refer to differences between a given sludge-containing treatment and the control (one-way ANOVA, Tukey's HSD test, $n = 3$, $p < 0.05$). Data are presented as means ± SD and expressed on a dry weight basis in mg kg^{-1}.

Parameter	C	O5	O10	O20	O30	B5	B10	B20	B30	P5	P10	P20	P30
Arsenic	2.5 ± 0.5	2.3 ± 0.4^{cB}	2.7 ± 1.5^{cB}	8.5 ± 0.9^{bB}	$14.9 \pm 1.8^{aB*}$	3.4 ± 1.7^{cB}	7.0 ± 0.9^{cAB}	13.4 ± 0.8^{bB}	$22.7 \pm 2.6^{aB*}$	8.7 ± 0.5^{cA}	12.4 ± 3.8^{cA}	$40.3 \pm 6.4^{bA*}$	$60.3 \pm 8.9^{aA*}$
Cadmium	2.7 ± 0.8	$0.8 \pm 0.1^{aB*}$	$0.4 \pm 0.2^{abA*}$	$0.2 \pm 0.1^{bB*}$	$0.2 \pm 0.1^{bB*}$	$0.9 \pm 0.0^{aAB*}$	$0.6 \pm 0.1^{bA*}$	$0.3 \pm 0.1^{cA*}$	$0.2 \pm 0.0^{cA*}$	$1.1 \pm 0.1^{aA*}$	$0.7 \pm 0.1^{bA*}$	$0.3 \pm 0.1^{cA*}$	$0.2 \pm 0.1^{cA*}$
Chromium	<0.05	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$	$<0.05^{aA}$
Copper	5.6 ± 0.5	4.6 ± 0.4^{aB}	3.5 ± 2.0^{aA}	5.6 ± 0.6^{aA}	7.0 ± 1.0^{aA}	5.5 ± 1.1^{aAB}	6.2 ± 0.7^{aA}	6.2 ± 1.3^{aA}	5.4 ± 0.1^{aA}	7.2 ± 0.5^{aA}	5.9 ± 0.3^{aA}	6.9 ± 0.4^{aA}	6.1 ± 1.3^{aA}
Lead	0.17 ± 0.1	0.15 ± 0.2^{aA}	0.12 ± 0.0^{aB}	0.16 ± 0.1^{aA}	0.20 ± 0.1^{aA}	0.16 ± 0.0^{aA}	0.13 ± 0.0^{aB}	0.17 ± 0.0^{aA}	0.20 ± 0.1^{aA}	0.21 ± 0.1^{aA}	0.18 ± 0.0^{aA}	0.19 ± 0.0^{aA}	0.26 ± 0.1^{aA}
Mercury	0.14 ± 0.0^{A}	0.10 ± 0.0^{aA}	0.16 ± 0.0^{aA}	0.17 ± 0.1^{aA}	0.17 ± 0.0^{aA}	0.14 ± 0.0^{aA}	0.15 ± 0.0^{aA}	0.12 ± 0.0^{aA}	0.12 ± 0.0^{aA}	0.14 ± 0.0^{aA}	0.16 ± 0.0^{aA}	0.16 ± 0.0^{aA}	0.16 ± 0.0^{aA}
Nickel	<0.05	$0.3 \pm 0.0^{aA*}$	$0.4 \pm 0.2^{aA*}$	$0.7 \pm 0.3^{aA*}$	$0.7 \pm 0.1^{aA*}$	$0.4 \pm 0.1^{aA*}$	$0.6 \pm 0.2^{aA*}$	$0.7 \pm 0.2^{aA*}$	$0.8 \pm 0.1^{aA*}$	$0.4 \pm 0.1^{aA*}$	$0.6 \pm 0.2^{aA*}$	$0.5 \pm 0.2^{aA*}$	$0.7 \pm 0.3^{aA*}$
Zinc	110 ± 7	100 ± 11^{aB}	80 ± 44^{aA}	130 ± 13^{aA}	135 ± 8^{aA}	122 ± 10^{aAB}	117 ± 10^{aA}	121 ± 10^{aA}	111 ± 6^{aA}	138 ± 9^{aA}	119 ± 6^{aA}	126 ± 13^{aA}	123 ± 19^{aA}

Table 5
Heavy metal concentrations of the two most commonly used fish diets (Efico Enviro 920 3 mm and Orbit 929 4.5 mm, BioMar A/S, Denmark, 3 different batches) and the dominant market-sized fish (a single fillet from three specimens of rainbow trout, *Oncorhynchus mykiss*) reared at the farm from which RAS sludge originated. Data are presented as means ± SD and expressed on a dry weight basis in mg kg^{-1}.

	Efico Enviro 920	Orbit 929	Rainbow trout
Arsenic	1.6 ± 0.3	2.1 ± 0.3	2.7 ± 0.2
Cadmium	0.25 ± 0.02	0.15 ± 0.01	<0.02
Chromium	0.25 ± 0.01	0.25 ± 0.06	<0.05
Copper	5.3 ± 0.2	4.9 ± 0.4	0.8 ± 0.1
Lead	0.05	<0.05	<0.02
Mercury	0.02 ± 0.0	0.02 ± 0.0	0.06 ± 0.0
Nickel	0.70 ± 0.06	0.55 ± 0.02	<0.05
Zinc	148 ± 9	137 ± 8	13 ± 1

and resultant vermicomposts (Table 3) contained substantial amounts of zinc. Relatively high zinc levels are known to occur in RAS sludge (Bergheim et al., 1998). Zinc concentrations in earthworms were well regulated, occurring at a consistent level (Table 4). This concurs with the findings observed between fish diets and fish muscle (Table 5), confirming organisms are well capable of regulating this metal (Kouba et al., 2010). Despite a much lower cadmium concentration in straw compared to RAS sludge (Table 1), its concentrations in earthworms were negatively related with the sludge inclusion levels, being highest in the control animals (Table 4). This suggests a protective role of elevated zinc concentrations against cadmium accumulation in organisms as documented on earthworms and fish in this study.

For a comprehensive evaluation of production systems involving vermicomposting of aquaculture sludge, consideration must be given to how end-products (vermicomposts and earthworm biomass) are further utilised. Maximum content of a potentially harmful substance is usually determined and often focuses on concentrations of selected heavy metals. The Czech national (Decree of the Ministry of the Environment of the Czech Republic No. 437/2016 on the Code, 2016), European Union (EU, 1986) as well as the United States (Brinton, 2000) criteria for use in agriculture were fulfilled for both the original RAS sludge and the final vermicomposts (Tables 1 and 3). Among the metals evaluated, zinc may be found to be too high for some more strict regulations, e.g. limits of 300 mg kg^{-1} dw (Bergheim et al., 1998). In these cases, the inclusion level and composition of bulking material must be considered. Zinc is thought to originate from fish diets in these aquaculture systems. In addition to its natural occurrence in feeds, it is further elevated via additional supplementation (Table 5). The necessity of this supplementation appears questionable, considering that dietary requirements of zinc for rainbow trout range between 15 and 30 mg kg^{-1} according to Ogino and Yang (1978), which is in line with those defined for other fish and crustaceans involved in intensive aquaculture (National Research Council, 2011). Since zinc is well regulated at relatively low and constant levels in fish (Table 5), excess quantities accumulate in the RAS. Taking this as a whole, our findings suggest that RAS sludge and particularly vermicomposts are applicable for soil fertilisation and remediation. The potential for using RAS-derived vermicomposts for fertilisation in pond based aquaculture, often focused on cyprinid production, has already been documented (Chakrabarty et al., 2009).

In pond aquaculture, vermicomposts which have not had earthworms removed provide an additional source of food for the cultured organisms. Live earthworms were successfully given to a number of fish species and other livestock e.g. chickens and pigs. Their processed form, earthworm meal, represents another valuable feed which can be incorporated into the diets of an even wider range of animals (Edwards, 1985). Vermiculture together with aquaculture are integrated components of organic and natural farming in some regions, e.g. India (Chakrabarty et al., 2009). Utilisation of earthworm biomass as a fish feed in less developed regions, where the availability of high-quality fish feed is low, or the price is unacceptably high, is reasonable. Diminishing the need of commercial, usually fish meal-based, diets may also partly reduce pressure on this commodity. Fish meal and fish oil are in permanent shortage in view of ever-increasing aquaculture due to overfishing of seas. Increased economic sustainability may be achieved by using earthworms for feeding pets, as a fish bait or stocking material for vermicomposts.

In terms of hygienic quality, earthworm biomass (Table 4) was found to be a suitable feed for fish, compared to provided fish diets (Table 5) containing no chromium and equal copper, nickel and zinc concentrations. Levels were well below EU limits for lead (11.4 mg kg^{-1}) and mercury (0.57 mg kg^{-1}). Cadmium limits (2.3 mg kg^{-1}) were exceeded only for the control (values recalculated as dw; EU, 2002, 2003, 2012), suggesting a positive role of zinc present in sludge (see discussion above, Tables 1 and 3). Earthworms derived from treatments P20 and 30 (Table 4) were above the limit of 28.4 mg kg^{-1} for arsenic (EU, 2012). High-performing commercial fish diets usually depend on the inclusion of feeds of marine origin that are relatively rich in arsenic. The main portion of total arsenic is comprised of organic forms with largely reduced toxicity. Inorganic forms constituted less than 1.2% of the total arsenic concentration in feedstuffs for fish and fish meals according to Sloth et al. (2005). They advocate that there is no or minimal transformation of chemical arsenic species during the processing of complete feedstuffs and fish digestion. It is expected that the same is true for the process of vermicomposting and biosynthesis in earthworms, making earthworms a safe animal feed. Confirmation of this assumption is an issue for further research.

4. Conclusions

The first comprehensive evaluation of a production system incorporating vermicomposting of sludge from intensive aquaculture and the possibility of the further utilisation of obtained vermicomposts and earthworms is provided. High survival of initial stocks, their individual growth and reproduction indices (cocoon and juvenile production) were documented. Complying with limits for heavy metals, vermicomposts were found to be suitable for use in agriculture. Only zinc limits may be occasionally exceeded when compared with the strictest regulations, given that it is probably overused in the commercial fish diets and accumulates in the sludge. Considering the protein content of earthworms, they are a valuable feed for livestock including fish. Utilisation of earthworm biomass as a fish feed is particularly reasonable in regions where the availability of commercial, usually fish meal-based, diets is low, or the price is unacceptably high. It may partly reduce pressure on this commodity, which is in permanent shortage in view of ever-increasing aquaculture due to overfishing of the seas. Further possible applications include feeding pets, fish bait and further stocking. Arsenic was the only potentially problematic metal, but its concentrations were considered safe, presuming that its bulk is as organic forms possessing largely reduced toxicity. Future research is needed to confirm this assumption. Integration of freshwater aquaculture and vermicomposting is recommended.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2017.12.216.

References

Albanell, E., Plaixats, J., Cabrero, T., 1988. Chemical changes during vermicomposting (*Eisenia fetida*) of sheep manure mixed with cotton industrial wastes. Biol. Fert. Soils 6, 266–269.

Bergheim, A., Cripps, S.J., Liltved, H., 1998. A system for the treatment of sludge from land-based fish-farms. Aquat. Living Resour. 11, 279–287.

Blancheton, J.-P., Piedrahita, R., Eding, E., Lemarie, G., Bergheim, A., Fivelstad, S., Roque D'Orbcastel, E., 2007. Intensification of landbased aquaculture production in single pass and reuse systems. In: Bergheim, A. (Ed.), Aquacultural Engineering and Environment, Research Signpost, Kerala, India, pp. 21–47.

Brinton, W.F., 2000. Compost Quality Standards and Guidelines. Woods End Research Laboratory, Inc., Mt Vernon, Maine, USA.

Bureau, D.P., Gunther, S.J., Cho, C.Y., 2003. Chemical composition and preliminary theoretical estimates of waste outputs of rainbow trout reared in commercial cage culture operations in Ontario. N. Am. J. Aquacult. 65, 33–38.

Buřič, M., Bláhovec, J., Kouřil, J., 2016. Feasibility of open recirculating system in temperate climate – a case study. Aquacult. Res. 47, 1156–1167.

Chakrabarty, D., Das, S.K., Das, K., Biswas, P., Karmegam, N., 2009. Application of vermitechnology in aquaculture. Dyn. Soil, Dyn. Plant 3, 41–44.

Decree of the Ministry of the Environment of the Czech Republic No. 437/2016 of the Code, 2016. www.zakonyprolidi.cz/cs/2016-437. (Accessed 10 October 2017).

del Campo, L.M., Ibarra, P., Gutiérrez, X., Takle, H., 2010. Utilization of Sludge from Recirculation Aquaculture, 9/2010, Nofima Marin AS, Norway.

Domínguez, J., Velando, A., Ferreiro, A., 2005. Are *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* (Oligochaeta, Lumbricidae) different biological species? Pedobiologia 49, 81–87.

Dvořák, J., Mančíková, V., Pižl, V., Elhottová, D., Šilerová, M., Roubalová, R., Škanta, F., Procházková, P., Bilej, M., 2013. Microbial environment affects innate immunity in two closely related earthworm species *Eisenia andrei* and *Eisenia fetida*. PLoS ONE 8, e79257.

Edwards, C.A., 1985. Production of feed protein from animal waste by earthworms. Phylos. T. Roy. Soc. B 310, 299–307.

Edwards, C.A., 2004. Earthworm Ecology. CRC Press LLC, Boca Raton, Florida, USA.

EU, 1986. Council Directive 86/278/EEC of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge Is Used in Agriculture.

EU, 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed – Council Statement.

EU, 2003. EU Directive (2003) Commission Directive 2003/100/EC of 31 October 2003 Amending Annex I of Directive 2002/32/EC of the European Parliament and of the Council on Undesirable Substances in Animal Feed.

EU, 2012. Commission Regulation (EU) No 744/2012 of 16 August 2012 Amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as Regards Maximum Levels for Arsenic, Fluorine, Lead, Mercury, Endosulfan, Dioxins, Ambrosia spp., Diclazuril and Lasalocid a Sodium and Action Thresholds for Dioxins.

Fallah, A.A., Saei-Dehkordi, S.S., Nematollahi, A., Jafari, T., 2011. Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (*Oncorhynchus mykiss*) using ICP-OES technique. Microchem. J. 98, 275–279.

FAO, 2016. The State of World Fisheries and Aquaculture 2016. Food and Agriculture Organization of the United Nations, Rome, Italy. www.fao.org/3/a-i5555e.pdf (accessed 16.08.17).

Horn, M.A., Mertel, R., Gehre, M., Kästner, M., Drake, H.L., 2006. *In vivo* emission of dinitrogen by earthworms via denitrifying bacteria in the gut. Appl. Environ. Microb. 72, 1013–1018.

Kouba, A., Buřič, M., Kozák, P., 2010. Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Poll. 211, 5–16.

Koubová, A., Goberna, M., Šimek, M., Chroňáková, A., Pižl, V., Insam, H., Elhottová, D., 2012. Effects of the earthworm *Eisenia andrei* on methanogens in a cattle-impacted soil: a microcosm study. Eur. J. Soil Biol. 48, 32–40.

Lim, S.L., Lee, L.H., Wu, T.Y., 2016. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. J. Clean. Prod. 111, 262–278.

Mariotti, F., Tomé, D., Mirand, P.P., 2008. Converting nitrogen into protein—beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. 48, 177–184.

Marsh, L., Subler, S., Mishra, S., Marini, M., 2005. Suitability of aquaculture effluent solids mixed with cardboard as a feedstock for vermicomposting. Bioresour. Technol. 96, 413–418.

Morais, F., Queda, C., 2003. Study of storage influence on evolution of stability and maturity properties of MSW composts. Advances for a sustainable Society Part II. In: Proceedings of the Fourth International Conference of ORBIT Association on Biological Processing of Organics. Perth, Australia.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC, USA.

Ndegwa, P.M., Thompson, S., 2000. Effects of C-to-N ratio on vermicomposting of biosolids. Bioresour. Technol. 75, 7–12.

Nigussie, A., Kuyper, T.W., Bruun, S., de Neergaard, A., 2016. Vermicomposting as a technology for reducing nitrogen losses and greenhouse gas emissions from small-scale composting. J. Clean. Prod. 139, 429–439.

Ogino, C., Yang, Y.G., 1978. Requirement of rainbow trout for dietary zinc. B. Jpn. Soc. Sci. Fish. 44, 1015–1018.

Reid, G., Liutkus, M., Robinson, S., Chopin, T., Blair, T., Lander, T., Mullen, J., Page, F., Moccia, R., 2009. A review of the biophysical properties of salmonid faeces: implications for aquaculture waste dispersal models and integrated multi-trophic aquaculture. Aquacult. Res. 40, 257–273.

Sloth, J.J., Julshamn, K., Lundebye, A.-K., 2005. Total arsenic and inorganic arsenic content in Norwegian fish feed products. Aquacult. Nutr. 11, 61–66.

Taylor, M., Clarke, W., Greenfield, P., 2003. The treatment of domestic wastewater using small-scale vermicompost filter beds. Ecol. Eng. 21, 197–203.

Tomsovy žížaly, 2008. www.hnojnizizaly.cz/ (accessed 07 August 2017).

van Rijn, J., 1996. The potential for integrated biological treatment systems in recirculating fish culture – a review. Aquaculture 139, 181–201.

Zbíral, J., 2011. Soil Analyses, Part II, third ed. Czech Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic (in Czech).

Zbíral, J., Honsa, I., 2010. Soil Analyses, Part I, third ed. Czech Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic (in Czech).

Zbíral, J., Malý, S., Váňa, M., 2011. Soil Analyses, Part III, third ed. Czech Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic (in Czech).

Zhenjun, S., Xianchun, L., Lihui, S., Chunyang, S., 1997. Earthworm as a potential protein resource. Ecol. Food Nutr. 36, 221–236.

CHAPTER 4

RECYCLING BIOFLOC WASTE AS NOVEL PROTEIN SOURCE FOR CRAYFISH WITH SPECIAL REFERENCE TO CRAYFISH NUTRITIONAL STANDARDS AND GROWTH TRAJECTORY

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Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory

Roman Lunda[1,2], Koushik Roy[1,2], Petr Dvorak[1], Antonin Kouba[1] & Jan Mraz[1]✉

Screening of novel feedstuffs, that too for data-deficient (nutritionally) animals, is somewhat ambiguous or problematic. Through systematic meta-analyses, the present study formulated most up-to-date crayfish nutritional standards, against which a recyclable waste (biofloc biomass, BM) from intensive aquaculture systems was assessed as a novel protein source. Growth trajectory dependencies and thermal growth coefficient qualifying for good growth in crayfish (TGC 0.5–0.64 units) were benchmarked. Using these standards and a 7-week growth trial, BM's suitability as a novel protein source for red swamp crayfish *Procambarus clarkii* was evaluated through its graded inclusions in a commercial feed. Results suggest that BM can elevate growth at 33–66% inclusion in existing feed formulations. Beyond 66% inclusion, BM can deteriorate growth in crayfish due to high ash content (exceeding physiological limit > 14%), arginine deficiency (~ 14–20% lower than an optimum requirement), and insufficient non-protein energy: protein ratio (3.7 cal mg^{-1}). Arginine is perhaps the most critical amino acid in dietary protein for crayfish, and deficient in BM. Although no critical bioaccumulation levels of heavy metals were breached by feeding 100% BM to crayfish, a mineral and heavy metal (Hg) stress seemed plausible. Crayfish raised solely on biofloc may not realize full growth potential.

Abbreviations

BM	Biofloc meal (biomass)
BFT	Biofloc technology aquaculture system
TGC	Thermal growth coefficient
EAA	Essential amino acid
IR	Interquartile range
GAM	Generalized additive model
LWG	Live-weight gain
CP	Crude protein
CL	Crude lipid

Freshwater crayfish, mostly endemic to the continents of North America, Australia-Oceania, and Europe[1], account for 1.71 million tons of global aquaculture production with a worth of 14.46 billion € as of 2018[2]. Presently they contribute a negligible fraction in the global aquaculture scenario (~ 3.5% of total freshwater aquaculture production) but having great potential ahead. During the last half-decade alone (2013–2018), freshwater crayfish production, and its commercial valuation have tripled[2]. In terms of crayfish nutrition research, efforts have been quite limited compared to other commercially important crustaceans (like penaeids and palaemonids)[3,4]. Therefore, screening of novel feedstuffs, that too for crayfish, is somewhat ambiguous or problematic. A brief prologue in this regard is provided in the supplementary text. On the other hand, aquaculture nutrition research has focused on developing feed substitution strategies with a minimal supply of fishmeal and fish oil

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in recent times. One potential ingredient could be a microbial biomass meal from biofloc technology systems (BFT)[4]. BFTs are a modern, intensive aquaculture system that evolved from the classic 'activated-sludge based sewage bioremediation' in wastewater treatment plants. The system essentially operates on the rationale of maintaining an optimum C: N ratio (6:1 to 15:1) by daily purging with carbohydrate (carbon) source[5,6]. It is done to support the blooming of microbial biomass (flocs). These microbial flocculants, known as 'bioflocs' bioremediate the nitrogenous wastes generated by fish and uneaten feed into consumable microbial protein for cultured animals[7,8]. Although they are consumed by the fish or shrimp stock, the biofloc biomass (as measured in Imhoff cones) or total suspended solids (TSS) may often exceed the recommended values for fish (25–50 ml L^{-1}; TSS up to 1000 mg L^{-1}) and shrimp (10–15 ml L^{-1}; TSS 400–600 mg L^{-1})—posing problems for the cultured animals[7,9–11]. It is advisable to drain part of the biofloc biomass daily through sedimentation or fractionation of biofloc system water[10,12–14]. Such thinning (filtering) of culture water generates a large amount of biofloc biomass as waste, quite frequently. This drained biofloc is often of limited use. In general, they can be used as an alternative to synthetic polymers for wastewater treatment[15], fertilizer, or inoculum to start a new system[16].

Our research intervenes in recycling this waste for aquatic animal nutrition. Since conventional protein sources in aquafeed (e.g., fishmeal) are becoming expensive and scarce, there has been a growing impetus in testing biofloc as an unconventional protein source for aquatic animals[8,17–19]. Few commercial floc meals are generically marketed under 'single-cell protein (SCP)' or 'microbial protein' category—Profloc (Nutrinsic), FeedKind (Calysta), and Novacq/OBM (Ridley, Maritech) with pricing (as of 2018) between 1.1–3.3 USD kg^{-1}[17,18]. One of these is listed in IAFFD (international aquaculture feed formulation database), with complete nutrient spectrum data, including essential amino acids[20]. So far, crayfish are not included in these mentioned researches. The novelty here is its potential use as a feedstuff (protein source) in the crayfish diet. In general, the protein (12–49%), lipid (0.5–12.5%), and ash (13–46%) contents in biofloc can vary substantially depending on several factors (reviewed by[22]). To the best of our knowledge, nutritional evaluation of biofloc as a feedstuff ingredient for artificial crayfish diets has not been done so far. Although rearing of crayfish in BFT system, where the animals co-fed on commercial feed pellets (primarily) and bioflocs suspended in the system, are recently being explored[23,24]. Our objective was to understand—(a) nutritional optima of freshwater crayfish from the available literature in the absence of centralized recommendations (see supplementary material); (b) growth trajectory and nutritional dependencies in crayfish (supplementary material); (c) response of red swamp crayfish to biofloc meal in their diet, in terms of nutrition, growth, and survivability; (d) the risk of heavy metals bioaccumulation or mineral stress in crayfish from feeding on biofloc, and; (e) evaluate nutritional strengths and bottlenecks associated with using biofloc meal in crayfish diet. The first two objectives (a and b) were rather a methodological and necessary step (placed in supplementary material) to the second part of our research related to the use of biofloc meal for crayfish (objectives c to e).

Results and discussion

Nutritional optima, growth trajectory, and nutritional dependencies of crayfish. Based on our meta-analyses, crayfish' optimum dietary nutritional requirement is tabulated as crayfish standards in Table 1. It is also compared with established standards of penaeid shrimps, often assumed as a template for most crustacean diets. Detailed information in this regard can be found in the supplementary material. In terms of crayfish growth trajectory, their thermal growth coefficient (TGC) may vary from 0.07–1 unit (interquartile range, IR 0.32–0.64 units). Results suggest any TGC in the range of 0.5–0.64 units may be regarded as 'reasonably good growth' in crayfish. Further insights into crayfish growth trajectory and its nutritional dependencies are presented in detail in the supplementary material. The information synthesized and approach used may serve as a template for future researchers exploring three less-established or unknown dimensions simultaneously (as in the present study)—novel feedstuff, optimum nutrition, and data-deficient (nutritionally) animals.

Growth response of crayfish to biofloc protein. Following a 9-week growth trial with graded BM levels in the diet, differential growth response by crayfish was realized (Fig. 1). Except for control and BM_{33} groups, crayfish' final body weight showed a significant deviation from the normal distribution. Further examining the skewness of final body weight distribution in BM_{66} and BM_{100} groups, it was apparent that these groups were dominated by runts (smaller sized individuals) with large size deviations from the handful of bigger individuals. The size heterogeneity showed a significant and negative correlation with BM inclusion in the diet (Pearson's 2-tailed $r = -0.63$, $p < 0.05$). Size heterogeneity in crayfish may aggravate community aggression[25]. However, the diet-driven size heterogeneity was not significantly correlated with mortality. The dietary treatments did not cause significant differences ($p > 0.05$) in survivability, confirmed by *post-hoc* analyses. Overall, the survivability remained > 70% through the experimental period in all groups (Table 2). It implies—BM does not pose a significant mortality risk to crayfish stocks irrespective of inclusion levels, but it has implications on growth (presented below).

The growth in terms of TGC, live-weight gain (LWG), and body weight (BW) were significantly depressed ($p < 0.05$) in the BM_{100} fed group. In contrast, the growth in control, $BM_{33,}$ and BM_{66} groups were higher with insignificant differences among them ($p > 0.05$) (Table 2, Fig. 1, 2). A statistically insignificant dampening of growth rate over time ($p > 0.05$) was observed in groups BM_{66} and BM_{100} (Fig. 2). At the end of culture (63 days), the realized TGC in crayfish fed on BM_{100} was on an average two times lower ($p < 0.05$) than the growth exhibited on control, $BM_{33,}$ or BM_{66} diets (Table 2, Fig. 2). In terms of feed utilization, the feed conversion ratio (FCR) and protein efficiency ratio (PER) were linearly related to increasing BM inclusion in the diet. The FCR increased with increasing share of BM in diet: $FCR = 1.156 + 0.006 \times BM$ (Adj. R^2 0.95, $p < 0.05$). The PER decreased with an increasing BM inclusion: $PER = 1.922 - 0.006 \times BM$ (Adj. R^2 0.95, $p < 0.05$). It means, for every 10% inclusion of BM, FCR increased by + 0.06 units, and PER decreased by − 0.066 units (Fig. 3). The results from the growth

Parameter	Crayfish standard (calculated)	NRC[4] standards for penaeid shrimps
Macronutrient and energy (based on *Cherax* sp. and *Procambarus* sp.)—crude		
Crude protein	29–34% (44%)*	33–42%**
Crude lipid	6.5–9%	5–6%
Crude NFE (nitrogen-free extract)	40–47%	–
Dietary fiber	Up to 7%	–
Total ash	7.8–10.8%	–
Gross energy	3590–4205 kcal kg^{-1}	3666–4888 kcal kg^{-1}**
Protein: Energy	72–91 mg $kcal^{-1}$ (113–119 mg $kcal^{-1}$)*	85–90 mg $kcal^{-1}$
Non-protein energy: Protein ratio	5.3–8.5 cal mg^{-1} (4.4–4.8 cal mg^{-1})*	–
Essential amino acids (based on *P. clarkii* only)—digestible		
Leucine	1.8–2.5%	1.8%
Valine	1.2–1.6%	1.4%
Threonine	0.3–1.5%	1.3%
Isoleucine	1.2–1.7%	1.2%
Arginine	**2.1–2.7%**	1.8%
Phenylalanine	0.8–1.5%	1.4%
Lysine	1.2–2.4%	1.8%
Methionine	1.1–4.9%	0.7%
Histidine	0.6–0.9%	0.7%
Tryptophan	0.4%	–
Essential minerals (based on *Astacus* sp., *Ornectes* sp., and *Procambarus* sp.)—available		
Calcium	3000–4000 mg kg^{-1}	–
Phosphorus	164–235 mg kg^{-1}	3000–7000 mg kg^{-1}
Iron	27–125 mg kg^{-1}	–
Zinc	10–14 mg kg^{-1}	15 mg kg^{-1}
Copper	6–9 mg kg^{-1}	10–32 mg kg^{-1}
Manganese	14.2–17.8 mg kg^{-1}	–

Table 1. Optimum dietary nutritional requirement of freshwater crayfish and its comparison with NRC (2011) standards for penaeid shrimps (usually adopted as *status quo*). *In parentheses—proposed reconsideration of calculated standards, based on high TGC obtained in the present trial. **Digestible values converted to crude values assuming 90% apparent digestibility.

trial are summarized in Table 2, and the relationship of feed utilization parameters in response to BM inclusion is depicted in Fig. 3. Interestingly, the calculated FCR(s) of our respective diets, when multiplied with the dietary arginine content, seem to 'hit the target' of arginine requirements by crayfish (e.g., FCR of BM_{100} × Arginine in BM_{100} = Fulfillment of arginine requirement).

As per the crayfish growth trajectory (quantified in the previous section), the group fed on 100% BM failed to show reasonably good growth. They were dominated by smaller-sized runts, poorest of the FCR and PER, but no significant mortality. Among the limited studies testing flocculated microbial meals in crustacean diets [reviewed in 8], BM inclusions were mostly up to 10–30% (of the total diet) or 30% (of fishmeal replacement). Good results in terms of growth were usually obtained at the maximum inclusion levels[8]. Like the present study, two previous studies had tested BM (on *Litopenaeus vannamei*) at a broader inclusion level from 17 to 84% of the total diet[17,26]. Despite different target species, the results seem close to that of the present study. Above 41–53% BM inclusion, the growth advantages were gradually lost[17,26]. Looking deeper into the aspects of our BM_{100}-protein compared to control, BM_{33}, or BM_{66}-protein, the arginine seems to be a bottleneck for reasonably good growth (Tables 2, 4). Other EAAs, which could also be critical (e.g., methionine and lysine), were comparable-to-higher in BM_{100} than in other diets (Table 4). Although methionine and lysine levels in diets fell short of our formulated crayfish nutritional standard (Table 1), at least it fulfilled penaeid EAA standards of NRC[4]. It hints that NRC's penaeid EAA standards cover well for most of the EAA requirements in crayfish, except for arginine (and tryptophan could not be judged). Arginine levels in BM (Table 4) neither fulfilled crayfish nor penaeid standards (Table 1).

Biofloc has been previously criticized for being partly deficient in arginine[27–29]. The arginine coefficient (proportion of total protein, in %) of biofloc meals, be it commercial ones like Novacq (2.38%[19]), FeedKind (2.54%[20]), or in the present study (2.73%) seem to have close resemblance (CV 5.5%). If we consider the mean arginine coefficient of BM from these data (2.55%) and tally it to fulfill the optimum arginine requirement of crayfish (minimum 1.8%), the crude protein level of such BM should be at least 70%. It is beyond the expected range of ordinary bioflocs[22]. BM harvested from high TSS systems (due to infrequent sedimentation or water exchange) can have lower protein content[10]. For example, the crude protein content of a biofloc can drop by – 34.5% if the

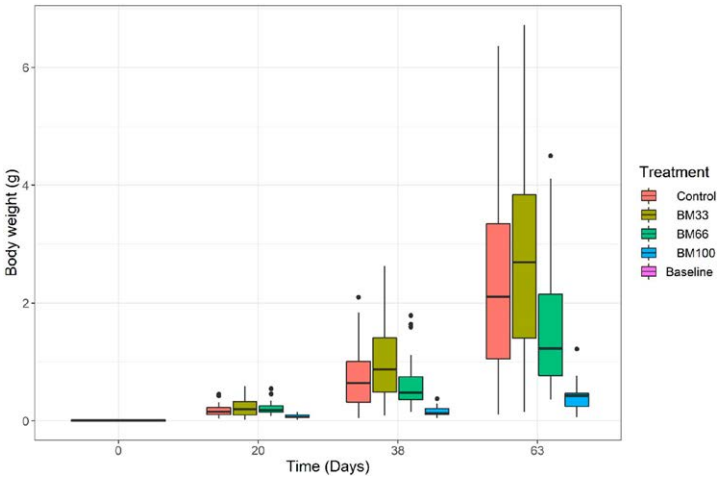

Figure 1. Body weight distribution in red swamp crayfish *Procambarus clarkii* fed graded level of biofloc meal (BM) in diets over 9 weeks of experimental duration. Measured on 20th, 38th and 63rd days post stocking. 'Baseline' indicates stocked stage-3 juveniles (0.007–0.008 g individual^{-1}). Size heterogeneity (measured by coefficient of variance, CV) seems maximum and comparable in control (mean CV = 67%), BM_{33} (mean CV = 67.5%) and BM_{66} (mean CV = 63.4%) groups but significantly suppressed ($p < 0.05$) in BM_{100} (mean CV = 51%). BM_{100} showed poor size throughout the experiment.

Diet group	Survival (%)	Final body weight (g)	Live weight gain (mg day^{-1})	Food conversion ratio	Protein efficiency ratio	Thermal growth coefficient
Control	70[a]	1.06–3.34 (2.44 ± 1.79)[a]	17–53 (39 ± 15)[a]	1.2*	2	0.60–0.94 (0.84 ± 0.14)[a]
BM_{33}	70[a]	1.40–3.84 (2.80 ± 1.86)[a]	22–61 (44 ± 16)[a]	1.4	1.6	0.68–0.99 (0.89 ± 0.13)[a]
BM_{66}	80[a]	0.77–2.15 (1.62 ± 1.19)[a]	12–34 (26 ± 9)[a]	1.5	1.5	0.53–0.79 (0.72 ± 0.11)[a]
BM_{100}	83[a]	0.25–0.47 (0.41 ± 0.25)[b]	4–7 (6 ± 1)[b]	1.8*	1.3	0.32–0.42** (0.40 ± 0.04)[b]

Table 2. Response of the red swamp crayfish *Procambarus clarkii* (initial body weight 7–8 mg) under 9-week growth trial (21.8 °C) fed experimental diets. Values presented in interquartile range with mean ± standard deviation in parentheses. [a,b]Superscripts denote statistically different ($p < 0.05$) groups. *Pattern: FCR multiplied by Arginine content of feeds ≈ fulfillment of Arginine requirement (as per crayfish or penaeid standards). **Below reasonably good growth (TGC 0.47–0.59) for crayfish standards.

TSS of the system is let to increase from ≤ 200 mg L^{-1} to 800–1000 mg L^{-1}[10]. As such, BM harvested from a low TSS system would have higher arginine (0.72%) compared to a high TSS system (0.47% arginine) (recalculated from[10]; using mean arginine coefficient = 2.55% of total protein). Even with aging biofloc, the content of arginine (also other EAAs) may decline. For example, from the 10th day to the 30th day of a biofloc culture, the arginine levels can decrease by 25–41% (recalculated from[27]). However, some specially produced commercial flocculated meals can have a high arginine coefficient (e.g., 5.3% of the protein in ProFloc[17]). Among all the EAAs, arginine content in red swamp crayfish seems maximum[3,21,30,31], indicating a supposedly higher arginine demand in crayfish. The same is true for marbled crayfish *Procambarus virginalis*[32]. Arginine is perhaps the most limiting EAA in most crustacean diets and is required between 1.6–2.7% of diet[33]. Due to the poor activity of the urea cycle in crustaceans, arginine is indispensable for growth[33,34]. Arginine functions as a phosphagen in crustaceans, being the only amino acid providing amidino group for the synthesis of creatine—a major reserve of high-energy phosphate for ATP regeneration[33,35].

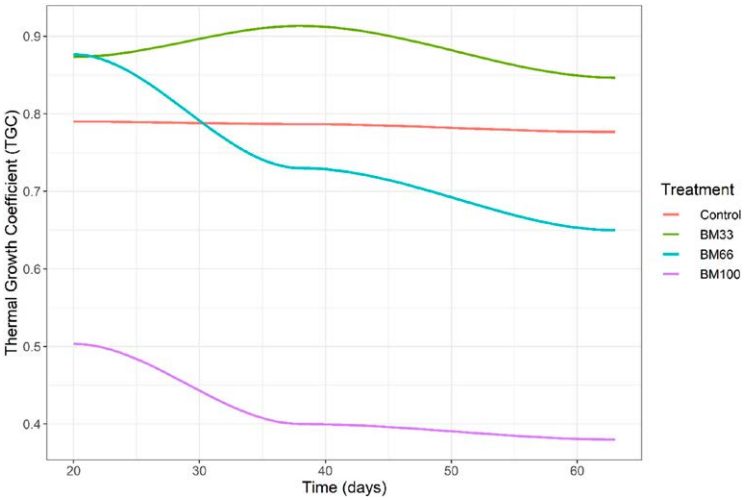

Figure 2. Growth pattern (TGC: thermal growth coefficient) of red swamp crayfish *Procambarus clarkii* fed different experimental diets over 9 weeks. A dampening of growth over time gradually setting-in at higher BM inclusion in the crayfish diet (from BM_{66} to BM_{100}). At the end of culture, BM_{100} resulted in twice less growth ($p < 0.05$) than achievable on other diets (control or BM_{33} and BM_{66}—statistically comparable TGC).

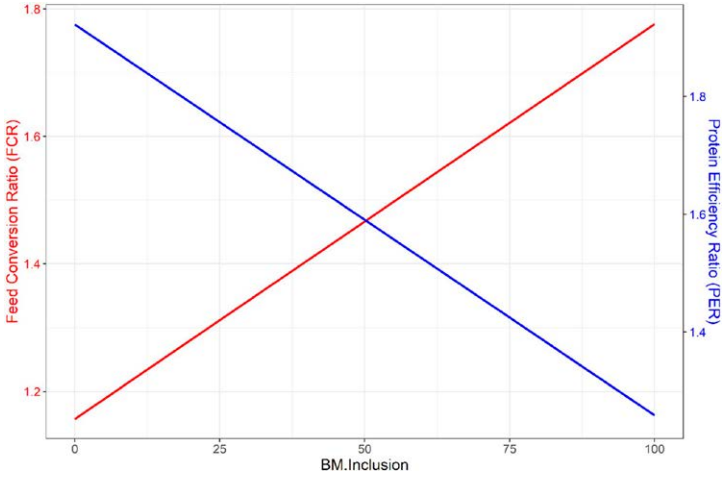

Figure 3. Feed utilization pattern (FCR in red and PER in blue) of red swamp crayfish *Procambarus clarkii* in response to the level of biofloc meal (indicated by BM.Inclusion, in %) in the diet. More feed is required per unit weight gain of crayfish with an increasing share of BM in the diet because protein utilization is lowered at higher BM inclusion.

Group	Hg (μg kg^{-1})	Mn (mg kg^{-1})	Cd (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Muscle					
Control	9.4 ± 0.9^{a}	BDL	0.008 ± 0.01^{a}	11.4 ± 1.4^{a}	4.1 ± 2.7^{a}
BM_{33}	10.5 ± 1^{b}	BDL	BDL	11.8 ± 0.9^{a}	7.0 ± 5.7^{b}
BM_{66}	10.4 ± 1.4^{b}	BDL	BDL	10.4 ± 1.0^{b}	3.2 ± 2.4^{a}
BM_{100}	12.8 ± 1.2^{c}	BDL	BDL	8.5 ± 0.5^{c}	BDL
Hepatopancreas					
Control	4.6 ± 1.0^{a}	2.2 ± 0.1^{a}	0.17 ± 0.05^{a}	46.1 ± 30.0^{a}	54.6 ± 13.0^{a}
BM_{33}	5.4 ± 0.6^{b}	2.9 ± 0.4^{ab}	0.13 ± 0.03^{b}	72.4 ± 26.3^{b}	90.4 ± 13.4^{b}
BM_{66}	5.4 ± 0.7^{b}	3.2 ± 0.8^{b}	0.13 ± 0.01^{b}	67.7 ± 34.8^{ab}	88.0 ± 6.0^{b}
BM_{100}	11.0 ± 1.2^{c}	3.6 ± 2.2^{b}	0.19 ± 0.01^{a}	76.3 ± 28.9^{b}	82.4 ± 12.0^{b}

Table 3. Heavy metals and mineral content (mean ± SE; dry matter basis) in the tail muscle and hepatopancreas of red swamp crayfish *Procambarus clarkii* fed graded levels of biofloc meal. BDL = below detection limit (Mn and Fe: < 2 mg kg^{-1}, Cd: < 0.002 mg kg^{-1}); different letters in superscript denote groups with significant differences as derived from Tukey's HSD multiple range test (α = 0.05).

Proximate fraction	Basal	BM_{33}	BM_{66}	BM_{100}
Crude protein (CP) (%)	44.2	44.1	44	43.9
Crude lipid (%)	7.8	6.7	5.6	4.5*
Crude NFE (%)	35.5	33.8	32.1	30.3
Crude Fibre (%)	2.7	3.4	4.2	4.9
Total Ash (%)	9.8	12	14.2	16.4*
Gross energy (kcal kg^{-1})	3890	3719	3549	3373
Protein: Energy ratio (mg kcal^{-1})	113.6	118.6	124	130.2
Non-protein energy: Protein ratio (cal mg^{-1})	4.8	4.4	4.1	3.7*
Essential amino acids (%)				
Leucine	2.3	2.3	2.2	2.2
Valine	1.3	1.5	1.6	1.8
Threonine	1.1	1.3	1.4	1.6
Isoleucine	1	1.1	1.1	1.2
Arginine	1.5	1.4	1.3	1.2**
Phenylalanine	1.4	1.5	1.7	1.8
Lysine	1.7	1.7	1.8	1.8
Methionine	0.6	0.6	0.7	0.7
Histidine	0.8	0.8	0.8	0.8
Tryptophan	–	–	–	–
Minerals and heavy metals (mg kg^{-1})				
Arsenic (As)	< 0.21	< 0.21	< 0.21	< 0.21
Cadmium (Cd)	0.41	0.6	0.7	0.90
Chromium (Cr)	2.06	3.9	5.8	7.72
Copper (Cu)	11.70	110.1	208.6	310$^{\#}$
Iron (Fe)	185	2437.3	4689.5	7010$^{\#}$
Mercury (Hg)	0.01	0.03	0.04	0.06
Manganese (Mn)	59.60	220.4	381.3	547$^{\#}$
Nickel (Ni)	2.06	4.2	6.4	8.67
Lead (Pb)	2.06	3.5	4.9	6.32
Zinc (Zn)	93.30	306.4	519.5	739$^{\#}$

Table 4. Proximate composition of biofloc meal, basal and treatment diets (dry matter basis). *Matching the values with crayfish standards (Table 1)—hints under-supply (lipid, NPE:P) or excessive supply (ash). **Matching the values with crayfish standards (Table 1) and optimistic assumption of biofloc protein digestibility (~ 90%)—hints under-supply of amino acid. $^{\#}$Matching the values with crayfish standards (Table 1) and most conservative assumption of mineral retention (~ 10% retention)—hints mineral stress due to over-supply.

Risk of heavy metals bioaccumulation or mineral stress from biofloc meal. The contents of heavy metals in BM were below the critical pollution limits. No critical limits were breached in the crayfish body that could qualify BM as a feedstuff capable of inducing unsafe heavy metal biomagnification, rendering them unfit for consumption. Content of Cd and Mn were mostly below the detection limits (Table 3). Except for mercury, hepatopancreas contained a higher amount of heavy metals (and minerals) than muscle. Hepatopancreas of crayfish, like most crustaceans, have been reported to be major storage of minerals, including heavy metals[3,37]. With increasing BM fraction in the diet, the concentration of Hg significantly increased in hepatopancreas (control → BM_{33} and $BM_{66} \rightarrow BM_{100}$; $p < 0.05$), while other metals did not show any significant trend (Table 3). Except for Cd, all metals were significantly higher ($p < 0.05$) in the hepatopancreas of BM_{100} fed crayfish compared to the control group. Such accumulation of heavy metal in hepatopancreas is capable of impairing metabolism in crayfish[37]. The concentration of Fe exhibits a rather 'bell curve' pattern, peaking at BM_{33} and receding thereafter, only in the muscle (Table 3). Cd and Zn did not exhibit any pattern as such. The heavy metal contents in crayfish and BM are given in Tables 3 and 4, respectively.

Globally, the total ash content in biofloc may range between 13–46% (reviewed by[22]), also applicable in our case. The problem of high ash content in most biofloc, limiting its inclusion in diets (despite good protein content), has been briefly discussed in Sabry Neto et al.[38]. One previous study, which studied BM at a high enough inclusion level, attributed high ash and probable toxic effects of trace minerals to retarded growth in *Litopenaeus vannamei* fed > 60% BM in a diet[26]. Owing to high ash content in BM, mineral stress seems plausible in the present study as well (*see* Tables 1, 4). By mineral stress, we imply even if 10% of the ash or minerals from BM are digested by crayfish, it is potentially much higher 'bioavailable minerals' in the body than their optimum physiological limits. Information on this aspect have been limited for shrimps [reviewed in 39, 40] and none for crayfish[3,36]. In shrimps (*Penaeus monodon, P. japonicus*), retarded growth was observed when excessive mineral premixes were supplemented in a practical diet[39], or more specifically, when trace minerals like Fe and Mn exceeded levels of 0.01% each in the diet[40]. The BM_{100} had all these factors (ash, Fe, and Mn) in excess (Table 4). Heavy metal stress could also be plausible. Any significant absorption of Hg in the body (presented above) is capable of impairing crayfish metabolism[37], provoking hyper-osmoregulation in crustaceans[41], with repercussions on aggravated energy expenditure[42]. Our metadata derived models show TGC in crayfish deteriorates at dietary ash levels > 14% (also in BM_{100}), during which the retention of ash is merely < 10% of total dietary intake (see supplementary material and Fig S2, S3). Thus ≥ 90% of the ingested ash (exceeding physiological limits) are excreted through digestive and osmoregulatory (metabolic) pathways. It has its own energy cost, which could have been utilized for protein-sparing or growth[42].

Recycling biofloc waste as a novel feedstuff for crayfish: Strengths and bottlenecks. Comparing the nutritional standards for crayfish with observed performance in growth trials, few strengths and bottlenecks of BM were realized (Tables 1 and 4). In terms of advantages: (a) BM has a high crude protein content (43.9%); (b) crude fiber content in BM (4.9%) was in the optimum range for crayfish, and; (c) BM is a rich supplier of minerals. However, there are more bottlenecks than limited advantages. BM has excessive total ash detrimental to crayfish growth, with probable manifestations on hyper-osmoregulation and energy expenditure (discussed above). A mediocre crude lipid content (4.5%) is another bottleneck for supplying non-protein energy. These, in combination, render the non-protein energy: protein ratio (NPE: $P = 3.7$ cal non-protein energy per 1 mg protein) in BM insufficient for effective protein sparing (≈growth). At such low NPE:P, the proteins are catabolized for meeting energy demand (even after oxidizing carbohydrates and lipids), rather than building biomass[42]. It is further compounded by arginine deficiency in BM (~ 14–20% less than an optimum requirement)—probably the most critical essential amino acid for crayfish (discussed above).

A retrospective evaluation of BM_{100} or BM (as a feedstuff for crayfish) applying our metadata derived 'growth-retention models' (supplementary Fig S3, S4, S6) could explain few nutrient utilization scenarios behind low growth in BM_{100}. The ash, protein, and lipid retentions from BM should be less than 5%, 10%, and 3% of dietary intakes, respectively (predicted). For control, BM_{33}, and BM_{66} diets, these retentions were well above the identified thresholds qualifying for reasonably good growth in crayfish (refer to supplementary material). Comprehensively, the retarded growth problem with solely feeding on biofloc biomass could be a synergistic effect of— (a) arginine deficiency, (b) mineral and heavy metal stress, and, (c) low non-protein energy to protein ratio.

Methods

Calculation of crayfish nutritional standards, growth trajectory, and its nutritional dependencies. In the absence of centralized nutrition recommendations for freshwater crayfish species, unlike other commercially important crustaceans (e.g., penaeid shrimps, *see* NRC[4]), available literature was meta-analyzed. Peer-reviewed and published articles (in English or at least with English abstract) were searched online (search engines: Web of Science, Scopus, and Google Scholar) using keywords like 'growth trials', 'crayfish', 'nutrition', 'proximate composition', 'body composition', 'amino acids', 'heavy metals', 'optimum requirement' were used in different combinations (depending on target information). Altogether 27 articles were sourced and data extracted for meta-analyses. Detailed methodology on each meta-analysis (*i.e.,* formulation of nutritional standards, calculation of growth trajectory and feed utilization parameters, quantification of nutritional dependencies on growth) are provided in the supplementary material.

Collection of biofloc biomass. Biofloc biomass was obtained from a well-established indoor, freshwater biofloc system, stocked with Nile tilapia *Oreochromis niloticus* at a stocking density of 35 kg m^{-3}. Commercial pellets (TILAPICO 3 mm, Coppens, The Netherlands) were used as standard feed for fish. Fish feed was given twice daily based on a feed amount equivalent to 2.5% of the fish body weight. Wheat flour (35.56% C; 2.38% N)

served as a carbon source which was applied daily with feed (22.05% C; 7.07% N) in a ratio of 1:0.6 (feed: flour). Assuming a 30% retention of nutrients from feed to fish, the projected C: N ratio was ≈6:1. Such a low C: N ratio favored frequent harvest of young and N-rich wet biofloc biomass[6,10] to be converted to dry matter for the ensuing experiment. Biofloc biomass was drained daily through a pump and a vortex separation device so that the suspended solids level stayed between 25 and 50 ml L^{-1} in the system. After separation, biofloc was filtered through a nylon screen (mesh size 60 µm) to drain the excess water. The filtrate was then dried at 80 °C to obtain a material of solid consistency. After obtaining enough dried biofloc, the samples were grounded by a hammer mill to yield finer particles and hereinafter referred to as the biofloc meal (BM).

Preparation of experimental feed. Commercial pellets (TILAPICO 3 mm, Coppens, The Netherlands) were used as the basal diet due to its similar protein content with our test ingredient (BM). The commercial 'fish feed' was chosen due to a lack of established 'crayfish feeds' in the market. Even the available ones appeared to be random feed mixtures targeted for ornamental crayfish keeping. Inclusion of BM by replacing basal diet was done on a weight by weight basis. All feeds were isonitrogenous. The graded inclusion levels were 0% (basal diet = control diet), 33% (67% basal + 33% BM; diet BM_{33}), 66% (34% basal + 66% BM; diet BM_{66}) and 100% (only BM; diet BM_{100}). Feed pellets (pellet size 2 mm) were cold extruded, dried (12 h; 45 °C), vacuum sealed, and stored at 4 °C till further use. The diet samples were analyzed in an accredited third-party laboratory (AGRO-LA, spol. s.r.o., https://www.agrola.cz/zemedelske-a-potravinarske-sluzby/) employing analytical methods (ISO verified and certified protocols in the Czech Republic) for proximate composition, essential amino acids (EAAs; except tryptophan due to analytical error), heavy metals, and essential mineral contents. Detailed composition of basal diet, treatment diets and the biofloc meal are summarized in Table 4.

Crayfish keeping. A total of 120 juvenile red swamp crayfish (*Procambarus clarkii;* conservation status: least concern) having a mean weight of 7.8 ± 0.7 mg at the onset of exogenous feeding (developmental stage 3), were used as experimental animals (10 individuals per tank; 4 group x triplicate). The experiment lasting for nine weeks was conducted in a series of indoor glass aquaria (54 × 36 × 30 cm, volume 46 L) with aeration and attached to a recirculating aquaculture system. Two baked clay bricks (28.5 × 13.5 × 6.5 cm), each with 39 cross holes (26 and 13 holes with a profile of 1 × 3 cm and 1 × 1 cm, respectively), were placed in each aquarium to provide shelters/refugia for the stocked crayfish[43]. After three weeks, a block of joined polypropylene tubes containing five tubes (length 10 cm, inner diameter 35 mm) was added to each aquarium as an additional shelter for on-growing animals. The bases were represented by three longitudinally joined tubes with a further two tubes positioned pyramidal in the second layer[44]. Altogether, 12 tanks were used and subjected to stable indoor climatic conditions with natural photoperiod (12L:12D).

Growth trial and feed utilization parameters. Crayfish were fed twice a day to apparent satiation (roughly corresponding 5–6% of the body weight) with the abovementioned diets for nine weeks. Uneaten feed, feces, and other wastes were siphoned out manually every morning. Dissolved oxygen (7.9 ± 0.3 mg L^{-1}), pH (7.6 ± 0.2), and temperature (21.8 ± 0.3 °C) were measured daily using Oxi 3205 and pH 720 m (WTW GmbH, Weilheim, Germany), respectively. Every three weeks, the body weight was measured using an electronic balance (lowest sensitivity 1 mg) and the number of survivors counted. The feed rationing was revised accordingly. Body weight measurements were taken before feeding. After the trial, final body weight and total length were recorded, including the number of survivors. The animals were not fed before the day of the final measurement.

The food conversion ratio (FCR, units), protein efficiency ratio (PER, units), and survivability (%) were determined for each diet following the formulas in Cortes-Jacinto et al.[45]. Live weight gain (LWG) was calculated applying the formula, LWG = final—initial weight (in mg)/ days reared. Coefficient of variance (CV) of body weight (standard deviation × 100/mean) was calculated as a measure of size heterogeneity. To eliminate statistical biasedness in the data due to hierarchical size distribution in crayfish groups, other measures of central dispersion like interquartile range (IR) and median were included besides the mean. The abovementioned parameters were calculated from the IR, median, and mean estimates of each treatment. All graphical models were generated using the ggplot2 package in R. Statistically significant differences (α level set at 0.05) in body weight, growth, and survivability of crayfish fed on different dietary treatments were tested. The grouped data were first subjected to a Shapiro–Wilk's normality test; then following the *p value*, either one-way ANOVA with *post-hoc* Tukey HSD (parametric test), or, Kruskal–Wallis *post-hoc* Dunn's test with Bonferroni correction (non-parametric test) was selected. The tests were performed using default commands in RStudio v1.2.5042.

Assessment of heavy metals risk from biofloc biomass. At the end of the experiment, tail muscle and hepatopancreas samples from representative crayfish of each group were collected and frozen (−20 °C). Selected heavy metals (Hg, Cd, Zn; following high bioaccumulation affinity realized in Kouba et al.[46]) and some additional minerals (Fe, Mn) were analyzed from these samples in the same accredited third-party laboratory. Body (muscle + hepatopancreas) heavy metal levels were compared with maximum permissible limits (Cd or Hg 0.5 mg kg^{-1} wet weight basis) given in the European Commission[47] for aquatic meat products (in the context of safety for consumption). In the context of agricultural use safety (as fertilizers), the heavy metal content of biofloc meal was determined and compared with Czech EPA limits (Cd 5 mg kg^{-1}, Hg 4 mg kg^{-1} dry matter basis) (Decree of Ministry of Environmental of the Czech Republic No. 437/2016 on the Code, 2016).

Ethics approval. All procedures performed in studies involving animals (*Oreochromis niloticus* and *Procambarus clarkii*) were in accordance with the ethical standards approved by the institutional ethics committee (Jihočeská univerzita v Českých Budějovicích Fakulta rybářství a ochrany vod).

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References

1. Richman, N.I. et al. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140060 (2015).
2. FAO FishStat. Global aquaculture production 1950–2018. FIGIS: FishStatPlus–universal software online query panel for fishery statistical time series. FAO Fisheries and Aquaculture Department, Rome. (2020) https://www.fao.org/fishery/statistics/global-aquaculture-production/query/en (accessed on: 06/2020)
3. Saoud, I.P., Garza De Yta, A. & Ghanawi, J. A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquac. Nutr.* **18**, 349–368 (2012).
4. NRC (National Research Council). Nutrient requirements of fish and shrimp. National Academies Press (2011). https://doi.org/10.17226/13039
5. Crab, R., Defoirdt, T., Bossier, P. & Verstraete, W. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* **356–357**, 351–356 (2012).
6. Arantes, R., Schveitzer, R., Quadros Seiffert, W., Lapa, K. R. & Vinatea, L. Nutrient discharge, sludge quantity and characteristics in biofloc shrimp culture using two methods of carbohydrate fertilization. *Aquac. Eng.* **76**, 1–8 (2017).
7. Avnimelech, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **176**, 227–235 (1999).
8. Gamboa-Delgado, J. & Márquez-Reyes, J. M. Potential of microbial-derived nutrients for aquaculture development. *Rev. Aquac.* **10**, 224–246 (2018).
9. Kuhn, D. D., Boardman, G. D., Lawrence, A. L., Marsh, L. & Flick, G. J. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture* **296**, 51–57 (2009).
10. Schveitzer, R. *et al.* Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. *Aquac. Eng.* **56**, 59–70 (2013).
11. Poli, M.A., Schveitzer, R. & de Oliveira Nuñer, A.P. The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. *Aquac. Eng.* **66**, 17–21 (2015).
12. Hargreaves, J.A. Biofloc production systems for aquaculture. Southern Regional Aquaculture Center, Publication no. 4503, 12 pp (2013).
13. Arantes, R., Schveitzer, R., Magnotti, C., Lapa, K. R. & Vinatea, L. A comparison between water exchange and settling tank as a method for suspended solids management in intensive biofloc technology systems: effects on shrimp (*Litopenaeus vannamei*) performance, water quality and water use. *Aquac. Res.* **48**, 1478–1490 (2017).
14. Schveitzer, R. *et al.* The role of sedimentation in the structuring of microbial communities in biofloc-dominated aquaculture tanks. *Aquaculture* **514**, 734493 (2020).
15. Ben Rebah, F., Mnif, W. & M. Siddeeg, S. Microbial flocculants as an alternative to synthetic polymers for wastewater treatment: A Review. *Symmetry (Basel)* **10,** 556 (2018).
16. van Rijn, J. Waste treatment in recirculating aquaculture systems. *Aquac. Eng.* **53**, 49–56 (2013).
17. Gamboa-Delgado, J. *et al.* Comparing the assimilation of dietary nitrogen supplied by animal-, plant- and microbial-derived ingredients in Pacific white shrimp *Litopenaeus vannamei*: A stable isotope study. *Aquac. Rep.* **17**, 100294 (2020).
18. Jones, S. W., Karpol, A., Friedman, S., Maru, B. T. & Tracy, B. P. Recent advances in single cell protein use as a feed ingredient in aquaculture. *Curr. Opin. Biotechnol.* **61**, 189–197 (2020).
19. Glencross, B. *et al.* Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp Penaeus monodon. *Aquaculture* **431**, 12–19 (2014).
20. IAFFD, International Aquaculture Feed Formulation Database. (2020). Feed Ingredients Composition Database (FICD) v5.1. Available online at https://www.iaffd.com/ (accessed on 29 September 2020)
21. Reigh, R. C. & Ellis, S. C. Utilization of animal-protein and plant-protein supplements by red swamp crayfish *Procambarus clarkii* fed formulated diets. *J. World Aquac. Soc.* **25**, 541–552 (1994).
22. Emerenciano, M., Gaxiola, G. & Cuzo, G. Biofloc Technology (BFT): A review for aquaculture application and animal food industry. In: Biomass now - cultivation and utilization (InTech, 2013). https://doi.org/10.5772/53902
23. Azhar, M. H. *et al.* Biofloc-based co-culture systems of Nile tilapia (*Oreochromis niloticus*) and redclaw crayfish (*Cherax quadricarinatus*) with different carbon–nitrogen ratios. *Aquac. Int.* **28**, 1293–1304 (2020).
24. Li, J. *et al.* Juvenile *Procambarus clarkii* farmed using biofloc technology or commercial feed in zero-water exchange indoor tanks: a comparison of growth performance, enzyme activity and proximate composition. *Aquac. Res.* **50**, 1834–1843 (2019).
25. Gruber, C., Tulonen, J., Kortet, R. & Hirvonen, H. Resource availability and predation risk influence contest behavior and dominance hierarchies in crayfish. *Behav. Ecol. Sociobiol.* **70**, 1305–1317 (2016).
26. Gamboa-Delgado, J. *et al.* Assessment of the relative contribution of dietary nitrogen from fish meal and biofloc meal to the growth of Pacific white shrimp (*Litopenaeus vannamei*). *Aquac. Res.* **48**, 2963–2972 (2017).
27. Fimbres-Acedo, Y.E. *et al. Oreochromis niloticus* aquaculture with biofloc technology, photoautotrophic conditions and *Chlorella* microalgae. *Aquac. Res.* are.14668 (2020). https://doi.org/10.1111/are.14668
28. Ekasari, J. *et al.* The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture* **426–427**, 105–111 (2014).
29. Ju, Z. Y. *et al.* Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquac. Res.* **39**, 118–133 (2008).
30. Zaglol, N. F. & Eltadawy, F. Study on chemical quality and nutrition value of fresh water crayfish (*Procambarus clarkii*). *J. Arab. Aquacul. Soc.* **4**, 1–18 (2009).
31. Amine, T., Aiad, A. & Abu El-Nile, M. Evaluation of chemical quality and nutrition value of fresh water cray fish (*Procambarus clarkii*). *J. High Inst. Public Heal.* **38**, 1–13 (2008).
32. Tönges, S. et al. Physiological properties and tailored feeds to support aquaculture of marbled crayfish in closed systems. bioRxiv (2020). https://doi.org/10.1101/2020.02.25.964114
33. Zhou, Q. C. *et al.* Dietary arginine requirement of juvenile Pacific white shrimp Litopenaeus vannamei. *Aquaculture* **364–365**, 252–258 (2012).
34. Wu, G. & Morris, S. M. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* **336**, 1–17 (1998).
35. Evoy, D., Lieberman, M. D., Fahey, T. J. & Daly, J. M. Immunonutrition: the role of arginine. *Nutrition* **14**, 611–617 (1998).
36. D'Abramo, L. R. & Robinson, E. H. Nutrition of crayfish. *Rev. Aquat. Sci.* **1**, 711–728 (1989).
37. Brown, P. B. Physiological adaptations in the gastrointestinal tract of crayfish. *Am. Zool.* **35**, 20–27 (1995).
38. Sabry Neto, H., Santaella, S.T. & Nunes, A.J.P. Bioavailability of crude protein and lipid from biofloc meals produced in an activated sludge system for white shrimp, *Litopenaeus vannamei*. *Rev. Bras. Zootec.* **44**, 269–275 (2015).

39. Piedad-Pascual, F. Mineral requirements of Penaeids. In: Advances in Tropical Aquaculture, Workshop at Tahiti, French Polynesia AQUACOP IFREMER. *Actes de Colloque* **9**, 309–318 (1989).
40. Kanazawa, A., Teshima, S. & Sasaki, M. Requirements of the juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese, and iron. *Mem. Fac. Fish. Kagoshima Univ* **33**, 63–71 (1984).
41. Pequeux, A. Osmotic regulation in crustaceans. *J. Crustac. Biol.* **15**, 1 (1995).
42. Bureau, D.P., Kaushik, S.J. & Cho, C.Y Bioenergetics. In: J.E. Halver & R.W. Hardy (eds) Fish Nutrition, 3rd edition (Academic Press/Elsevier, Amsterdam), 2–61. (2002).
43. Kouba, A., Hamáčková, J., Buřič, M., Policar, T. & Kozák, P. Use of three forms of decapsulated Artemia cysts as food for juvenile noble crayfish (*Astacus astacus*). *Czech J. Anim. Sci.* **56**, 114–118 (2011).
44. Veselý, L. *et al.* Salinity tolerance of marbled crayfish *Procambarus fallax* f. *virginalis*. *Knowl. Manag. Aquat. Ecosyst.* (2017). https://doi.org/10.1051/kmae/2017014
45. Cortes-jacinto, E., Villarreal-colmenares, H., Civera-cerecedo, R. & Naranjo-paramo, J. Effect of dietary protein level on the growth and survival of pre-adult freshwater crayfish *Cherax quadricarinatus* (von Martens) in monosex culture. *Aquac. Res.* **35**, 71–79 (2004).
46. Kouba, A., Buřič, M. & Kozák, P. Bioaccumulation and effects of heavy metals in crayfish: a review. *Water, Air, Soil Pollut.* **211**, 5–16 (2010).
47. European Commission. Commission Regulation No 466/2001 of March 2001 setting maximum levels for certain contaminants in foodstuffs. European commission 2001R0466/20051129, 17p (2005). https://op.europa.eu/s/n7e6

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Author contributions
R.L. and K.R. (contributed equally to the work): Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Visualization, Writing—original draft. P.D.: Investigation, Resources, Project administration. A.K.: Conceptualization, Resources, Investigation, Funding Acquisition, Validation, Writing—review & editing. J.M.: Conceptualization, Resources, Supervision, Funding Acquisition, Validation, Writing—review & editing.

Competing interests
The authors declare no competing interests.

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CHAPTER 5

General discussion

Fish stocking density, total fish biomass, selection of feed, feed input rate, water quality and water management regimes are known to have a decisive impact on the assimilation of nutrients in RAS and ultimate wastewater production. The main source of nutrients being – uneaten feed, fish feces, soluble excreta, pH buffer input and in-system solids or bioflocs (Ebeling and Timmons, 2012; Goddek et al., 2015). Valorisation of aquaculture waste in the form of sludge is a well-presented topic in the environmental field. In Chapter 1 we presented well-known technologies with supplementary summarization. But what are the most beneficial techniques for waste valorisation? Commercial recirculating systems generally replace 5–15% of the total production system's volume per day (Martins et al., 2010). With a 5–15% daily water exchange rate, a commercial facility carrying an average biomass of 100 metric tons (with a fish density of 80 $kg.m^{-3}$) would typically produce 62–188 m^3 of wastewater per day (Timmons et al., 2002; van Rijn, 2013).

Direct application of aquaculture sludge on fields for fertilization

Applying dewatered aquaculture sludge to fields is the simplest way to utilize TSS. This application can be achieved directly or in ratios. Geotextile bag systems are typically sized to capture a certain amount of waste solids. Considering the passive treatment effects of the geotextile bag system, sizing criteria should also consider the fate of the treated effluent to minimize the cost of RAS effluent treatment. The final handling of the captured solids remains to be determined; however land application of solids will likely be the most cost effective means of handling (Guerdat et al., 2013). Brod et al. (2017) applied dried fish sludge from RAS on 'agricultural' land and achieved a relative agronomic efficiency compared with mineral fertilizer of 50–80%. Sludge from RAS, used for vermicomposting (Chapter 3) was generally applicable to the field, as it met all the regulations under the existing legislation. Danaher et al. (2013) presented the significant effect of composted substrate (60% dewatered aquaculture sludge and 40% shredded guinea grass as carbon source) on tomato (*Lycopersicon esculentum*) seeds. They compared their own mixture with commercial fertilizer generally used for tomato seeds. Rakocy et al. (2009) used the same type of substrate for lettuce seedlings (*Lactuca sativa*) and sweet basil plants (*Ocimum basilicum*). Sediment from catfish (*undefined species*) breeding culture was used as fertilization for maize (*Zea mays*) production with positive effect (Van Tung et al., 2020). The compost derived from the sediment played an important role in supplying the nutrients for cultivating the plants. Adding compost from 10 to 20 t/ha to the cornfield could improve yield by around 10–15%. Strauch et al. (2018) reported concentrations of macro and micronutrients that were shown to have adequate plant growth in the sludge of recirculating aquaculture systems for *C. gariepinus* production. However, the concentrations were less than those of common chemical fertilizers used in traditional agriculture. In addition, the ideal destination of the sludge can decrease the environmental impact since it reduces the accumulation of organic matter. The sludge is considered suitable to fertilize crops in land based agriculture given that fish sludge is one of the most nutrient rich wastes among livestock sources, showing nitrogen and phosphorus contents of approximately 4.45% and 2.35%, respectively (Khiari et al., 2019).

It is important to mention that the same idea has been proven in marine aquaculture systems. Shrimp biosolids (SB) are composed of shrimp fecal matter and decomposed shrimp feed and remain as debris in the bottoms of drained ponds used to culture shrimp. These biosolids are considered waste and are usually disposed of in landfill. Nutrient tests of SB indicated that the material is rich in nutrients especially nitrates (196 $mg.kg^{-1}$), with a 6.8 pH, and a cation exchange capacity of 8.0. Hopkins et al. (1994) removed SB deposits from aquaculture ponds

during the shrimp growing season and estimated the yield of nitrogen was approximately 700 $kg.ha^{-1}$. SB, however, contains high sodium levels and dissolved solids indicating that this material must be diluted in the soil solution to prevent salinity problems. Dufault et al. (2000, 2001) determined that SB needs to be supplemented with inorganic fertilizer to sustain bell pepper (*Capsicum annuum*) and broccoli (*Brassica oleracea italica*) plant growth, and only low rates of SB should be used with salt sensitive crops grown in medium compost of 10% SB with 90% soilless medium. SB is a valuable source of N, P, K and a variety of other useful plant nutrients; however, SB contains high levels of Na.

Super-intensive shrimp culture has a high potential to be used as a raw material for organic fertilizer. It has a high nutrient content, such as total N (0.58%), P_2O_5 (3.33%), K_2O (0.8%), 9.94% of organic carbon (Suwoyo et al., 2019). Joesting et al. (2016) used solid waste from aquaculture in the production of *Spartina alterniflora* and *Juncus roemerianus* seedlings. The results of this study indicate that *J. roemerianus* is a suitable plant species which can be used for marine aquaculture solid waste utilization. The positive benefits of using aquaculture effluents (tank water and sludge from Nile tilapia production systems) in pepper production have been presented by Palada et al. (1999). In the first year, yield of peppers applied with sludge was lower than the yield of fertigated peppers. In the second year, yield from the sludge treatment was higher than yields from conventional treatments of fertigation and commercial fertilizers. That study showed that it is possible to grow vegetable crops using effluents from intensive tilapia culture in tanks without external fertilizer inputs. It is important to mention that the positive effect of aquaculture waste fertilization was proven not only on blooming plants but also on typical field species such as common wheat (*Triticum aestivum*) by Al-Jaloud et al. (1993) and barley (*Hordeum vulgare*) (Hussain and Al-Saati, 1999). Aquaculture sludge extract as an enrichment medium for microalgae growth is possible and can enhance the growth to maximum levels compared with the artificial culture medium. Arumugam et al. (2020) identified the influence of aquaculture sludge extract on four microalgae species: *Chlorella vulgaris, Neochloris conjuncta, Nannochloropsis ocenica* and *Nephroclamys subsolitaria*.

Biogas production

Strauch et al. (2018) found that 5 to 10% of the energy input (by feed) is recovered in the deposited solid wastes. Mirzoyan and Gross (2013) stated that 2 to 4% of the RAS energy demands could be covered by using the energetic potential of the remaining sludge by AD. During thermophilic composting of shrimp aquaculture sludge, the emission of nitrogen as NH_3 gas at 60 and 70 °C was 14.7% and 15.6%, respectively, which is much higher than that at 50 °C (9.0%). The nitrogen mass balance analysis revealed that higher temperature enhanced the solubilization of non-dissolved nitrogen and evaporation of NH_4^+ as NH_3 gas. Microbial community analysis clarified the change of dominant bacteria from *Bacillus* to *Geobacillus* group, with the rise of composting temperature. Generally speaking, thermophilic composting of shrimp aquaculture sludge at 60–70 °C is the most favorable condition for enhancing NH_3 recovery (Koyama et al., 2018). Nhut et al. (2019) presented biogas composition (CH_4 52,7% and 43,7% CO_2) from RAS´s sludge composting. The percentage of CH_4 in RAS sludge biogas was 6% higher than that in pond sludge biogas. One kilogram of striped catfish (*Pangasianodon hypophthalmus*), produced in RAS, could produce 33.5 L of CH_4, which represents 0.33 kW h potential energy yield per kg of fish produced. Nevertheless, sludge from striped catfish has a lower quality and quantity of methane than that from animal manures, and the resulting electricity yield is low. Therefore, composting is presently considered as the best option to reuse part of the nutrients that are trapped in the sludge from striped catfish in RAS.

Sludge in the form of microbial biomass

BFT offers a new alternative way of shrimp and fish culture. This technology has benefits in many ways. The primary production of BFT is microbial biomass. Aquatic organisms stocked in BFT receive feed in parallel with biomass, thus reducing the cost of overall production (Emerenciano et al., 2017). Moreover, the microbial biomass is still growing, and it is important to drain it out of the system (Hargreaves, 2013). There is a simple method of filtering and drying to get biofloc meal from microbial biomass. There is potential in the nutrient composition of this matter. Biofloc meal can partially replace the total diet. The partial replacement of conventional protein sources (such as fish meal and soybean meal (SBM) with BM in shrimp diets has received attention from many researchers in recent years. Studies have confirmed that the inclusion of BM can significantly reduce supplemental protein content of the feeds, enhance growth performance of shrimp reared in BFT systems and, in turn, reduce feed costs (El-Sayed, 2021). Bauer et al. (2012) used BM from *L. vannamei* culture with soybean protein for replacing fishmeal in shrimp diets – with a successful replacement of 100% (14% BM). Microbial flocs, produced in sequencing batch reactors (SBR) using sucrose as a carbon source, can replace up to 15.6% of SBM or FM protein in *L. vannamei* diets (Kuhn et al., 2009). A higher substitution level (up to 30%) was reported when bioflocs produced in SBR were used (Kuhn et al., 2010). In Chapter 4 were presented the successful results – with 33% BM replacement in feed for crayfish (*Procambarus clarkii*). Higher supplementation in the diet had a negative effect on growth. The nutritional quality of biofloc biomass from *O. niloticus* BFT was also well reported in Binalshikh-Abubkr et al. (2021). In their study, they recommend future studies of BM as a supplement or replacement for feed ingredients in fish diets because of its ability to improve the growth performance of cultured animals.

Using sludge for vermicomposting

Vermicomposting could present the most beneficial waste valorisation technique. But it is important to mention that the fusion of aquaculture and vermicomposting – and especially using aquaculture sludge for vermicomposting – is not a well-reported issue. Chapter 3 presented promising results in the utilization of RAS aquaculture sludge by vermicomposting. Over the least two decades, only five papers were presented. Rynk et al. (1998), Marsh et al. (2005), Birch et al. (2010), Yeo et al. (2010) and Kouba et al. (2018) – Chapter 3 presented the direct application of aquaculture sludge on vermiculture systems. Chakrabarty et al. (2009) presented a review about the application of aquaculture sludge but without proven results. It is hard to directly compare the results from Chapter 3 with similar research. Again, waste composition is the main topic in how to utilize it. Marsh et al. (2005) collected sludge from a highly intensive RAS, and Buyuksonmez et al. (2005) from FTS, both of which filter water and create smaller volumes of concentrated waste. Birch et al. (2010) used sediment from land pond with low nutrient composition. In Chapter 3 sludge was used from a different part of RAS and an adjacent pond – which was used for sedimentation of effluent water. Expanding on the pilot study by Marsh et al. (2005) which utilised the earthworm *E. fetida*, the study (Chapter 3) provides a comprehensive evaluation of the successful use of earthworms for vermicomposting sludge from RAS by means of *E. andrei*. These earthworms are widely distributed throughout temperate regions and are the most commonly used species in vermicultures (Edwards, 2004). Literature suggests that *E. andrei* is the preferred candidate for use in temperate zones, due to its higher reproduction indices (Domínguez et al., 2005) as well as elevated innate defence mechanisms (Dvořák et al., 2013) which are beneficial when exposed to RAS sludge that is rich in potentially pathogenic microorganisms. Nevertheless, in Chapter 3 the highest survival rate of earthworms (exceeding 90% among treatments) was proven, compared to 82% reported by Marsh et al. (2005). High survival of initial stocks, their individual growth and

reproduction indices (cocoon and juvenile production) were documented. Complying with limits for heavy metals, vermicomposts were found to be suitable for use in agriculture. For the optimization of aquaculture, sludge utilization for vermicomposting is still important future research. The fusion of aquaculture and vermicomposting is a promising idea. Chakrabarty et al. (2009) recorded significantly higher plankton production and fish (common carp) growth in vermicompost treated ponds in comparison to traditionally used organic manures and inorganic fertilizers. It might be possible to use pond bottom sludge for vermicomposting and the final product for reintegration. Deolalikar and Mitra (2004) have reported comparable efficacy of vermicompost with other commercial manures used in aquaculture. An increased net productivity with better growth of *L. rohita* has been found when using vermicompost. As well as being used for carp culture, vermicompost can also be used for catfish species. Ghosh (2004) recorded better growth of *Clarias batrachus* and higher water retention capacity in vermicompost manured ponds compared to inorganic fertilizer treated ponds. The study of Kaur and Ansal (2010) shows the utilization of vermicompost directly as feed (higher growth of *C. carpio*) and indirectly as manure for fish culture ponds. Thus, along with the additional manure value, the expenditure on the feed can also be reduced with the use of vermicompost. Vermicompost has also been reported to result in the higher survival and growth of aquatic organisms – including fish and prawn (Kumar et al., 2007) – without adversely affecting the water quality. It is also important to mention that the final product of vermicomposting is not just suitable fertilization matter, but also a valuable source of protein and feed in the form of worms.

Tacon et al. (1983) reported that the rainbow trout which were fed 100% worm meal (*E. foetida*) protein in the form of frozen slices did not yield any encouraging growth and dietary performance. Similar to their results, Óscar Pereira and Gomes (1995) also reported that the growth rate and feed utilization efficiency were adversely affected when the diets containing a high level of frozen worms were fed to rainbow trout. According to Edwards and Niederer (1988), worm meal is able to substitute fish meal for monogastric animals and fish; 25–50% of dietary protein could be supplied from worm meal. Stafford and Tacon (1985) evaluated the dried earthworm meal derived from *E. foetida* in the diet of rainbow trout and reported that there was no adverse effect on the growth performance or feed utilization efficiency in fish that were fed diets containing low levels of earthworm meal. Ganesh et al. (2003) observed better growth and nutrient utilization in carp (*Cirrhinus mrigala*) muscle that were fed a diet containing *E. foetida* worm meal than the fish that were fed a meal-based diet. The results of Istiqomah et al. (2009) showed no difference in weight gain in carp (*Catla catla*) fry diet (30% inclusion of earthworm meal) compared to control (30% inclusion of fish meal). Further, survival was higher in the former (75.75%) than the latter (66.66%). Kostecka and Paczka (2006) reported that the aquarium fish *Poecilia reticulata* fed by earthworm (*E. foetida*) biomass, showed a significant increase of brood number (twice as many offspring as control). Zhenjun et al. (1997) presented how common nutrient analysis showed that *E. foetida* meal has a high protein content in the range of 54.6 to 71.0% dry matter. Protein content and amino acid composition were close to that of fish meal and hen egg, and higher than that of cow milk powder and soybean meal. Casts of *E. foetida* had a protein content of 7.9% dry matter, which is similar to that of corn meal, and hence worm casts could be used for partial replacement of corn meal or wheat bran in animal diets. Worm body fluids contained 9.4% protein and 78.79 free amino acid per litre and were found to be rich in vitamins and minerals, particularly Fe. The same *E. foetida* meal was used as dietary protein source for *L. rohita* advanced fry by Mohanta et al. (2016). In Chapter 3 we found that, in terms of hygienic quality, earthworm biomass could be a suitable feed for fish. Compared to a fish diet, there was no chromium, and equal copper, nickel and zinc concentrations in biomass. Levels were well below EU limits for lead (11.4 $mg.kg^{-1}$) and mercury (0.57 $mg.kg^{-1}$).

Wastewater valorisation for the growing of plants

The recycling and application of aquaculture wastewater directly into the soil to produce crops of interest is the simplest and most efficient way to use this effluent. Aquaculture effluents must be used in a manner that avoids environmental degradation by considering site conditions (soil, topography, vegetation), time of application, application rates, nutrient absorption rates of the crops and the total area that can receive the effluents (Timmons et al., 2002). Chapter 2 presented the advantages and disadvantages of aquaculture wastewater utilization. It is important to note that wastewater from average RAS usually has high pH (compared to plant requirements), a low concentration of potassium, and the high level of sodium concentration depends on pH adjustment. Despite these problems, the use of wastewater for aquaponics or as a nutrient solution for hydroponic plant growing is well documented. As mentioned in Chapter 1, wastewater composition from RAS depends on many factors. According to Bosma et al. (2017), the choice of vegetables for an aquaponic system is based on three parameters, namely; the market demand, the convenience for growing fish and vegetables in an aquaponic system, and the match between nutrient input and requirements. Hence only a few plants have been successfully grown in aquaponic systems. These include *Lactuca sativa, Cucumis sativus, Capsicum annum, Solanum lycopersicum, Solanum melongena* (with some extra care) and root crop such as carrot.

Different requirements for pH value of water (fish vs. plants) is mostly compromised in the one-loop system. Goddek (2017) mentioned that for optimal growth of all organisms in aquaponic systems, multi-loop compilation is more suitable. Certain nutrients are affected by the pH of nutrient solution, either by influencing the ionic form of the nutrient in solution or by influencing the nutrient's uptake and assimilation into plant tissue. Nitrogen transformations could be significantly affected by low pH in the aquaponic system (Zou et al., 2016). Low pH values could present a health problem for some pH sensitive fish types, whilst higher pH could be harmful for plants' growth (Rakocy et al., 2004). Although Blanchard et al. (2020) did not observe a significant effect of pH range 4–7 from tilapia wastewater on *C. sativus* growth. A similar conclusion (pH 5–8) was reported by Tyson et al. (2008).

The supplementation of K is necessary to reach adequate levels for plant growth, which are approximately 180–400 $mg.L^{-1}$ depending on the plant species (Resh, 2012; Bittsanszky et al., 2016). The concentrations of K in all studies that focused on wastewater quality were well below those recommended for vegetables grown in hydroponics 26.41 $mg.L^{-1}$ (Lenz et al., 2021), 50.8 $mg.L^{-1}$ (Bittsanszky et al., 2016); probably due to the absence of supplementation throughout the cultivation cycles. Chapter 2 presented a potassium range of 7.96–155 and iron 0.3–14.3 $mg.L^{-1}$ in wastewater from three different RAS's. Some studies did not even mention the potassium concentration in wastewater (Goddek et al., 2015). Hussain et al. (2014) presented normal water spinach growth in wastewater from carp with only 20 $mg.L^{-1}$ of potassium. Or it is necessary to add potassium in the most natural form of fertilizer (Yep and Zheng, 2020). Plants and fish, however, have different nutrient requirements, for example, they have different potassium requirements (Graber and Junge, 2009). Fish feed may not be rich in certain nutrients, such as potassium and iron that are required by plants. These may need to be supplemented to meet the needs of plants. Another way to increase potassium concentration in wastewater for plant cultivation is the addition of KCl and KDF to the feed. Guwa et al. (2020) reported that the addition of 5.1 $g.kg^{-1}$ KCL, 9 $g.kg^{-1}$ KDF and 30 $mg.kg^{-1}$ of iron sulphate ($FeSO_4$) in feed, increases potassium and iron in wastewater and sludge from *C. gariepinus* culture. Iron concentration in *L. sativa* after experiment was 0.29 $g.kg^{-1}$ compared with control 0.22 $g.kg^{-1}$, and iron concentration in water was 0.15 $mg.L^{-1}$ compared with control 0.06 $mg.L^{-1}$. In aquaponic and hydroponic systems, iron is usually supplemented in a chelated form as iron ethylenediaminetetraacetic acid (Fe-EDTA) or iron

diethylenetriaminepentaacetic acid (Fe-DTPA). However, the chelated iron needs to be added continuously and is unstable at pH >7 (Rakocy et al., 2006). In this study, the addition of Fe through the feed additive $FeSO_4$ in the fish feed resulted in consistent improved growth of fish and plants under favourable conditions without requiring the continuous addition of Fe in any part of the system (Guwa, 2020). Ng et al. (2009) reported no adverse effects and significant differences in proximate composition when tilapia were fed blended organic acids and KDF at 2 $g.kg^{-1}$. Similarly, the whole body proximate composition of grass carp (*Ctenopharyngodon idella*) was significantly affected when KCl was included in the diet, where the moisture content increased as the lipid content decreased (Zhu et al., 2014). Furthermore, the inclusion of KDF in the diet significantly improved the protein efficiency ratio in *O. niloticus* culture (Elala and Ragaa, 2015).

Potassium and sodium values in aquaculture wastewater presented the most important issue and the next step to optimizing wastewater to optimal plant cultivation nutrient solution. Since the pH value in RAS has an unstable tendency to decrease (be acidic), it is necessary to constantly replenish the system with a buffer (Ebeling and Timmons, 2012). Chapter 1 explained the toxic effects of sodium on plants. According to the wide research in Chapter 2, sodium concentration in wastewater from RAS with sodium bicarbonate adjustment was 383–407 $mg.L^{-1}$. A similar concentration of sodium 336–340 $mg.L^{-1}$ was observed by Shete et al. (2015) with a negative effect on mint (*Mentha arvensis*) growth. The common way to desalinate wastewater from Na is by using plant section with halophytes plants. Or again to use high sodium concentration in water to grow special species. An obvious plant candidate for this, is marsh samphire (*Salicornia europaea*) and potentially other halophytes such as sea kale (*Crambe maritima*), sea aster (*Tripolium pannonicum*) and sea purslane (*Atriplex portulacoides*). Gunning (2016) noted that in the most arid regions of the word the cultivation of halophytes as an alternative to conventional crops is gaining significant popularity – and *Salicornia europea* is becoming increasingly popular on the menus of restaurants and the counters of health-food stores across the country (Kotzen et al., 2019). Doncato and Costa (2021) even combined BFT with saline aquaponics. They use one-year-old biofloc water from breeding stock tanks of the shrimp *Litopenaeus vannamei* to grow Brazilian halophytes *Salicornia neei, Apium graveolens* and *Paspalum vaginatum* in saline aquaponics. Plants were established in hydroponic units and clarified saline water from a BFT system was recirculated and replaced weekly over 30 days. *Paspalum urvillei* can tolerate high iron concentrations and increased growth performance (de Araújo et al., 2014). Problems of high iron concentration BFT were reported in Chapter 4. Marques et al. (2017) and their halophyte aquaponics (*Halimione portulacoides*) for the bio mitigation of a super intensive marine fish farm effluent revealed a considerable potential for the mitigation of dissolved nitrogen (67% decrease efficiency).

There exists two ways to approach plants in aquaculture. It is possible to use constructed wetlands; basically, plant systems to remove elements from the water and treat it like filtration. Or one can use aquaponics to have fish and plant yield production (Rakocy et al., 2006). The major element in RAS´s wastewater is nitrogen in all its forms (Timmons et al., 2002). Jobling (2012) recommended that NO_3-N concentrations do not exceed 50 $mg.L^{-1}$ in water used for the culture of fish and shellfish, since high nitrate concentrations typically result in algae blooms (in outdoor systems), which over time can result in the lowering of pH (Watson and Hill, 2006). Van Tung et al. (2020) referred to the use of wastewater from *C. gariepinus* breeding culture for water spinach (*Ipomoea aquatic*), thanks to plant natural filtration, 38.78% total organic carbon, 27.07% N and 58.42 P was drained from wastewater. Enduta et al. (2011) removed 79.17% NO_3-N after 4 weeks and then 87.10% after 12 weeks for the water spinach system. It was about 66.67% after week 4 and then 80.65% after week

12 for the mustard green (*Brassica juncea*) from RAS wastewater. Similar nitrate removal from Nile tilapia RAS (80.69%) by water spinach was reported (Salam et al., 2014). The study of de Farias Lima et al. (2019) presented the performance of an aquaponic system using constructed semi-dry wetland with lettuce planted on treating wastewater from shrimp *Macrobrachium amazonicum* culture. Their results suggested that the aquaponic recirculation system, using constructed semi-dry wetlands with lettuces, was satisfactorily efficient like water treatment filters at the densities tested in removing the main pollutants from shrimp culture water. Sánchez (2014) even investigated the idea of using aquaponics for aquaculture wastewater treatment and also human urine treatment. He used 0.02% of human urine as a source of ammonia for the growing of herbs, lettuce and tomatoes. Human source of ammonia was used by Leng et al. (1995) in separate tanks to grow duckweed (*Araceae lemnoideae*) as an alternative source of fish food. Vegetable (tomato, cucumber, and aubergine) production removed 0.52, 0.11 and 0.8 $g.m^{-2}d^{-1}$ for N, P and K in hydroponics and 0.43, 0.07 and 0.4 $g.m^{-2}d^{-1}$ for N, P and K in system. In aquaponics, 69% of nitrogen removal by the overall system could thus be converted into edible fruit (Graber and Junge, 2009). When using wastewater from a RAS that includes a biofilter, the nitrogen concentration in the water may be low for plant production (Rakocy, 2012). However, Mentha species can also perform well under low nitrogen concentrations. In *Mentha arvensis*, good growth was observed under very low levels of ammonia nitrogen (0.81 $mg.L^{-1}$) and nitrate (0.22 $mg.L^{-1}$) combined with *C. carpio* (Shete et al., 2016). According to Knaus et al. (2020), even under the relatively low levels of nitrate from the aquaculture unit combined with relatively high NH_4^+-N in the plant nutrient tanks and low phosphorus (3.3 $mg.L^{-1}$) the intensive production of *C. gariepinus* provided adequate nutrients for *Mentha spicata* cultivation during experiments. Much lower P values between 1.55 $mg.L^{-1}$ and 1.71 $mg.L^{-1}$were reported in the cultivation of *O. niloticus* and *C. carpio* in co-cultivation with cucumber, tomato, and lettuce (Knaus and Palm, 2017). Phosphorus deficits in wastewater for plant cultivation were observed in samples of different RAS (Chapter 2). Consequently, aquaponic experiments report a range of 1–17 $mg.L^{-1}$ PO_4-P (Rakocy et al., 2004; Lennard and Leonard, 2006; Endut et al., 2010; Robinson et al., 2011). However, recommended concentrations in standard hydroponics are generally between 40 and 60 $mg.L^{-1}$ PO_4-P (Sonneveld and Voogt, 2009; Sikawa and Yakupitiyage, 2010; Resh, 2012). This discrepancy suggests that phosphate should be added to aquaponic systems, especially for blooming vegetables that do not yet show satisfying yields in aquaponics (Nichols and Savidov, 2011).

Aquaponics or hydroponics are the main goals in the issue of wastewater valorisation. The most used fusion organisms in aquaponics are *O. niloticus* and non-bloom plants like lettuce, spinach, or herbs (Rakocy et al., 2004; Liang and Chien, 2013; Espinosa Moya et al., 2016; Forchino et al., 2017; Pinho et al., 2017; Forchino et al., 2018; Quagrainie et al., 2018; Setiadi et al., 2018; Chen et al., 2020). Nuwansi et al. (2019) reported utilization of phytoremediated *C. carpio* wastewater for growing gotukola (*Centella asiatica*). Common or ornamental colour forms of carps were frequently used in aquaponic systems in combination with water celery (*Oenanthe javanica*), spinach (*Beta vulgaris* var. *bengalensis*), *I. aquatica, M. arvensis*, *O. basilicum, L. sativa*, vetiver grass (*Chrysopogon zizanioides*), watercress (*Nasturtium officinale*) and even *C. annum* or *S. lycopersicum* bloom plants with high 200–400 $mg.L^{-1}$ potassium (Resh, 2012) requirement (Roosta and Hamidpour, 2011; Roosta and Mohsenian, 2012; Shete et al., 2013, 2016; Hussain et al., 2014, 2015; Filep et al., 2016; Nuwansi et al., 2016, 2017, 2019; Sirakov et al., 2018; Maucieri et al., 2019; Irhayyim et al., 2020; Ajijah et al., 2021; Luo et al., 2021). African catfish and its related species are mostly used in aquaponics for many reasons. No significant differences were observed in survivability, mortality, FCR, SGR of fish, bred in aquaponic systems compared to standard RAS culture. The stocking density

of catfish is very high (50–200 $kg.m^{-3}$), they are also not sensitive to low pH value or the addition of nutrients (Enduta et al., 2011; Knaus and Palm, 2017; Palm et al., 2018; Baßmann et al., 2020; Knaus et al., 2020; Oladimeji et al., 2020; Pasch et al., 2021). Palm et al. (2018) reported that increasing stocking density affected survival rate. In their experiment, the stocking density was extensive, and the mortality of approximately 6%, including escapees, was in a similar range. A general pH tolerance range for fish was stated at 6.0–9.5. In RAS the pH value potentially declines through bacterial nitrification (Masser et al., 1992). Wurts and Durborow (1992) indicated that fish, in general, may become stressed and die when the pH value decreases to <5. Losordo et al. (1999) affirmed this statement but indicated that a lower pH threshold of 4.5 was dangerous to fish. Baßmann et al. (2017) for the first time, recorded significantly fewer external injuries on aquaponic-raised fish and suggested a positive effect of the aquaponics rearing condition for fish welfare. The increased number of wounds on control fish was thought to be caused by behavioural alteration, possibly diminishing welfare (Huntingford et al., 2007). However, agonistic behaviour inside the husbandry does not inevitably lead to more wounds. Van de Nieuwegiessen et al. (2009) reported higher aggression during the induced stress phase, consequently increasing skin lesions in groups from higher stocking densities. Moreover, under aquaponics conditions, the fish show calmer behaviour, less activity, and fewer external injuries (Baßmann et al., 2020). Guwa (2020) reported high (1.23–1.39) FCR compared to other studies of African catfish in aquaponic systems using water spinach (Endut et al., 2009, 2010), mustard green (1.13–1.32) (Enduta et al., 2011) and herbs (0.61) (Knaus and Palm, 2017). The growth rate was in a comparable range to other African catfish grown in aquaponic systems in combination with basil, cucumber, lettuce, and tomato (Palm et al., 2018). Moreover, the growing of plants is not the only option in wastewater valorisation. Microalgae cultivation has become popular as an alternative and beneficial addition in fish feed.

Wastewater for microalgae cultivation

Typical yield expected in microalgae cultivation due to the low concentration of nutrients is 0.3–0.6 $g.L^{-1}$. Nitrogen is an important macro element contributing to biomass production (1–10% of the total mass) and is also a critical factor in regulating algal lipid content (Becker, 1994). Phosphorus is essential for growth and many cellular processes such as energy transfer and the biosynthesis of nucleic acids in algal cells (Chen et al., 2011). The alga grows well in fish water with a specific growth rate of 0.026 h^{-1} (0.623 day^{-1}) and a doubling time of 28 h. These growth parameters compare favourably with those reported elsewhere (Göksan et al., 2007; Madkour et al., 2012), indicating the suitability of cultivating *S. platensis* in fish water. The marine alga, *Platymonas subcordiformis* could remove 87–95% nitrogen and 98–99% phosphorus in the flounder aquaculture wastewater. The biomass of algae was 8.9 times higher than the initial level. However, further studies are required to make these technologies economically viable for algae biofuel production (Guo et al., 2013). The biomass productivities of *Chaetoceros calcitrans, Nannochloris maculate*, and *Tetraselmis chuii* cultured in aquaculture wastewater were not significantly different ($p>0.05$) from their biomass productivities in special Conway medium, indicating that aquaculture wastewater could be reused for microalgal cultivation and the resulting biomass could be used as an aquaculture feed (Khatoon et al., 2016). In addition, the growth of *S. platensis* could be coupled with aquaculture wastewater remediation and its biomass could be used as an agricultural fertilizer (Wuang et al., 2016). The biomass of *Desmodesmus armatus* cultivated in recirculating aquaculture systems increased continuously (Cheban et al., 2015). Based on the results of these studies and this study, aquaculture wastewater is a suitable replacement for fresh water used in microalgal cultivation. Additionally, aquaculture wastewater remediation and increased

CO_2 fixation could be obtained with microalgal cultivation (Kuo et al., 2016). The usefulness of *S. platensis* in aquaculture wastewater treatment was studied and the subsequent application of algal biomass in fertilizer studies was demonstrated. The cultivation of *S. platensis* was done indoors, under an illuminance of not more than 1,000 lx. At these conditions, the algae were able to remove the ammonia and nitrate concentrations in fish water, indicating its ability to treat the water despite its inadequacy in removing nitrate. Potentially, the efficacy of water treatment can be much higher under sunlight where illuminance is typically about 100,000 lx. The supplementation of *S. platensis* for leafy vegetables led to enhanced plant growth in all tested vegetables, when compared to the controls. When compared to the performance of chemical fertilizer, the *Spirulina*-based fertilizer performed comparably in most plant growth parameters, and favourably for one tested species — *Arugula*. Seed germinat;ion (when measured by seedling's dry weight) also improved for all tested vegetables except White Crown. This work has evidenced the usefulness of *S. platensis* in fish water treatment and its applicability as agricultural fertilizer (Wuang et al., 2016). The application of *Chlorella vulgaris* was also found to be beneficial to the growth of *L. sativa* (Faheed and Fattah, 2008).

In this Ph.D. thesis, aquaponics, vermicomposting and biofloc technology were selected as main candidates technologies for aquaculture waste valorisation for several reasons. A RAS located in university indoor room was used for all tests during the whole experimental period. Therefore, we tried to test all systems in the environment of RAS as a pre-test of several technologies. As mentioned, several times, we have used waste products from RAS in various forms. The sludge from the mechanical drum filter was constantly added to the testing vermicompost located in the same room as RAS. Wastewater from the RAS has been used many times for analysis and also as a source for variety of hydroponic systems. The BFT system was also a part of the aquaculture room. Therefore, it was possible to compare all the features of both systems, including the health of the fish. In my point of view, these three technologies do not require as complex operation as the other technologies mentioned and therefore may be a part of RAS. By combining these technologies, future visions may arise for the processing of waste from the aquaculture production system.

Conclusion

- Based on the results of this thesis, several technologies – aquaponics, hydroponics, vermicomposting, biofloc technology can be recommended for intensive aquaculture in order to increase economic efficiency and contribute to the protection of the environment.
- Implementation of a detailed analysis of composition of sludge from different aquaculture systems (Chapter 2, 3, 4).
- Detailed analyses of composition of sludge and especially wastewater from RAS utilization for plant growth under aquaponic condition (Chapter 2).
- The absolute absence of the required amount of potassium for plant cultivation was demonstrated in all monitored RAS´s wastewater samples (Chapter 2).
- Daily use sodium bicarbonate for pH adjustment in RAS led to high concentrations of sodium in the wastewater, which are toxic to plants (Chapter 2).
- The positive effect of a production system incorporating vermicomposting of sludge from intensive aquaculture on the growth of earthworms with no toxicity was provided (Chapter 3).
- Complying with limits for heavy metals, vermicomposts were found to be suitable for use in agriculture (Chapter 3).
- The effective use of excess microbial biomass from biofloc system has been demonstrated. Particularly (33%) effective replacement of feed by sludge (old biomass from biofloc technology) in crayfish diet can be recommended (Chapter 4).

References

Ajijah, N., Apriyana, A.Y., Sriwuryandari, L., Priantoro, E.A., Janetasari, S.A., Pertiwi, T.Y.R., Suciati, A.M., Sembiring, T., 2021. Beneficiary of nitrifying bacteria for enhancing lettuce (*Lactuca sativa*) and vetiver grass (*Chrysopogon zizanioides* L.) growths align with carp (*Cyprinus carpio*) cultivation in an aquaponic system. Environ. Sci. Pollut. Res. 28, 880–889.

Al-Jaloud, A.A., Hussain, G., Alsadon, A.A., Siddiqui, A.Q., Al-Najada, A., 1993. Use of aquaculture effluent as a supplemental source of nitrogen fertilizer to wheat crop. Arid Land Res. Manag. 7, 233–241.

Arumugam, K., Ahmad, M.F., Yaacob, N.S., Ikram, W.M., Maniyam, M.N., Abdullah, H., Katayama, T., Komatsu, K., Kuwahara, V.S., 2020. Enhancement of targeted microalgae species growth using aquaculture sludge extracts. Heliyon 6.

Baßmann, B., Brenner, M., Palm, H.W., 2017. Stress and welfare of African catfish (*Clarias gariepinus* Burchell, 1822) in a coupled aquaponic system. Water 9, 504.

Baßmann, B., Harbach, H., Weißbach, S., Palm, H.W., 2020. Effect of plant density in coupled aquaponics on the welfare status of African catfish, *Clarias gariepinus*. J. World Aquacult. Soc. 51, 183–199.

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, Jr W., Poersch, L.H., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342, 112–116.

Becker, E.W., 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, ISBN 0521350204.

Binalshikh-Abubkr, T., Hanafiah, M., Das, S.K., 2021. Proximate chemical composition of dried shrimp and tilapia waste bioflocs produced by two drying methods. J. Mar. Sci. Eng. 9, 193.

Birch, S., Bell, R., Nair, J., Cao, P., 2010. Feasibility of vermicomposting of aquaculture solid waste on the Mekong Delta, Vietnam: A pilot study. Global Science Books, Ikenobe, Japan, pp. 127–134.

Bittsanszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B., Kőmíves, T., 2016. Nutrient supply of plants in aquaponic systems. Ecocycles 2, 17–20.

Blanchard, C., Wells, D.E., Pickens, J.M., Blersch, D.M., 2020. Effect of pH on cucumber growth and nutrient availability in a decoupled aquaponic system with minimal solids removal. Horticulturae 6, 10.

Bosma, R.H., Lacambra, L., Landstra, Y., Perini, C., Poulie, J., Schwaner, M.J., Yin, Y., 2017. The financial feasibility of producing fish and vegetables through aquaponics. Aquac. Eng. 78, 146–154.

Bringezu, S., Schütz, H., Pengue, W., O'Brien, M., Garcia, F., Sims, R., Howarth, R.W., Kauppi, L., Swilling, M., Herrick, J., 2014. Assessing global land use: balancing consumption with sustainable supply. United Nations Environment Programme, Nairobi, Kenya, 131 pp. ISBN 978-92-807-3330-3.

Brod, E., Oppen, J., Kristoffersen, A.Ø., Haraldsen, T.K., Krogstad, T., 2017. Drying or anaerobic digestion of fish sludge: Nitrogen fertilisation effects and logistics. Ambio 46, 852–864.

Buyuksonmez, F., Rynk, R., Hess, T., Fornshell, G., 2005. Composting characteristics of trout manure. J. Residuals Sci. Technol. 2, 149–157.

Danaher, J.J., Shultz, R.C., Rakocy, J.E., Bailey, D.S., 2013. Alternative solids removal for warm water recirculating raft aquaponic systems. J. World Aquacult. Soc. 44, 374–383.

de Araújo, T.O., de Freitas-Silva, L., Santana, B.V.N., Kuki, K.N., Pereira, E.G., Azevedo, A.A., da Silva, L.C., 2014. Tolerance to iron accumulation and its effects on mineral composition and growth of two grass species. Environ. Sci. Pollut. Res. 21, 2777–2784.

de Farias Lima, J., Duarte, S.S., Bastos, A.M., Carvalho, T., 2019. Performance of an aquaponics system using constructed semi-dry wetland with lettuce (*Lactuca sativa* L.) on treating wastewater of culture of Amazon River shrimp (*Macrobrachium amazonicum*). Environ. Sci. Pollut. Res. 26, 13476–13488.

Deolalikar, A., Mitra, A., 2004. Effect of vermicompost on growth of fish *Labeo rohita* (Hamilton). Journal of Aquaculture in the Tropics.

Domínguez, J., Velando, A., Ferreiro, A., 2005. Are *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* (Oligochaeta, Lumbricidae) different biological species? Pedobiologia 49, 81–87.

Doncato, K.B., Costa, C.S.B., 2021. Micronutrient supplementation needs for halophytes in saline aquaponics with BFT system water. Aquaculture 531, 735–815.

Dufault, R.J., Korkmaz, A., 2000. Potential of biosolids from shrimp aquaculture as a fertilizer in bell pepper production. Compost Sci. Util. 8, 310–319.

Dufault, R.J., Korkmaz, A., Ward, B., 2001. Potential of biosolids from shrimp aquaculture as a fertilizer for broccoli production. Compost Sci. Util. 9, 107–114.

Dvořák, J., Mančíková, V., Pižl, V., Elhottová, D., Šilerová, M., Roubalová, R., Škanta, F., Procházková, P., Bilej, M., 2013. Microbial environment affects innate immunity in two closely related earthworm species *Eisenia andrei* and *Eisenia fetida*. PloS one 8.

Ebeling, J.M., Timmons, M.B., 2012. Recirculating Aquaculture Systems, Aquaculture Production Systems. Wiley-Blackwell, pp. 245–277.

Edwards, C., Niederer, A., 1988. Production and processing of earthworm protein. In: Edwards, C.A., Neuhauser, E.F. (Eds), Earthworms in waste and environmental management. SPB Academic Publishing, 391 pp.

Edwards, C.A., 2004. Earthworm Ecology. CRC Press, 456 pp.

El-Sayed, A.F.M., 2021. Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. Rev. Aquac. 13, 676–705.

Elala, N.M.A., Ragaa, N.M., 2015. Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. J. Adv. Res. 6, 621–629.

Emerenciano, M.G.C., Martínez-Córdova, L.R., Martínez-Porchas, M., Miranda-Baeza, A., 2017. Biofloc technology (BFT): A tool for water quality management in aquaculture. Water Quality 5, 92–109.

Endut, A., Jusoh, A., Ali, N., Nik, W., Hassan, A., 2009. Effect of flow rate on water quality parameters and plant growth of water spinach (*Ipomoea aquatica*) in an aquaponic recirculating system. Desalin. Water Treat. 5, 19–28.

Endut, A., Jusoh, A., Ali, N., Nik, W.B.W., Hassan, A., 2010. A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresour. Technol. 101, 1511–1517.

Enduta, A., Jusoh, A., Ali, N.a., Wan, Nik, W., 2011. Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. Desalin. Water Treat. 32, 422–430.

Espinosa Moya, E.A., Angel Sahagún, C.A., Mendoza Carrillo, J.M., Albertos Alpuche, P.J., Álvarez-González, C.A., Martínez-Yáñez, R., 2016. Herbaceous plants as part of biological filter for aquaponics system. Aquac. Res. 47, 1716–1726.

Faheed, F.A., Fattah, Z.A., 2008. Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. J. Agri. Soc. Sci. 4, 165–169.

FAO, 2020. The State of World Fisheries and Aquaculture: Opportunities and Challenges.

Filep, R.M., Diaconescu-, Ş., Costache, M., Stavrescu-Bedivan, M.M., Bădulescu, L., Nicolae, C.G., 2016. Pilot aquaponic growing system of carp (*Cyprinus carpio*) and basil (*Ocimum basilicum*). Agriculture and Agricultural Science Procedia 10, 255–260.

Forchino, A., Lourguioui, H., Brigolin, D., Pastres, R., 2017. Aquaponics and sustainability: The comparison of two different aquaponic techniques using the Life Cycle Assessment (LCA). Aquac. Eng. 77, 80–88.

Forchino, A.A., Gennotte, V., Maiolo, S., Brigolin, D., Mélard, C., Pastres, R., 2018. Eco-designing Aquaponics: a case study of an experimental production system in Belgium. Procedia CIRP 69, 546–550.

Ganesh, P., Mohan, M., Subha, R., Vijayalakshmi, G., 2003. Earthworm meal for fish feed formulation and its influence on growth of mrigal fingerlings. Journal of Ecobiology 15, 181–184.

Ghosh, C., 2004. Integrated vermi-pisciculture an alternative option for recycling of solid municipal waste in rural India. Bioresour. Technol. 93, 71–75.

Goddek, S., 2017. Opportunities and challenges of multi-loop aquaponic systems. Ph.D. thesis. Wageningen University. 170 pp. DOI: 10.18174/412236

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

Göksan, T., Zekeriyaoglu, A., Ak, İ., 2007. The growth of *Spirulina platensis* in different culture systems under greenhouse condition. Turkish Journal of Biology 31, 47–52.

Graber, A., Junge, R., 2009. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. Desalination 246, 147–156.

Guerdat, T.C., Losordo, T.M., DeLong, D.P., Jones, R.D., 2013. An evaluation of solid waste capture from recirculating aquaculture systems using a geotextile bag system with a flocculant-aid. Aquac. Eng. 54, 1–8.

Gunning, D., 2016. Cultivating *Salicornia europaea* (marsh samphire). Dublin, Ireland: Irish Sea Fisheries Board, 92pp.

Guo, Z., Liu, Y., Guo, H., Yan, S., Mu, J., 2013. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. J. Environ. Sci. 25, S85–S88.

Guwa, O., 2020. Optimisation of nutrient input to integrated aquaponics systems through mineral supplementation by way of fish feed additives. Stellenbosch: Stellenbosch University.

Hargreaves, J.A., 2013. Biofloc production systems for aquaculture. Southern Regional Aquaculture Center 4503, 11.

Hopkins, J.S., Sandifer, P.A., Browdy, C.L., 1994. Sludge management in intensive pond culture of shrimp: Effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. Aquac. Eng. 13, 11–30.

Huntingford, F., Adams, C., Braithwaite, V., Kadri, S., Pottinger, T., Sandøe, P., Turnbull, J., 2007. Current issues in fish welfare. J. Fish Biol. 68, 332–372.

Hussain, G., Al-Saati, A.J., 1999. Wastewater quality and its reuse in agriculture in Saudi Arabia. Desalination 123, 241–251.

Hussain, T., Verma, A.K., Tiwari, V.K., Prakash, C., Rathore, G., Shete, A.P., Nuwansi, K.K.T., 2014. Optimizing Koi Carp, *Cyprinus carpio* var. Koi (Linnaeus, 1758), stocking density and nutrient recycling with spinach in an aquaponic system. J. World Aquacult. Soc. 45, 652–661.

Hussain, T., Verma, A.K., Tiwari, V.K., Prakash, C., Rathore, G., Shete, A.P., Saharan, N., 2015. Effect of water flow rates on growth of *Cyprinus carpio* var. koi (*Cyprinus carpio* L., 1758) and spinach plant in aquaponic system. Aquac. Int. 23, 369–384.

Chakrabarty, D., Das, S.K., Das, K., Biswas, P., Karmegam, N., 2009. Application of vermitechnology in aquaculture. Dynamic Soil, Dynamic Plant 3, 41–44.

Cheban, L., Malischuk, I., Marchenko, M., 2015. Cultivating *Desmodesmus armatus* (Chod.) Hegew. in recirculating aquaculture systems (RAS) waste water. Fisheries & Aquatic Life 23, 155–162.

Chen, M., Tang, H., Ma, H., Holland, T.C., Ng, K.S., Salley, S.O., 2011. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresour. Technol. 102, 1649–1655.

Chen, P., Zhu, G., Kim, H.-J., Brown, P.B., Huang, J.-Y., 2020. Comparative life cycle assessment of aquaponics and hydroponics in the Midwestern United States. J. Clean Prod. 275, 122888.

Irhayyim, T., Feher, M., Lelesz, J., Bercsenyi, M., Barsony, P., 2020. Nutrient removal efficiency and growth of watercress (*Nasturtium officinale*) under different harvesting regimes in integrated recirculating aquaponic systems for rearing common carp (*Cyprinus carpio* L.). Water 12, 1419.

Istiqomah, L., Sofyan, A., Damayanti, E., Julendra, H., 2009. Amino acid profil of earthworm and earthworm meal (*Lumbricus rubellus*) for animal feed stuff. J. Indones. Trop. Anim. Agric. 34, 253–257.

Jobling, M., 2012. JS Lucas and PC Southgate (Eds), Aquaculture: Farming aquatic animals and plants. Springer, 612 pp.

Joesting, H.M., Blaylock, R., Biber, P., Ray, A., 2016. The use of marine aquaculture solid waste for nursery production of the salt marsh plants *Spartina alterniflora* and *Juncus roemerianus*. Aquacult. Rep. 3, 108–114.

Kaur, V.I., Ansal, M.D., 2010. Efficacy of vermicompost as fish pond manure – Effect on water quality and growth of *Cyprinus carpio* (Linn.). Bioresour. Technol. 101, 6215–6218.

Khatoon, H., Banerjee, S., Syakir Syahiran, M., Mat Noordin, N.B., Munafi Ambok Bolong, A., Endut, A., 2016. Re-use of aquaculture wastewater in cultivating microalgae as live feed for aquaculture organisms. Desalin. Water Treat. 57, 29295–29302.

Khiari, Z., Kaluthota, S., Savidov, N., 2019. Aerobic bioconversion of aquaculture solid waste into liquid fertilizer: Effects of bioprocess parameters on kinetics of nitrogen mineralization. Aquaculture 500, 492–499.

Knaus, U., Palm, H.W., 2017. Effects of fish biology on ebb and flow aquaponical cultured herbs in northern Germany (Mecklenburg Western Pomerania). Aquaculture 466, 51–63.

Knaus, U., Wenzel, L.C., Appelbaum, S., Palm, H.W., 2020. Aquaponics (sl) Production of Spearmint (*Mentha spicata*) with African Catfish (*Clarias gariepinus*) in Northern Germany. Sustainability 12, 8717.

Kostecka, J., Pączka, G., 2006. Possible use of earthworm *Eisenia fetida* (Sav.) biomass for breeding aquarium fish. Eur. J. Soil Biol. 42, S231–S233.

Kotzen, B., Emerenciano, M.G.C., Moheimani, N., Burnell, G.M., 2019. Aquaponics: Alternative types and approaches. In: Aquaponics Food Production Systems. Springer, Cham, pp. 301–330.

Kouba, A., Lunda, R., Hlaváč, D., Kuklina, I., Hamáčková, J., Randák, T., Kozák, P., Koubová, A., Buřič, M., 2018. Vermicomposting of sludge from recirculating aquaculture system using *Eisenia andrei*:Technological feasibility and quality assessment of end-products. J. Clean Prod. 177, 665–673.

Koyama, M., Nagao, N., Syukri, F., Abd Rahim, A., Kamarudin, M.S., Toda, T., Mitsuhashi, T., Nakasaki, K., 2018. Effect of temperature on thermophilic composting of aquaculture sludge: NH_3 recovery, nitrogen mass balance, and microbial community dynamics. Bioresour. Technol. 265, 207–213.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick, Jr G.J., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296, 51–57.

Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, Jr G.J., 2010. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303, 28–33.

Kumar, P., Sagar, V., Choudhary, A., Kumar, N., 2007. Vermiculture: Boon for fish farmers. Fishing Chimes 27, 40–42.

Kuo, C.-M., Jian, J.-F., Lin, T.-H., Chang, Y.-B., Wan, X.-H., Lai, J.-T., Chang, J.-S., Lin, C.-S., 2016. Simultaneous microalgal biomass production and CO_2 fixation by cultivating *Chlorella sp*. GD with aquaculture wastewater and boiler flue gas. Bioresour. Technol. 221, 241–250.

Leng, R., Stambolie, J., Bell, R., 1995. Duckweed-a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development 7, 36.

Lennard, W.A., Leonard, B.V., 2006. A Comparison of Three Different Hydroponic Sub-systems (gravel bed, floating and nutrient film technique) in an Aquaponic Test System. Aquac. Int. 14, 539–550.

Lenz, G.L., Loss, A., Lourenzi, C.R., de Alcantara Lopes, D.L., de Matos Siebeneichler, L., Brunetto, G., 2021. Common chicory production in aquaponics and in soil fertilized with aquaponic sludge. Sci. Hortic. 281, 109946.

Liang, J.-Y., Chien, Y.-H., 2013. Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia-water spinach raft aquaponics system. Int. Biodeterior. Biodegrad. 85, 693–700.

Losordo, T.M., Masser, M.P., Rakocy, J.E., 1999. Recircultaing Aquaculture Tank Production Systems: A Review of Component Options. Southern Regional Aquaculture Center 453.

Luo, X.L., Rauan, A., Xing, J.X., Sun, J., Wu, W.Y., Ji, H., 2021. Influence of dietary Se supplementation on aquaponic system: Focusing on the growth performance, ornamental features and health status of Koi carp (*Cyprinus carpio* var. Koi), production of Lettuce (*Lactuca sativa*) and water quality. Aquac. Res. 52, 505–517.

Madkour, F.F., Kamil, A.E.-W., Nasr, H.S., 2012. Production and nutritive value of *Spirulina platensis* in reduced cost media. The Egyptian Journal of Aquatic Research 38, 51–57.

Marques, B., Calado, R., Lillebø, A.I., 2017. New species for the biomitigation of a super-intensive marine fish farm effluent: Combined use of polychaete – assisted sand filters and halophyte aquaponics. Sci. Total Environ. 599, 1922–1928.

Marsh, L., Subler, S., Mishra, S., Marini, M., 2005. Suitability of aquaculture effluent solids mixed with cardboard as a feedstock for vermicomposting. Bioresour. Technol. 96, 413–418.

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. Aquac. Eng. 43, 83–93.

Masser, M.P., Rakocy, J.E., Losordo, T.M., 1992. Recirculating aquaculture tank production systems – management of recirculating systems. Southern Regional Aquaculture 452, 1–12.

Maucieri, C., Nicoletto, C., Zanin, G., Birolo, M., Trocino, A., Sambo, P., Borin, M., Xiccato, G., 2019. Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. PloS one 14.

Mirzoyan, N., Gross, A., 2013. Use of UASB reactors for brackish aquaculture sludge digestion under different conditions. Water Res. 47, 2843–2850.

Mohanta, K.N., Subramanian, S., Korikanthimath, V.S., 2016. Potential of earthworm (*Eisenia foetida*) as dietary protein source for rohu (*Labeo rohita*) advanced fry. Int. Biodeterior. Biodegrad. 2, 1138594.

Ng, W.K., Koh, C.B., Sudesh, K., Siti-Zahrah, A., 2009. Effects of dietary organic acids on growth, nutrient digestibility and gut microflora of red hybrid tilapia, *Oreochromis* sp., and subsequent survival during a challenge test with *Streptococcus agalactiae*. Aquac. Res. 40, 1490–1500.

Nhut, N., Hao, N., Bosma, R., Verreth, J., Eding, E., Verdegem, M.J., 2019. Options to reuse sludge from striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) ponds and recirculating systems. Aquac. Eng. 87, 102020.

Nichols, M., Savidov, N., 2011. Aquaponics: a nutrient and water efficient production system. II International Symposium on Soilless Culture and Hydroponics 947, pp. 129–132.

Nuwansi, K., Verma, A., Prakash, C., Tiwari, V., Chandrakant, M., Shete, A., Prabhath, G., 2016. Effect of water flow rate on polyculture of koi carp (*Cyprinus carpio* var. koi) and goldfish (*Carassius auratus*) with water spinach (*Ipomoea aquatica*) in recirculating aquaponic system. Aquac. Int. 24, 385–393.

Nuwansi, K., Verma, A., Tiwari, V., Prakash, C., Chandrakant, M., 2017. Standardization of the stocking density ratios of koi carp (*Cyprinus carpio* var. koi): Goldfish (*Carassius auratus*) in polyculture aquaponic recirculating system. Turk. J. Fish. Quat. Sci. 17, 1271–1278.

Nuwansi, K., Verma, A., Rathore, G., Prakash, C., Chandrakant, M., Prabhath, G., 2019. Utilization of phytoremediated aquaculture wastewater for production of koi carp (*Cyprinus carpio* var. koi) and gotukola (*Centella asiatica*) in an aquaponics. Aquaculture 507, 361–369.

Oladimeji, S.A., Okomoda, V.T., Olufeagba, S.O., Solomon, S.G., Abol-Munafi, A.B., Alabi, K.I., Ikhwanuddin, M., Martins, C.O., Umaru, J., Hassan, A., 2020. Aquaponics production of catfish and pumpkin: Comparison with conventional production systems. Food Sci. Nutr. 8, 2307–2315.

Óscar Pereira, J., Gomes, E.F., 1995. Growth of rainbow trout fed a diet supplemented with earthworms, after chemical treatment. Aquac. Int. 3, 36–42.

Palada, M.C., Cole, W.M., Crossman, S.M., 1999. Influence of effluents from intensive aquaculture and sludge on growth and yield of bell peppers. J. Sustain. Agric. 14, 85–103.

Palm, H.W., Knaus, U., Appelbaum, S., Goddek, S., Strauch, S.M., Vermeulen, T., Jijakli, M.H., Kotzen, B., 2018. Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. Aquac. Int. 26, 813–842.

Pasch, J., Ratajczak B., Appelbaum S., Palm H.W., Knaus U., 2021. Growth of basil (*Ocimum basilicum*) in DRF, raft, and grow pipes with effluents of African catfish (*Clarias gariepinus*) in decoupled aquaponics. Agri Engineering 3, 92–109.

Pinho, S.M., Molinari, D., de Mello, G.L., Fitzsimmons, K.M., Emerenciano, M.G.C., 2017. Effluent from a biofloc technology (BFT) tilapia culture on the aquaponics production of different lettuce varieties. Ecol. Eng. 103, 146–153.

Quagrainie, K.K., Flores, R.M.V., Kim, H.-J., McClain, V., 2018. Economic analysis of aquaponics and hydroponics production in the US Midwest. J. Appl. Aquaculture 30, 1–14.

Rakocy, J., Shultz, R., Bailey, D., Pantanella, E., Danaher, J., 2009. Alternative Media Types for Seedling Production of Lettuce and Basil, International Symposium on Growing Media and Composting 891, pp. 257–264.

Rakocy, J.E., 2012. Aquaponics – Integrating Fish and Plant Culture, Aquaculture Production Systems. Wiley-Blackwell, pp. 344–386.

Rakocy, J.E., Shultz, R.C., Bailey, D.S., Thoman, E.S., 2004. Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. In: Nichols M.A. (Ed.), Proceedings of the South Pacific Soilless Culture Conference. International Society Horticultural Science, Leuven 1, pp. 63–69.

Rakocy, J.E., Masser, M.P., Losordo, T.M., 2006. Recirculating aquaculture tank production systems: aquaponics-integrating fish and plant culture. Southern Regional Aquaculture 454, 1–16.

Resh, H.M., 2012. Hydroponic Food Production: A Definitive Guidebook for the Advanced Home Gardener and the Commercial Hydroponic Grower, Seventh Edition. Taylor & Francis, ISBN 9781439878675.

Robinson, M.V., Alvariño, J.R., Duran, J., 2011. Aquaponics: integrating fish feeding rates and ion waste production for strawberry hydroponics. Span. J. Agric. Res. 9, 537–545.

Roosta, H.R., Hamidpour, M., 2011. Effects of foliar application of some macro-and micro-nutrients on tomato plants in aquaponic and hydroponic systems. Sci. Hortic. 129, 396–402.

Roosta, H.R., Mohsenian, Y., 2012. Effects of foliar spray of different Fe sources on pepper (*Capsicum annum* L.) plants in aquaponic system. Sci. Hortic. 146, 182–191.

Rynk, R., Fornshell, G., Buyuksonmez, F., Hess, T., 1998. Composting and vermiculture: alternative practices for managing manure and mortalities on aquaculture farms. Book of Abstracts, Aquaculture '98. World Aquaculture Association, Baton Rouge, Louisiana, 464.

Salam, M., Hashem, S., Asadujjaman, M., Li, F., 2014. Nutrient recovery from in fish farming wastewater: an aquaponics system for plant and fish integration. World Journal of Fish and Marine Sciences 6, 355–360.

Sánchez, H.J.A., 2014. Aquaponics and its potential aquaculture wastewater treatment and human urine treatment. M.Sc. thesis. Faculdade de Ciências e Tecnologia, Universidae Nova de Lisboa, Portugal, 65 pp.

Setiadi, E., Widyastuti, Y.R., Prihadi, T.H., 2018. Water quality, survival, and growth of red tilapia, *Oreochromis niloticus* cultured in aquaponics system, E3S Web of Conferences. EDP Sciences, 02006.

Shete, A., Verma, A., Tandel, R., Prakash, C., Tiwari, V., Hussain, T., 2013. Optimization of water circulation period for the culture of goldfish with spinach in aquaponic system. J. Agric. Sci. 5, 26.

Shete, A., Verma, A., Chadha, N., Prakash, C., Chandrakant, M., 2015. A comparative study on fish to plant component ratio in recirculating aquaponic system with common carp and mint. Journal of Environment and Bio-Sciences 29, 323–329.

Shete, A.P., Verma, A.K., Chadha, N.K., Prakash, C., Peter, R.M., Ahmad, I., Nuwansi, K.K.T., 2016. Optimization of hydraulic loading rate in aquaponic system with common carp (*Cyprinus carpio*) and Mint (*Mentha arvensis*). Aquac. Eng. 72–73, 53–57.

Sikawa, D.C., Yakupitiyage, A., 2010. The hydroponic production of lettuce (*Lactuca sativa* L) by using hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) pond water: Potentials and constraints. Agric. Water Manage. 97, 1317–1325.

Sirakov, I., Velichkova, K., Stoyanova, S., Kaymakanova, M., Slavcheva-Sirakova, D., Atanasova, R., Staykov, Y., 2018. Effect of synbiotic dietary supplementation on growth, physiological and immunological parameters in common carp (*Cyprinus carpio* L.) fingerlings and on yield and physiological parameters in lettuce (*Lactuca sativa* L.), cultivated in mesocosmos aquaponic system. Bulgarian J. Agric. Sci. 24, 140–149.

Sonneveld, C., Voogt, W., 2009. Nutrient management in substrate systems, Plant nutrition of greenhouse crops. Springer, pp. 277–312.

Stafford, E.A., Tacon, A.G., 1985. The nutritional evaluation of dried earthworm meal (*Eisenia foetida*, Savigny, 1826) included at low levels in production diets for rainbow trout, *Salmo gairdneri* Richardson. Aquac. Res. 16, 213–222.

Strauch, S.M., Wenzel, L.C., Bischoff, A., Dellwig, O., Klein, J., Schüch, A., Wasenitz, B., Palm, H.W., 2018. Commercial African catfish (*Clarias gariepinus*) recirculating aquaculture systems: Assessment of element and energy pathways with special focus on the phosphorus cycle. Sustainability 10, 1805.

Suwoyo, H.S., Tuwo, A., Haryati, H., Anshary, H., 2019. Potential, characteristics and utilization of shrimp pond solid waste as organic fertilizer. International Journal of Environment, Agriculture and Biotechnology 4, 411–421.

Tacon, A., Stafford, E., Edwards, C., 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 35, 187–199.

Timmons, M.B., Ebeling, J.M., Wheaton, F.V., Summerfelt, S.T., Vinci, B.J., 2002. Recirculating Aquaculture Systems. Cayuga Aqua Ventures 2nd edition, 769 pp.

Tyson, R., Simonne, E., Treadwell, D., Davis, M., White, J., 2008. Effect of water pH on yield and nutritional status of greenhouse cucumber grown in recirculating hydroponics. J. Plant Nutr. 31, 2018–2030.

van de Nieuwegiessen, P.G., Olwo, J., Khong, S., Verreth, J.A., Schrama, J.W., 2009. Effects of age and stocking density on the welfare of African catfish, *Clarias gariepinus* Burchell. Aquaculture 288, 69–75.

van Rijn, J., 2013. Waste treatment in recirculating aquaculture systems. Aquac. Eng. 53, 49–56.

Van Tung, T., Tran, B.Q., Thao, N.T.P., Hieu, T.T., Le, S., Tuan, N.Q., Sonne, C., Lam, S.S., Van Le, Q., 2020. Recycling of aquaculture wastewater and sediment for sustainable corn and water spinach production. Chemosphere, 129329.

Watson, C.A., Hill, J.E., 2006. Design criteria for recirculating, marine ornamental production systems. Aquac. Eng. 34, 157–162.

Wuang, S.C., Khin, M.C., Chua, P.Q.D., Luo, Y.D., 2016. Use of *Spirulina biomass* produced from treatment of aquaculture wastewater as agricultural fertilizers. Algal Research 15, 59–64.

Wurts, W.A., Durborow, R.M., 1992. Interactions of pH, carbon dioxide, alkalinity and hardness in fishponds. Southern Regional Aquaculture Center publication no. 464. Liming Fishponds 3, Auburn University For. Citeseer.

Yeo, S.E., Binkowski, F.P., 2010. Processing aquaculture system biosolids by worm composting-vermicomposting. Great Lakes Water Institute, University of Wisconsin-Milwaukee, 17 pp.

Yep, B., Zheng, Y., 2020. Potassium and micronutrients fertilizer addition in aquaponic solution for drug-type *Cannabis sativa* L. cultivation. Canadian Journal of Plant Science.

Zhenjun, S., Xianchun, L., Lihui, S., Chunyang, S., 1997. Earthworm as a potential protein resource. Ecol. Food Nutr. 36, 221–236.

Zhu, W., Liu, M., Chen, C., Wu, F., Yang, J., Tan, Q., Xie, S., Liang, X., 2014. Quantifying the dietary potassium requirement of juvenile grass carp (*Ctenopharyngodon idellus*). Aquaculture 430, 218–223.

Zou, Y., Hu, Z., Zhang, J., Xie, H., Guimbaud, C., Fang, Y., 2016. Effects of pH on nitrogen transformations in media-based aquaponics. Bioresour. Technol. 210, 81–87.

English summary

Aquaculture waste valorisation

Roman Lunda

With the global population estimated to reach 8.3–10.9 billion people by 2050, the sustainable development of the aquaculture and agricultural sectors requires optimization in terms of production efficiency, but also reductions in the utilization of limited resources, in particular, water, land and fertilizers. Water treatment technology has undergone a dynamic development in recent years with new treatment methods rapidly emerging. Also, in the field of RAS, a choice can be made from many different treatment methods. In addition to a proper cost/benefit analyses, the choice of a suitable treatment method depends largely on factors directly or indirectly related to the location of the recirculating system. Climatic conditions, water availability, discharge regulations, and land availability are the kind of location-dependent factors which are major determinants of the type of treatment methods to be used. These factors, together with the market value of the cultured organisms, may justify the use of sophisticated treatment methods in some cases; while in others, optimal economic benefit is accomplished with relatively simple water treatment techniques at the expense of water savings and production intensity. The conventional and novel technologies for aquaculture solid waste management are analysed and summarized in this thesis. Constructed wetlands, aerobic composting, anaerobic treatment, enzymatic or chemical hydrolysis, and aquaponics are conventional and well-known technologies used in aquaculture waste reduction, valorisation, and recycling. Novel technologies are mainly applied to recycle resources or produce valuable by-products, including biodiesel, fish silage, biochar, lactic acid, hydrogen, and insect larvae growth from aquaculture sludge, as well as phytoremediation and biofloc technology for aquaculture sludge treatment. Finally, future directions of aquaculture solid waste management are proposed. Creating valuable fertilizer matter by vermicomposting represents a very promising technique for the future. This technology does not require unnecessarily high costs. Chapter 3 documents aquaculture sludge composition and utilization for earthworm vermiculture. This chapter also includes toxicity and other possibilities for aquaculture sludge utilization as direct field application. This gives sludge from standard freshwater RAS a great opportunity and opens the doors for its direct use in the vermicomposting sector. Biogas and biochar production are just the next fragment of the aquaculture sludge valorisation issue. The potential to reuse fuel from waste to actuate RAS (from which sludge was produced) is an awesome environmental advancement. Insects, in all their forms (larvae, adults, and meal), represent a future solution to the problem of protein nutrition throughout the world. Aquaculture sludge offers the perfect substrate for the culture of insects. The composition of used sludge also means there is the possibility of direct application on fields as a source of nutrients for plant growth. It is well known that aquaculture solids treatment can be profitable thanks to biofloc technology. This technology presents a pillar in aquaculture waste valorisation. Leveraging nutrient rich biomass from biofloc technology as a potential feed source was presented in Chapter 4. It has been proven that almost 33% of feed could be replaced by biofloc meal for crayfish culture. The biggest problems of aquaculture waste valorisation by plenty of possible technologies is its variability. Chapter 2 documented the composition of sludge and wastewater from several RASs. The results show a possibility of wastewater utilization for plant production in the form of aquaponic or hydroponic systems. The benefits of aquaponics relate not only to the efficient uses of land, water and nutrient resources, but also allow for the increased integration of smart energy opportunities such as biogas and solar power.

In this regard, aquaponics is a promising technology for producing both high-quality fish protein and vegetables in ways that can use substantially less land, less energy and less water – while also minimizing chemical and fertilizer inputs that are used in conventional food production. Chapter 2 also evaluated nutrient concentration according to RAS adjustment and the problem of high sodium concentrations. All aquaculture waste can be valorised by several technologies. There is no perfect composition of sludge or wastewater for the requirements of plants. But its nutrient value can reduce costs for hydroponics nutrient solution production. To achieve the ideal composition, it is necessary to choose the right approach in the aquaculture system. Given the fact that aquaponics follows nutrient and water reuse principles, it seems to be a promising solution for sustainable aquaculture and hydroponics practices. However, further research and developments are needed, as demonstrated by the challenges described in this thesis.

Czech summary

Zhodnocení odpadu z akvakultury

Roman Lunda

S odhadovanou celosvětovou populací 8,3–10,9 miliard lidí do roku 2050 vyžaduje odvětví akvakultury a zemědělství optimalizaci z hlediska efektivity výroby, ale také snížení využití omezených zdrojů, zejména vody, půdy a hnojiv v rámci udržitelného rozvoje. Technologie pro úpravu vody prošly v posledních letech dynamickým vývojem spolu s novými objevy a metodami. Také v oblasti RAS je nyní možné využít mnoho nových metod a postupů při úpravě vod. Navíc při správné analýze nákladů a výnosů závisí volba vhodné metody zpracování do značné míry na faktorech přímo nebo nepřímo souvisejících s umístěním recirkulačního systému. Klimatické podmínky, dostupnost vodního zdroje, regulace vypouštění a dostupnost půdy jsou druhem faktorů závislých na lokalitě, které jsou hlavními determinanty typu použitých metod čištění. Tyto faktory spolu s tržní hodnotou kultivovaných organismů mohou v některých případech ospravedlnit použití sofistikovaných metod čištění, zatímco v jiných je dosaženo optimálního ekonomického přínosu relativně jednoduchými technikami úpravy vody na úkor úspor vody a intenzity výroby. Konvenční a nové technologie pro nakládání s pevným odpadem z akvakultury jsou v posledních letech středem zájmu. Uměle vybudované mokřady, aerobní kompostování, anaerobní čištění, enzymatická nebo chemická hydrolýza a akvaponie jsou dnes již dobře známé technologie používané při snižování, zhodnocování a recyklaci odpadu z akvakultury. Nové technologie se používají hlavně k recyklaci zdrojů nebo k produkci cenných vedlejších produktů, včetně biopaliv, rybí siláže, biouhlí, kyseliny mléčné, vodíku a kalu jako média pro růst larev hmyzu, stejně tak jako technologie fytoremediace a bioflok pro zpracování kalů z akvakultury. Nakonec jsou navrženy možné směry pro nakládání s pevným odpadem z akvakultury. Vytváření hodnotného substrátu pro hnojení pomocí vermikompostování představuje velmi slibnou techniku pro budoucnost využití kalu. Tato technologie nevyžaduje zbytečně vysoké náklady. Kapitola 3 dokumentuje složení a využití kalů z akvakultury pro využití ve vermikompostování. Tato kapitola zahrnuje další možnosti využití kalů z akvakultury jako například přímé použití na pole včetně nezávadnosti v oblasti toxicity. To dává kalu ze standardního sladkovodního RAS velkou příležitost pro jeho přímé použití v odvětví vermikompostování. Výroba bioplynu a biouhlí je jen dalším fragmentem možnosti zhodnocení a využití kalů z akvakultury. Potenciál použití paliva z odpadu, pro energetický provoz RAS (ze kterého byl kal vyroben) je úžasným environmentálním pokrokem. Hmyz ve všech svých formách (larvy, dospělci a hmyzí moučka) představuje budoucí řešení problému zajištění proteinové výživy na celém světě. Kal z akvakultury nabízí dokonalý substrát pro kultivaci hmyzu. Kal je možné, díky svému složení přímo aplikovat na pole jako zdroj živin pro růst rostlin. Je dobře známo, že zpracování pevných látek v akvakultuře může být díky využití technologii bioflok, velice výhodné. Tato technologie představuje pilíř zhodnocování odpadu z akvakultury. Využití biomasy bohaté na živiny z technologie bioflok jako potenciálního zdroje krmiva bylo vysvětleno v Kapitole 4. Bylo prokázáno, že téměř 33 % krmiva pro chov raků může být nahrazeno bioflok biomasou. Největší překážkou při zhodnocování odpadu z akvakultury je nepřeberné množství všech možných technologií a jejich variabilita. Kapitola 2 uvádí složení kalů a odpadních vod z několika různých akvakulturních systémů RAS. Výsledky této studie dokazují možnost využití odpadních vod pro rostlinnou výrobu ve formě akvaponických nebo hydroponických systémů. Výhody akvaponie se netýkají pouze efektivního využívání půdy, vody a zdrojů živin, ale také umožňují větší integraci inteligentních energetických možností, jako je bioplyn a solární energie.

V tomto ohledu představuje akvaponie slibnou technologii pro produkci ryb a zeleniny za pomocí způsobů, které nejsou náročné na množství půdy, vyžadují méně energie a vody – a zároveň minimalizují chemické přísady a hnojivové doplňky, které se používají při konvenční výrobě potravin. Kapitola 2 také hodnotí koncentraci živin podle jednotlivých typů úpravy RAS a problém nadměrného množství sodíku v systémech. Veškerý odpad z akvakultury lze zhodnotit pomocí několika technologií. Neexistuje však dokonalé složení kalu či odpadní vody pro úplnou potřebu rostlin. Ovšem živiny obsažené v kalu a odpadní vodě mohou snížit náklady na výrobu živných roztoků v hydroponii. K dosažení ideálního složení je nutné zvolit správný přístup v akvakulturních systémech. Vzhledem k tomu, že akvaponie dodržuje zásady opětovného používání živin a vody, zdá se být slibným řešením pro udržitelné akvakulturní a hydroponické systémy. Je však zapotřebí dalšího výzkumu a vývoje, jak dokazují výzvy popsané v této práci.

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List of publications

Peer-reviewed journals with IF

Lunda, R., Roy, K., Dvořák, P., Kouba, A., Mráz, J., 2020. Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory. Scientific Reports 10: 19607. (IF 2019 = 3.998; AIS 2019 = 1.263)

Linhartová, Z., **Lunda, R.,** Másílko, J., Dvořák, P., Bárta, J., Bártová, V., Kadlec, J., Samková, E., Bedrníček, J., Pešek, M., Laknerová, I., Smole Možina, S., Smetana, P., Mráz, J. 2019. Influence of rosemary extract (*Rosmarinus officinalis*) Inolens to extend the shelf life of vakuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets stored under refrigerated conditions. Aquaculture International 27: 833–847 (IF 2019 = 1.363; AIS 2019 = 0.347)

Lunda, R., Roy, K., Másílko, J., Mráz, J., 2019. Understanding nutrient throughput of operational RAS farm effluents to support semi-commercial aquaponics: Easy upgrade possible beyond controversies. Journal of Environmental Management 245: 255–263. (IF 2019 = 5.647; AIS 2019 = 0.922)

Linhartová, Z., **Lunda, R.,** Másílko, J., Dvořák, P., Lundová, K., Stejskal, V., Matoušek, J., Mráz, J., 2018. Impact of photostimulation for delayed maturity on flesh quality of brook trout (*Salvelinus fontinalis*) stored under refrigerated conditions. Aquaculture Research 49: 3817–3829. (IF 2018 = 1.502; AIS 2018 = 0.339)

Kouba, A., **Lunda, R.,** Hlaváč, D., Kuklina, I., Hamáčková, J., Randák, T., Kozák, P., Koubová, A., Buřič, M., 2018. Vermicomposting of sludge from recirculating aquaculture system using *Eisenia andrei*: Technological feasibility and quality assessment of end-products. Journal of Cleaner Production 117: 655–673. (IF 2018 = 6.395; AIS 2018 = 0.864)

Sterniša, M., Dvořák, P., **Lunda, R.,** Linhartová, Z., Smole Možina, S., Mráz, J., 2018. Bleeding of common carp (*Cyprinus carpio*) improves sensory quality of fillets and slows oxidative and microbiological changes during refrigerated aerobic storage. Food Technology and Biotechnology 56: 524–532. (IF 2018 = 1.517; AIS 2018 = 0.327)

Lunda, R., Linhartová, Z., Másílko, J., Dvořák, P., Smole Možina, S., Mráz, J., 2016. Effect of different types of descaling methods on shelf life of air-/vacuum-packaged common carp (*Cyprinus carpio* L.) fillets under refrigerated storage conditions. Aquaculture International 24: 1555–1568. (IF 2016 = 1.095; AIS 2016 = 0.317)

Technologies and Methodologies

Lunda, R., Stejskal, V., Prokešová, M., Mráz, J., 2019. Technologie biofloc. Edice Metodik, FROV, JU, Vodňany, č. 173, 40 s.

Mráz, J., **Lunda**, R., Stejskal, V., 2018. Konstrukce recirkulačních akvaponických systémů. Edice Metodik, FROV JU, Vodňany, č. 171, 72 s.

Linhartová, Z., Siglová, K., **Lunda, R.,** Dvořák, P., Mráz, J., Bárta, J., Bártová, V., Kadlec, J., Samková, E., Bedrníček, J., Pešek, L., Laknerová, I., Smetana, P., 2018. Vliv čtyř přírodních aditiv na prodloužení skladovatelnosti chlazených kapřích (*Cyprinus carpio*) paštik. Ověřená technologie, FROV JU, Vodňany, 18 s.

Abstracts and conference proceedings

Dvořák, P., **Lunda, R.,** Linhartová, K., Novikava, K., Mráz, J., Dvořáková Líšková, Z., 2017. The use of biofloc biomasss as a component of feed for juvenile grass carp *Ctenopharyngodon idella* in RAS. In: Book of abstracts "Aquaculture Europe 2017", 17–20 October 2017, Dubrovnik, Croatia.

Linhartová, Z., **Lunda, R.,** Másílko, J., Dvořák, P., Novikava, K., Stejskal, V., Matoušek, J., Mráz, J., 2017. Impact of photostimulation for delayed maturity on flesh quality of brook trout *Salvelinus fontinalis* stored under refrigerated conditions. In: Book of abstracts "Aquaculture Europe 2017", 17–20 October 2017, Dubrovnik, Croatia.

Lunda, R., Másílko, J., Mráz, J., 2017. Quality of wastewater and sludge from recirculating aquaculture systems in the Czech Republic as a source of nutrient for secondary use. In: Book of abstracts "Aquaculture Europe 2017", 17–20 October 2017, Dubrovnik, Croatia.

Lunda, R., Linhartová, Z., Másílko, J., Dvořák, P., Smole-Možina, S., Mráz, J., 2016. Effect of different types of descaling methods on shelf life of air-/vacuum-packaged common carp (*Cyprinus carpio* L.) fillets under refrigerated storage conditions. In: Book of abstracts "Aquaculture Europe 2016", 20–23 September 2016, Edinburgh, Great Britain.

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Biofloc as a source of protein in aquaculture feed	2017
Aquaculture waste valorisation	2021

International conferences	**Year**
Lunda, R., Linhartová, Z., Másílko, J., Dvořák, P., Smole Možina, S., Mráz, J., 2016. Effect of different types of descaling methods on shelf life of air-/vacuum-packaged common carp (*Cyprinus carpio* L.) fillets under refrigerated storage conditions. Aquaculture Europe 2016. 20–23 September 2016, Edinburgh, Great Britain, oral presentation	2016
Lunda, R., Másílko, J., Mráz, J., 2017. Quality of wastewater and sludge from recirculating aquaculture systems in the Czech Republic as a source of nutrient for secondary use. Aquaculture Europe 2017. 17–20 October 2017, Dubrovnik, Croatia, poster 137	2017

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