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Assessing Nutrient Removal Efficiency in an Integrated Recirculating Aquaculture System and its Applicability at Different Conditions in Fish Culture

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List of Abbreviations

µm	Micrometre (micron)
ALDU	Aquaculture Laboratory of Debrecen University
ANOVA	Analysis of Variance
BF	Biological Filter
d	Day
DO	Dissolved Oxygen
EMF	Electromagnetic field
FB	Final Biomass
FCR	Feed conversion ratio
FT	Fish Tank
g	Gram
GARL	Georgikon Aquatic Research Laboratory
h	Hour
IB	Initial Biomass
IRAS	Integrated Recirculating Aquaculture System
kg	Kilogram
kHz	kilohertz
L	Litre
m^2	Square meter
m^3	Cubic meter
MF	Magnetic field
mg L^{-1}	Milligram per litre
min^1	Minute

mT	Millitesla
N	Nitrogen
NH_3	unionized ammonia
NH_3-N	Ammonia nitrogen
NH_4-N	Ammonium nitrogen
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
NR	Nutrient Removal
NRR	Nutrient Removal Rates
P	Phosphorus
PO_4-P	Orthophosphate
RAS	Recirculation Aquaculture System
SE	Standard Error
SGR	Specific Growth Rate of fish
SGRP	Specific Growth Rates of Plants
SPSS	Statistical Package for the Social Science
TAN	Total Ammonia Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
W	Watt
WCT	Waste Collection Tank

List of Some Common and Scientific Names Mentioned in This Dissertation

Common Carp	*Cyprinus carpio carpio*
Koi Carp	*Cyprinus carpio haematopterus*
Koi Carp	*Cyprinus carpio var. koi*
Duckweed plants	*Lemna minor*
Asian Watergrass plants	*Hygroryza aristata*
Red Root Floater, Floating Spurge	*Phyllanthus fluitans*
Pennywort Plants	*Hydrocotyle rotundifolia*
Watercress Plants	*Nasturtium officinale*
Watercress Plants	*Rorippa nasturtium-aquaticum*
Water Spinach	*Ipomoea aquatica*
Duckweed	*Lemna gibba*
Great Duckweed, Common Duckmeat	*Spirodela oligorhiza*
Water Fern	*Azolla filiculoides*
Water Hyacinth	*Eichhornia crassipes*
Nile Tilapia	*Oreochromis niloticus* L.
European Seabass	*Dicentrarchus labrax*
Eurasian Watermilfoil	*Myriophyllum spicatum*
Rainbow Trout	*Oncorhynchus mykiss*
Red Hybrid Tilapia	*Oreochromis sp.*

ABSTRACT

In integrated recirculating aquaculture systems (IRASs), several factors such as fish species, fish size, fish density, temperature, plant species, and harvesting rate of plants can affect the nutrient removal rates and growth of cultured species. In order to recycle wastes and produce plant biomass in the IRASs, it is necessary to optimize the recycling rates of nitrogen and phosphorus. Therefore, the research has evaluated the nutrient removal capacities at different modules of the IRAS for rearing common carp (*Cyprinus carpio*), considering the effects of different plant species (*Lemna minor*, *Hygroryza aristata,* and *Phyllanthus fluitans*), size of fish, harvesting biomass of plants, and magnetic water treatment technique.

In all experiments, the experimental units have the same concept of structure. One experimental unit of an IRAS consisted of three tanks: a fish tank, a waste collection tank, and a plant based biofilter unit. The fish and waste collection tanks were set on the floor, while the plant based biofilter unit was installed above the fish tank. Water from the waste collection tank was pumped to the plant based biofilter unit by a submerged pump, circulated to the fish tank and, then returned to the waste collection tank by gravity. A series of experiments were conducted under laboratory conditions to investigate the nutrient cycling efficiency in the IRASs. The water quality parameters and growth of both fish and plants were measured in all the systems, and then the nutrient removal capacities of the plant based biofilters were calculated.

The research results proved that the use of plant based biofilters in the IRASs was effective in maintaining water quality, removing nutrients, and providing good conditions for common carp growth and survival. The nutrient uptake capacities of tested plant species differ and are strongly influenced by the growth rate of plants, which is affected by environmental conditions. The research revealed that *H. aristata* was the strongest plant in removing nutrients among the tested plant species, followed by *L. minor*. While the bacterial biofilm in the moving-bed filter was the superior filter to reduce high concentrations of ammonium nitrogen and nitrite nitrogen.

The research also exposed that the increase in the initial body size of stocked fish significantly decreased the total ammonia nitrogen excreted into the fish tank. However, the increase in the initial body size of stocked fish did not affect the removal efficiencies of total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen; and both the bacterial biofilm filter

and plant based biofilter *(Hydrocotyle rotundifolia)* were independent of the fish size. The initial body size of fish also was negatively correlated with the specific growth rate of fish in the IRAS.

In the IRASs (Aquaponics), increasing the biweekly harvested biomass of watercress plants (*Nasturtium officinale*) decreased the growth of the plants, while it did not affect the growth of the common carp. Increasing the harvested biomass of the plants also decreased the nitrate nitrogen and orthophosphate removal efficiencies of the IRASs, while it did not affect the ammonia and nitrite nitrogen removal efficiencies.

The research results also showed that the use of magnetized water in the IRASs increased the growth of plants (*L. minor*) and the specific growth rate of common carp. However, the magnetized water had no effects on the concentrations of ammonium nitrogen, nitrite nitrogen and nitrate nitrogen in the IRASs.

The present research suggests that the use of plant based biofilters in the IRASs can be beneficial in removing nutrients and adding harvestable products. Based on the results obtained from the four experiments, *Hygroryza aristata* is a more suitable plant that can be used in removing nutrients in the IRASs. The present research also suggests that the small size of fish (initial body size of 33-46 g) should be stocked into the IRASs at the beginning of the rearing season to achieve better performance in fish. In IRASs, the biweekly harvesting of less than 25% of the biomass of the growing watercress is recommended for efficient nutrient removal and sustainable growth of both fish and plants. The present research also suggests that the use of magnetized water treatment in the IRASs can improve the growth of both fish and plant based biofilter and this could be beneficial as a cost-effective technique to increase the profitability of these systems.

1. INTRODUCTION

1.1. Background Information

The aquaculture industry is the fastest growing sector for animal food production since 1970, contributing to almost 50% of the global fish supply (FAO, 2018). Wastewater of intensive aquaculture contains considerable amounts of nutrients, is well documented as a serious problem to environmental deterioration (Timmons et al., 2002; Endut et al., 2011; Martins et al., 2010; Hussain et al., 2015). In order to mitigate the environmental impacts and maintain sustainable aquaculture farming, various methods have been proposed to solve the issue of nutrients discharged from intensive aquaculture. Recirculating aquaculture systems (RAS) have been established in response to the increasingly strict environmental regulations and limited access to land and water (Timmons et al., 2002). The RAS offers many benefits in terms of reducing water requirements, recycling nutrients, improving waste management and better disease management (Timmons et al., 2002; Martins et al., 2010). However, RAS is typically used for commercially important species with high stocking densities and low water exchange rates in order to cover the high investment costs. Due to increasing concerns about the problems of setup and operation costs, diseases, animal welfare, and the accumulations of the nitrate and phosphorus concentrations in the RASs, the research and developments in the RASs tend to focus on: (1) technical improvements within the recirculation loop and (2) recycling of nutrients through integrated farming (Martins et al., 2010).

Accordingly, integrated recirculating aquaculture systems (IRASs) have received a lot of attention as promising practices. These systems are considered as alternative solutions for the efficient utilization of available resources, recycling nutrients, managing water quality, reducing environmental problems of aquaculture and maintaining ecological balance. These systems can also be operated at a comparatively lower cost in terms of water use, space and management. The IRASs refer to integrated systems where additional separated units are integrated into a RAS (Endut et al., 2011; Ardiansyah and Fotedar, 2016). These separated units have the ability to convert nutrients into harvestable products and higher nutrient retention can be achieved by the primary and secondary products while having positive impacts on water quality (Schneider et al., 2005). In these systems, wastewater from aquatic rearing units containing major nutrients such as nitrogen and phosphorus compounds is not only treated by typical nitrification and denitrification processes, but also by the uptake of vegetable/ornamental or aquatic plants (Lin et al., 2002; Hargreaves, 2006; Endut et al., 2011;

Estim et al., 2019). Numerous studies have confirmed that water quality is improved in IRASs compared with systems containing no plants (Redding et al., 1997; Lin et al., 2003; Jo et al., 2002; Neori et al., 2004; Rakocy, 2007; Endut et al., 2011; Ardiansyah and Fotedar, 2016; Nakphet et al., 2017; Estim et al., 2019).

The IRAS is now accepted as an alternative solution to the conventional practice of farming, as the system is known to decrease the expenses involved in operations and reduce the environmental problems of aquaculture (Hargreaves, 2006; Estim et al., 2019; Goddek et al., 2019). However, optimizing growth conditions for both plants and fish is the biggest challenge to profitability because the amount of nutrients from different integrated modules differs and depends on the nutritional values of the feed, which in turn depends on the specific demands of the cultured species (Schneider et al., 2005; Delaide et al., 2016; Goddek and Vermeulen, 2018). Nutrients in the system such as nitrogen and phosphorus can have direct effects on water quality, plant growth, and nutrient removal capacity, and then indirectly affect fish growth (Schneider et al., 2005; Goddek et al., 2019). It is necessary to maintain the balance of nutrient production and uptake in order to ensure effective nutrient removal (Buzby and Lin, 2014). However, several factors such as fish species, fish size, fish density, temperature, plant species, harvesting rate of plants and the microbial community can have a pronounced effect on the growth of cultured species and nutrient removal rates of the IRASs (Schneider et al., 2005; Hu et al., 2015; Goddek et al., 2019). These factors should be at an optimum to maximize the nutrient removal efficiency of these systems and growth conditions of cultured species. However, little information is available on the optimum of these factors in the IRASs. The integrated technology that combines the elements of the RAS and plant production is still under development (Popp et al., 2018), and there is a strong need to optimize the recycling rates of nutrients to achieve efficient nutrient removal in the overall system (Goddek et al., 2019). Therefore, it is of prime necessity to understand the functioning of the nutrient cycles in the IRAS and optimise the recycling rates of nitrogen and phosphorus concerning the following criteria: (1) cultivated plant species, (2) fish size, (3) harvesting biomass of plants and (4) magnetic water treatment technique.

1.2. Aim and Objectives of the Research

The research aimed to assess the nutrient cycling efficiency in the IRASs and evaluate the nutrient removal capacities at different modules of the IRAS for rearing common carp (*Cyprinus carpio*) considering the effects of different plant based biofilters (*Lemna minor*, *Hygroryza aristata*, and *Phyllanthus fluitans*), size of fish, harvesting biomass of plants and magnetic water treatment technique.

The above aim is achieved by meeting the following objectives:

1- Evaluate and compare the growth and nutrient removal efficiencies of three plant-based biofilters (*Lemna minor, Hygroryza aristata* and *Phyllanthus fluitans)* with the bacterial biofilms of a moving-bed filter in the IRASs culturing common carp.
2- Investigate the effect of different plant species (*L. minor, H. aristata* and *P. fluitans)* as biofilters in the IRASs on the growth and survival rates of the common carp.
3- Assess the effect of the initial body size of stocked common carp on water quality and the removal capacities of dissolved inorganic nitrogen in the IRASs.
4- Investigate the effect of harvesting different biomasses of watercress (*Nasturtium officinale*) on water quality and nutrient removal capacity in an IRAS for rearing common carp.
5- Examine the growth performance of both watercress and common carp in an IRAS (Aquaponics) under different plant harvesting regimes.
6- Identify the potential of using magnetic water treatment to improve water quality and the growth performance of plant-based biofilter *(L. minor)* in the IRAS.
7- Investigate the impacts of magnetized water on the feeding efficiency and growth performance of common carp in the IRAS.

2. LITERATURE REVIEW

2.1. Development of Aquaculture

Aquaculture has been practiced for centuries, particularly in Asia, however, the industry has grown dramatically in the last half-century. Aquaculture has gone through major changes, growing from small-scale activities to large-scale commercial farming (Karnai and Szűcs, 2018). World aquaculture production has increased substantially from less than 1 million tonnes of annual production in 1950 to 110.2 million tonnes in 2016 (FAO, 2018). The average annual supply of food fish from aquaculture for human consumption has increased by ten times, from 0.7 kg in 1970 to 7.8 kg in 2015 (FAO, 2018). In 2016, the total production included 80.0 million tonnes of food fish (USD 231.6 billion) and 30.1 million tonnes of aquatic plants (USD 11.7 billion). Farmed food fish production included 54.1 million tonnes of finfish (USD 138.5 billion), 17.1 million tonnes of molluscs (USD 29.2 billion), 7.9 million tonnes of crustaceans (USD 57.1 billion) and 938 500 tonnes of other aquatic animals (USD 6.8 billion). The contribution of aquaculture to the global production of capture fisheries and aquaculture has risen continuously, reaching 46.8% in 2016, up from 25.7% in 2000 (FAO, 2018). Inland aquaculture was the source of 64.2% of the world's farmed food fish production, as compared with 57.9% in 2000. Finfish farming still dominates inland aquaculture, accounting for 92.5% (47.5 million tonnes). Aquaculture is also the main source of edible aquatic plants, accounting for 96% of the total of 31.2 million tonnes of aquatic plants (FAO, 2018).

The expansion of aquaculture production, especially for species such as shrimps, salmon, tilapia, carp and catfish, is evident in the relative growth rates of per capita consumption of different species groups in recent years. Since 2000, average annual growth rates have been most significant for freshwater fish (3.1%). In 2015, global per capita consumption of freshwater fish was 7.8 kg, or 38% of the total, as compared with 17% in 1961 (FAO, 2018). Freshwater species, such as carp, catfish (including *Pangasius* spp.) and tilapia are expected to represent about 62% of total world aquaculture production in 2030, as compared with 58% in 2016 (FAO, 2018).

Carps are considered the preferred species in aquaculture production and have a major share within global aquaculture. Common carp is the third most significant fish species of the world's aquaculture production and 97.3% of its global production is originated from aquaculture (Karnai and Szűcs, 2018). Common carp production has increased significantly during the last decades (Figure 1.1), and contributed about 4.557 million tonnes in 2016,

accounting for 8% of total major species produced in world aquaculture (FAO, 2018). In Europe, common carp contributed 1.8% (170,000 tonnes) of the total inland fisheries production (9.42 million tonnes) during 2015–2016. It is a major farmed species in European freshwater aquaculture with production localized in central and eastern European countries, represents about 70% of carp production in Europe during 2016 (Russian Federation: 60,000 tonnes; Poland: 20,000 tonnes; Czech Republic: 20000 tonnes; Hungary: 10,000 tonnes; Ukraine: 10,000 tonnes) (Roy et al., 2019). The land-locked central European countries rely heavily on common carp aquaculture in fishponds. The European common carp production reached its peak (180,000 tonnes) during 2009–2010 and has been declining since (Roy et al., 2019). This reduction of common carp aquaculture in Europe may be attributed to several factors: (i) diversification of alternative aquaculture species (ii) decreasing popularity of common carp among farmers and consumers, (iii) recent eutrophication concerns associated with carp farming (Rahman, 2015; Roy et al., 2019). Despite the reduction in European production, the global common carp production during the last fifty years has increased significantly (Figure 1.1). Asia is considered the main producing region of common carp in the world. Common carp production in Asia increased from 697,982 tonnes in 1990 to 3.860 million tonnes in 2015. The main common carp production countries are China (75.5%) and Indonesia (10.7%), the combined production of which amounted to 86.2% of the total common carp production in 2015 (Karnai and Szűcs, 2018).

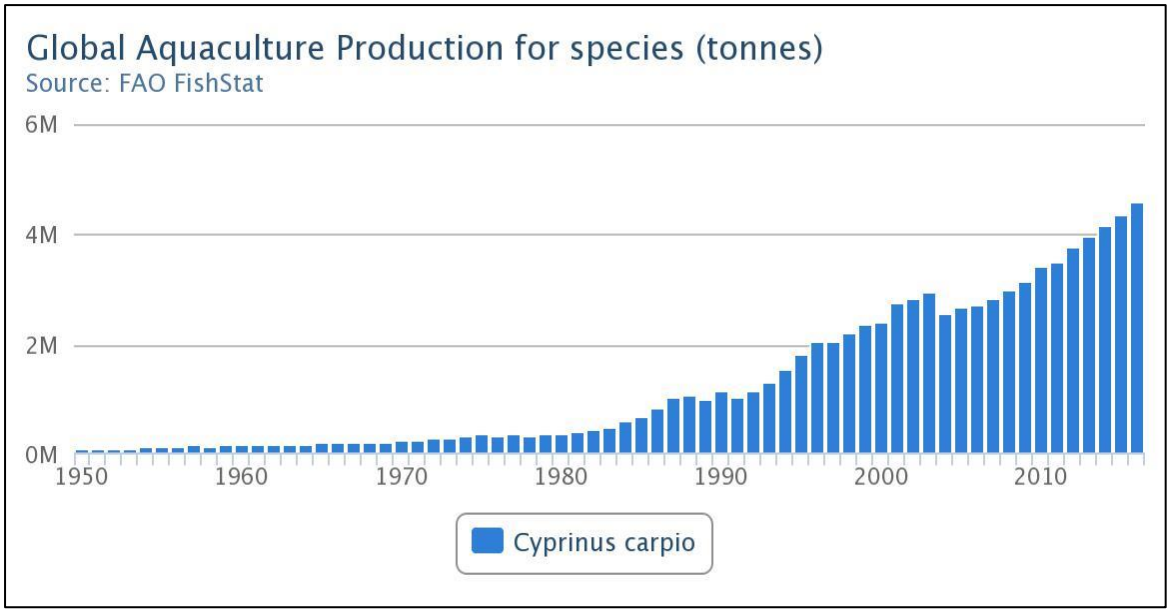

Figure 1.1. Global aquaculture production of common carp (*Cyprinus carpio*), 1950-2016 (FAO)

2.2. Ecological Characteristics and Production Systems of Common Carp

Common carp is the largest member of the Cyprinidae family and the most important cultured fish in the world. It has four subspecies: *Cyprinus carpio carpio* of the European-Transcaucasian area; *C. c. aralensis* of the mid-Asian region; *C. c. haematopterus* of the Amur-Chinese or Far Eastern region and *C. c. viridivio-laceus* of North Vietnam (Jhingran and Pullin, 1985). Currently, common carp is represented by the two subspecies: the East Asian subspecies of *C. c. haematopterus* (Amur River basin, and rivers and lakes of southeast China) and the eastern European subspecies of *C. c. carpio*, which is located in the Caucasus region, central Asia and across Europe (Spasić et al., 2010; Vandeputte, 2003).

Common carp is considered a hardy fish species because it can survive in poor water quality, as well as rapid fluctuation of temperature. The optimal temperature is between 18 to 25 °C, and fish can survive at temperatures between 0 to 35 °C. Temperatures outside the optimal range may decrease feeding and growth (Horváth et al., 2002; Watson et al., 2004). The pH should be kept as close to 7 as possible and fish may tolerate a pH between 5-9 (Horváth et al., 2002; Watson et al., 2004). Oxygen level should be at least 5 mg L^{-1}, while unionized ammonia levels should be below 0.05 mg L^{-1} (Horváth et al., 2002). Common carp can reach 0.6 to 1.0 kg body weight in the first year in natural subtropical and tropical conditions. While growth in a temperate climate is slower, the fish reach between 1-2 kg body weight in the third rearing season (Kestemont, 1995; Billard, 1999). The female carp is the homogametic sex, and the age at first maturity depends mainly on the rearing temperature. For example, the first maturity in the natural environment is 4-5 years old in the Volga, 3-4 years in Poland, 2 years in France (Camargue) and 1 year in the Middle East (Billard, 1999). Males generally mature earlier than females in temperate regions. In France, the male can mature at 500 g body weight and the female at 800-1000 g. In cold regions such as central Europe, males usually mature at 2-3 years old (1kg) and females at 4 years (3-5 kg) (Billard, 1999).

Carps are considered one of the preferred species in aquaculture production systems due to their tolerance to high variations in water quality and diseases, high survival and growth performances under culture conditions, and feeding habits at a low level of the food chain (Kestemont, 1995). Carps are cultured for food or as ornamental fish throughout most of the world regions, except for Australia and North America, where the fish are considered unpalatable (Hulata, 1995; Jhingran and Pullin, 1985). Common carp can be farmed to market size in extensive or semi-intensive ponds, in monoculture or polyculture with other cyprinids, tilapias and mullets. Also, carp can be cultured on natural food with supplementary feeding in

ponds; or in intensive systems on complete feeds in cages, irrigation reservoirs and running water ponds, as well as in recirculating production systems. Integrated systems with animal husbandry and/or plant products are also used to culture carp fish, such as carp-cum-duck in central and Eastern Europe (Kestemont, 1995; Pillay and Kutty, 2005).

2.2.1. Extensive common carp monoculture production in earthen ponds

Common carp can be reared in earthen ponds. The degree of culture intensity ranges from highly extensive to relatively highly intensive. Extensive common carp monoculture is the traditional method and natural production based on the productivity of the pond (Pillay and Kutty, 2005). In temperate regions, the stocking density of common carp varies from 300 to 600 fish ha^{-1} in unfed unfertilized ponds, to 900 fish ha^{-1} in fertilized ponds, and 4000 fish ha^{-1} in ponds that received formulated feed (Kestemont, 1995). The average productivity of carp culture systems in central European countries ranges between 0.3 and 1 tonne/ha depending on water quality, fertilization, and climate (Roy et al., 2019).

2.2.2. Intensive monoculture in ponds

Intensive monoculture is feeding fish completely with artificial feed and supplying water with strong aeration or running water (Pillay and Kutty, 2005). Monoculture ponds may range in density from 1000 to 20000 fish ha^{-1} (Kestemont, 1995). Economic analysis of common carp production showed that the most profitable stocking density for intensive monoculture was 16000 fish ha^{-1} (Kestemont, 1995).

2.2.3. Net cage culture

Floating-net-cage culture has many advantages such as a high yield per unit area, ease of management and harvesting (Pillay and Kutty, 2005). Cage capacities range in an area from 25 to 100 m^2 (2.5 m deep) and are constructed to float to a depth of 1.5 m (Pillay and Kutty, 2005). Stocking density in cages should be at no more than 75 fish m^{-2} (initial weight of stocked fish ranges between 100-200 g). One cage can produce marketable carps (1 kg) in 6 months (from April to October) fed on a high-quality feed (Kestemont, 1995). The annual production yield between 100-200 kg m^{-2} (Kestemont, 1995).

2.2.4. Farm or irrigation pond culture

Common carp can be cultured in irrigation reservoir ponds which are used for agriculture. The reservoir ponds range from 0.5 to 30 ha, and each pond is provided with floating piers and aerators (Kestemont, 1995). In this system, approximately 100 g of fish are stocked into the reservoir from April through May and the fish are reared to the market size of approximately 800 g by harvest time in autumn (Ikuta and Yamaguchi, 2005). The production

in this system is between 5-10 tonnes ha^{-1} per year and can be increased to 20-30 tonnes by increasing the number of aerators (Kestemont, 1995).

2.2.5. Running water ponds and raceways

The ponds are generally small (20-100 m^2 and 1.2-1.5 m deep), and the water is supplied from the river at an inflow rate varying from 300 to 800 litres per second (Kestemont, 1995). In this system, approximately 150-250 g of carp can be stocked in April at a density of 3-l1 kg m^{-2}. Fish are fed several times per day with both formulated feed and silkworm pupae (Kestemont, 1995). Usually, carp reach marketable size (1 kg) in autumn and the annual production in this system is approximately 100-200 kg m^{-2} (Kestemont, 1995). In Japan, similar systems are constructed in stone or concrete raceways with running water (Ikuta and Yamaguchi, 2005). The total annual production in Japan is about 300 kg m^{-2} (Ikuta and Yamaguchi, 2005).

2.2.6. Integrated fish farming

Traditional carp monoculture has been associated with agriculture (rice, cereals) or farming (duck) in several regions, such as Europe, Japan, Southeast Asia (Pillay and Kutty, 2005). In France, carp production is integrated with crop rotation. Usually, after drainage and harvesting the fish, the bottom of the pond is cultivated one year for producing a crop of cereal, such as barley and corn, and then the pond is refilled with water to produce fish for 2 or 3 years (Kestemont, 1995). In Japan and Southeast Asia, common carp are commonly reared with rice in the same area. The production of this integration in Japan is approximately 1800 kg ha^{-1} per year (Pillay and Kutty, 2005).

2.3. Wastes Production and Environmental Impacts of Aquaculture

Waste generated by the metabolism of cultured organisms and uneaten feed; and mainly depends on species and its size, temperature, rearing methods, feeding level, feeding practices, feed composition and its assimilation rate (Schneider et al., 2005). Wastewater from aquaculture contains high levels of organic matter, nitrogenous compounds, phosphorus and suspended solids waste from overfeeding and waste excretion. Approximately 25-30% of the nutrient added through the feed returned as the biomass of products at harvest; while, a large amount of nutrients (about 75% of nitrogen and phosphorus in feed) are released into the water in the form of excretory products and uneaten feed, leading to deterioration in water quality (Table 1.1). The main nitrogen waste (60–90%) is in the dissolved form and about 9–27% is urea; whereas, a larger proportion (25–85%) of phosphorus excreted within the fecal waste (Van Rijn, 2013).

Table 1.1. Percentage of nitrogen recovered by different fish species and discharged to the environment in different aquaculture production systems

Fish species	Culture system	Recovered by fish		Discharged					References
				Dissolved		Solid		Total	
		N	P	N	P	N	P		
Tilapia hybrid *Oreochromis Niloticus*× *O. Aureus*	Tanks	21-22	18.8	59-72	60-62	3.6-5.4	19-22		(Siddiqui and Al-Harbi, 1999)
Channel catfish *Ictalurus punctatus*	Pond	27						73	(Boyd, 1985)
Gilthead seabream *Sparus aurata*	Pond	26		66		7			(Neori and Krom, 1991)
Gilthead sea bream *Sparus aurata*	Pond	30		60		10			(Porter et al., 1987)
Rainbow trout *Oncorhynchus mykiss*	Raceway	19		74		7			(Foy and Rosell, 1991)
Bighead catfish *Clarias macrocephalus*	Cages	24						76	(Lin et al., 1993)
Atlantic salmon *Salmo salar*	Cages	25		62		13			(Folke and Kautsky, 1989)

Table extracted from Piedrahita (2003)

Wastewater from aquaculture systems contains a large amount of nutrients, is well documented as a serious problem to environmental deterioration (Lee and Shoda, 2008). The degree of impacts depends on many factors such as the level of technology and management during operation farms, scales of production, the location of farms and capacity of the receiving ecosystem (Pa´ez-Osuna, 2001; Troell, 2009). The negative environmental impacts of aquaculture activities are: (1) pollution of surface and ground waters by effluent release, (2) disease transmission (3) destruction of the natural ecosystem and (4) declined biodiversity of a natural population of fish by the escape of non-native fish species (Boyd, 2003). Additionally, the consumption of organic matter which is the preferred source of many microorganisms in ecosystems can cause a deficiency of oxygen, leading to severe risk to many aquatic lives. Ammonia is the major end product of nitrogen metabolism, and high levels of ammonia can be toxic to aquatic life (Corpron and Armstrong, 1983). The toxicity of nitrate is also reported on aquatic organisms when nitrate reacts with haemoglobin causing a deficiency of oxygen in their body (methaemoglobin) and subsequently death (Camargo et al., 2005). Nitrogen and phosphorus can stimulate the growth of photosynthetic aquatic life such as algae, leading to adverse changes and excessive eutrophication in aquatic ecosystems (Aure and Stigebrandt, 1990). It is well known that nutrient loading can cause eutrophication in receiving water bodies and a decrease in farm production or even collapse of the aquaculture industry, for instance, fish culture in the Philippines (Rodrigueza and Montaño, 2007) and the prawn industry in China (Msuya et al., 2006).

2.4. Recirculating Aquaculture Systems (RASs)

Along with aquaculture development, the environmental impacts of this industry have also increased. Recirculating aquaculture systems (RASs) have been developed in response to increasingly strict environmental regulations and limited access to land and water (Martins et al., 2010). The RAS offers many benefits in terms of reducing water requirements, recycling nutrients, improving waste management and better disease management (Timmons et al., 2002; Martins et al., 2010). The RAS from the environmental protection point of view is considered less environmental impact about 26–38% less eutrophication potential and 93% less on the water source dependence from flow-through systems. This reduction of eutrophication potential means that each fed kilogram of feed releases 8.1 g less suspended solid, 5.7 g less total nitrogen and 0.8 g less phosphorus (Roque d'Orbacastel et al., 2009).

The RASs often consist of an organized set that allows at least a portion of the water leaving a fish culture tank to be reconditioned and then reused; they should contain a fish culture tank, a particulate filter for solids removal, a biological filter for ammonia removal, a pump for water circulation and an oxygenation device (Timmons et al., 2002). The RASs show a better efficiency to control the quality of water and reduce the negative environmental impacts of aquaculture. However, nutrients in RASs are not converted to valuable products; they are destroyed and converted in a nontoxic form by nitrification and denitrification processes (van Rijn and Shnel, 2001; Eding et al., 2003). The RASs reduce the amount of water release; but they are not typically zero-waste systems (Piedrahita, 2003). In the RASs, solid wastes should be treated before water transferred to the biological filter; and finally, these solid wastes are released to the environment (Chen et al., 1995). Bacterial biofilm filters can take about 4–8 weeks to establish a healthy and effective population of bacteria to remove ammonia concentrations (Timmons et al., 2002). The bacterial biofilm filters also can be affected by different factors that cause inhibition of the nitrification process, such as temperature, pH value, ammonia concentration, salinity, dissolved oxygen, and organic loading (Timmons et al., 2002; Tseng and Wu, 2004; Ling and Chen, 2005; Malone and Pfeiffer, 2006).

Additionally, a high capital investment cost and a long period to return this cost (on average 8 years) are the biggest restraints of the RASs (Badiola et al., 2012). The RASs are typically used for commercially important species with high stocking densities and low water exchange rates to cover the high capital and operation costs (Badiola et al., 2012). One strategy to minimize costs has been involved by reducing total production costs by increasing the production rate, which in turn can be achieved by either increasing the stocking density or

the growth rate. However, there are physical and engineering restrictions that limit increasing stocking densities. High stocking density can negatively affect growth rates in many species of fish (Ruane et al., 2002). Increasing stocking density also can be associated with a higher risk to spread infectious agents to a larger fish stock (Iguchi et al., 2003) as well as increasing concern for animal welfare and this may limit the intensity to which producers may be permitted to operate (Ormandy et al., 2011).

Due to increasing concerns about the problems associated with bacterial biofilm filters, nutrient overloading, diseases, and animal welfare as well as the problems of setup and operation costs of the RASs, the research and developments in the RASs tend to focus on: (1) technical improvements within the recirculation loop and (2) recycling of nutrients through integrated farming (Martins et al., 2010). Accordingly, an integrated recirculating aquaculture system (IRAS) has received a lot of attention as a promising practice due to its low cost and low environmental impacts.

2.5. Integrated Recirculating Aquaculture Systems (IRASs)

In order to deal with the problems associated with nutrient overloading in the environment as well as the problems of the RASs (setup and operation costs, diseases, and animal welfare), integrated recirculating aquaculture systems (IRASs) are getting popularity. The IRASs refer to integrated systems where additional separated units are combined into a RAS (Chien and Tsai, 1985). These separated units can take nutrients and have positive impacts on water quality (Redding et al., 1997; Jo et al., 2002; Akinbile and Yusoff, 2011) and finally on the carrying capacity of the system. Usually, the wastewater treatment unit involves an additional cost; while, the additional separated unit in the IRASs can produce income, which can increase the profitability of the systems (Troell, 2009). The subordinate species in the IRASs are usually more than just biofilters; they are harvestable products, and higher nutrient retention can be achieved by the main and subordinate products. Schneider et al. (2005) reported that between 20–50% feed nitrogen and 15–65% feed phosphorous converted to fish biomass if fish cultured alone; while the integration of fish culture with phototrophic conversion raises nutrient retention of feed nitrogen by 15–50% and feed phosphorous by up to 53%. The IRASs offer a great benefit in terms of maximizing the use of resources (water, land, and feed) and production of the system, as well as minimizing the waste and negative impacts on the surrounding environment (Popp et al., 2018).

Several combinations have been applied at different levels according to size, complexity, and types of species grown. For instance, the University of the Virgin Islands has applied a

freshwater approach, where the effluent of a tilapia recirculating system has been integrated with lettuce sub-systems (Rakocy, 1999). Schneider et al. (2005) also referred to the integrated recirculation system included aquatic plants (tilapia- duckweed- tilapia), where the trickling filter was replaced by duckweed reactor and the duckweed was fed to tilapia. Seaweeds have also been applied as biofilters for marine integration systems because they can absorb a significant amount of nitrogen and phosphorus (Van Khoi and Fotedar, 2011). More advanced integration is the integrated multi-trophic aquaculture (IMTA) which refers to the integration of species from various trophic or nutritional levels in the same system (Popp et al., 2018). The combination in the IMTA refers to the more intensive farming of the different species in the proximity to each other, linked by nutrient and energy transfer through the water. In this integration technology, the production of fed species is incorporated with extractive species including phototrophic conversions such as macro-algae, microalgae, and plants (Popp et al., 2018).

The IRAS is now accepted as an alternative to the conventional practice of farming as the system is known to decrease the expenses involved in operations and reduce environmental problems of aquaculture (Hargreaves, 2006; Estim et al., 2019). The IRASs can be classified as integrated marine systems (Neori et al., 2004), aquaponic systems (Rakocy, 2007), high rate algal ponds (Pagand et al., 2000), constructed wetlands (Zachritz et al., 2008), active suspension ponds based on bio-flocs technology (Crab et al., 2007 and 2010) and periphyton systems (Schneider et al., 2005). In these systems, wastewater from aquatic rearing units containing major nutrients such as nitrogen and phosphorus compounds is not only treated by typical nitrification and denitrification processes, but also by the uptake of vegetable/ornamental or aquatic plants (Lin et al., 2002; Hargreaves, 2006; Endut et al., 2011; Estim et al., 2019). The biological and chemical processes should be balanced and this can be achieved when the suitable ratios and selection of cultured species are achieved. Martinez-Porchas et al. (2010) indicated that the biological requirements of subordinate species of integrated systems have to be provided by the main culture units, otherwise serious risks may occur. The selection of suitable organisms can be based on their functions and roles in the ecosystem, acceptance by consumers, and economic value.

Numerous studies have confirmed that water quality is improved in integrated systems compared with systems containing no plants (Redding et al., 1997; Lin et al., 2002; Jo et al., 2002; Neori et al., 2004; Rakocy, 2007; Endut et al., 2011; Ardiansyah and Fotedar, 2016; Nakphet et al., 2017; Estim et al., 2019). However, optimizing growth conditions for both plants and fish is the biggest challenge to profitability because the amount of nutrients from

different integrated modules differs and depends on the nutritional values of the feed which in turn depends on the specific demands of the cultured species (Schneider et al., 2005; Delaide et al., 2016; Goddek and Vermeulen, 2018). Nutrients in the system can have direct effects on water quality, plant growth and nutrient removal efficiency and then indirectly affect fish growth (Schneider et al., 2005). In order to recycle aquaculture wastes to produce plant biomass, it is necessary to optimize the recycling rates of nitrogen and phosphorus (Goddek et al., 2019). However, several factors such as fish species, fish size, fish density, temperature, plant species, harvesting rate of plants and the microbial community can affect the nutrient removal rates of the integrated system and growth rate of cultured species (Schneider et al., 2005; Hu et al., 2015; Goddek et al., 2019). The integration technology is still under development (Popp et al., 2018), and there is a need to design the integrated system to maintain the balance of nutrient production and uptake in order to ensure effective nutrient removal (Buzby and Lin, 2014) as well as the sustainability of the whole system.

2.5.1. Integrated Recirculating Aquaponic systems (Aquaponics)

Aquaponic systems are the integrated recirculating aquaculture systems that combine aquaculture (fish) and hydroponic sub-systems (plants) (Rakocy et al., 2016; Love et al., 2015). These systems are gaining popularity because they offer many advantages in terms of reducing water consumption, increasing the profitability of the system, recycling nutrients, and reducing the environmental impacts of aquaculture (Wongkiew et al., 2017; Estim et al., 2019). A typical aquaponic system contains a fish tank (aquaculture), a biofilter (for nitrification) and a plant grow bed (hydroponics). Aquaponics is a symbiotic production system between fish, microbes and plants. The removal of nutrient waste in these systems could occur by several mechanisms, such as the absorption by vegetable planted in the hydroponic tank, the removal of dissolved solids via microbial assimilation by microorganisms in the water column and adsorption on biofilms formed within the root system of vegetable planted in the hydroponic tank (Timmons et al., 2002). In brief, after fish digest food, ammonia nitrogen is excreted into the water. Nitrifying bacteria utilise ammonia nitrogen and convert it into nitrite and then to nitrate. Finally, plants absorb and utilise nitrate for growth (Wongkiew et al., 2017).

There are three types of aquaponic systems based on the types of grow bed; nutrient film technique (NFT), floating-raft (deep water culture) and media-filled (flood and drain) (Engle, 2015). Each type has its advantages and disadvantages, for example, NFT and floating-raft aquaponic systems require a biofilter for nitrification and a sedimentation tank for solid

removal (Engle, 2015); while, the media-filled type is considered the simplest aquaponic system that does not require separate biofilters because it contains media (pumice stones or clay beads) in the grow bed for nitrification (Zou et al., 2016). However, the floating-raft type is most commonly used in aquaponic systems because it allows the plant roots to freely absorb the nutrients from water without clogging the water channel (Engle, 2015; Timmons et al., 2002). Many types of plants have been grown in aquaponic systems such as lettuce, basil, mint, watercress, water spinach, tomatoes, peppers, cucumbers and cabbage (Timmons et al., 2002). The plants growing in aquaponics take nitrate as the main nitrogen source because nitrate concentration in these systems is higher than ammonium and nitrite concentrations (Rakocy et al., 2003). However, the preference for ammonium and nitrate depends on their concentrations, growth stages and plant's genetic factors. The nitrogen uptake rate of the plant is influenced by many factors such as nutrient concentrations, light intensity, humidity, temperature and ambient carbon dioxide concentration (Tiaz and Zeiger, 2002).

Sustainable aquaponic production requires optimal environmental conditions and nutritional requirements for fish, bacteria and plants. It is complex to determine the exact fish to plant ratio because fish and plant species have different nutritional requirements that are dependent on the growth stage and external factors such as system design (Gichana et al., 2019). Lam et al. (2015) investigated the influence of component ratio (hydroponic tank to fish rearing tank volumes) on fish growth, vegetable yield and nutrient removal in a recirculating aquaponic system containing *Oxyeleotris marmorata* and a hydroponic tank grown with *Ipomoea aquatica*. They found that a high ratio (3 m^3 /m^3) was effective in reducing nutrients and associated with a high production of both plants and fish (Table 1.3).

Plant density is another factor that affects nutrient concentrations in aquaponics (Gichana et al., 2019). The density of plants per unit area is the most important factor to optimize plant growth in any production system. If the density of plants is too high, the concentration of nutrients in the aquaponic system decreases to levels that may be too low to sustain plant growth and result in nutrient deficiencies. Low plant density may increase nutrient production while nutrient uptake remains the same, resulting in nutrient accumulation (Gichana et al., 2019).

In aquaponics, three strategies have been adopted for producing crops: staggered cropping, intercropping and batch cropping (Rakocy et al., 2016). A staggered production system is one where a group of the plant is harvested at different stages of growth. This allows the crop to be harvested repeatedly and keeps the nutrient uptake in the culture system relatively constant. This system is most effective for crops that can be grown continuously such as leafy

green vegetables and herbs (Rakocy et al., 2016). However, if the harvesting of the plants is too much, the number of plants in the bed will reduce and the uptake of the nutrients in the aquaponic system may decrease, resulting in nutrient accumulation and, eventually, fish mortality.

Despite the intensive and sustainable production in aquaponic systems, there are only a few commercial operations of aquaponic systems. However, lower requirements for resources such as water and land will encourage research to continue developing the aquaponic systems. Aquaponic systems will have potential in arid and semiarid areas due to their water reuse efficiency and conservation characteristics.

2.6. Wastewater Treatment Units and Sub-systems

Wastewater treatment units and sub-systems can be classified into two categories: (1) units used for treating wastewater within the recirculating loop and (2) units for treating the effluents. Generally, the main purpose of any treatment system is to remove solid wastes and nutrients. The solid waste can be removed by using settleable solids, sedimentation tanks (Chen et al., 1994), or mechanical filtration (suspended and fine solids). Screen filtration and expendable granular media filtrations are commonly applied as mechanical filtration methods for solids removal (Timmons et al., 2002). Foam fractionation (protein skimming) is usually applied for fine solids removal (Timmons et al., 2002). For nutrient removal, various units and sub-systems have been applied to reduce nutrient levels in aquaculture systems; popular methods for nutrient removal are shown in Table 1.2.

Three techniques have been recently used to remove nutrient waste are (1) autotrophic bacterial conversion by nitrification process in bacterial biofilms filters, (2) heterotrophic bacterial conversion of ammonia–nitrogen directly to microbial biomass (Bio-floc) and (3) photoautotrophic conversions by using seaweed or plant uptake of ammonium or nitrate and subsequent harvesting (Ebeling et al., 2006). It is reported by Ebeling et al. (2006) that the wastewater within the recirculating loop can be controlled by using the bacterial biofilm filters (nitrification process), plants/algae uptake, and immobilization by bacteria, while the effluents of the systems can be treated by integrating with the sub-systems of wetlands, and hydroponics. These methods have been confirmed to diminish nutrients by many researchers in different operating conditions (Table 1.3). However, methods that use the nitrification process do not increase the retention of nutrients; they convert nutrients to less toxic forms and do not reduce the output of nutrients to the environment as well as they are often expensive and involve advanced technology. Other methods such as integrating the plants or

seaweeds into the treatment units can be useful to turn nutrient-rich wastewater into harvestable products, and higher nutrient retention can be achieved by primary and secondary products as well as they are low cost and low environmental impacts.

Table 1.2. Wastewater treatment methods

Methods	References
Earthen ponds or reservoirs	(Chin et al., 1993)
Sedimentation	(Chen et al., 1994)
Nitrifying bacteria (Nitrification process in biofilters)	(Malone and Pfeiffer, 2006; Timmons et al., 2002)
De-nitrification	(Menasveta et al., 2001)
Filter feeder bivalves	(Jones et al., 2002)
Using microalgae	(Chuntapa et al., 2001)
Using seaweeds as biofilters	(Neori et al., 2003; Khoi and Fotedar, 2011)
Constructed wetlands	(Lin et al., 2003; Zachritz et al., 2008)
Bio-floc technology	(Crab, 2010)
Aquaponic systems	(Rakocy, 2007)
Macrophytes as bio-filter	(Redding et al., 1997, Ardiansyah and Fotedar, 2016)

2.7. Nutrients Removal Efficiency of Some Wastewater Treatment Units

2.7.1. Bacterial Biofilm Filters (Autotrophic bacterial conversions)

Aquaculture operations often rely on biological treatments (nitrification process) to remove nitrogen compounds from production systems. In this process, nitrifying bacteria convert ammonia nitrogen to nitrite, and then to nitrate, which is less toxic to fish. Nitrifying bacteria grow on either a wetted or submerged media surface (Timmons et al., 2002). Typical media used to carry out the nitrification process in biological filters are river gravel, crushed rock, sand, some plastic media, or ceramic material shaped as small beads or large ball, ring and saddles (Timmons et al., 2002). The size and capacity of bacterial biofilm filters to remove ammonia is mostly based on the total surface area that is accessible for the growth of bacteria on the media surface (Timmons et al., 2002). The nitrifying bacteria population are affected by many factors that can cause stress for bacteria during initiation and activation phases of biological filters, such as salinity changes (Tseng and Wu, 2004) and temperature changes (Malone and Pfeiffer, 2006), ammonia concentrations, organic loading, dissolved oxygen and pH value (Timmons et al., 2002). The nitrification process is executed in a variety of bacterial biofilm filters that are commonly used in RASs such as rotating biological contactors,

downflow micro-bead filter, fluidized sand biofilters, trickling filters, and moving bed biofilm filter (Malone and Pfeiffer, 2006; Timmons et al., 2002; Kamstra et al., 2017).

Rotating biological contactors have been widely employed in aquaculture because they need little hydraulic head, have low operation costs, provide gas stripping, tend to be more self-cleaning and can maintain a consistently aerobic treatment environment (Brazil, 2006). The major weaknesses of these filters are the large weight gain due to biomass loading of media and the mechanical nature of its operation; which, resulting in loading on the shaft and bearings (Brazil, 2006). Brazil, (2006) tested the rotating biological contactor in RAS of tilapia fish and the average rate of TAN areal removal was approximately 0.42 g m^{-2} per day (Table 1.3).

A downflow micro-bead filter is another type of biological filter that has been used in RASs. These filters have a specific surface area between 1150 and 3936 m^2 m^{-3}. The filters are easy to set up and operate, as well as they can be used as a hybrid filter for both solids waste removal and nitrification (Timmons et al., 2002). Timmons et al. (2006) reported that the average TAN removal rate is around 0.30 g m^{-2} per day.

Fluidized sand biofilters have been commonly employed in the RASs. These filters have a high specific surface area range between 4000 -20000 m^2 m^{-3} and have a reasonable cost compared to other types of biofilters (Summerfelt, 2006). The main weaknesses are the high cost of pumping water through the biofilter and the additional aeration. These biofilters are not easy to operate and can have serious maintenance problems (Timmons et al., 2002). Fluidized sand biofilters can remove 86–88% of the TAN, 66–82% of the BOD5 and 15–41% total phosphorus (Davidson et al., 2008).

Trickling filter also has been extensively used in the RASs due to its simplicity of setup and operation, self-aerating, and can remove carbon dioxide from the water. Also, it has a moderate capital cost, low maintenance and a huge range of tolerance to variations in hydraulic and organic loads (Timmons et al., 2002). The major weaknesses are low volumetric removal rates with the big size of biofilter resulting in a high cost of nitrification systems and the risk of clogging when not properly designed and operated. The specific surface area of media in trickling filters ranges between 100-300 m^2 m^{-3}, and TAN removal rates range from 0.1 to 0.9 g m^{-2} per day (Timmons et al., 2002).

The moving bed biofilm reactor was developed in Norway, based on the conventional activated sludge process and biofilter process. It is widely used in the RASs due to its advantageous properties, including sufficient mixing, effective mass transfer, avoidance of clogging, the high removal rate of pollutants, and relatively small spatial requirements (Li et

al., 2020; Kamstra et al., 2017). Such reactors are filled with suspended carriers, which move and circulate while providing an attachable surface for slow-growing microorganisms such as nitrifying bacteria. These reactors enable operation in continuous mode without backwashing or sludge return. Li et al. (2020) found that the moving bed biofilm reactor can remove 86% of the TAN; while Kamstra et al. (2017) found the TAN removal rates range from 0.98-2.63 g m^{-2} per day (Table 1.3).

Table 1.3. The efficiency of some wastewater treatment units and sub-systems in removing nutrients

Water source system	Reactor unit/ Species	Removal rate		References
RAS with Tilapia	Rotating biological contactors	TAN	0.42 g / m^2 /day	(Brazil, 2006)
Tilapia wastewater	Down flow microbead	TAN	0.30 g/ m^2 /day	(Timmons et al., 2006)
Rainbow trout wastewater	Fluidized sand biofilters	TAN TP	86- 88% 15-41%	(Davidson et al., 2008)
RAS with hybrid striped bass	Trickling filters	TAN	0.64 g / m^2 /day	(Lyssenko and Wheaton, 2006)
Hybrid African catfish wastewater	moving bed biofilm reactor	TAN	0.98-2.63 g / m^2 /day	Kamstra et al. (2017)
Bio-floc technology in Tilapia pond		TAN	95% C/N ratio is 20	(Crab et al., 2009)
IRAS with western king prawns (*Penaeus latisulcatus*)	Seaweeds (*Ulva lactuca*)	TAN PO_4	59-81% 50-55%	(Van Khoi and Fotedar, 2011)
IRAS with common carp (*Cyprinus carpio* L.), tank combined with mechanical filter and a biofilter contains two floating aquatic plants	Aquatic plants combination *(Lemna minor* and *Wolffia arrhiza)*	NH_4-N NO_2-N NO_3-N TN TP PO_4	0.15 mg / l 0.08 mg / l 16.07±8.8 mg / l 13.9±15 mg / l 0.48 mg / l 0.3 mg / l	(Velichkova and Sirakov, 2013)
Intensive African catfish operation	Wetland	TAN NO_3-N PO_4 TN TP	90% 38% 90% 65-80% 65-80%	(Kerepeczki et al., 2003)
Aquaponic recirculation system	Aquatic plants *Ipomoea aquatica*	TAN NO_2-N NO_3-N PO_4	78.32–85.48% 82.93–92.22% 79.17–87.10% 75.36–84.94%	(Endut et al., 2011)
Recirculating aquaponic system (RAS) with Marble goby (*Oxyeleotris marmorata Bleeker*)	Hydroponic tank with water spinach (*Ipomoea aquatica*)	TAN NO_2-N NO_3-N TP BOD5 TSS	83% 87% 70% 60% 63% 88%	(Lam et al., 2015)

Table 1.3. Continued. The efficiency of some wastewater treatment units and sub-systems in removing nutrients

Water source system	Reactor unit/ Species	Removal rate			References
RAS with Nile tilapia (*Oreochromis niloticus*) and sedimentation, biofiltration unit and aquatic plant unit	Aquatic plant *(Azolla filiculoides)*	NH_4-N NO_3-N PO_4	4.35% 3.3% 2.6%		(Redding et al., 1997)
	Aquatic plant *(Elodea nuttallii)*	NH_4-N NO_3-N PO_4	8.19% 5.92% 7.81%		
	Aquatic plant *(Rorippa nasturtiumaquaticum*)	NH_4-N NO_3-N PO_4	10.66% 15.43% 8.63%		
Wastewater obtained from an intensive RAS stocked with Arctic charr (*Salvelinus alpinus*)	Water Hyacinth (*Eichhornia crassipes*)	TS COD NH_4-N NO_2-N NO_3-N PO_4-P	39.5- 48% 84.4-89.5% 76% 76.4- 90.6% 43.7-54.4% 66.2-76.8%		(Snow and Ghaly, 2008)
	Water Lettuce *(Pistia stratiotes*)	TS COD NH_4-N NO_2-N NO_3-N PO_4-P	29.3- 45.6% 82.5% 68-72% 65-74.5% 41.8-52.9% 65-75.3%		
	Parrot's Feather *(Myriophyllum aquaticum*)	TS COD NH_4-N NO_2-N NO_3-N PO_4-P	21.4- 36.5% 78.7-84.4% 55.9- 64% 49.6-61.4% 34.5- 50.9% 64.5-66.8%		
Water from Intensive Bioproduction Korean (IBK) Recirculating Aquaculture System		mg / l	initial	After 24 h	(Jo et al., 2002)
	Aquatic plant *(Pistia stratiotes)*	NH_4-N NO_2-N NO_3-N	2.3 0.197 21.4	1.1 0.029 20.1	
	Aquatic plant *(Hygrophila angustifolia)*	NH_4-N NO_2-N NO_3-N	2.3 0.197 21.4	1.7 0.17 20.1	
	Aquatic plant *(Eichhornia crassipes)*	NH_4-N NO_2-N NO_3-N	2.3 0.197 21.4	1.4 0.057 20.8	
	Aquatic plant *(Hydrocotyle leucocephala*)	NH_4-N NO_2-N NO_3-N	2.3 0.197 21.4	1.7 2.91 28.4	

2.7.2. Aquatic Plant Species (photoautotrophic conversions)

Phytoremediation is one of the biological wastewater treatment methods that utilise aquatic plants to remove nutrients from water bodies. The use of macrophytes for removing nutrients from wastewater effluents and water bodies has been well studied (Lin et al., 2002; Sooknah, 2000; Vymazal, 2007; Jianbo et al., 2008). Treatment technology that uses aquatic plants to treat nutrients is currently attracting much attention due to its low cost and low environmental impacts. It is considered an alternative technology for recycling nutrients (Cheng et al., 2009). In aquaculture, aquatic plants can be used for nutrients removal in two different ways: (1) plants in sub-systems (aquaponic systems or wetlands); where nutrients released from a RAS into sub-systems and converted as a valuable by-product (Rakocy, 2007) or as wildlife habitat (Lin et al., 2003); (2) plants in a biological filter unit of the RAS; where nutrients from rearing units in the RAS are recycled through mechanical solids removal and nutrient assimilations units (biofilters), and eventually converted into harvestable products (van Rijn, 1996). In this system, nutrients assimilations can be macrophytes (aquatic plants) (Redding et al., 1997; Ardiansyah and Fotedar, 2016); or seaweeds (Neori et al., 2004; Msuya et al., 2006).

Using aquatic plants in a wastewater treatment unit can offer many benefits in terms of removing nutrients, managing water quality, and producing a valuable by-product that can be utilised as a source of feed for humans and animals or fish species as well as for biogas, biofertilizer and biomaterial (Singhal and Rai, 2003; Nhan et al., 2019). Using aquatic plant based biofilters as a water treatment unit in the RASs can also help in reducing the delay of introducing cultured species to the system during the activation phases of the biological filters; as well as reducing the accumulations of the nitrate and phosphorus concentrations in the systems (Corpron and Armstrong, 1983). However, limited investigations have been done in the field of raising fish in the RASs that are purified by plant based biofilters. Table 1.3. shows the efficiency of some wastewater treatment units and sub-systems in removing nutrients. For example, Rakocy and Allison (1981) proposed a combination of two floating aquatic plants, (*Eichhornia crassipes* and *Spirodela oligorhiza)* and two submerged plants, *(Vallisneria* sp. and *Egeria densa)* with *Tilapia aurea* in the recirculating fish system. In this study, the combined plant population removed between 12-16% of the nitrogen waste. Porath and Pollock (1982) also studied the feasibility of duckweed (*Lemna gibba)* for stripping ammonia from fish effluent that was taken from a tank containing tilapia fish. They found that the circulation of the effluent under a duckweed mat resulted in decreasing the ammonia level by 50% within 24 h after initiation of the circulation, and 80% within less than 48 h. Corpron and Armstrong (1983) tested *Elodea densa* plants to reduce ammonia levels in recirculating

Macrobrachium rosenbergii systems (Table 1.3). The results showed that the submerged aquatic plants *E. densa* were capable of reducing ammonia levels. Redding et al. (1997) also investigated the ability of three different species of aquatic plants *(Azolla filiculoides*, *Elodea nuttallii*, and *Rorippa nasturtium-aquaticum*) to remove nutrients from untreated wastewater of recirculating *Oreochromis niloticus* culture systems. They found that the concentrations of ammonia, nitrate and phosphorus were significantly reduced in all systems compared to untreated wastewater (Table 1.3). Velichkova and Sirakov (2013) proposed a combination of the IRAS. In this combination, a common carp (*Cyprinus carpio* L.) tank is combined with a mechanical filter and a biological filter that contains two floating aquatic plants *(Lemna minor* and *Wolffia arrhiza*). This plant based biofilter was significantly able to reduce ammonium, nitrite, nitrate, total nitrogen, total phosphorus and orthophosphate concentrations in the system (Table 1.3). Nakphet et al. (2017) also tested different aquatic plants in a recirculating red hybrid tilapia system. They found that aquatic plant species (*Canna generalis*, *Typha angustifolia*, *Cyperus involucratis* and *Echinodorus cordifolius*) removed (90%) nitrogenous waste in seven days and were much better at removing nutrients than an unplanted tank. The results show that aquatic plants are a promising alternative to the method of wastewater treatment in recirculating red hybrid tilapia systems.

2.8. Effects of Plant Species Selection

Nutrients removal through plant uptake depends on species, removed capacity of the plant, culture density and plant growth rate, as well as environmental conditions such as temperature and solar radiation (Sooknah, 2000). Plant selection is one of the most significant factors in aquatic plant treatment systems; the economic achievement and nutrients removal efficiency of these systems mainly rely on the growth rate of plants, which is strongly influenced by photosynthetic activity and environmental conditions (Roongtanakiat et al., 2007; Xia and Ma, 2006). Sooknah (2000) reported that the removal of nutrients by macrophytes increases as standing crop density increases.

Appropriate plants that can be used in aquatic plant treatment systems should have high removal rates of both organic and inorganic compounds as well as a high growth rate (Roongtanakiat et al., 2007). The degree of purification of aquatic plants depends not just on the ability of plants to uptake nutrients, but also on their capability to change the environment of wastewater to improve the removal of organic materials by biochemical processes. Plant roots provide physical support as a living substrate for aerobic bacteria, which actively degrades organic matter (Stowell et al., 1981). The scientific foundation for the integration technology of intensive production is the symbiotic growth between plants and

microorganisms attached to plants. Once microorganisms are attached to aquatic plant roots, they formulate a cooperative relationship with plants.

The selection of aquatic plant species in the IRASs depends on several criteria: growth rate and concentration of nutrients in tissues, the simplicity of harvest and control of life cycle, disease resistance, and a match between the physiological characteristics and the environmental growth (Neori et al., 2004). Depend on these criteria; the selection of aquatic plant species will be influenced by planned purpose. If aquatic plants will be used to produce the high value of biomass, then the decision is based on the quality of tissue; but, if the main reason is for bioremediation, then nutrient removal and growth rates are the main determinations (Neori et al., 2004).

The capacity of removal nutrients differs from plant to plant and also from species to species within a genus (Singh et al., 2003). There are variations between aquatic plant species in the growth rate, nutrients removal capacity and response to harvesting, and these variations can lead to a difference in the amount of nutrients removed from the system in the same environment (Vymazal, 2007). Nutrient uptake by plants also depends on nutrient availability in the system, which can have direct effects on plant growth (Xie et al., 2013), and eventually on the quantity of nutrients removed from the system by harvesting plants biomass.

According to the mentioned criteria, the plants in this research were chosen due to their potential to recover nutrients into useful products, rapid growth rates and simplicity of harvest, for example:

Duckweed *(Lemna minor)* can be used as animal feed and ornamental plants as well as an alternative source for bio-energy (Duan et al., 2013). The reduction in the nitrate nitrogen concentrations has been reported previously for *Lemna* sp. by Ferdoushi et al. (2008), who found that the introduction of *Lemna* sp. in a fish pond efficiently removed nitrate nitrogen and improved water quality. Ardiansyah and Fotedar (2016) also investigated the duckweed (*Lemna minor*) as a biofilter medium in the IRAS. They found that the capacity of the IRAS to eliminate the nitrogenous waste depends on the capacity of the *L.minor* compartment, which in turn directly determines the carrying capacity of the IRAS.

Asian watergrass *(Hygroryza aristata)* can also be used as a cattle feed as well as a diuretic, emollient, galactagogue, strangury, diarrhea, fatigue and general debility (Malik et al., 2014). Tan et al. (2014) found that *H. aristata* removed the excessive amount of nitrogen under real operational conditions in a canal, while Han et al. (2013) reported that *H. aristata* removed the concentration of nitrate nitrogen from the pond water. However, no information is available on using *H. aristata* for treating nutrients in the IRASs.

Pennywort plant (*Hydrocotyle rotundifolia*) was chosen because the genus of *Hydrocotyle* has an extensive root system and a rapid growth rate. *Hydrocotyle* species have a high ability to remove nutrients from water bodies (McChesney, 1994). It is reviewed in McChesney (1994) that *H. ranunculoides* and *H. umbellate* plants are useful as a substitute for water hyacinth plants during the cooler months. However, no information is available on using *H. rotundifolia* for treating nutrients, and most of the information is related to using *H. ranunculoides* and *H. umbellate* for wastewater treatment and biomass harvesting for fuel production (McChesney, 1994).

Watercress (*Nasturtium officinale*) is another plant that can be consumed by humans as a salad green and medicinal herb. The plant has relatively large quantities of beta carotene (vitamin A), ascorbic acid (vitamin C), folic acid, iron, iodine, calcium and phosphorous. It also contains a high level of amino acids (arginine, glycine, lysine and tryptophan) and antioxidants (Smith, 2007). Recently, Nhan et al. (2019) found that watercress can reduce the concentrations of TAN and nitrate in a floating-draft aquaponic recirculating system compared to the system without watercress. However, little published information is available on the optimum harvesting biomass of watercress in IRASs.

2.9. Effects of Harvesting Plants

Harvesting of plant biomass is usually implemented as an effective tool for plant management and removing the nutrients absorbed in plant tissues. In theory, harvesting plants by using the same technique and method could reduce a large amount of nutrients from the system; and plants can maintain their growth rate once the system is rich in nutrients. If aquatic plants are left to die and decompose within the water, almost all of the nutrients in the plant tissues will go back to the water (Verhofstad et al., 2017; Hastie, 1992). Harvest is necessary to achieve the permanent uptake of nutrients by plants in systems being applied for removing nutrients. The systems that are harvested frequently to sustain the growth rate of plants have better removal of phosphorus (Hastie, 1992). Verhofstad et al. (2017) concluded that harvesting at an intermediate frequency is better when intending to remove the highest portion of nutrients under a moderately low nutrient loading. For the best system performance, the standing crop needs to be kept healthy and at the desired density and growth rate. However, the quantity and method of harvest can have effects on the performance of the system to maintain the density of plants and remove nutrients. Leaving a clean edge when harvesting the system can make slow re-growth of plants while remaining small clumps in the edges of the system will help to recover much faster (Hastie, 1992). Moreover, harvesting

higher than 20% of the aquatic plant biomass can create open spaces in the system and this may let sufficient sunlight enter the water leading to significant algal growth (Hastie, 1992).

Additionally, in an integrated recirculating aquaponic system, harvesting plants can affect the nutrient uptake efficiency and development of plants especially in a staggered production system, where a group of plants is harvested at different stages of growth. This production system allows the crop to be harvested repeatedly and keeps the nutrient uptake in the culture system relatively constant. However, harvesting too much density of plants can decrease the number of plants in the bed and the uptake of the nutrients in the system, resulting in nutrient accumulation. Therefore, the selection of the appropriate biomass of plants to be harvested can help in sustaining plant development and optimizing nutrient uptake efficiency in systems. Moreover, several studies have suggested that overall nutrient removal could be improved if a harvesting regime is applied (Vymazal et al., 2010; Yang et al., 2016; Verhofstad et al., 2017), but others reveal that harvesting can negatively affect nutrient removal (Kim and Geary, 2001; Wang et al., 2014; Zheng et al., 2015). The importance of harvest management for the nutrient removal, as well as the growth and development of plants, has always been highly controversial (Álvarez and Bécares, 2008; Vymazal et al., 2010; Zheng et al., 2015; Zheng et al., 2018; Sun et al., 2019). Despite the idea of nutrient reduction through harvesting having gained more attention over the last decade (Bartodziej et al., 2017), little published information is available on the requirement of the optimum harvesting of the plant biomass in the IRASs (aquaponics).

2.10. Effect of Fish Size

The economic values of most either consumption fish or ornamental fish are influenced by fish sizes. The size of fish also has a great influence on the growth and survival of fish, especially in recirculating production systems where the food is available in adequate quantity and quality (Houlihan et al., 2001). Usually, small fish tend to use feed more efficiently in order to satisfy their energetic requirements for growth, than larger fish (Houlihan et al., 2001). Scientists believe that the growth rate of fish depends on many factors, such as fish species and their protein requirements, the size and sex of the fish, the different protein sources used, and different dietary energy levels (Houlihan et al., 2001). Talbot (1993) reported that the specific growth rate declined with the increasing body weight of many salmonid fish species. Franco-Nava et al. (2004) also found that the specific growth rate was significantly different between small and big sizes of European seabass (*Dicentrarchus labrax*) reared in recirculating aquaculture systems. However, the results of a study by Enache

et al. (2011) showed no significant differences in growth of biomass between the two sizes of common carp fish in a recirculating aquaculture system, using a trickling filter as a biological filter, (average weight of 65 g were compared with 152 g fish, and fish weight of 66 g were compared with 150 g fish).

The size of fish also affects the survival rates of fish. Thurston and Russo (1983) noted that the susceptibility of rainbow trout to ammonia decreased as the fish developed from the yolk-sac fry to juveniles and increased afterward. However, Abbas (2006) concluded that ammonia toxicity is independent of common carp size and age; Abbas (2006) found that the 96h LC50 of unionized ammonia for different sizes (5, 10 and 15 g) of common carp were 0.34-0.91 mg L^{-1}, 0.47-1.03 mg L^{-1} and 0.50-0.99 mg L^{-1} respectively. Thurston et al. (1983) also reported the same conclusion after studying the toxicity of ammonia for fathead minnows fish (0.09-2.3 g).

Nitrite toxicity to fish depends on water quality (pH, temperature, and oxygen concentration), chloride concentration in water, length of exposure, fish size and age, fish species, and individual fish susceptibility (Kroupova et al., 2005). Lewis and Morris (1986) reviewed that small fish, even larvae, are unlikely to be more sensitive to nitrite than larger fish. There is definite evidence that very small fish of some species are less vulnerable to toxicity than fish of intermediate or large size. Palachek and Tomasso (1984) concluded that fathead minnows (*Pimephales promelas*) weighing between 0.3 and 0.8 g were more tolerant to nitrite than fish weighing from 0.9 to 3.3 g. Bartlett and Neumann (1998) came to the same conclusion after studying the sensitivity of brown trout alevins (*Salmo trutta*) to nitrite. Atwood et al. (2001) also found that the 96h LC50 of nitrite for small Nile tilapia (*Oreochromis niloticus*) fish (average weight 4.4±1.50 g) was 81 mg L^{-1} compared with 8 mg L^{-1} for large fish (90.7±16.43 g).

It appears that there is still considerable debate regarding the effects of fish size on the growth and survival of cultured species. Additionally, many studies only focused on a single size of fish when they examined the efficiency of different water filtration techniques, fish growth, and survival (Redding et al., 1997; Jo et al., 2002; Tseng and Wu, 2004; Guerdat et al., 2010; Velichkova and Sirakov, 2013; Nakphet et al., 2017); while still there is limited information on the relationship between the initial size of stocked fish and the efficiencies of water filtration techniques, common carp growth and survival in the IRASs.

2.11. Effects of Magnetic Water Treatment

Magnetized water is used successfully for improving water properties in different sectors such as farming and agriculture, wastewater treatment and scale elimination (Ali et al., 2014). Cai et al. (2009) reported that the magnetic field changes the physicochemical properties of water, and results in decreasing the surface tension and increasing the viscosity of water. Ali et al. (2014) cited that magnetized water can improve irrigation water quality, water-saving and scale elimination, as well as the health of livestock, plant growth and crop yield. The positive effect of magnetized water was also reported on the germination rates of the rice (Carbonell et al., 2000) and lettuce seeds (Reina and Pascual, 2001).

Previous studies revealed that the magnetic field can change the surface tension, density, viscosity, hardness and conductivity of water as well as the solubility of solid matter; and these changes in water properties can affect the biological activities of the organisms (Gabrielli et al., 2001; Krzemieniewski et al., 2003; Krzemieniewski et al., 2004). It was shown that the magnetic field reorganizes the water molecules into tiny and homogeneous clusters easing their travel through the pathways in plant and animal cell membranes (Ali et al., 2014). Magnetic fields change osmotic processes, affect the permeability of the cellular membrane and disturb the hydration ability of tissues in animals (Ibraheim and Khater, 2013) and plants (Reina and Pascual, 2001). The additional magnetic field can bring effect to the metabolism of living organisms, namely the magnetic biologic effect, which may affect the enzyme activity, cell membrane permeability and cell metabolism (Liu et al., 2008). The magnetic field may influence the metabolism of living organisms by modifying the synthesis of carbohydrates, proteins and the accumulation of essential amino acids. All metabolic reactions are based on the difference in electrical charges and system ions. Electromagnetic forces cause changes in biological cell metabolism and the movement of electrons and ions may cause changes in biomolecules concentration, such as protein, carbohydrate, and lipid. Therefore, it can modify free radical activities, cell metabolism, cell membrane characteristics, cell growth and enzymatic activity (Santos et al., 2017; Hassan et al., 2018a). In all animals, there is no common mechanism has been implicated regarding the effect of the magnetic field on the growth performance of the animals. Brizhik (2014) suggested that the magnetic field can cause a hierarchy of changes from the primary effect on the dynamics of electrosolitons, to the changes of the macromolecules state, to the effects on the respiration rate and, finally, to the effect on the whole metabolism of the system. Another mechanism reported by Rodriguez et al. (2002) in the dairy cattle, is related to the increase in the level of

insulin-like growth factor-I that plays an essential function in the regulation of growth hormone actions in every cell in the body.

Although the applications of magnetic water treatment have been successfully used in different fields, limited investigations have been done in aquaculture on different species. Some authors reported that the magnetic water treatment had positive effects on the growth of fish (Hassan et al., 2018a; Nofouzi et al., 2017), and the water quality of the systems (Krzemieniewski et al., 2003; Hassan and Rahman, 2016; Hassan et al., 2018b). However, other authors revealed no effect of using the magnetic water treatment on the fish growth (Krzemieniewski et al., 2004; Hassan et al., 2019), and water quality (Krzemieniewski et al., 2004; Hassan et al., 2019). It appears that there is still a significant debate regarding the effects of magnetic water treatment on the growth of cultured species and the water quality of rearing systems. Besides that, there are currently no publications regarding the impacts of magnetic water treatment on the common carp growth in an IRAS.

3. EXPERIMENTS PERFORMED

3.1. Effects of Bio-filter Type and Plant Species in an Integrated Recirculating Aquaculture System

Paper accepted in the Iranian Journal of Fisheries Science. (IF = 0.711)

3.1.1. Introduction

Production systems in the aquaculture sector differ in their capacity to increase production and reduce the negative environmental impacts while achieving food security. Recirculating aquaculture systems (RASs) have been developed in response to increasingly strict environmental regulations and limited access to land and water (Martins et al., 2010). However, RASs are usually characterized by high stocking densities for marketed and higher-price species with large quantities of feed and low water exchange rates to cover the high investment cost (Timmons et al., 2002). Approximately, 20–50% nitrogen and 15–65% phosphorus supplied through the feed is converted into fish biomass when harvest, while large amounts of nutrients in the form of uneaten feed and excretory products are discharged into the water, leading to deterioration in water quality (Schneider et al., 2005). The accumulation of nutrients can be a major concern in RASs if not properly managed. Aquaculturists often rely on bacterial biofilm filters to control nutrient concentrations in the RASs. However, in biological filters, natural colonization of nitrifying bacteria works well for initiating a biological filter but can take about 4–8 weeks to establish a healthy and effective population of bacteria (Timmons et al., 2002). The population of nitrifying bacteria can be affected by many factors that cause inhibition of the nitrification process, such as temperature (Malone and Pfeiffer, 2006), pH value and ammonia concentration (Groeneweg et al., 1994), salinity (Tseng and Wu, 2004), dissolved oxygen (Hao and Huang, 1996) and organic loading (Ling and Chen, 2005). Nitrifying bacteria also gradually acidify the system (van Rijn, 1996).

In order to deal with the problems associated with bacterial biofilm filters in the RASs and nutrient overloading in the environment; integrated recirculating aquaculture systems (IRASs) are getting popularity. The IRASs refer to integrated systems where additional separated units are integrated into a RAS (Chien and Tsai, 1985). These separated units that have the ability to convert nutrients could be aquatic plants (Redding et al., 1997; Jo et al., 2002). The utilization of aquatic plants as a biofilter in the RAS can have positive impacts on water quality (Jo et al., 2002) and add significant income, for example as food for humans, animal feed, fibre and ornamental plants (Wersal and Madsen, 2012). The plants in this study were chosen because of their potential to recover nutrients into useful products as well as their rapid growth and simplicity of harvest. *Lemna minor* and *Phyllanthus fluitans* can be used as

animal feed and ornamental plants. *Hygroryza aristata* also can be used as cattle feed and as a diuretic, emollient, galactagogue, strangury, diarrhea and general debility (Malik et al., 2014).

Although numerous studies have been proven the usefulness of bacterial biofilm filtration (van Rijn, 1996; Guerdat et al., 2010; Ling and Chen, 2005; Malone and Pfeiffer, 2006; Timmons et al., 2002) and aquatic plants (Redding et al., 1997; Jo et al., 2002; Ardiansyah and Fotedar, 2016; Nakphet et al., 2017) to remove nutrients in RASs, none of them have evaluated and directly compared the efficacy of such systems under greenhouse laboratory conditions and the comparison between these systems remains unclear. Therefore, the present study aimed to evaluate and compare the nutrient removal efficacy of bacterial biofilm in a moving-bed filter with three aquatic plant species (*L. minor, H. aristata* and *P. fluitans*) as biological filters in RASs culturing *Cyprinus carpio* L. under greenhouse conditions.

3.1.2. Materials and Methods

3.1.2.1. Collection of fish and plants

Common carp (*Cyprinus carpio* L.) with an average weight of 45.81 ± 0.06 g were collected from a local farm and transported to Georgikon Aquatic Research Laboratory (GARL), Keszthely, Hungary. The fish were held in 12 plastic tanks for one week to adapt them to the laboratory conditions. The fish were fed a commercially formulated feed, Nutra MP T (50% protein, 20% fat, 1% fibre, 8.5% ash, 0.5 Na, 1.8% Ca and 1.4% P) (Skretting a Nutreco Co., Mozzecane, Italy) until the start of the experiment.

Lemna minor, *Hygroryza aristata* and *Phyllanthus fluitans* were obtained from Interaqua-Flora Ltd., Hungary, cleaned and put in 9 plastic tanks for two weeks as an acclimatization period.

3.1.2.2. Biological filters

A moving-bed biological filter was designed and established as one of the experimental treatments. Media with established biofilms were obtained from an operating recirculating system in GARL. Plastic media in the form of "bio-balls" with a specific surface area of 400 m^2 m^{-3} were used to place in the moving-bed filter tanks. In order to determine the appropriate design of biofilters, the calculation of the size of the moving-bed filter was based on the calculations published by Timmons et al. (2002). Hence, 0.003 m^3 of plastic media was placed in each moving-bed filter tank.

L. minor, H. aristata and *P. fluitans* were also used as biofilters in this study. The biomass of the plants to be used for the trial was estimated by their ammonium uptake rates, which was estimated during a preliminary experiment. In that experiment, three beakers (1L) were filled with water and ammonium nitrate (NH_4NO_3) to give an initial ammonium nitrogen

concentration (NH_4-N) of 0.32 mg L^{-1}. Approximately 20.7 g (wet weight) of each plant was added to each beaker and the ammonium uptake rate was calculated based on the reduction in NH_4-N after one hour and 24 hours. The NH_4-N uptake rates of the plants were approximately 5.80 mg NH_4-N $kg^{-1}h^{-1}$, 3.14 mg NH_4-N $kg^{-1}h^{-1}$ and 1.45 mg NH_4-N $kg^{-1}h^{-1}$ for *L. minor*, *P. fluitans* and *H. aristata* respectively. Therefore, biomasses of 162 g of *L. minor*, 299 g of *P. fluitans* and 647 g of *H. aristata* were stocked into biofilter tanks which had the same surface area of 0.41 m^2.

3.1.2.3. Design of recirculating systems

The trial comprised 12 independent experimental units; each unit consisted of three tanks: a fish tank, a waste-collection tank and a biological filter tank. The fish and waste-collection tanks were put on the floor, while the biological filters were installed on a wooden stand to elevate it higher than the fish tanks. Water from the bottom of the fish tank was drained through a PVC pipe to the waste-collection tank, and water from the waste-collection tank was pumped through a plastic tube to the biological filter tank by a submerged pump. Water from the biological filter tank was circulated back to the fish tank by gravity (Figure 2.1). The flow rate of water was set at 3 L min^{-1}. The water volumes in the fish and waste-collection tanks were kept at 55 and 36 litres respectively, while the water volume in each biofilter was kept at 60 litres. The tank of the moving-bed filter was provided with two air stones and covered with a black plastic cover to prevent algae from growing. The fish tank was also aerated with two air stones to supply dissolved oxygen for fish. A polyethylene mesh (1.0–1.5 cm in diameter) was put above the fish tank to prevent fish from jumping outside.

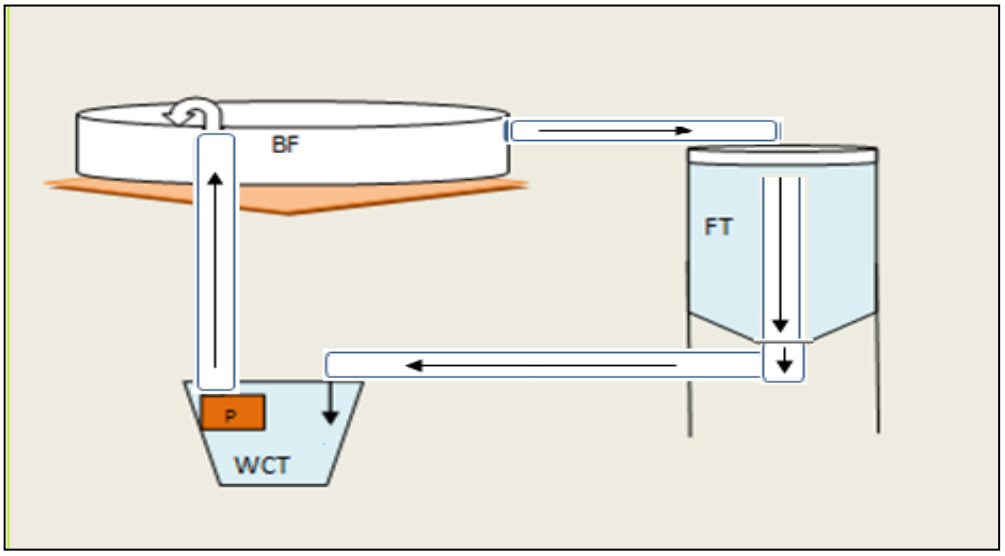

Figure 2.1. Diagram of the experimental recirculating units (the arrows show the direction of water flow), (FT): Fish tank, (WCT): Waste collection tank, (BF): Biological filter, (P): Pump.

3.1.2.4. Experimental setup and rearing conditions

The trial was conducted for five weeks in an insulated greenhouse, in which the environmental conditions were recorded but not completely controlled, and the only natural light was used throughout the study. The trial was designed as four treatments with three replicates in a random arrangement. The biofilter tank of each of the four treatments was stocked with one type of filtration (bacterial biofilm filtration, *L. minor* filtration, *P. fluitans* filtration and *H. aristata* filtration). The fish were initially stocked at 7.5 kg m^{-3}, and the total biomass was approximately 412 g $tank^{-1}$. All fish were fed by hands twice a day at 09:00 and 16:00 hours with a commercial diet (pellet size 2 mm). The feeding rate of fish was 2.5% of body weight per day and the amount of feed after two weeks was adjusted according to the actual weight of the fish until the end of the trial. The uneaten feed was collected one hour after feeding, while faeces were removed daily before the feeding commenced through a filter net with a mesh size of 100 µm and the remaining water returned into the waste-collection tank of the same system. A weekly amount of 40 litres of water was siphoned out of the waste collection tanks and replaced with new water, amounting to 27% of the water system. Also, new water was added to compensate for the water lost by evaporation which was 2–3% of the water system per week.

3.1.2.5. Sample collection and analysis

Survival and growth rates of the fish were recorded after the first two weeks and at the end of the experiment for each tank. Dissolved oxygen (DO), temperature and pH of water in the fish tanks were measured once a day before feeding commenced. Temperature and DO were measured by using the OxyGuard Handy Polaris meter (OxyGuard International A/S, Denmark), while pH was measured by using the Lovibond Senso Direct pH 110 meter (Tintometer Group, Germany). Ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N), total nitrogen (TN), orthophosphate (PO_4-P) and total phosphorus (TP) were measured weekly in the fish tanks and in the influent and effluent waters of each biofilter to obtain the removal rates. Water samples were analysed under laboratory conditions, using the MSZ EN ISO 11732:2005 method to determine NH_4-N levels and the MSZ EN ISO 13395:1999 method for NO_2-N and NO_3-N, while TN was determined by using the MSZ EN ISO 11905-1:2000 method. PO_4-P and TP levels were determined using the MSZ EN ISO 15681-1:2005 C Annex and the MSZ EN 1189:1998 methods respectively. Water quality measurements were measured according to the European standard methods and the recommendations of the International Organization for Standardization.

3.1.2.6. Data calculation and statistics

Fish biomass, specific growth rates (SGR) and survival rates were calculated using the following formulas: Fish biomass (g) = Sum of individual fish weights (g); SGR (% day) = $100 \times (\ln W_t - \ln W_0)/t$ and survival rate (%) = $100 \times (n_t / n_0)$, where W_t and W_0 are the weight of fish at the testing time and the start of the trial respectively and (t) is the number of rearing days. The n_t is the number of fish at the testing time and n_0 is the number of fish at the start of the trial. The feed conversion ratio (FCR) was calculated as follows: FCR = WF (g) / WG (g), where WF is the weight of feed given to the fish (g) and WG is the weight gain (g).

Nutrient removal rates were used to determine the nutrient removal cycle of biofilters. A nutrient removal rate is defined as the percentage of a particular nutrient that is reduced after passing through the biofilter (Tseng and Wu, 2004). The nutrient removal (NR %) was calculated using the following equation: $NR\% = [(N_I - N_E)/ N_I] \times 100$, where N_I and N_E are the amounts of a particular nutrient in influent and effluent waters of biofilter respectively. The removal rates of NH_4-N, NO_2-N, NO_3-N, PO_4-P and TP were calculated using the same equation.

The plants were harvested from the biofilter tanks at the end of the experiment and the weight of the plants was recorded. The specific growth rates of plants (SGRP) were calculated as follows: SGRP (% day) $=100 \times (\ln B_f - \ln B_i)/t$; Where B_f and B_i are the final biomass of the plant and the initial stocked biomass respectively, while (t) is the number of rearing days.

All statistical analyses were performed using the SPSS version 22.0 for the windows package. All of the data obtained were tested for normality of distribution and homogeneity of variance. One-way analysis of variance (ANOVA) was conducted to test the differences in parameters amongst treatments. Significant ANOVAs were followed by Duncan's multiple range tests to recognize specific differences amongst treatments. The 5% level of probability was considered to be the significance level.

3.1.3. Results

3.1.3.1. Water quality parameters in fish tanks

There were no significant differences ($P>0.05$) in the overall means of dissolved oxygen, pH and temperature amongst treatments (Table 2.1). Dissolved oxygen in all treatments showed a slight decline from 6.99 to 6.5 mg L^{-1}, whereas the mean temperature increased as the trial progressed; from 21°C to 30°C. The mean pH in all treatments fluctuated between 7.0 and 7.9.

The overall means of NH_4-N and NH_3 in fish tanks of systems using bacterial biofilm filtration and *H. aristata* were significantly lower ($P<0.05$) than those stocked with *L. minor* and *P. fluitans* (Table 2.1). The lowest mean was recorded in fish tanks of systems using bacterial biofilm throughout the study (Figure 2.2. A, D). However, no significant differences ($P>0.05$) were found between systems using bacterial biofilm filtration and those stocked with *H. aristata* (Table 2.1).

The mean NO_2-N in fish tanks of systems stocked with *P. fluitans* was significantly higher ($P<0.05$) than in the other three treatments (Table 2.1). The mean NO_2-N in fish tanks of systems using bacterial biofilm filtration remained below 0.44±0.11 mg L^{-1} over the entire period of the trial (Figure 2.2. B).

The mean NO_3-N and TN in fish tanks of systems using bacterial biofilm filtration were significantly higher ($P<0.05$) than in the other three treatments (Table 2.1). The mean NO_3-N and TN concentrations in all treatments increased as the trial progressed (Figure 2.2. C, F). No treatments significantly affected ($P>0.05$) the PO_4-P and TP means (Table 2.1). In all treatments, the PO_4-P concentrations increased gradually while the TP concentrations fluctuated over time (Figure 2.2. E, G).

Table 2.1 Overall mean water quality parameters of tanks used for culturing *Cyprinus carpio* in recirculating aquaculture systems using different biological filters

	Lemna minor	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
NH_4-N (mg L^{-1})	1.50±0.33^{a}	0.72±0.16^{b}	1.71±0.12^{a}	0.32±0.01^{b}
NH_3 (mg L^{-1})	0.0091±0.002^{a}	0.0031±0.001^{b}	0.0093±0.002^{a}	0.0019±0.0004^{b}
NO_2-N (mg L^{-1})	1.16±0.37^{b}	1.07±0.19^{b}	2.51±0.33^{a}	0.39±0.03^{b}
NO_3-N (mg L^{-1})	8.68±1.43^{b}	8.96±1.76^{b}	8.53±0.88^{b}	20.11±2.34^{a}
TN (mg L^{-1})	16.01±1.46^{b}	18.14±2.19^{b}	18.08±1.64^{b}	28.55±3.07^{a}
PO_4-P (mg L^{-1})	0.059±0.004^{a}	0.062±0.003^{a}	0.057±0.003^{a}	0.059±0.004^{a}
TP (mg L^{-1})	0.219±0.023^{a}	0.243±0.027^{a}	0.186±0.021^{a}	0.172±0.019^{a}
DO (mg L^{-1})	6.71±0.04^{a}	6.72±0.05^{a}	6.69±0.04^{a}	6.64±0.03^{a}
pH	7.43±0.07^{a}	7.41±0.06^{a}	7.44±0.05^{a}	7.52±0.07^{a}
Temperature (°C)	26.08±0.93^{a}	26.37±0.96^{a}	26.34±0.97^{a}	26.67±0.99^{a}

Values (means ± SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; $P<0.05$); data are the means of three replicates.

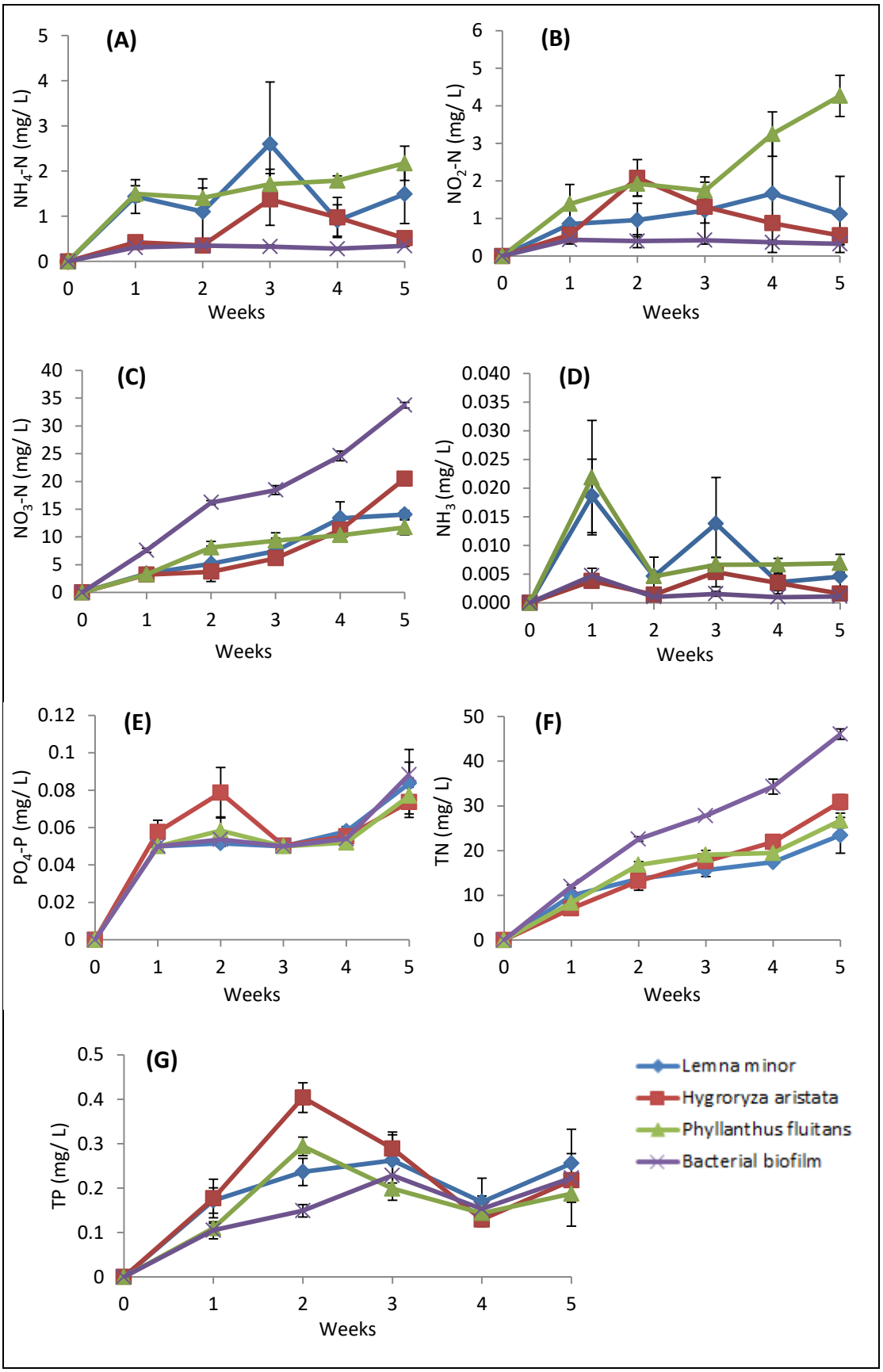

Figure 2.2. Weekly mean concentrations of (A) NH_4-N, (B) NO_2-N, (C) NO_3-N, (D) NH_3, (E) PO_4-P, (F) TN, and (G) TP in tanks of *Cyprinus carpio* reared in recirculating aquaculture systems during the 5-week trial (error bars indicate the standard error)

3.1.3.2. Removal rates of biological filters

The mean NH_4-N removal rates of the bacterial biofilm filters were significantly higher ($P<0.05$) than the values obtained with *L. minor* and *P. fluitans* filters. The NH_4-N removal rates in the *H. aristata* filters were significantly higher ($P<0.05$) than those in the *P. fluitans* filters and comparable with those in the *L. minor* and bacterial biofilm filters (Table 2.2). The bacterial biofilm filter had the highest NO_2-N removal rates and lowest NO_3-N removal rates, which significantly differed ($P<0.05$) from those of other biofilters. The mean removal rates of NO_2-N and NO_3-N in the *H. aristata* filters were significantly higher ($P<0.05$) than those in the *P. fluitans* filters and comparable with those in the *L. minor* filters (Table 2.2). However, no significant differences ($P>0.05$) in the mean removal rates of PO_4-P and TP were found between any of the treatments (Table 2.2). The mean PO_4-P removal rates by *H. aristata* and *P. fluitans* increased in the early stage of the trial and decreased afterward, while the mean removal rates of bacterial biofilm and *L. minor* filters increased over time (Figure 2.3. D). The mean removal rates of TP increased gradually in all filters (Figure 2.3. E).

Table 2.2 Overall mean removal rates of four biological filters used in recirculating *Cyprinus carpio* systems during the 5 weeks trial

	Lemna minor	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
NH_4-N (%)	10.96 ± 2.80^{bc}	17.96 ± 3.20^{ab}	6.67 ± 0.78^{c}	25.00 ± 2.51^{a}
NO_2-N (%)	9.25 ± 1.70^{bc}	11.30 ± 3.51^{b}	2.14 ± 0.62^{c}	25.95 ± 4.29^{a}
NO_3-N (%)	7.53 ± 1.22^{ab}	8.15 ± 0.90^{a}	5.07 ± 1.02^{b}	2.27 ± 0.31^{c}
PO_4-P (%)	4.05 ± 1.16^{a}	4.97 ± 0.83^{a}	2.51 ± 0.69^{a}	2.94 ± 0.91^{a}
TP (%)	19.04 ± 4.64^{a}	11.50 ± 3.42^{a}	17.73 ± 3.62^{a}	14.49 ± 4.36^{a}

Values (means ± SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; $P<0.05$); data are the means of three replicates.

3.1.3.3. Growth and survival rates

After five weeks, the growth rate of common carp increased in all treatments. The mean biomass gain, SGR and weight gain of fish reared in systems stocked with *P. fluitans* were significantly lowest ($P<0.05$) than in the other three treatments (Table 2.3). However, the mean biomass gain, SGR and fish weight gain did not differ significantly ($P>0.05$) between fish reared in systems stocked with *L. minor*, *H. aristata* and bacterial biofilm filtrations (Table 2.3). The highest mean biomass gain, SGR and fish weight gain were achieved for fish reared in systems using bacterial biofilm filtration, followed by systems with *H. aristata* (Table 2.3). The mean FCR of fish reared in systems stocked with *P. fluitans* was significantly higher ($P<0.05$) than in the other three treatments (Table 2.3). In all treatments, the survival rates of fish were the same ($P>0.05$), and 100% survival rates were achieved in

all systems (Table 2.3). The mean SGRP of plants in systems stocked with *H. aristata* was significantly higher (P<0.05) than in the other treatments (Table 2.3).

Table 2.3 Effects of bio-filtration types on growth and survival rates of *Cyprinus carpio* reared in recirculating aquaculture systems

	Lemna minor	*Hygroryza aristata*	*Phyllanthus fluitans*	Bacterial biofilm
Stocking fish				
Fish biomass (g tank^{-1})	412.667±1.28^{a}	412.133±0.81^{a}	411.533±1.34^{a}	412.833±1.62^{a}
Stocking density (kg m^{-3})	7.503±0.023^{a}	7.493±0.014^{a}	7.482±0.024^{a}	7.506±0.029^{a}
Number of fish	9	9	9	9
Mean fish weight (g fish^{-1})	45.85±0.14^{a}	45.79±0.09^{a}	45.73±0.15^{a}	45.87±0.18^{a}
Harvesting fish				
Final fish biomass (g tank^{-1})	731.23±4.05^{a}	740.37±7.59^{a}	690.37±10.97^{b}	753.80±11.54^{a}
Final stocking density(kg m^{-3}	13.295±0.07^{a}	13.461±0.14^{a}	12.552±0.20^{b}	13.705±0.21^{a}
Number of surviving fish	9	9	9	9
Final fish weight (g fish^{-1})	81.25±0.45^{a}	82.26±0.84^{a}	76.71±1.29^{b}	83.76±1.28^{a}
Fish weight gain (g fish^{-1})	35.40±0.52^{a}	36.47±0.92^{a}	30.98±1.10^{b}	37.89±1.32^{a}
Biomass gain (g tank^{-1})	318.57±4.72^{a}	328.23±8.30^{a}	278.83±9.90^{b}	340.96±11.90^{a}
SGR (% d^{-1})	1.61±0.23^{a}	1.66±0.33^{a}	1.46±0.33^{b}	1.70±0.57^{a}
Daily growth rate (g fish^{-1})	1.00±0.00^{a}	1.07±0.032^{a}	0.87±0.033^{b}	1.07±0.031^{a}
Feed consumption (g fish^{-1} d^{-1})	1.308±0.017^{a}	1.314±0.009^{a}	1.335±0.009^{a}	1.328±0.012^{a}
Feed conversion ratio	1.29±0.012^{b}	1.26±0.043^{b}	1.49±0.084^{a}	1.23±0.032^{b}
Survival (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
Stocking plant biomass (g biofilter tank^{-1})	162±0.00^{c}	647±0.00^{a}	299±0.00^{b}	0.0±0.00^{d}
Final plant biomass (g biofilter tank^{-1})	894.80±10.07^{b}	5429.90±117.32^{a}	466.76±15.52^{c}	0.0±0.00^{d}
Plant biomass gain (g biofilter tank^{-1})	732.8±10.07^{b}	4782.90±117.32^{a}	167.76±15.52^{c}	0.0±0.00^{d}
Specific growth rate of plant (SGRP % d^{-1})	4.86±0.033^{b}	6.10±0.57^{a}	1.26±0.08^{c}	0.0±0.00^{d}

Values (means ± SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; P<0.05); data are the means of three replicates

3.1.4. Discussion

In RASs, water quality parameters should be maintained within recommended limits for optimum fish growth and survival. The results indicated that all types of biofilters used in the current study provided acceptable water quality parameters and good conditions for common carp growth and survival in RASs. The temperature, pH and DO concentrations in the fish tanks of all systems remained within the tolerance range for common carp growth and survival (Horváth et al., 2002). The maximum concentrations of NH_3, NO_2-N, and NO_3-N in all systems were lower than the lethal level reported by various authors for common carp

(Tarazona et al., 1987; Biswas et al., 2006; Solbé et al., 1985; Kroupova et al., 2010; Iqbal et al., 2004; Kim et al., 2013) (Table 2.4).

Table 2.4. Comparative description of the maximum values of some water quality parameters with the lethal levels of common carp

The maximum values/system		Concentration levels (mg L^{-1})			Reference
	mg L^{-1}	No Effect	Lowest Effect	Lethal level	
NH_3	0.042 (*P. fluitans)*			1.3	(Tarazona et al., 1987)
		0.0286	0.034	0.043	(Biswas et al., 2006)
NO_2-N	5.29 *(P. fluitans)*			16	(Solbé et al., 1985)
		7	28	88	(Kroupova et al., 2010)
NO_3-N	34.7 (*bacterial biofilm)*			865	(Iqbal et al., 2004)
Phosphate			No toxicity reported		(Kim et al., 2013)

Although the water quality parameters were maintained at the levels recommended for fish in all treatments, the concentrations of NH_4-N, NO_2-N, and NO_3-N in the fish tanks were different between treatments (Table 2.1). These variations between treatments in the ability to maintain the concentrations of water quality could be attributed to differences in mechanisms for the uptake of nutrients, specific environmental requirements and biomass (Redding et al., 1997; Cahill et al., 2010). Redding et al. (1997) concluded that purification in culture systems could be processed by different mechanisms such as nitrification, denitrification, microbial assimilation and sedimentation as well as plant uptake and the growth form of plants can play an important role in the design criterion. Our results also indicated that the bacterial biofilm filter was a powerful filter to maintain NH_4-N concentrations within the lowest level and this was due to the highest removal rates of NH_4-N which tended to increase as the trial progressed. The increasing trend was probably due to the increase in the number of nitrifying bacteria in response to the rise in ambient ammonia concentrations as a consequence of increasing fish biomass and the amount of feed given to fish. Previous studies have also shown the removal efficiency of biofilm filter improved with increasing ambient ammonia concentrations (Zhu and Chen, 1999; Brazil, 2006). In contrast, the higher concentrations of NH_4-N in the *P. fluitans* and *L. minor* systems were possibly due to the lower removal rates of NH_4-N which tended to decrease over time (Figure 2.3. A). The decreasing trend could be attributed to differences in the growth performance of plants, since different plant species have different growth performance in the current study. Vymazal (2007) reported that the potential rate of nutrient uptake by plants is influenced by their growth rate and the concentration of nutrients in the plant tissue. Another explanation for the decreasing trend of

NH_4-N removal rates in plant based systems may be related to biological factors, such as the age of the plant, its nutritional history and interplant variability (Ahn et al., 1998).

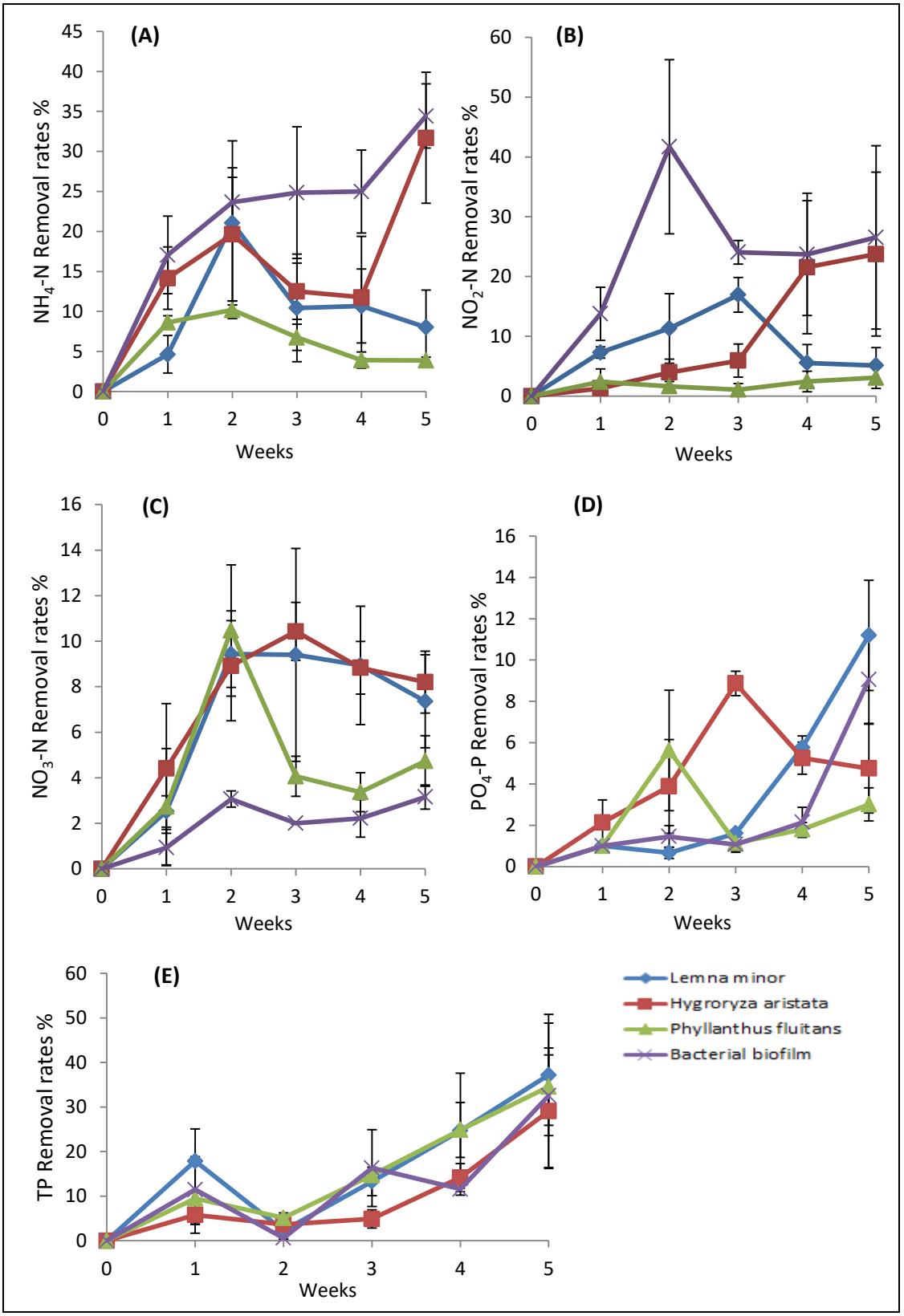

Figure 2.3. Weekly mean removal rates by four biological filters for (A) NH_4-N, (B) NO_2-N, (C) NO_3-N, (D) PO_4-P, and (E) TP in recirculating *Cyprinus carpio* systems during the 5-week trial (error bars indicate the standard error)

The highest removal rates of NO_2-N by bacterial biofilm filters corresponded to the NH_4-N removal rate and the nitrification process. However, the NO_2-N removal rates remained relatively constant in the later stages of the trial (Figure 2.3. B); suggesting that the nitrite-oxidizing bacteria were probably limited in responding to the increase in nitrite concentrations in the systems (Guerdat et al., 2010) since the ammonia-oxidizing bacteria have a higher kinetic reaction rate than the nitrite-oxidizing bacteria in the nitrification process (Timmons et al., 2002). In contrast, the lower NO_2-N and higher NO_3-N removal rates by plant based systems could be attributed to the fact that ammonium and nitrate are directly taken up by plants as a nitrogen source (Fang et al., 2007), and it is most likely that nitrifying bacteria were responsible for the NO_2-N removal rate in the plant based systems (Wei et al., 2011). The reduction in the nitrate nitrogen concentrations has been reported previously for *Lemna* sp. by Ferdoushi et al. (2008), who found that the introduction of *Lemna* sp. in a fish pond efficiently removed nitrate nitrogen and improved water quality. Tan et al. (2014) also found that *Hygroryza aristata* removed the excessive amount of nitrogen under real operational conditions in a canal, while Han et al. (2013) reported that *Hygroryza aristata* removed the concentration of nitrate nitrogen from the pond water. In the current study, different trends in NO_3-N removal rates between selected plants (Figure 2.3. C), could be related to differences in the nutrient utilization capacity and growth performance of plants, since different plant species have different nutrient utilization capacity and growth characteristics. Hu et al. (2015) investigated the effect of plant species on nitrogen recovery and concluded that plant species had a significant influence on nitrogen transformations, and the higher plant biomass translates to a higher plant uptake rate resulting in higher nitrate removal efficiency. Different growth performance between the selected plant species in the current study may be attributed to the continuous water flow and/or specific environmental requirements for each plant (Redding et al., 1997; Van der Steen et al., 1998). Crowding of plants and the limited surface

area also may slow down the growth of plants, (Driever et al., 2005) since no harvesting regime was applied in the current study to give the plants more space for extending and increased their growth.

In the present trial, the artificial feed was the only source of phosphorus and a large part of it was removed by removing uneaten food and fish faeces; which resulted in a large portion of soluble phosphorus and suspended particles in the water column. The removal of phosphate in culture systems can be processed through the plant uptake and the mechanism of sedimentation (Redding et al., 1997). In fact, the biofilter tank bottoms had little sediments which can play an important role in removing phosphate. Midlen and Redding (1998) reported that over half of the phosphorus inputs are bound in the soils of the pond bottom in a relative insoluble form. Although the differences in the removal rate of PO_4-P between treatments were not significant; the plant systems exhibited different trends; which could be related to differences in the concentration of nutrients in plant tissue (Vymazal, 2007).

Previous studies indicated that the growth and survival of fish are influenced by water quality parameters in the culture system (Colt, 2006; Ridha and Cruz, 2001; Timmons et al., 2002; Ardiansyah and Fotedar, 2016). Different growth performance of fish between treatments in the present trial was probably due to differences in water quality during the trial period. The elevated but acceptable level of NH_4-N, NH_3 and NO_2-N may be one reason for the lowest growth performance and highest FCR of fish reared in the system stocked with *P. fluitans*. The SGRs of common carp in the present trial ranging from 1.46 to 1.70% d^{-1} were higher than the 1.03-1.06% d^{-1} reported by Karakatsouli et al. (2010) for (52 g) mirror common carp and the 0.84% d^{-1} obtained by Ridha and Cruz (2001) for (62 g) Nile tilapia (*Oreochromis niloticus* L.). However, the SGRs of fish were lower than those (1.6-2.14% d^{-1}) achieved by Ahmed et al. (2013) for common carp reared in RAS on different diets. The lower SGRs may be related to the lower feeding rates and feeding frequency used in this trial

(2.5% body weight twice a day) compared to those used by Ahmed et al. (2013) (3% body weight, four times a day). In the present trial, no fish mortality was found in any of the treatments. Fish were probably unaffected because a lower feeding rate was used in this trial. Huisman (1976) suggested 3% of the body weight per day as a suitable amount of feed for (42.1 g) common carp. In addition, the stocking density of fish was probably below the carrying capacity of these systems and did not reach the threshold at which survival rates would be affected.

The results presented in this trial demonstrate that the use of plant based biofilters was effective in maintaining water quality, removing nutrients, adding harvestable products and providing good conditions for fish growth and survival. However, bacterial biofilm in the moving-bed filter was the strongest filter to reduce high concentrations of NH_4-N and NO_2-N; and had generally the highest removal rates of NH_4-N and NO_2-N; whereas plant based filters had higher NO_3-N removal rates. The nutrient uptake capacities of plant based systems were different and are strongly influenced by the growth rate of plants, which is affected by environmental conditions. *H. aristata* was the strongest plant in removing nutrients among the tested plant species, followed by *Lemna minor*. The use of plant based biofilters in this filtration technique can be beneficial in decreasing the high investment and operation costs associated with RASs; however, from a technical point of view, the bacterial biofilm filter is the strongest biofilter to be used to reduce high concentrations of NH_4-N and NO_2-N. Regardless of the suitability of bacterial biofilm and plant based filters, several factors must be considered when choosing appropriate biological filters, such as space, cost and benefit analyses, system location, climatic conditions and discharge regulations.

3.2. Effect of the Initial Size of Fish and Bio-filtration Types in Integrated Recirculating Aquaculture Systems

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3.2.1. Introduction.

Recirculating aquaculture systems (RASs) offer many advantages in terms of reducing water consumption, better disease management, nutrient recycling, and improving opportunities for waste management (Timmons et al., 2002). If not properly managed, the accumulation of nitrogenous wastes can be a major concern in RASs. In an intensive aquaculture system, approximately 20-50% nitrogen supplied through the feed is converted into fish biomass when harvest, while large amounts of nitrogen in the form of uneaten feed and excretory products are directly discharged into the water (Schneider et al., 2005). Ammonia is the major end product of nitrogen metabolism, excreted by aquatic animals and high levels of ammonia can have negative effects on fish growth and survival (Guan et al., 2010). Ammonia can be controlled by using the nitrification process, plants uptake and immobilization by bacteria (Hargreaves, 2006). The removal efficiency of any type of filtration technique is dependent on the production and distribution of nitrogenous waste which in turn mainly relies on fish species and its size, temperature, rearing methods, feeding level, feeding practices, feed composition and feed utilization efficiency by animals (Houlihan et al., 2001; Schneider et al., 2005).

In aquaculture, the body mass of fish is a key factor that determines the level of feed provided. Young fish need large quantities of feed in proportion to their body mass to satisfy their energy requirements for growth than adult fish (Houlihan et al., 2001). Furthermore, the fish size in the ornamental fish industry is considered one of the critical factors that can determine the variety and price of ornamental fish such as koi carp (*Cyprinus carpio haematopterus*) (Watson et al., 2004). Koi carp was used as a model species in the current trial because it has a wide distribution throughout the world as an expensive ornamental fish; and can take a long time to reach market size or preferred quality, for example, the deep red colour can take more than a year for development (Watson et al., 2004). *Hydrocotyle rotundifolia* plant also was chosen because it has a rapid growth rate with an extensive root system, and appears more cold-tolerant (McChesney, 1994).

The economic performance of a RAS mainly depends on the cost of the water treatment components and the selection of marketed species (Timmons et al., 2002). The selection of the proper size of cultured species and the type of filtration techniques are critical to the technical and economic success of the RAS. A large number of studies only focused on a

single size of fish when they examined the efficiency of different water filtration techniques in RASs such as bacterial biofilm filtration or plants uptake (Redding et al., 1997; Lekang and Kleppe, 2000; Ridha and Cruz, 2001; Jo et al., 2002; Tseng and Wu, 2004; Guerdat et al., 2010; Velichkova and Sirakov, 2013; Nakphet et al., 2017); while still there is limited information on the relationship between the initial size of fish and the efficiencies of these filtration techniques. This trial aimed to evaluate and compare the effect of the initial size of fish and bio-filtration types on fish growth performance, water quality and the efficiencies of two biological filters: an aquatic plant (*H. rotundifolia)* and bacterial biofilm of a trickle-down filter in recirculating koi rearing systems.

3.2.2. Materials and Methods

3.2.2.1. Experimental organisms

All koi carp and *H. rotundifolia* plants were obtained from a local farm in Armadale, Western Australia, and then stocked in the acclimation tanks in Curtin Aquatic Research Laboratory, Perth, Australia (CARL) for two weeks before the experiment.

3.2.2.2. Two biological filters

Trickle-down filters were designed and established in the experimental treatments. Media in the form of "bio-balls" with a specific surface area of 200 m^2 m^{-3} and established biofilms were obtained from an operating RAS in CARL. To determine the appropriate quantity of bio-balls, the computations were based on the calculations published by Timmons et al. (2002). Thus, six tanks of the biofilters placed with 0.02 m^3 of bio-balls per tank; three tanks were prepared with small fish and three with large fish.

H. rotundifolia plant was also used as a biofilter and the biomass of the plants to be used for the experiment was estimated by their ammonia uptake rates, which was estimated during a preliminary experiment. In that experiment, an aquarium (40 L) was filled with water and ammonium chloride to give initial total ammonia nitrogen (TAN) concentration of 2 mg L^{-1}. Then 500 g (wet weight) of plants were added to the aquarium and the ammonia uptake rates calculated based on the reduction in the TAN over 24 hours. The ammonia uptake rate of the plant was 5.20 mg ammonia kg^{-1} h^{-1}. Therefore, six tanks of the biofilters (0.60 m^2 surface area) stocked with 4.5 kg of plants per tank; three tanks were stocked with small fish and three with large fish systems.

3.2.2.3. Experimental systems and rearing conditions

The trial was performed whereas both sizes of fish had both types of biological filters. Three replicated tanks were randomly designed for each treatment. The total biomass of fish was approximately 1.5 kg per tank, with an initial mean weight of 9.43±0.46 g and

107.64±9.0 g for small and large fish respectively. The trial comprised of 12 independent systems; each system consisted of three tanks: a biological filter tank, a rearing fish tank and a waste-collection tank. The waste-collection and fish tanks were placed on the floor, while the biological filter tanks were placed above the waste-collection tanks. Water from 20 cm below the water surface in the waste-collection tank was pumped through a plastic tube to the biological filter by a submerged pump. Water from the biological filter was then circulated back to the fish tank by gravity and water from the bottom of the fish tank was drained through a PVC pipe to the waste-collection tank. The water volumes in the rearing fish, waste-collection and biological filter tanks were maintained at approximately 200, 50 and 70L respectively. The water flow rates were set at 3 L per minute and illumination was provided 12 hours a day. The fish tanks were supplied with one automatic heater (Sonpar, Model: HA-200, China) to maintain the water temperature at 20-22ºC and two air stones suspended mid-depth in the water column. The feeding rate of fish was 2.5% of the body weight per day with a commercial feed, Nova MF (50% protein, 22% fat and 0.5% fibre) (Skretting Co., Cambridge, Tasmania, Australia). Fish were fed twice a day and the feeding rate was adjusted monthly according to the weight gain and mortality of fish. The uneaten feed and faeces were siphoned out daily before the feeding started through a filter net with a mesh size of 100 µm and the remaining water returned back into the waste-collection tank of the same experimental unit. Approximately 30% of the system water was siphoned out weekly and replaced with new water.

3.2.2.4. Data collection and statistical analysis

Survival and growth rates of fish were recorded monthly for each tank. Dissolved oxygen (DO), temperature and pH of water in the fish tanks were measured once a day. DO was measured by using the Milwaukee SM600 meter (Milwaukee Instruments, Romania); while temperature and pH were measured by using the Cyber Scan pH 300 meter (Eutech Instruments, Singapore). Total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) were measured weekly in the fish tanks and in the influent and effluent waters of each biofilter to obtain the removal rates. The TAN, NO_2-N and NO_3-N were measured using the HACH DR/890 colourimeter (Hach Co., Loveland, Colorado, USA). The water samples were analysed following the methods in DR/890 colourimeter procedures manual (Hach, 2009), using the salicylate method for TAN, the diazotization method for NO_2-N, and the cadmium reduction method for NO_3-N.

Fish biomass, specific growth rates (SGR) and survival rates were calculated using the following formulas: Fish biomass (g) = sum of individual fish weight (g); SGR (% day) = 100

× ($\ln W_t$ - $\ln W_0$) / t; and survival rate % = 100 × (n_t / n_0). Where W_t and W_0 are the weight of fish at sampling time and the start of the trial respectively, and (t) is the number of rearing days. The n_t is the number of fish at the sampling time and n_0 is the number of fish at the start of the trial. The feed conversion ratio (FCR) was calculated as follows: FCR = WF / WG, where WF is the weight of feed given to the fish (g) and WG is the weight gain (g).

The TAN removal rate (NR%) was used to determine the removal cycle of biofilters. The NR% was calculated as follows: NR% = [(TAN_I - TAN_E) / TAN_I] × 100; where, TAN_I and TAN_E are the total ammonia nitrogen in the influent and effluent waters of biofilter respectively (Tseng and Wu, 2004). The same equation was used to calculate the NO_2-N and NO_3-N removal rates of each biofilter.

All statistical analyses were performed using SPSS version 20.0 for the Windows package. All of the data obtained were tested for normality of distribution and homogeneity of variance. One-way analysis of variance (ANOVA) was conducted to test the differences in parameters amongst treatments. Significant ANOVAs were followed by Duncan's multiple range tests to identify specific differences among treatments. The 5% level of probability was considered to be the significance level.

3.2.3. Results

3.2.3.1. Water quality parameters in fish tanks

Temperature, DO and pH among all treatments were not significantly different ($p > 0.05$) during the trial period (Table 3.1). The DO and pH ranged from 8.4 to 6.0 mg L^{-1} and 7.4 to 6.0 respectively. Water temperatures were constant in all treatments and ranged from 20 to 22°C. With respect to the biofilter type used in the system, all tanks of large fish had significantly lower ($p < 0.05$) means of TAN than the tanks stocked by small fish (Table 3.1). Small fish tanks that connected with *H. rotundifolia* filter had significantly higher ($p < 0.05$) means of TAN than the other treatments over the entire period of the trial (Figure 3.1. A). The highest mean of NO_2-N was found in small fish tanks connected with bacterial biofilm, which was significantly different ($p < 0.05$) than the other treatments (Table 3.1). The mean NO_2-N in both tanks of small and large fish connected with *H. rotundifolia* remained constant below 0.80±0.021 mg L^{-1} (Figure 3.1. C). The mean NO_3-N in both tanks of small and large fish connected with bacterial biofilm filters were significantly higher ($p < 0.05$) than those connected with *H. rotundifolia* filters (Table 3.1). However, there were no significant differences ($p > 0.05$) in the mean NO_3-N of small fish tanks connected with *H. rotundifolia* filter and large fish tanks connected with the same biofilter (Table 3.1). Similarly, both tanks

of small and large fish had the same mean of NO_3-N ($p > 0.05$) when they connected with a bacterial biofilm filter (Figure 3.1. D).

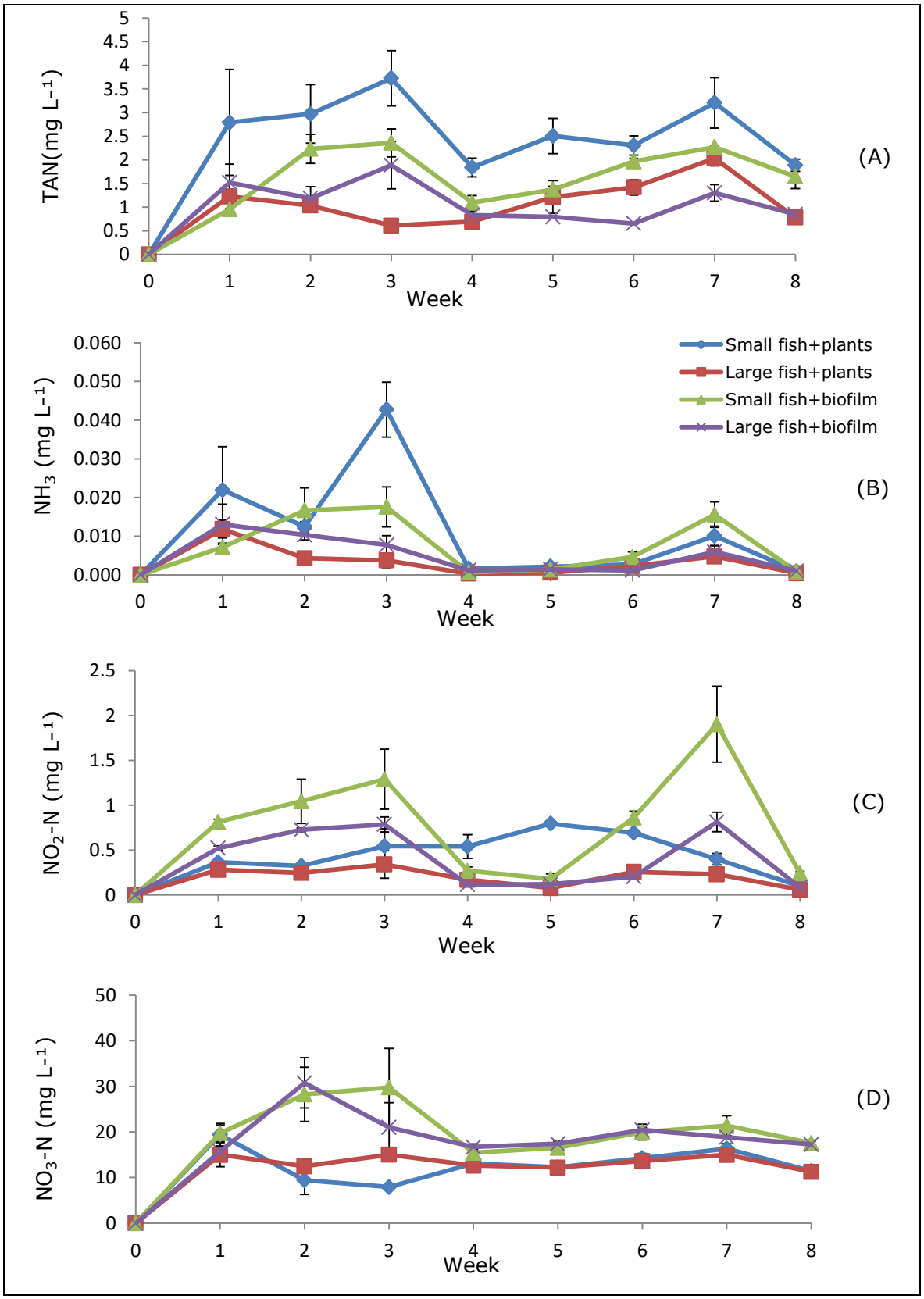

Figure 3.1. Weekly mean concentrations of (A) TAN, (B) NH_3, (C) NO_2-N and (D) NO_3-N in tanks of *C. carpio haematopterus* reared in RAS for 8 weeks trial (error bars indicate the standard error).

Table 3.1. Overall mean water quality parameters of tanks used for culturing different size of koi carp in recirculating aquaculture systems using different biological filters

Parameter	*Small fish + H. rotundifolia*	*Small fish + bacterial biofilm*	*Large fish + H. rotundifolia*	*Large fish + bacterial biofilm*
TAN (mg L^{-1})	2.65±0.20^{a}	1.74±0.12^{b}	1.12±0.10^{c}	1.13±0.11^{c}
NH_3 (mg L^{-1})	0.011±0.003^{a}	0.008±0.002^{ab}	0.003±0.001^{b}	0.005±0.001^{b}
NO_2-N (mg L^{-1})	0.42 ±0.04^{b}	0.95±0.18^{a}	0.21±0.03^{b}	0.42±0.06^{b}
NO_3-N (mg L^{-1})	13.02±0.86^{b}	21.07±1.53^{a}	13.40±0.49^{b}	19.76±1.24^{a}
DO (mg L^{-1})	6.9±0.12^{a}	6.9±0.14^{a}	7.2±0.11^{a}	7.1±0.13^{a}
pH	6.69±0.10^{a}	6.68 ±0.11^{a}	6.6±0.11^{a}	6.74±0.09^{a}
Temperature (°C)	20.9±0.15^{a}	20.8±0.09^{a}	20.6±0.15^{a}	20.7±0.17^{a}

Values (mean ±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; $p < 0.05$); data are means of three replicates.

3.2.3.2. Removal efficiency of biofilters

There were no significant differences ($p > 0.05$) in the mean removal rates of TAN, NO_2-N and NO_3-N in systems stocked by bacterial biofilm filters with small fish and those systems stocked by large fish with the same filters. Similarly, systems with *H. rotundifolia* filters responded in the same way to remove TAN, NO_2-N and NO_3-N when they stocked with any sizes of fish (Table 3.2). However, bacterial biofilm filters with both sizes of fish had significantly higher ($p < 0.05$) mean removal rates of TAN and NO_2-N than *H. rotundifolia*; while *H. rotundifolia* filters showed significantly higher removal rates of NO_3-N ($p < 0.05$) than bacterial biofilm filters (Table 3.2). The mean TAN removal rate of all filters stocked with *H. rotundifolia* decreased as the trial progressed (Figure 3.2. A); while the mean NO_2-N removal rates increased over time (Figure 3.2. B). All filters stocked with bacterial biofilm showed a decreasing trend in the NO_3-N removal rates with the progression of the trial (Figure 3.2. C).

Table 3.2. Overall mean removal rates of *H. rotundifolia* and bacterial biofilm filtration used with different size of koi carp in recirculating aquaculture systems for 8 week trial

Removal rates (%)	*Small fish + H. rotundifolia*	*Small fish + bacterial biofilm*	*Large fish + H. rotundifolia*	*Large fish + bacterial biofilm*
TAN	30.05±2.40^{b}	42.60±3.43^{a}	32.14±2.52^{b}	45.8±1.70^{a}
NO_2-N	6.2±0.56^{b}	36.03±1.35^{a}	5.2±0.59^{b}	33.1± 1.72^{a}
NO_3-N	25.45±1.30^{a}	11.62±1.37^{b}	24.72±1.54^{a}	12.90±1.48^{b}

Values (mean ±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; $p < 0.05$); data are means of three replicates.

3.2.3.3. Growth and survival rates of koi fish

Small fish had significantly higher ($p < 0.05$) means of biomass, biomass gain, SGR and weight gain than large fish with respect to the biofilter type used in the systems. However, there were no significant differences ($p > 0.05$) in the mean biomass gain, SGR and weight gain of small fish reared in systems stocked with *H. rotundifolia* and small fish in systems using bacterial biofilm filter. Similarly, large fish responded in the same way ($p > 0.05$) for growth when they stocked with any type of biofilters (Table 3.3). The mean FCR of small fish was significantly lower ($p < 0.05$) than large fish (Table 3.3). Even though there were no statistically significant differences in the mean of FCR between the small fish with both biofilters, the best mean of FCR was achieved in the systems connected with the bacterial biofilm filters (Table 3.3). After eight weeks of culture, there was no significant difference in the survival rates of small fish reared with the *H. rotundifolia* and small fish reared with the bacterial biofilm filter; which was significantly lower ($p < 0.05$) than the survival rates of the large fish stocked with any type of biofilters. No mortality was recorded with the larger fish reared with any type of biofilters (Table 3.3).

Table 3.3. Effects of the initial size of fish on growth and survival rates of koi carp reared in recirculating aquaculture systems with different biological filters

Growth Parameters	*Small fish + H. rotundifolia*	*Small fish + bacterial biofilm*	*Large fish + H. rotundifolia*	*Large fish + bacterial biofilm*
Fish biomass (kg)	1.504±0.001^{a}	1.505±0.002^{a}	1.504±0.001^{a}	1.507±0.001^{a}
Stocking density (kg m^{-3})	7.52	7.53	7.52	7.54
Number of fish	159.67±7.35	159.67±7.35	14±1.15	14±1.15
Initial mean fish weight (g $fish^{-1}$)	9.42±0.46^{b}	9.43±0.46^{b}	107.43±9.0^{a}	107.64±9.0^{a}
Harvesting	----------	----------	----------	----------
Final biomass (kg)	2.759±0.03^{a}	2.837±0.02^{a}	2.499±0.04^{b}	2.522±0.02^{b}
Final stocking density (kg m^{-3})	13.795	14.185	12.495	12.61
Surviving fish number	150±6.00	152.67±8.30	14±1.15	14±1.15
Final mean fish weight (g $fish^{-1}$)	18.45±0.7^{b}	18.70±1.12^{b}	181.41±17.7^{a}	182.39±13.5^{a}
Fish weight gain (g $fish^{-1}$ / 56 days)	8.98±0.27^{b}	9.24±0.66^{b}	72.11±8.97^{a}	72.83±4.73^{a}
Biomass gain (kg)	1.255±0.03^{a}	1.332±0.02^{a}	0.989±0.04^{b}	1.008±0.02^{b}
SGR (% day^{-1})	1.19±0.03^{a}	1.21±0.02^{a}	0.90±0.03^{b}	0.91±0.01^{b}
FCR	2.025±0.04^{b}	1.844±0.02^{b}	2.360±0.08^{a}	2.301±0.03^{a}
Survival rate %	94.0±0.66^{b}	95.5±0.93^{b}	100±0.00^{a}	100±0.00^{a}

Values (means±SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; $p < 0.05$); data are means of three replicates.

3.2.4. Discussion

Our results demonstrated that the size of fish did not affect the removal efficiencies of TAN, NO_2-N and NO_3-N and both biofilters were independent of the fish size. It is reported in Helfrich and Libey (1990) that the capacity of the biofilter is influenced by the surface area of the biofilter, hydraulic loading and the turnover time. In this trial, both biofilters were designed and sized hypothetically based on the total ammonia production rate, which was based on the fish feeding rate. The same nitrogen removal performance by the bacterial biofilm filter, when used with any size of fish was probably due to the same surface area used, allowing equivalent removal rates in the small and large fish systems. This hypothesis is confirmed by the findings of Ridha and Cruz (2001) and Lekang and Kleppe (2000) who found the removal efficiency of biofilters stocked with different media did not differ significantly under the same ratio of the surface area to the ammonia production rates.

However, the higher TAN removal rate by the bacterial biofilm filter was possibly due to the boost in the number of nitrifying bacteria in response to the rise in the concentrations of ammonia as a result of increasing fish biomass. Brazil (2006) found the increase in the ambient ammonia concentrations up to 3.5 mg L^{-1} improved the removal efficiency of the biofilm filter. In contrast, the decreasing trend in TAN removal rate by *H. rotundifolia* filters could be attributed to the conditions of continuous water flow used in this trial (Redding et al., 1997); or may be related to the biological factors, such as the age of the plant, its nutritional past history and the concentration of nutrients in the plant tissue (Ahn et al., 1998).

The NO_2-N removal rates by bacterial biofilm filters corresponded to the TAN removal rates and the highest removal rates of NO_2-N in the bacterial biofilm filters was due to the second step of the nitrification process and increase in the number of nitrite-oxidizing bacteria in responding to the increase in the nitrite concentration in the systems (Timmons et al., 2002). In contrast, lower removal rates of NO_2-N and higher removal rates of NO_3-N by *H. rotundifolia* filters (Figure 3.2. B, C) may present evidence that plants take ammonia and

nitrate as a nitrogen source by direct absorption and are incorporated into the plant biomass (Fang et al., 2007). It is most likely that the nitrifying bacteria attached to the plants were responsible for the slight increase in the NO_2-N removal rate in the later stages of the trial (Wei et al., 2011).

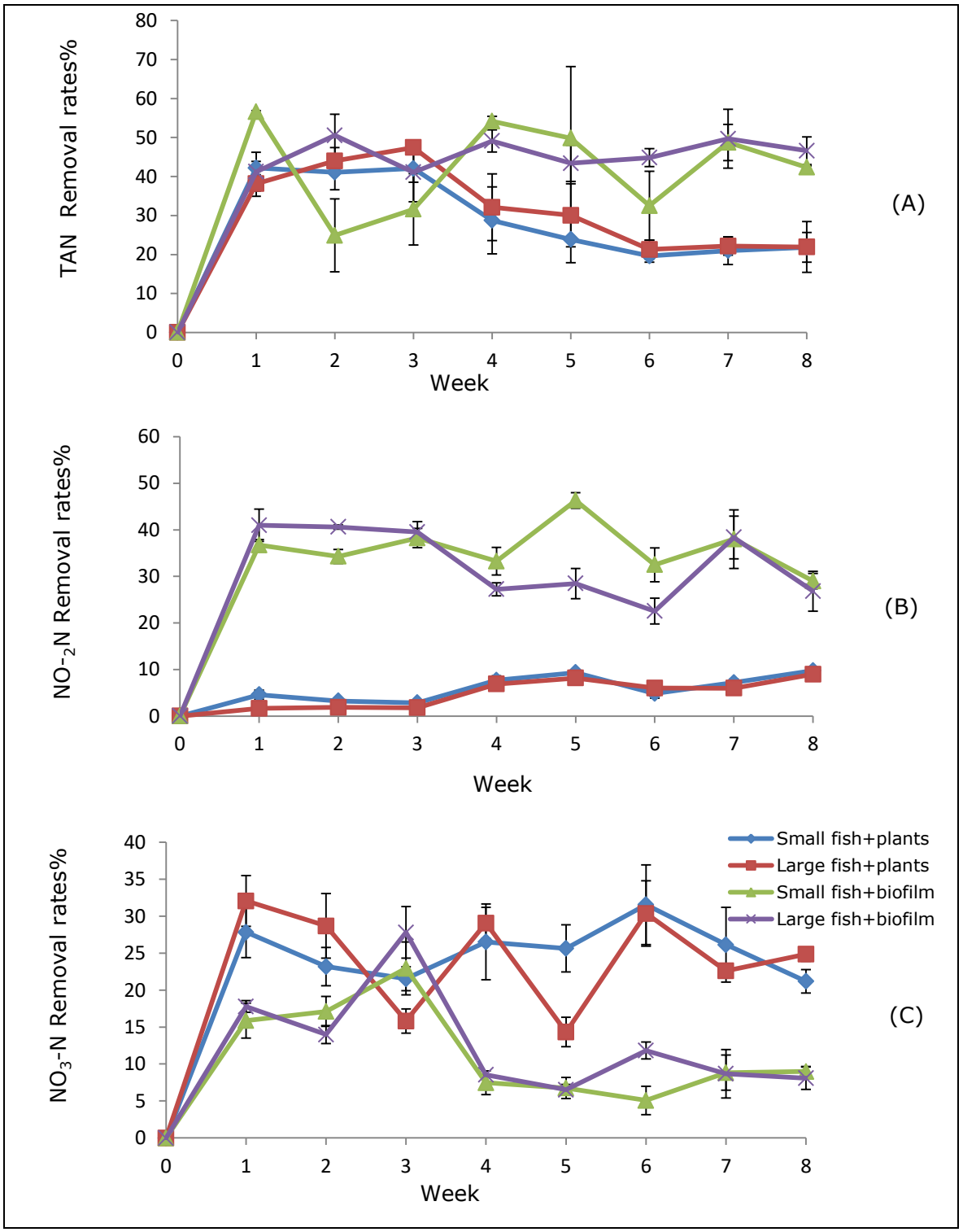

Figure 3.2. Mean removal rates of *H. rotundifolia* and bacterial biofilm for (A) TAN, (B) NO_2-N and (C) NO_3-N in recirculating koi systems during the 8 weeks trial (error bars indicate the standard error).

Fish excrete various nitrogenous wastes through gill diffusion, gill exchange, urine and faeces excretion. In our trial, a part of the nitrogenous waste source was removed from the fish tanks by removing fish faeces and uneaten food. Our results indicated that an inverse relationship between the initial fish size and the total ammonia nitrogen excreted into the fish tanks. Considering that smaller fish are undergoing increased oxidation of amino acids catabolism for energetic requirements compared to the large fish, and this may support the higher rates of protein catabolism in smaller fish. The negative relationship was also found with different fish species such as rainbow trout (Bucking, 2017), haddock (Lankin et al., 2008) and cobia (Feeley et al., 2007). This relationship has been partly explained in terms of the physiological changes during fish ontogeny and also be related to muscle development and the variations in the surface area of respiratory organs (Post and Lee, 1996). The small size fish have a relatively larger gill surface area per body weight compared to the large size fish. Robb and Abrahams (2003) observed a negative relationship for the mass-specific gill surface area, and they found smaller fish have more efficient gas exchange with their environment compared to larger fish.

In the present trial, all water quality parameters in the treatments were at the levels recommended for koi aquaculture throughout the trial (Timmons et al., 2002; Watson et al., 2004). However, the highest values of TAN and unionized ammonia (NH_3) was recorded in the systems stocked by small fish with *H. rotundifolia* filter (Table 3.1); this could have been a result of the interaction between higher ammonia excretion by small fish and lower removal efficiency by *H. rotundifolia* filter compared to other treatments. The maximum value of the NH_3 (0.050 mg L^{-1}) in the systems stocked by small fish with *H. rotundifolia* filter (Figure 3.1. B) was much less than the lethal levels of 1.23 mg L^{-1} reported by Hasan and Macintosh (1986) for common carp (6-8 g), and the 1.3 mg L^{-1} reported by Tarazona et al. (1987) for common carp (125-260 g). The maximum NO_2-N concentrations of 3.74 mg L^{-1} in the

systems stocked by small fish with bacterial biofilm filter was much less than the concentrations reported by Kroupova et al. (2010) who concluded that the larvae and embryos of common carp have three different concentration levels of nitrite: the lethal concentration (88 mg L^{-1}), the lowest observed effect (28 mg L^{-1}) and no observed effect (7 mg L^{-1}). The maximum NO_3-N concentrations of 31.5 mg L^{-1} in the systems stocked by small fish with bacterial biofilm, was less than lethal values of 865 mg L^{-1} reported by Iqbal et al. (2004) for common carp and 1484 mg L^{-1} reported by Tilak et al. (2002) for the Indian major carp.

Our results demonstrated that the SGR declines with the increasing initial body size of fish and it was the best at the systems stocked by small size fish with any type of biofilters. Previous studies indicated that the growth and survival of fish are influenced by water quality parameters (Timmons et al., 2002; Jha and Barat, 2005; Colt, 2006) as well as fish size, stocking density, access to food, and water exchange (Jobling, 1993). In our trial, the differences in the SGR between treatments were not essentially due to the changes in the water quality. The differences were more directly related to the differences in the food consumption and feed utilization efficiency by fish; because our results also indicated that the FCR increases with increasing fish weight, and it was the best in the small size group. Houlihan et al. (2001) reported that feed efficiency depends on the size and sex of the fish; and small fish tend to use feed more efficiently to satisfy their energetic requirements for growth, than larger fish. In line with our results, Franco-Nava et al. (2004) found the SGR of 66.88 g European seabass *(Dicentrarchus labrax)* (1.05% d^{-1}) was significantly higher than the 510.86 g fish (0.4% d^{-1}).

The SGRs of large koi in the present trial (0.90–0.91% d^{-1}) were higher than the 0.39-0.43% d^{-1} reported by Papoutsoglou et al. (2000) for (116 g) common carp (*Cyprinus carpio L.*) reared in the closed circulated systems and were lower than the 1.03-1.06% d^{-1} reported by Karakatsouli et al. (2010) for (51.88 g) mirror common carp. Moreover, the SGRs of small

koi (1.19-1.21% d^{-1}) were comparable with those reported by Velichkova and Sirakov (2013) for (8.18 g) common carp reared in a RAS; but higher than the 1.03-1.06% d^{-1} reported by Karakatsouli et al. (2010) for (51.88 g) mirror common carp and the 0.84% d^{-1} obtained by Ridha and Cruz (2001) for (62 g) Nile tilapia (*Oreochromis niloticus* L.). The higher SGR achieved with small fish in this trial was probably due to the younger fish (9.4 g) used, and this provides more evidence that there is a negative relationship between growth performance and the initial stocking size of fish. In the present trial, no mortality was found between large koi with both biofilters. The large koi fish were probably unaffected because the stocking density of fish was below the carrying capacity of these systems and did not reach the threshold which affected survival rates. However, the survival rates of small koi (94.0-95.5%) were higher than the 92% reported by Knaus and Palm (2017) for (36.3 g) common carp and the 62-93% obtained by Jha and Barat (2005) for (0.14 g) koi carp.

Based on the findings of the present trial, the increase in the initial size of fish is negatively correlated to the total ammonia nitrogen excretion and the SGR of fish, while positively correlated with the FCR. The fish size did not affect the TAN, NO_2-N, and NO_3-N removal efficiencies of both biological filters. The bacterial biofilm filter had generally higher removal rates of TAN than the *H. rotundifolia* filter; whereas *H. rotundifolia* plant had higher NO_3-N removal rates when stocked with any size of fish.

3.3. Effects of Harvesting Different Biomasses of Plants in an Integrated Recirculating Aquaponic System

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3.3.1. Introduction

Aquaponic systems are the integrated recirculating aquaculture systems (IRASs) that combine aquaculture and hydroponics (Rakocy et al., 2016; Love et al., 2015). These systems are gaining popularity because they offer many advantages in terms of reducing water consumption, increasing the profitability of primary and secondary products, recycling nutrients, and reducing the environmental impacts of aquaculture (Wongkiew et al., 2017; Estim et al., 2019). Aquaponics is a symbiotic production system between fish, microbes, and plants. After fish digest food, ammonia nitrogen is excreted into the water, and high levels of ammonia can negatively affect fish growth and survival. Nitrifying bacteria can utilise ammonia nitrogen and convert it into nitrite and then nitrate. Finally, plants can absorb and utilise nitrate for growth (Wongkiew et al., 2017).

Among the most economical plant species that can grow in the aquaponic systems are leafy greens such as basil, spinach, chives, mint, and watercress (Nhan et al., 2019). Watercress (*Nasturtium officinale*) is an aquatic, perennial herb consumed by humans as a salad green and medicinal herb. The plant has relatively large quantities of beta carotene (vitamin A), ascorbic acid (vitamin C), folic acid, iron, iodine, calcium, and phosphorous. It also contains a high level of amino acids (arginine, glycine, lysine, and tryptophan) and antioxidants (Smith, 2007). Watercress is in high demand and has a high economic value in urban areas, which makes it a very suitable crop for commercial or small scale farming. The demand for watercress is greater than the amount that businesses can supply to the market (Nhan et al., 2019).

The common carp (*Cyprinus carpio* L.) was also chosen as a model species in the present trial because it has a wide distribution throughout the world and can survive in poor water quality. The majority of carp production in Central and Eastern Europe comes from extensive and semi-intensive fishpond operations, where untreated pond water discharged into the environment causes environmental problems (Woynarovich et al., 2011). In response to the increasingly strict environmental regulations in the region, improving carp farming practices and reducing their environmental impact would be a new desirable trend for ecological approaches and sustainability.

In aquaponic systems, three strategies have been adopted for producing crops: staggered cropping, intercropping, and batch cropping (Rakocy et al., 2016). A staggered production

system is one where a group of plants is harvested at different stages of growth. This allows the crop to be harvested repeatedly and keeps the nutrient uptake in the culture system relatively constant. This system is most effective for crops that can be grown continuously such as leafy green vegetables and herbs (Rakocy et al., 2016). However, if the harvesting of the plants is too much, the number of plants in the bed will reduce and the uptake of the nutrients in the aquaponic system may decrease, resulting in nutrient accumulation and, eventually, fish mortality. Therefore, the selection of the appropriate biomass of plants to be harvested can optimise nutrient uptake efficiency and sustain plant development in aquaponic systems.

Additionally, several studies have suggested that overall nutrient removal could be improved if a harvesting regime is applied (Vymazal et al., 2010; Yang et al., 2016; Verhofstad et al., 2017), but others reveal that harvesting can negatively affect nutrient removal (Kim and Geary, 2001; Wang et al., 2014; Zheng et al., 2015). The importance of harvest management for the nutrient removal, as well as the growth and development of plants, has always been highly controversial (Álvarez and Bécares, 2008; Vymazal et al., 2010; Zheng et al., 2015; Zheng et al., 2018; Sun et al., 2019). Despite the idea of nutrient reduction through harvesting having gained more attention over the last decade (Bartodziej et al., 2017), little published information is available on the requirement of the optimum harvesting of the biomass of plants in integrated recirculating aquaponic systems. Therefore, this trial aimed to investigate the effects of harvesting different biomasses of watercress on water quality, nutrient removal efficiency, and the growth of both watercress and the common carp in an integrated recirculating aquaponic system.

3.3.2. Materials and Methods

3.3.2.1. Experimental fish and plants

A total number of 144 common carp (*C. carpio* L.) with an average weight of 33.67 ± 0.012 g were used for the experiment. Fish were collected from a stock tank at the Aquaculture Laboratory of Debrecen University (ALDU), Hungary. Watercress (*N. officinale*) was also taken from a growing hydroponic bed in an operating aquaponic system at ALDU. Healthy seedlings (144 seedlings) that already had white roots with an average height of 6 cm and a weight of 20 g were transplanted into 12 hydroponic units. Each hydroponic unit was stocked with twelve seedlings of watercress.

3.3.2.2. Design of systems

The trial comprised 12 independent experimental systems; each system consisted of a 200 L fish tank, a 20 L waste collection tank, and a 0.086 m^3 hydroponic unit with expanded clay.

The fish and waste collection tanks were placed on the floor, while the hydroponic unit was installed on a plastic stand to elevate it above the fish tank. Water from the waste collection tank was pumped through a plastic tube at a flow rate of 3 L/min to the hydroponic unit by a submerged pump. Water from the hydroponic unit was circulated to the fish tank and then returned back through a PVC pipe to the waste collection tank by gravity (Figure 4.1). The outlet of each hydroponic unit was constructed as a bell siphon with a maximum water level of 15 cm and an auto-mechanical water out movement, initiating the ebb under water pressure. The water volumes in the fish and waste collection tanks were maintained at 100 L, and 17 L, respectively. The fish tank was supplied with one air stone to provide dissolved oxygen for the fish, and a polyethylene mesh was put above the fish tank to prevent the fish from jumping outside. All the experimental systems were operated with fish and plants for four days before the commencement of the experiment to acclimate the fish and plants to the experimental systems.

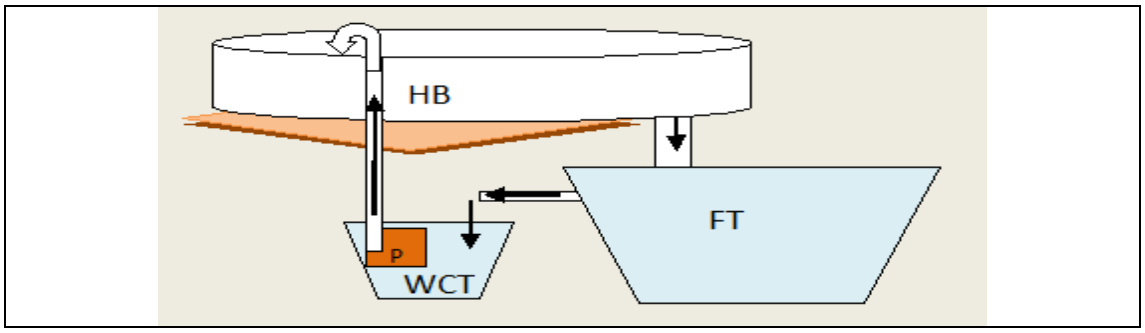

Figure 4.1. Diagram of the experimental units (the arrows show the direction of water flow); FT: Fish tank; WCT: Waste collection tank; HB: Hydroponic bed; P: Pump.

3.3.2.3. Experimental setup and rearing conditions

The trial was conducted for 58 days in an insulated greenhouse at ALDU, and only natural light was used to provide uniform conditions for fish and plant growth. Initially, 12 seedlings of plants with a total biomass of 240 g were transplanted into each hydroponic unit (0.43 m^2 surface area). The effect of harvesting different biomass ratios of above-ground plants was evaluated by a random design with three replicates. There were four treatments: harvesting 0%, 25%, 33%, and 50% of plants biweekly from the surface area of each hydroponic bed. Harvesting was carried out in such a way that the five centimetre above-ground portion of the plants remained in place to allow the plants to regrow again. The fish were initially stocked at 2.02 kg/m^3, and the total biomass was approximately 404 g/tank. The feeding rate for the fish was 2% of body weight per day, and all the fish were fed by hand twice a day at 09:00 and 15:00 hours with the commercially formulated feed (pellet size 2 mm) Aller Master (35% crude protein, 9% crude fat, 4.7% crude fibre, 7% crude ash, and 1.1% P) (Aller Aqua Group,

Allervej, Christiansfeld, Denmark). The uneaten feed and faeces were siphoned out daily before feeding and separated from the siphoned water by a 100 µm mesh size net, and then the water was returned back into the fish tank of the same system. Depending on the loss of technological water, the necessary amount of new water was added (~20–30 L) every ten days (including the water to compensate that lost by evaporation).

3.3.2.4. Water quality parameters

The dissolved oxygen (DO), temperature, and pH were measured in the fish tanks and hydroponic beds once a day before feeding using a Hach HQ30d portable meter (HACH CO., Loveland, Colorado, USA). Triplicate water samples were collected every 10 days from the fish tanks, as were the influent and effluent waters of each hydroponic unit to determine the nutrient removal rates. Ammonia nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), orthophosphate (PO_4-P), and total phosphorus (TP) were measured with the HACH Lange DR/3900 spectrophotometer (HACH CO., Loveland, Colorado, USA), using spectrophotometric methods outlined by the HACH company. The levels of NH_3-N, NO_2-N, and NO_3-N were determined using the Nessler method (Method 8038), diazotisation method (Method 8507), and cadmium reduction method (Method 8039), respectively (Hach, 2019). The TP and PO_4-P were determined by the phosphor-molybdenum blue method (HACH, Lange, LCK349, Phosphate Ortho/Total cuvette test), according to the ISO 6878_2004, DIN EN 6878/D11 standard (Hach, 2019).

The nutrient removal rates (NRR) were used to determine the nutrient removal cycle in each system. The nutrient removal rate (NRR%) was calculated using the following equation (Gichana et al., 2019):

$$NRR\% = [(C_I - C_E) / C_I] \times 100 \quad (1)$$

where C_I and C_E are the concentrations of a particular nutrient in the influent and effluent waters of the grow beds, respectively.

3.3.2.5. Fish and plant growth parameters

The growth and survival rates of the fish were recorded at the end of the experiment for each tank. The specific growth rates (SGR), fish weight gain (WG), feed conversion ratio (FCR), and survival rates were calculated using the following formulas:

$$SGR\ (\%\ /\ day) = 100 \times (\ln Wt - \ln W0), \quad (2)$$

$$WG = Wt - W0, \quad (3)$$

$$FCR = WF\ (g)\ /\ WG\ (g\ ), \quad (4)$$

$$Survival\ rate\ (\%) = 100 \times (nt\ /\ n0), \quad (5)$$

where Wt and W0 are the weights of fish at the end and the start of the trial, respectively, and (t) is the number of rearing days. The WF is the weight of feed given to the fish (g), and WG is the weight gain of the fish (g). The nt and n0 are the numbers of fish at the end and the start of the trial, respectively.

At the end of the experiment, all the plants were harvested from the hydroponic beds and the weight of the plants was recorded. The final biomass (FB), biomass gain, and specific growth rates of plants (SGRP) were calculated using the following equations:

Final biomass production of plants (FB) = Cumulative amount of plants harvested during the harvestings throughout the trial (g), (6)

$$\text{Biomass gain of plants (g)} = FB - IB, \quad (7)$$

$$SGRP\ (\%\ /\ day) = 100 \times (\ln FB - \ln IB)/t, \quad (8)$$

where FB and IB are the final biomass of the plants and the initial stocked biomass, respectively, while t is the number of rearing days.

3.3.2.6. Statistical analyses

All statistical analyses were performed using SPSS version 22.0 for windows. All the data obtained were tested for normality of distribution and homogeneity of variance. One-way analysis of variance (ANOVA) was conducted to test the differences between the parameters amongst treatments. Significant ANOVAs were followed by Duncan's multiple range tests to recognize specific differences amongst treatments. A $P < 0.05$ was considered significant for all analyses.

3.3.3. Results

3.3.3.1. Water quality parameters in fish tanks

The means for all the water quality parameters in the fish tanks were similar ($P > 0.05$) amongst the treatments except for PO_4-P and TP (Table 4.1). The lowest means for PO_4-P and TP were recorded in the fish tanks of the unharvested systems (0%), which significantly differed ($P < 0.05$) from those of the other three treatments (Table 4.1). The dissolved oxygen in all the treatments never dropped below 7 mg/L, whereas the temperature decreased as the trial progressed from 16.7 °C to 11.5 °C. The mean pH values fluctuated in all the treatments between 7.01 and 7.65. The mean NH_3-N concentrations decreased in all the treatments as the trial progressed, while the mean NO_2-N concentrations fluctuated over time (Figure 4.2. a, c). The means for NO_3-N increased in all the treatments during the first 30 days and decreased after that, with the highest means in the 33% and 50% harvested systems (Figure 4.2. d). The mean PO_4-P and TP concentrations for all the treatments decreased as the trial progressed, and the highest means were recorded at the early stage of the culture period (Figure 4.2. e, f).

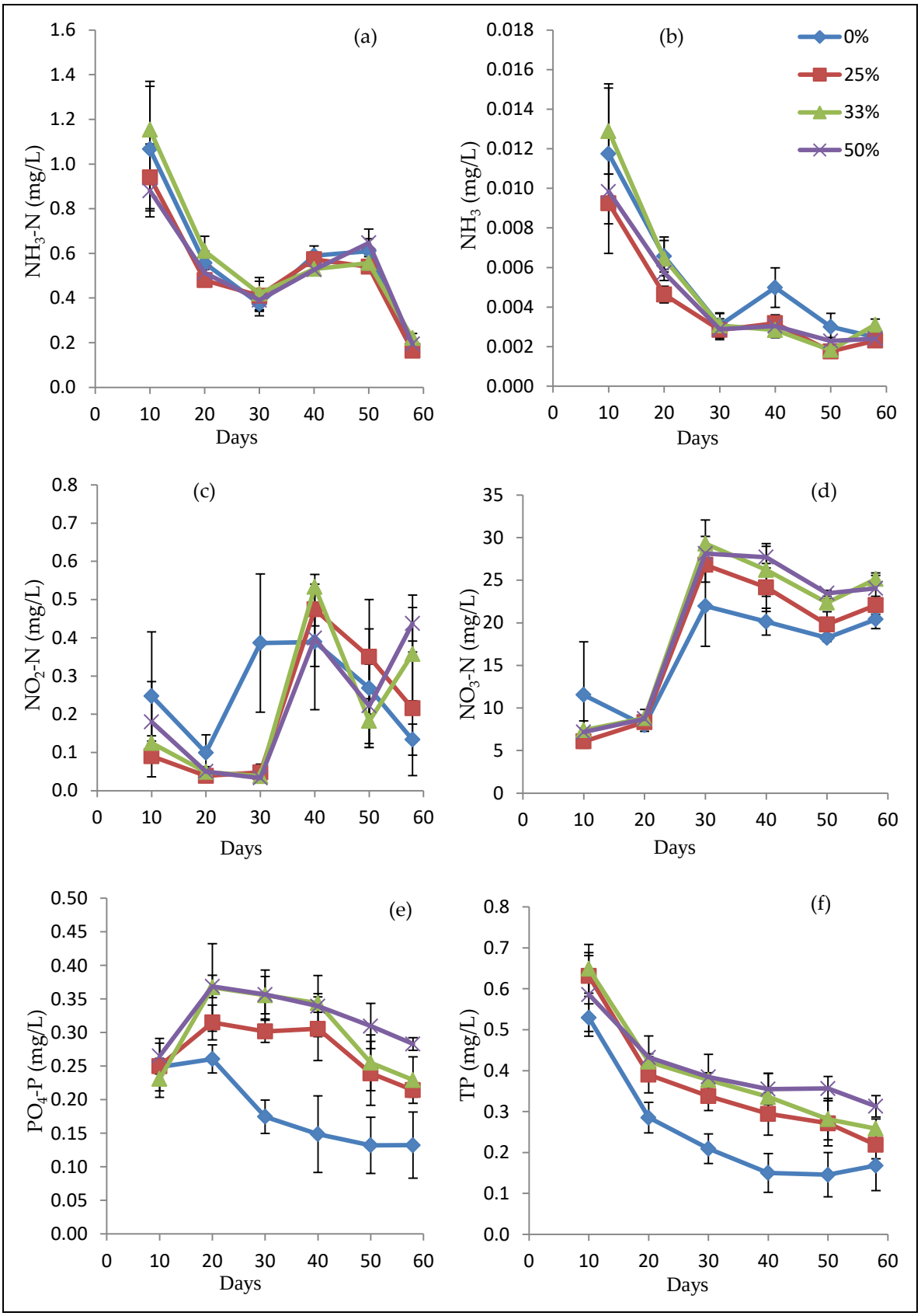

Figure 4.2. Concentrations of (**a**) NH_3-N; (**b**) NH_3; (**c**) NO_2-N; (**d**) NO_3-N; (**e**) PO_4-P; and (**f**) TP in tanks of *Cyprinus carpio* reared in an integrated recirculating aquaponic system for a 58 day trial (data are the means of three replicates (n = 3), and error bars indicate the standard errors).

Table 4.1. Overall mean water quality parameters in the fish tanks and hydroponic units of aquaponic systems under different plant harvesting regimes.

Water Parameters	Harvested Biomass			
	0%	25%	33%	50%
Fish Tanks				
NH_3-N (mg/L)	0.56 ± 0.08[a]	0.52 ± 0.06[a]	0.58 ± 0.07 [a]	0.53 ± 0.05[a]
NH_3 (mg/L)	0.005 ± 0.001[a]	0.004 ± 0.001[a]	0.005 ± 0.001[a]	0.004 ± 0.001[a]
NO_2-N (mg/L)	0.25 ± 0.05[a]	0.20 ± 0.05[a]	0.21 ± 0.04[a]	0.22 ± 0.04[a]
NO_3-N (mg/L)	16.71 ± 1.68[a]	17.87 ± 3.1.98[a]	19.86 ± 2.17[a]	19.87 ± 2.12[a]
PO_4-P (mg/L)	0.18 ± 0.02[b]	0.27 ± 0.01[a]	0.29 ± 0.02[a]	0.32 ± 0.01[a]
TP (mg/L)	0.25 ± 0.04[b]	0.36 ± 0.04[a]	0.39 ± 0.03[a]	0.40 ± 0.03[a]
Dissolved oxygen (mg/L)	8.16 ± 0.07[a]	8.19 ± 0.06[a]	8.24 ± 0.06[a]	8.34 ± 0.06[a]
pH	7.41 ± 0.02[a]	7.35 ± 0.04[a]	7.37 ± 0.03[a]	7.40 ± 0.04[a]
Temperature (°C)	14.71 ± 0.35[a]	14.45 ± 0.34[a]	14.29 ± 0.33[a]	14.21 ± 0.33[a]
Bed Units				
Dissolved oxygen (mg/L)	8.16 ± 0.07[a]	8.15 ± 0.07[a]	8.22 ± 0.07[a]	8.20 ± 0.08[a]
pH	7.41 ± 0.03[a]	7.38 ± 0.04[a]	7.37 ± 0.04[a]	7.40 ± 0.04[a]
Temperature (°C)	14.44 ± 0.34[a]	14.37 ± 0.34[a]	14.26 ± 0.33[a]	14.16 ± 0.33[a]

Values (means ± SE) in the same row with different superscript letters (a, b….) are significantly different (Duncan test; P <0.05); data are the means of three replicates (n = 3).

3.3.3.2. Nutrient removal rates

There were no significant differences (P >0.05) in the mean removal rates for NH_3-N and NO_2-N amongst any of the treatments (Table 4.2). The mean NH_3-N and NO_2-N removal rates increased in all the treatments over time (Figure 4.3. a, b). The highest removal rates for NO_3-N, PO_4-P, and TP were calculated in the unharvested system, which significantly differed (P <0.05) from those in the 33% and 50% systems (Table 4.2). However, the mean NO_3-N, PO_4-P, and TP removal rates in the 25% harvested systems were comparable with those in the unharvested and 33% harvested systems (Table 4.2). The mean NO_3-N removal rates in the unharvested systems increased over time, while the means for the other three treatments slightly decreased at the later stage of the culture period (Figure 4.3. c). The mean PO_4-P and TP removal rates increased in all treatments over time (Figure 4.3. d, e).

Table 4.2. Overall mean nutrient removal efficiency for each treatment during the 58 day trial.

Removal Rates	Harvested Biomass			
	0%	25%	33%	50%
NH_3-N (%)	36.20 ± 3.19[a]	35.06 ± 3.82[a]	31.80 ± 2.74[a]	31.88 ± 2.93[a]
NO_2-N (%)	42.43 ± 3.24[a]	41.67 ± 3.96[a]	47.14 ± 3.10[a]	42.58 ± 4.23[a]
NO_3-N (%)	63.58 ± 2.36[a]	59.67 ± 2.78[ab]	54.26 ± 2.33[bc]	49.49 ± 2.78[c]
PO_4-P (%)	31.09 ± 1.19[a]	28.44 ± 0.94[ab]	26.33 ± 1.34[bc]	24.97 ± 1.13[c]
TP (%)	47.31 ± 1.25[a]	46.56 ± 0.93[ab]	44.76 ± 0.83[b]	43.06 ± 1.17[b]

Values (means ± SE) in the same row having different superscript letters (a, b, c….) are significantly different (Duncan test; P<0.05); data are the means of three replicates (n = 3).

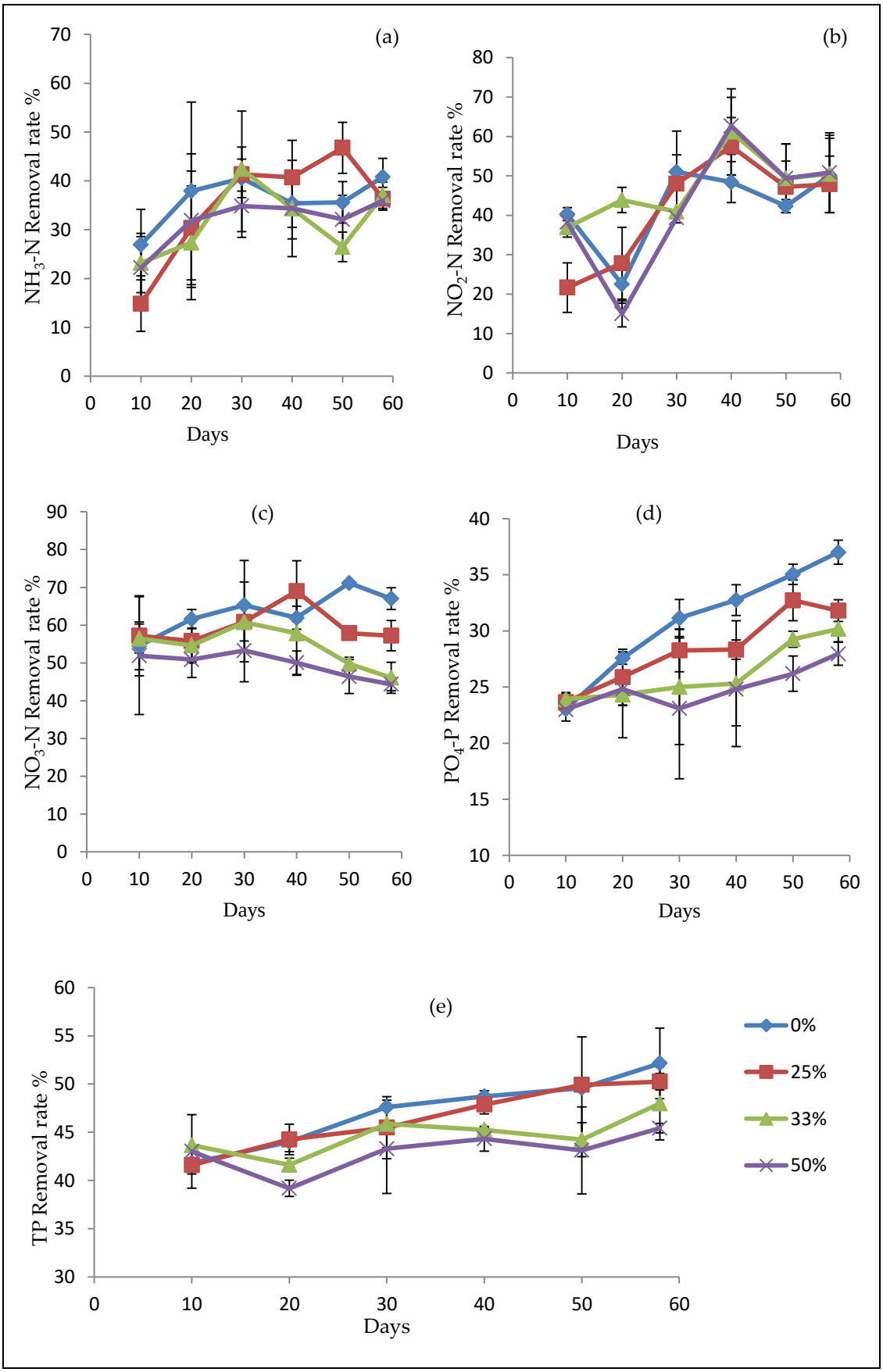

Figure 4.3. Removal rates for (**a**) NH_3-N; (**b**) NO_2-N; (**c**) NO_3-N; (**d**) PO_4-P; and (**e**) TP in aquaponic systems under different plant harvesting regimes (data are the means of three replicates (n = 3), and error bars indicate the standard errors).

3.3.3.3. Concentrations of nutrients before and after harvesting plants

The concentrations of NO_3-N and PO_4-P in the outlet of the bed units increased after two days of harvesting compared to the levels before two days of harvesting (Figure 4.4. b, c), while the concentration of NH_3-N started to increase after two days of the second harvesting (Figure 4.4 a).

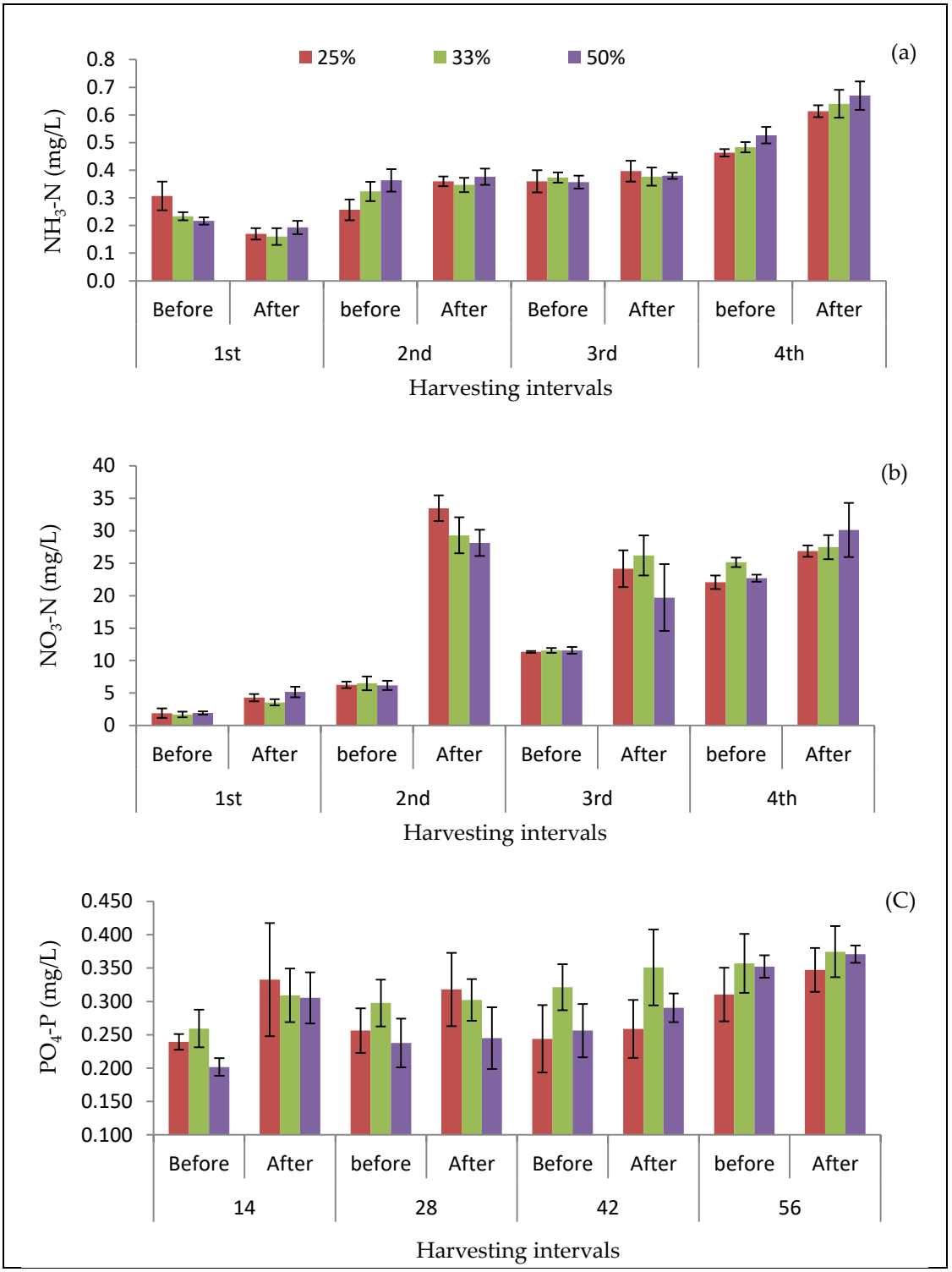

Figure 4.4. Concentrations of (**a**) NH_3-N; (**b**) NO_3-N, and (**c**) PO_4-P at the outlet of the hydroponic beds before and after two days of harvesting plants (data are the means of three replicates (n = 3), and error bars indicate the standard errors).

3.3.3.4. Fish and plant growth performance

After the experimental period, the growth rate of the common carp increased in all the treatments. The mean biomass gain, SGR, individual weight gain and FCR of the fish did not differ significantly (P >0.05) amongst any of the treatments (Table 4.3). The survival rates were also similar (P >0.05) in all treatments, and no mortality was recorded in any system (Table 4.3).

Table 4.3. Growth performance of *Cyprinus carpio* reared for 58 days in integrated recirculating aquaponic systems under different plant harvesting regimes.

Growth Parameters	Harvested biomass			
	0%	25%	33%	50%
Initial fish weight (g/fish)	33.71 ± 0.10[a]	33.75 ± 0.08[a]	33.64 ± 0.04[a]	33.55 ± 0.99[a]
Final fish weight (g/fish)	60.20 ± 0.83[a]	60.37 ± 1.42[a]	61.35 ± 1.68[a]	60.63 ± 1.13[a]
Fish weight gain (g/fish/58 days)	26.49 ± 0.73[a]	26.62 ± 1.39[a]	27.70 ± 1.71[a]	27.07 ± 1.03[a]
Specific growth rate (%/day)	1.00 ± 0.02[a]	1.00 ± 0.04[a]	1.03 ± 0.05[a]	1.02 ± 0.03[a]
Feed consumption (g/fish/day)	0.67 ± 0.00[a]	0.67 ± 0.00[a]	0.67 ± 0.00[a]	0.67 ± 0.00[a]
Fish weight gain (g/fish/day)	0.46 ± 0.01[a]	0.46 ± 0.03[a]	0.48 ± 0.03[a]	0.47 ± 0.02[a]
Feed conversion ratio	1.46 ± 0.04[a]	1.46 ± 0.07[a]	1.40 ± 0.08[a]	1.43 ± 0.06[a]
Survival (%)	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]

Values (means ± SE) in the same row with the same superscript letters are not significantly different (Duncan test; P<0.05); data are the means of three replicates (n = 3).

The mean biomass gain and specific growth rates of the plants (SGRP) in the 0% systems were significantly higher (P <0.05) than the values for the 33% and 50% harvested systems (Table 4.4). However, the mean biomass gain and SGRP of the 25% system were similar to the values for the unharvested and 33% harvested systems (Table 4.4). The amount of harvested plants for the treatments increased during the harvesting times, except for the decrease in the 50% harvested system at the third and fourth harvestings (Figure 4.5. a).

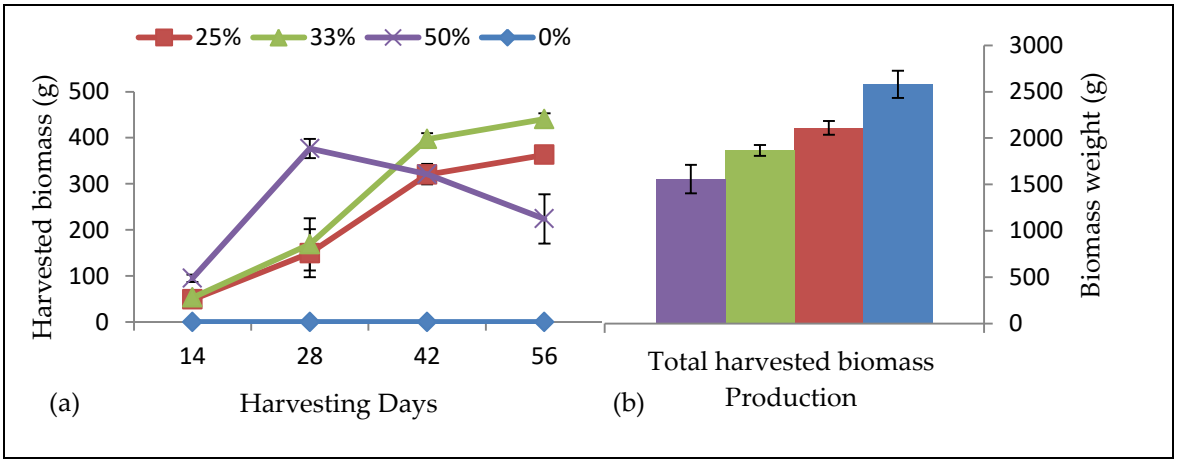

Figure 4.5. Harvested biomass of *Nasturtium officinale* in aquaponic systems under different harvesting regimes. (**a**) Amount of harvested plants on the 14th–56th days of the trial; (**b**) Total harvested biomass production in each treatment (data are the means of three replicates (n = 3), and error bars indicate the standard errors).

Table 4.4. Growth performance of *Nasturtium officinale* in integrated recirculating aquaponic systems under different harvesting regimes.

Growth Parameters	Harvested Biomass			
	0%	25%	33%	50%
Stocking biomass (g)	240 ± 0.00^{a}	240 ± 0.00^{a}	240 ± 0.00^{a}	240 ± 0.00^{a}
Final biomass (g)	2580.00 ± 180.00^{a}	2110.14 ± 8.36^{ab}	1865.97 ± 71.67^{bc}	1556.90 ± 187.95^{c}
Biomass gain (g)	2340.0 ± 180.0^{a}	1870.14 ± 8.36^{ab}	1625.97 ± 71.67^{bc}	1316.90 ± 187.95^{c}
Specific growth rate of the plants (%/day)	4.090 ± 0.120^{a}	3.745 ± 0.005^{ab}	3.535 ± 0.065^{bc}	3.210 ± 0.210^{c}

Values (means ± SE) in the same row with different superscript letters (a, b,….) are significantly different (Duncan test; $P < 0.05$); data are the means of three replicates (n = 3).

3.3.4. Discussion

The aquaponic systems designed with watercress plants (*N. officinale*) in this trial were efficient in removing nutrients because the plants take up the waste generated by the fish. The growth rate of the watercress increased exponentially in all treatments. However, our results showed that an increase in the harvested biomass of plants negatively affected the final biomass production (Table 4.4). Information relating to the influence of harvesting biomass on the growth performance and nutrient removal efficiency of plants in aquaponic systems is very limited; however, a very recent study found harvesting can cause damage to the plant tissues and that the plants after harvesting would not have the ability to transport nutrients and nonstructural carbohydrates from the stems and leaves to the storage organs, which could support the growth of new buds (Sun et al., 2019). Several studies with different plant species also found that repeated aboveground harvesting can slow down plant biomass development because harvesting does not allow sufficient opportunity for plants to absorb more nutrients for growth, consequently leading to very low biomass production compared to that in those not harvested (Kim and Geary, 2001; Jinadasa et al., 2008; Verhofstad et al., 2017; Jeke et al., 2019). In the present trial, the amount of harvested plants in the 50% harvested system decreased at the third and fourth harvestings (Figure 4.5. a), suggesting that harvesting can affect nutrient storage in plant tissues. Harvesting can alter the storage of rhizome

carbohydrates needed for early growth and stand strength, depending on the time of harvest and the type of biomass harvested (Jinadasa et al., 2008). Our results are in agreement with the results obtained by Verhofstad et al. (2017), who found that increasing harvesting frequency had a large effect on *Myriophyllum spicatum* cover, height, composition, and abundance. On the other hand, Zheng et al. (2015) found that harvesting plants improved shoot density and the biomass of plants in the system compared with those in the unharvested system, while Vymazal et al. (2010) found that the aboveground biomass was nearly identical at one and two time harvests.

After harvesting plants, the concentrations of NH_3-N, NO_3-N, and PO_4-P in the outlet of the bed units were higher compared to the levels before two days of harvesting (Figure 4.4. a, b, c), and this was probably due to a temporary reduction in the nutrient removal capacity of the system. The purification in rearing systems could be mediated by different mechanisms such as nitrification, denitrification, microbial assimilation, sedimentation, and plant uptake (Saeed and Sun, 2012). Plant uptake is one of the pathways for nutrient removal. When the plants are harvested, the microenvironment of the plant rhizosphere will be affected, leading to a decrease in the uptake of nutrients (Sun et al., 2019). This is the one reason why the concentrations in the effluents of the bed units temporarily increased after two days of harvesting plants, especially for NO_3-N and PO_4-P (Figure 4.4). Similarly, (Sun et al., 2019) reported that the concentrations of NO_3-N and NH_4-N in the effluents increased temporarily after harvesting plants, and the effect on NO_3-N was greater than that on NH_4-N, demonstrating that the harvesting of plant shoots would reduce the root exudates of the plants.

In aquaponic systems, nutrient removal capacity is influenced by the growth stage of the plants, nutrient needs of the plants, and the activity of nitrifying bacteria (Wongkiew et al., 2017; Estim et al., 2019; Gichana et al., 2019). Our results indicated that increasing the aboveground harvested biomass of plants had no effects on the ammonia and nitrite nitrogen

removal efficiencies (Table 4.2), and this could be due to the activity of the nitrifying bacteria in the systems. It is reported in (Wongkiew et al., 2017) that nitrifying bacteria are responsible for the major removal of ammonia and nitrite nitrogen in media-based aquaponic systems. Therefore, the activity of the nitrifying bacteria can describe the ammonia and nitrite nitrogen removal trends by all treatments in the present trial. The increasing trends with the treatments were possibly due to the boost in the number of nitrifying bacteria in response to the rise in ambient ammonia concentrations as a consequence of the increasing fish biomass. At the early stage of the trial, there were not enough nitrifying bacteria to perform the nitrification process efficiently (Estim et al., 2019; Knaus and Palm, 2017; Irhayyim and Fotedar, 2019). Similar findings were reported by Zheng et al. (2015), who found that the NH_3-N and NO_2-N removal efficiencies were comparable between the harvested and unharvested wetlands, and both wetlands in the second year showed higher nutrient removal than in the first year. On the other hand, Verhofstad et al. (2017) found that harvesting two times per growing season removed the highest amount of nitrogen from the system compared with the five times harvesting system. The results of another study revealed that harvesting during winter decreased NH_4-N removal (Wang et al., 2014), while the results obtained by Yang et al. (2016) revealed that the harvesting of shoots during summer could improve ammonia nitrogen removal.

Nitrates in aquaponic systems are taken up by plants as the main nitrogen source, and higher plant biomass translates to a higher plant uptake rate, resulting in a higher nitrate removal efficiency (Hu et al., 2015; Wongkiew et al., 2017). In the present trial, the unharvested treatment had the highest plant biomass production (Figure 4.5. b), resulting in the highest NO_3-N removal efficiencies, which tended to increase as the plant biomass increased over time (Figure 4.3. c). By contrast, the decreasing trends at the later stage of the experiment with the other treatments could be related to differences in the growth

performance of plants, cover, and abundance of plants in the bed unit after harvesting. It was concluded in Verhofstad et al. (2017) that an increase in harvesting frequency negatively affected macrophyte cover, height, abundance, and biomass production, as well as total nutrient removal from the system. They found that an increase in the harvesting of plants up to five times removed a lower amount of nutrients from the system compared to the system of harvesting two times per growing season. Furthermore, it should be mentioned that an increase in the dissolved oxygen in the water to more than 4 mg/L can inhibit the denitrification process (Zheng et al., 2015). In our trial, the dissolved oxygen in the hydroponic beds of all the treatments never dropped below 7.73 mg/L, and this may provide further evidence that nitrates are directly taken up by plants as a nitrogen source and are incorporated into the plant biomass (Fang et al., 2007).

The removal of phosphorus can be mediated through plant uptake and the mechanism of sedimentation (Bunce et al., 2018). In Midlen and Redding (1998), it is reported that over half of the phosphorus inputs are bound in the soils of the pond bottom in a relatively insoluble form. In the present trial, the artificial feed was the only source of phosphorus, and a large part of it was removed by the removal of uneaten food and fish faeces, which resulted in a large portion of soluble phosphorus and suspended particles in the water column. Our results indicated an inverse relationship between the harvested biomass of plants and the PO_4-P and TP removal capacities (Table 4.2). This was primarily due to the smaller plant biomass production in the systems (0% > 25% > 33% > 50%), as a result of the slow recovery of the plants and regrowth of biomass after harvesting (Figure 4.5. a, b). The lower PO_4-P and TP removal rates for all treatments at the beginning of the experiment could be attributed to the lower nutrient needs for the plants as a consequence of the smaller biomass of the plants (Figure 4.3. d, e). In Jones et al. (2015), it is reported that young plants have low nutrient requirements, which increase during the vegetative growth. Our findings were comparable to

the findings reported by Kim and Geary (2001), who found the productivity of macrophytes to be affected significantly by harvesting, and this resulted in lower phosphorus removal compared to that in systems that were not harvested. It is also reported in Verhofstad et al. (2017) that harvesting two times per growing season removed the highest amount of phosphorus from the system compared with the five times harvesting system.

In the present trial, all the water quality parameters in the fish tanks of the systems remained within the tolerance range for common carp growth and survival (Horváth et al., 2002, Timmons et al., 2002). This was due to the effects of the nitrification process and plant uptake. The maximum value of NH_3 (0.017 mg/L) at the beginning of the experiment (Figure 4.2. b) was lower than the concentrations reported by Biswas et al. (2006), who concluded that the common carp has three different concentration levels of NH_3: a favourable concentration (0.0286 mg/L), growth-inhibiting concentration (0.034 mg/L), and lethal concentration (0.043 mg/L). The maximum NO_2-N concentration of 0.58 mg/L in the present trial was much lower than the values reported by Kroupova et al (2010), who concluded that the common carp has three different concentration levels of nitrite: the lethal concentration (88 mg/L), that of lowest effect (28 mg/L), and that of no observed effect (7 mg/L). Furthermore, the maximum observed NO_3-N concentration (33.2 mg/L) was much lower than lethal values of 865 mg/L reported by Iqbal et al (2004) for the common carp. Phosphate has no toxic effects on aquatic organisms (Simplício et al., 2017; Kim et al., 2013). However, Suzuki et al. (2003) recommended that the orthophosphate concentration should be less than 15 mg/L in a closed recirculating system. In the present trial, the levels of ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen (Table 4.1) were within the acceptable levels for recirculating aquaculture systems (Timmons et al., 2002) and aquaponic systems (Rakocy et al., 2004). These concentrations were in agreement with the ranges of water quality variables

achieved by (Rakocy et al., 2004) during the staggered culture of basil production in aquaponics.

Our results also revealed that an increase in the aboveground harvested biomass of plants did not affect the common carp growth and survival in aquaponic systems. The growth and survival of fish are influenced by water quality parameters in the culture system (Colt, 2006; Ardiansyah and Fotedar, 2016; Estim et al., 2019; Wongkiew et al., 2017; Irhayyim and Fotedar, 2019). Thus, the similar growth performance between the fish in all the treatments was probably due to the equivalent water quality parameters during the trial period, particularly the ammonia and nitrite concentrations. The SGRs of common carp in the present trial, ranging from 1.00% to 1.03%, were higher than the 0.39% reported by Knaus and Palm (2017), and the 0.79% obtained by Maucieri et al. (2019) for the common carp in different media-based aquaponic systems. Our results also were higher than the 0.841% and the 0.83% obtained by Hussain et al. (2014 and 2015) for koi carp (*Cyprinus carpio var. koi*) fed with 2% body weight in aquaponic systems. However, the SGRs of the fish were lower than those (5.41%–5.50%) achieved by Shete et al. (2016 and 2017) for common carp reared in aquaponic systems. The lower SGRs may be related to the different temperature (14.14 °C), feeding rates (2%), and stocking density of the fish (2.02 kg/m^3) used in this trial compared to those used by Shete et al. (2016 and 2017), (25.78 °C, 5% body weight and 0.090 kg/m^3, respectively).

The findings of this trial reveal that increasing the biweekly harvested biomass of plants had negative effects on the growth performance of watercress in aquaponic systems, while it had no effects on the growth of common carp. The 0% and 25% systems were recorded to have the highest plant biomass production. In this trial, watercress plants were efficient in removing nutrients generated by fish in aquaponic systems. However, increasing the harvested biomass of the plants had negative effects on the NO_3-N and PO_4-P removal

efficiencies, while it had no effects on the NH_3-N and NO_2-N removal efficiencies. The 0% and 25% systems were recorded to have the highest NO_3-N and PO_4-P removal efficiencies. The present trial suggests that the biweekly harvesting of less than 25% of the growing area of watercress is recommended for improving nutrient removal efficiency and sustaining the growth of both fish and plants in aquaponic systems.

3.4. Impacts of Using Magnetic Water Treatment in an Integrated Recirculating Aquaculture System

Paper published in Journal of Central European Agriculture, https://doi.org/10.5513/jcea01/21.2.2672

3.4.1. Introduction

Recirculating aquaculture systems (RASs) offer many benefits in terms of reducing water requirements, nutrient recycling, improving waste management and better disease management (Timmons et al., 2002). The research and developments in the RASs tend to focus on: (1) technical improvements within the recirculation loop and (2) recycling of nutrients through integrated farming (Martins et al., 2010). Production systems that use plants to remove nutrients from wastewater have a promising future as an alternative technology for converting nutrients into valuable products and preventing nutrient overload in the environment (Schneider et al., 2005; Martins et al., 2010).

Recently, magnetized water is used successfully for improving water properties in different sectors such as farming and agriculture, wastewater treatment and scale elimination (Ali et al., 2014). Cai et al. (2009) reported that the magnetic field changes the physicochemical properties of water, and results in decreasing the surface tension and increasing the viscosity of water. Ali et al. (2014) stated that magnetized water improved irrigation water quality, water saving, and scale elimination. The positive effect of magnetized water was also reported on the germination rates of the rice (Carbonell et al., 2000) and lettuce seeds (Reina and Pascual, 2001). Moreover, it was shown that the magnetic field reorganizes the water molecules into tiny and homogeneous clusters easing their travel through the pathways in plant and animal cell membranes (Ali et al., 2014). Magnetic fields change osmotic processes, affect the permeability of the cellular membrane, and disturb the hydration ability of tissues in animals (Ibraheim and Khater, 2013) and plants (Reina and Pascual, 2001). Ali et al. (2014) reported that magnetized water improved the health of livestock as well as plant growth and crop yield.

Although the applications of magnetic water treatment have been successfully used in different fields, limited investigations have been done in aquaculture on different species. Some authors reported that the magnetic water treatment had positive effects on fish growth (Hassan et al., 2018a; Nofouzi et al., 2017), and water quality of the systems (Krzemieniewski et al., 2003; Hassan and Rahman, 2016; Hassan et al., 2018b). However, other authors revealed no effect of using the magnetic water treatment on fish growth (Krzemieniewski et al., 2004; Hassan et al., 2019), and water quality (Krzemieniewski et al., 2004; Hassan et al.,

2019). It appears that there is still a considerable debate regarding the effects of magnetic water treatment on the growth of cultured species and water quality of rearing systems. Besides that, there are currently no publications regarding the impacts of magnetic water treatment on common carp growth in an integrated recirculating aquaculture system. Therefore, the purpose of the present trial was to evaluate the effects of using electromagnetic field (EMF) on water quality parameters, feed utilization efficiency and growth performance of common carp in an integrated recirculating aquaculture system (IRAS).

3.4.2. Materials and Methods

3.4.2.1. Experimental systems

The experimental system was performed according to the prototype published by (Irhayyim and Fotedar, 2019). In brief, the trial comprised six independent experimental systems: each system consisted of three tanks: a rearing fish tank, a waste-collection tank and a biological filter tank. All three tanks were joined and operated under the theory of an IRAS. Water from the waste-collection tank was pumped through a plastic tube to the biological filter tank by a submerged pump (RESUN, Model: P-1500, Guangdong Risheng Group Co. Ltd., China) and water from the biological filter tank was then circulated to the fish tank by gravity. Water from the bottom of the fish tank was drained back through a PVC pipe to the waste-collection tank (Figure 5.1). The volumes of water in the waste-collection and fish rearing tanks were kept at 36 and 55 litres respectively, while the volume of water in the biological filter tanks was kept at 60 litres. The rearing fish tanks were provided with one air stone and covered with a polyethylene mesh (1.0–1.5 cm in diameter) to prevent fish from jumping outside. The biological filter tanks were also aerated with one air stone. The light was provided by snow LED light bulbs (Aaalite, 1430 lumen, E27, 15W, 3000 Kelvin), which were set on timers to a 12 h light: 12 h dark.

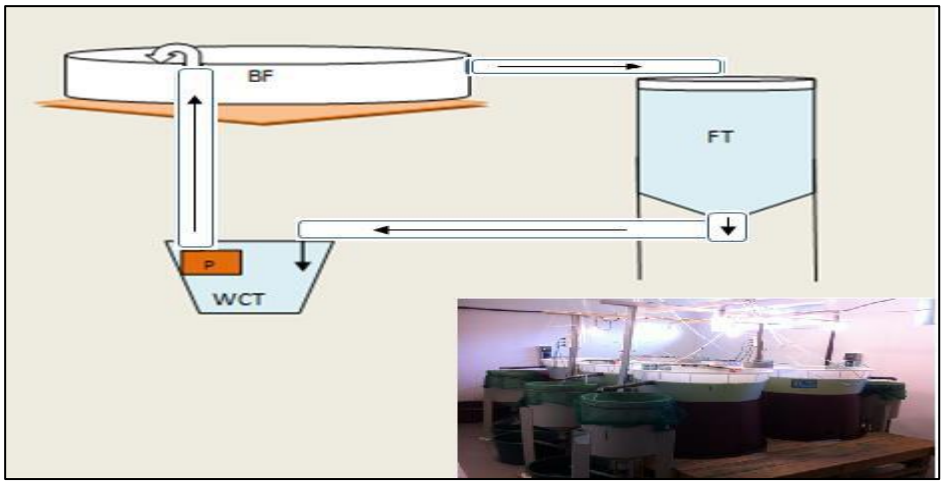

Figure 5.1. Diagram of experimental units (arrows show the direction of water flow), (FT): Fish tank (WCT): Waste collection tank, (BF): Biological filter, (P): Pump

3.4.2.2. Experimental biological filters

Six combined biological filters with the same surface area of 0.41 m^2 were designed to be used in this experiment. In order to qualify as an IRAS, each biological filter contains 0.0015 m^3 of plastic media and 70 g of duckweed (*Lemna minor*) as a biofilter medium (Figure 5.2). The plastic media in the form of bio-balls and a specific surface area of 400 m^2/m^3 were used to place in the biofilter tanks. The plastic media with established biofilms were obtained from an operating recirculating system in Georgikon Aquatic Research Laboratory (GARL), Keszthely, Hungary.

The duckweed (*L. minor)* plant was also chosen because of its potential to convert nutrients into high protein-enriched products as well as it has rapid growth and simplicity of harvest. Duckweed (*L. minor*) can be used as an organic feed for animals and many species of fish (Mukherjee et al., 2010). The plants were obtained from the aquatic research laboratory of Debrecen University, Hungary. The plants were cleaned and put in a stock tank for two weeks as an acclimatization period until the start of the trial.

3.4.2.3. Experimental fish

Common carp (*Cyprinus carpio* L.) were originated from natural reproduction in a pond at H & H Carpio fish farming Ltd., Ócsárd, Baranya, Hungary. A total number of 72 common carp with an average weight of 10.59 ± 0.06 g were collected from the same stock pond and transported to GARL, Keszthely, Hungary. The experimental IRASs were operated with fish for 7 days before the commencement to acclimate fish to the experimental conditions. Fish were fed a commercially extruded feed that declared by the manufactured company as, Nutra MP (50% crude protein, 18% crude fat, 1% crude fibre, 11% crude ash, 0.5% Na, 2% Ca and 1.5% P) (Skretting a Nutreco Co., Mozzecane, Italy).

3.4.2.4. Experimental design and rearing conditions

The trial was conducted over 28 days in GARL and designed as two treatments with three replicates in a random arrangement. The first treatment was supplied with a magnetic field device, which was placed before the biofilter tank, while the control treatment was set up without the device. The Electromagnetic field (EMF) was generated in a coil by currents using a commercial magnetic field generator with a frequency of 25 kilohertz (kHz) and an intensity of 0.8 millitesla (mT) (Magnetic Field Generator Multi Plus; manufactured by IVT Innovative Versorgungs-Technik GmbH, Hirschau, Germany) (Figure 5.2).

Fish were initially stocked at a density of 12 fish per tank (mean biomass: 127 g per tank, equal to 2.3 kg/m^3). All fish were fed by hands twice a day at 09:00 and 16:00 hours with a commercial diet (pellet size 1.5 mm) and the feeding rate was 3.5% of body weight per day.

The uneaten feed was collected one hour after feeding, while faeces were removed daily before the feeding commenced through a filter net with a mesh size of 100 µm and the remaining water returned back into the waste-collection tank of the same experimental unit. The water flow rate was set at 3 litres per minute, and approximately 30% of the system water was siphoned out weekly and replaced with new water. Every four days, 20% of the surface area of the duckweed biofilter was harvested from each system and the weight of the harvested plants was recorded.

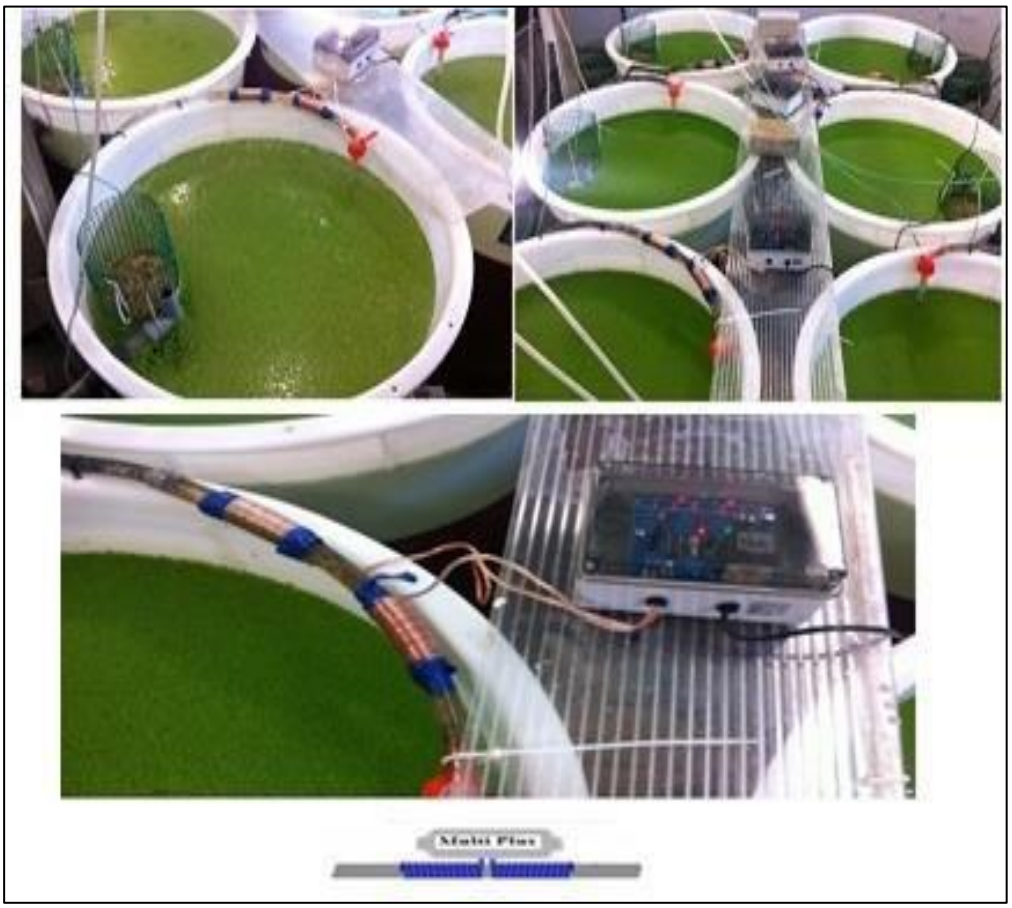

Figure 5.2. Magnetic field generator and the biological filters used in the experimental units

3.4.2.5. Sample collection and analysis

Dissolved oxygen (DO), temperature and pH of water in the fish tanks were measured once a day before feeding commenced. Dissolved oxygen and temperature were measured by using the OxyGuard Handy Polaris meter (OxyGuard International A/S, Denmark), while pH was measured by using the Milwaukee MW100 meter (Milwaukee Instruments, Romania). The concentrations of ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) in all fish tanks were measured weekly using a Lovibond photometer Multi Direct (Tintometer Group, Germany), following the methods in the instruction manual.

Survival and growth rates of the fish were recorded at the end of the trial for each tank. Specific growth rates (SGR), fish weight gain (WG), feed conversion ratio (FCR) and survival rates were calculated using the following formulas:

SGR (%/day) = $100 \times (\ln W_f - \ln W_i)/t$ WG = $W_f - W_i$

FCR = WF (g) / WG (g)

Survival rate (%): $S = 100 \times (n_f / n_i)$

Where W_f and W_i are the weight of fish at the end and the start of the trial respectively, while (t) is the number of rearing days. The WF is the weight of feed given to the fish (g) and WG is the weight gain (g). The n_f and n_i are the number of fish at the end and the start of the trial respectively.

At the end of the experiment, the plants were harvested from the biofilter tanks and the weight of the plants was recorded. The final biomass and specific growth rates of plants (SGRP) were calculated using the equations:

Final biomass of plants = Biomass of plants in biofilter tank at the end of the trial (g) + harvested biomass throughout the trial (g).

SGRP (%/day) $=100 \times (\ln B_f - \ln B_i)/t$;

Where: B_f and B_i are the final biomass of the plant and the initial stocked biomass respectively, while (t) is the number of rearing days.

3.4.2.6. Statistical analyses

All statistical analyses were performed using the SPSS version 22.0 for Windows package. All of the data obtained were tested for normality of distribution and homogeneity of variance. The independent t-test was conducted to determine any significant differences between treatment means, and the Mann-Whitney test was used to test the differences if the data did not have a normal distribution or homogeneous variance. The 5% level of probability was considered to be the significance level.

3.4.3. Results and Discussion

3.4.3.1. Plant growth performance

The results indicated that the SGRP of plants in the electromagnetic field systems of 0.8 mT and 25 kHz, was significantly higher ($P<0.05$) than in the control systems (Table 5.1). Previous studies revealed that the exposure of plants to continuous electromagnetic field induces different biological responses such as changes in enzyme activity, growth rate and gene expression (Vian et al., 2016; Tkalec et al., 2005). The results obtained in this trial are comparable with the results of Yaycili and Alikamanoglu (2005), who found that the weight, length, number of leaves and chlorophyll content of *Paulownia tomentosa* and *Paulownia*

fortune were positively affected by the magnetic field. The positive effect of the magnetic field also reported on the germination rates of the rice (Carbonell et al., 2000) and lettuce seeds (Reina and Pascual, 2001) exposed to the magnetic field. One explanation suggests that the ionic currents in the plant cell membrane can interact with the magnetic field, and this can change the ionic concentrations and osmotic pressure of the plant membrane which helps to regulate the water flow into the cell (Reina and Pascual, 2001). In contrast to the results obtained in this trial, Tkalec et al. (2005) found the growth of duckweed (*Lemna minor*) significantly decreased after the exposure to the electromagnetic field of 900000 kHz. The low frequency (25 kHz) used in the present trial could be one of the explanations for the higher SGRP of the plants compared to the results by Tkalec et al. (2005), who concluded that the effects of electromagnetic fields vary between different plants species according to the frequencies applied and field strength.

Table 5.1. Growth of *Lemna minor* under the exposure of electromagnetic field in an integrated recirculating aquaculture system

	EMF[1]	Control
Initial plant biomass (g per biofilter tank)	70±0.00[a]	70±0.00[a]
Final plant biomass (g per biofilter tank)	709.060±4.287[a]	612.483±11.41[b]
Plant biomass gain (g per biofilter tank)	639.060±4.29[a]	542.483±11.41[b]
SGRP (%/DAY)	8.27±0.021[a]	7.74±0.065[b]

Values (means ± SE) having the same superscript letters are not significantly different.
[1]EMF is the electromagnetic field system

3.4.3.2. Water quality parameters in fish tanks

There were no significant differences (P>0.05) in the means of DO, pH and temperature between the treatments over the entire period of the trial (Table 5.2). The temperature, pH and DO concentrations in the fish tanks of all systems remained within the tolerance range for common carp growth and survival (Horváth et al., 2002). However, DO in both treatments showed a slight decline as the trial progressed from 8.40 to 7.61 mg/L, and this could be related to an increase in the total biomass of fish and a consequent rise in the oxygen consumption rates (Jørgensen et al., 1993). The pH values remained relatively constant (ranged from 7.2 to 7.4), and did not change in any of the systems during the trial period. Cahill et al. (2010) suggested that a lack of change in pH between systems may be attributed to the systems being maintained under good aeration. Water temperature in the fish tanks was around the average of 24°C, which is within the optimum temperature of common carp foraging and growth (24 and 28 °C) reported by Oyugi, et al. (2012).

The overall means of NH_4-N, NO_2-N and NO_3-N concentrations in fish tanks did not differ significantly ($P>0.05$) between the electromagnetic field system and the control system (Table 5.2). The results of the present trial are comparable with the findings of various authors (Krzemieniewski et al., 2004; Hassan et al., 2018a; Hassan et al., 2019) who found no changes in ammonium concentrations between the magnetic field system and the control system. However, the results of this trial were in contrast with the findings obtained by Hassan and Rahman (2016), and Hassan et al. (2018b) who found a reduction in the ammonium concentrations of the magnetic treatments. The different findings could be related to the exposure time and/or lower magnetic intensity (0.8 mT) used in this trial, compared to those (100-200 mT) used by Hassan and Rahman (2016), and Hassan et al. (2018b). Tang et al. (2015) reported that the effect of the magnetic field is influenced by the exposure duration, field intensity and sensitivity of different species. Hassan et al. (2018a) suggested that the ammonium concentration in water from fish tanks could be reduced by increasing the magnetic intensity from 100 to 200 mT.

Table 5.2. Overall mean water quality parameters in the electromagnetic field and control systems

	EMF[1]	Control
NH_4-N (mg/L)	0.351±0.011[a]	0.379±0.008[a]
NO_2-N (mg/L)	0.148±0.046[a]	0.149±0.050[a]
NO_3-N (mg/L)	5.88±0.90[a]	6.69±1.01[a]
DO (mg/L)	7.97±0.066[a]	7.96±0.070[a]
pH	7.28±0.021[a]	7.29±0.020[a]
Temperature (°C)	23.97±0.28[a]	23.94±0.28[a]

Values (means ± SE) having the same superscript letters are not significantly different.
[1]EMF is the electromagnetic field system

The concentrations of NH_4-N, NO_2-N and NO_3-N in the fish tanks of both treatments were maintained at the levels recommended for common carp aquaculture (Horváth et al., 2002; Timmons et al., 2002), and this was due to the functions of different mechanisms to convert nutrients such as nitrification process and plants uptake. The means NH_4-N in both systems increased during the first 2 weeks and decreased after that (Figure 5.3). The decreasing trend in the NH_4-N concentrations in the later stages of the experiment could be related to the increase in the growth rate of plants, since the potential rate of nutrient uptake by plants is limited by their growth rate (Vymazal, 2007). Another explanation for this decreasing trend could be due to the increase in the number of nitrifying bacteria (*Nitrosomonas)* in response to the rise in ammonia concentrations as a consequence of increasing fish biomass (Brazil, 2006). The mean NO_2-N in both systems remained relatively constant during the course of the

experiment (Figure 5.4). This may be attributed to the second step of the nitrification process and the increase in the number of nitrite-oxidizing bacteria (*Nitrobacter*) (Timmons et al., 2002). In the same line, the mean NO_3-N in both systems increased in the first week and decreased thereafter (Figure 5.5). The decreasing trend may provide further evidence that ammonium and nitrate are directly taken up from the water culture by macrophytes as a nitrogen source and are incorporated into the plant biomass (Fang et al., 2007).

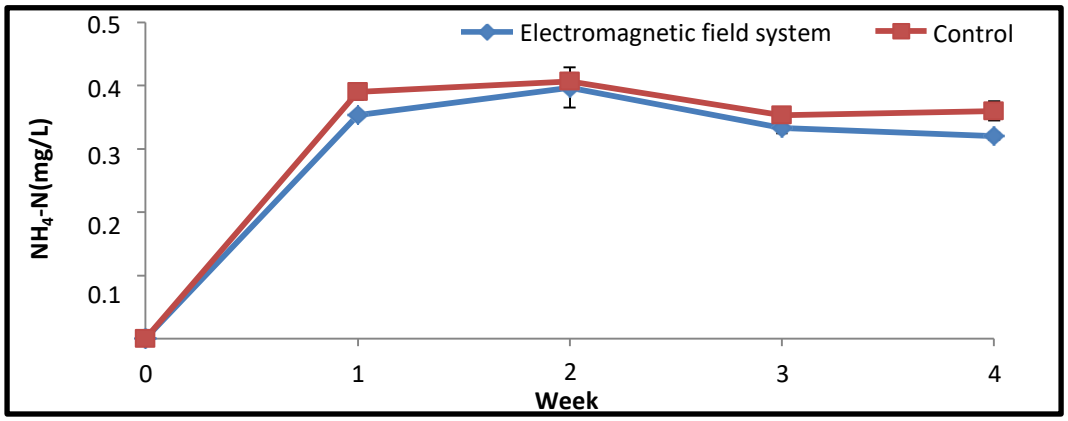

Figure 5.3. Weekly mean concentrations of ammonium nitrogen in fish tanks under the exposure of electromagnetic field (error bars indicate the standard error).

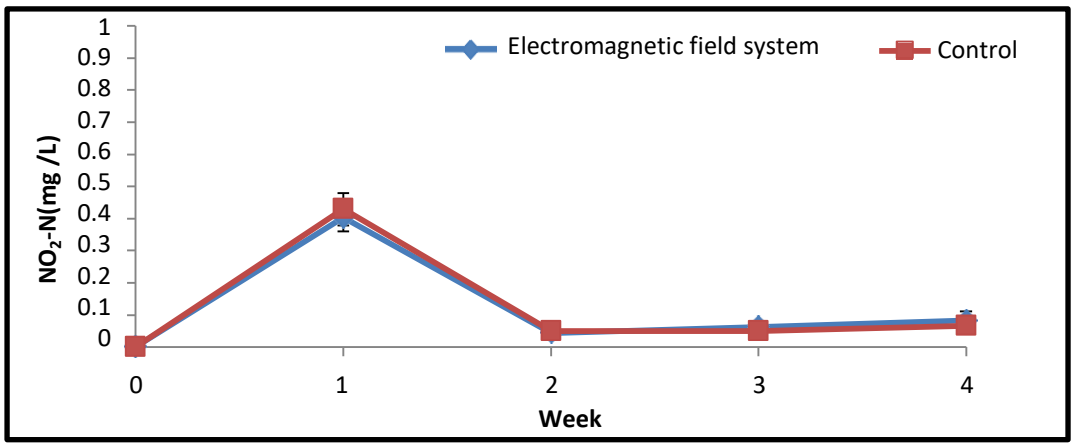

Figure 5.4. Weekly mean concentrations of nitrite nitrogen in fish tanks under the exposure of electromagnetic field (error bars indicate the standard error).

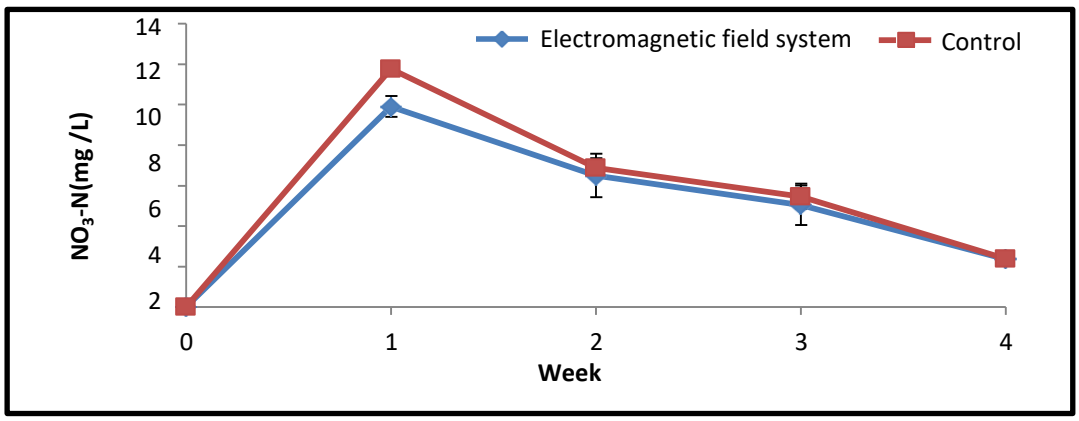

Figure 5.5. Weekly mean concentrations of nitrate nitrogen in fish tanks under the exposure of electromagnetic field (error bars indicate the standard error)

3.4.3.3. Growth and survival rates of fish

The magnetized water had significant effects ($P<0.05$) on the SGR and weight gain of common carp compared to the control system (Table 5.3). The lowest mean of FCR was also recorded with the fish reared in the electromagnetic field system, which was significantly lower ($P<0.05$) than those at the control system (Table 5.3). The differences in growth performance of fish in the present trial are more likely to be related to differences in the food consumption and feed utilization efficiency by fish; because the best FCR in this trial was achieved at the magnetic field group (Table 5.3). Previous studies revealed that the magnetic field has the ability to change the surface tension, density, viscosity, hardness and conductivity of water as well as the solubility of solid matter; and these changes in water properties can affect the biological activities of the organisms (Gabrielli et al., 2001; Krzemieniewski et al., 2003; Krzemieniewski et al., 2004). The additional magnetic field can bring effect to the metabolism of living organisms, namely the magnetic biologic effect, which may affect the enzyme activity, cell membrane permeability and cell metabolism (Liu et al., 2008). The magnetic field may influence the metabolism of living organisms by modifying the synthesis of carbohydrates, proteins and the accumulation of essential amino acids. All metabolic reactions are based on the difference in electrical charges and system ions. Electromagnetic forces cause changes in biological cell metabolism and the movement of electrons and ions may cause changes in biomolecules concentration, such as protein, carbohydrate, and lipid. Therefore, it can modify free radical activities, cell metabolism, cell membrane characteristics, cell growth and enzymatic activity (Santos et al., 2017; Hassan et al., 2018a). In all animals, there is no common mechanism has been implicated regarding the effect of the magnetic field on the growth performance of the animals. Brizhik (2014) suggested that the magnetic field can cause a hierarchy of changes from the primary effect on the dynamics of electrosolitons, to the changes of the macromolecules state, to the effects on

the respiration rate and, finally, to the effect on the whole metabolism of the system. Another mechanism reported by Rodriguez et al. (2002) in the dairy cattle, is related to the increase in the level of insulin-like growth factor-I that plays an essential function in the regulation of growth hormone actions in every cell in the body.

Although there are no published studies about the effect of magnetized water on common carp growth, the results of the present trial were in agreement with the findings obtained by Hassan et al. (2018a) with red hybrid tilapia (*Oreochromis sp.*) in RAS and Nofouzi et al. (2017) with rainbow trout (*Oncorhynchus mykiss*). However, the results were in contrast with the findings obtained by Krzemieniewski et al. (2004) who found no significant difference between the growth of wels catfish (*Silurus glanis* L.) larvae reared in the system modified by the constant magnetic field and the control group. In the present trial, the SGRs of 1.82 and 1.61%/day were calculated for common carp reared in the electromagnetic field and the control systems respectively, which are lower than those (6.22-6.32%/day) achieved by Hassan et al. (2018a) for red hybrid tilapia (*Oreochromis sp.*) reared in a RAS under the exposure of different magnetic field intensities. The lower SGR was probably due to the higher initial stocking rate (2.3 kg/m^3) and/or the lower magnetic intensity (0.8 mT) used in the present trial compared to those (0.213 kg/m^3 and 100-200 mT) used by Hassan et al. (2018a). However, the SGRs of fish in the present trial were higher than the 1.28-1.52%/day reported by Nofouzi et al. (2017) for rainbow trout (*Oncorhynchus mykiss*) and the 1.60%/day obtained by Hassan et al. (2019) for Jade Perch (*Scortum barcoo)* juveniles reared in a RAS under the exposure of different magnetic field intensities. Furthermore, the SGR of fish in the present trial was also higher than the 1.03-1.06%/day and 0.9-1.21%/day reported for common carp reared in the RASs without magnetic treatment by Karakatsouli et al. (2010) and Velichkova and Sirakov (2013) respectively.

Table 5.3. Growth, feeding efficiency and survival rates of *Cyprinus carpio* L. under the exposure of electromagnetic field in an integrated recirculating aquaculture system

	EMF[1]	Control
Mean stocked fish biomass per tank (g)	125.37±0.363[a]	128.166± 1.026[a]
Number of fish per tank	12	12
Mean initial weight of fish (g)	10.44±0.030[a]	10.68±0.084[a]
Mean final fish biomass per tank (g)	209.223± 1.88[a]	201.413± 0.69[b]
Number of surviving fish	12	12
Mean final weight of fish (g)	17.44±0.15[a]	16.78±0.058[b]
Biomass gain per tank (g)	83.85± 2.04[a]	73.24±0.98[b]
Mean fish weight gain (g/fish/28 days)	7.00±0.17[a]	6.10±0.08[b]
SGR (%/day)	1.82±0.037[a]	1.61±0.026[b]
Feed consumption (g/fish/28days)	10.24±0.03[a]	10.46±0.08[a]
FCR	1.46±0.037[b]	1.71±0.034[a]
Survival rate (%)	100±0.00[a]	100±0.00[a]

Values (means ± SE) having the same superscript letters are not significantly different.
[1]EMF is the electromagnetic field system

In the present trial, no fish mortality was recorded in both treatments (Table 5.3), and this could be related to the low magnetic intensity used. Moreover, the stocking density of fish was probably below the carrying capacity of these systems and did not reach the threshold at which survival rates would be affected. The survival rates of fish in the present trial (100%) were higher than the 80.9% survival rate reported by Krzemieniewski et al. (2004) for the larval of wels catfish (*Silurus glanis)* treated with the magnetized water in a RAS and the 88.89-95.83% reported by Hassan et al. (2019) for Jade Perch (*Scortum barcoo)* juveniles reared in a RAS under the exposure of different magnetic field intensities. The higher survival rate was achieved in the present trial might be related to the duration of exposure, the field intensity and the variation in the sensitivity of different species.

The results of this experiment revealed that the growth of common carp and feed utilization efficiency improved after the exposure to the electromagnetic field of 0.8 mT in IRASs. The growth of plants that used as a biofilter medium in the IRASs also improved when they exposed to the electromagnetic field of 0.8 mT. However, the electromagnetic field

had no significant effect on water quality parameters in this trial. According to these improvements, the use of the magnetic water technique and plant based biofilters in the IRAS can be valuable for increasing the profitability of these systems. Further investigations are necessary to determine the ideal intensity of the magnetic field, which can positively affect the water quality and the growth performance of cultured species since the responses of different species of fish differ under the exposure of the magnetic fields.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

Integrated recirculating aquaculture systems were developed to overcome the problems of nutrient overloading in the environment and problems associated with the recirculating systems (for example, setup and operation costs, the delay of introducing cultured species to the system during activation phases of biological filters, diseases, animal welfare, and the accumulations of the nitrate and phosphorus concentrations). However, several factors such as fish species, fish size, fish density, temperature, plant species, and harvesting rate of plants can affect the nutrient removal rates and growth of cultured species in IRASs. In order to recycle wastes and produce plant biomass in the IRASs, it is necessary to optimize the recycling rates of nitrogen and phosphorus. Thus, the research aimed to evaluate the nutrient removal capacities at different modules of the IRAS for rearing common carp (*Cyprinus carpio*), considering the effects of different plant species (*Lemna minor, Hygroryza aristata, and Phyllanthus fluitans*), size of fish, harvesting biomass of plants, and magnetic water treatment technique.

The research results proved that the use of plant based biofilters (*L. minor, H. aristata* and *P. fluitans*) in the IRASs was effective in maintaining water quality, removing nutrients, adding harvestable products and providing good conditions for common carp growth and survival. The nutrient uptake capacities of the tested plant based biofilters were different and strongly influenced by the growth rate of plants, which is affected by the environmental conditions. The research reveals that the *H. aristata* was the strongest plant in removing nutrients among the tested plant species, followed by *L. minor*. However, the bacterial biofilm in the moving-bed filter was the superior filter to reduce high concentrations of NH_4-N and NO_2-N.

The research results also showed that the increase in the initial body size of stocked fish did not affect the removal efficiencies of TAN, NO_2-N, and NO_3-N; and both the bacterial biofilm and plant based biofilters (*H. rotundifolia*) were independent of the fish size. However, the bacterial biofilm filter showed higher removal rates of TAN and NO_2-N; while, the plant based biofilter (*H. rotundifolia*) had higher removal rates of NO_3-N. The results also showed that the increase in the initial body size of fish significantly decreased the TAN excretion into the fish tank and the specific growth rate of fish.

The research results also revealed that increasing the biweekly harvested biomass of watercress plants (*Nasturtium officinale*) in the IRASs decreased the growth of the plants, while it did not affect the growth of the common carp. The 0% and 25% harvested systems were recorded to have the highest plant biomass production. Watercress plants were efficient in removing nutrients generated by fish in aquaponic systems. However, increasing the harvested biomass of watercress plants decreased the NO_3-N and PO_4-P removal efficiencies, while it did not affect the NH_3-N and NO_2-N removal efficiencies. The 0% and 25% harvested systems were recorded to have the highest NO_3-N and PO_4-P removal efficiencies.

The research results showed that the use of magnetized water in the IRASs increased the specific growth rate of common carp and decreased the feed conversion ratio. The growth of plants (*L. minor*) that used as a biofilter medium in the IRASs also improved after exposure to the magnetized water. However, the magnetized water had no significant effects on the concentrations of NH_4-N, NO_2-N, and NO_3-N in the IRASs.

4.2. Recommendations

Based on the outcomes from the present research, the following recommendations are made:

The *H. aristata* is a more suitable plant to be used in removing nutrients, adding harvestable products and reducing the overall cost of the production systems, followed by *L. minor*, while the bacterial biofilm filter from a technical point of view is the strongest biofilter to reduce high concentrations of NH_4-N and NO_2-N. Regardless of the suitability of the bacterial biofilm and plant based biofilters, several factors must be considered when choosing appropriate biological filters, such as space, cost and benefit analyses, system location, climatic conditions and discharge regulations.

The research also recommends that the small size of fish (initial body size of 33-46 g) should be stocked into the IRASs at the beginning of the rearing season to achieve better performance in fish. Moreover, the biweekly harvesting of less than 25% of the growing area of watercress plants is recommended for improving nutrient removal efficiency and sustaining the growth of both fish and plants in aquaponic systems. The research also suggests that the use of the magnetic water treatment technique in the IRAS can improve the growth of fish and plant, and this can increase the profitability, and makes these systems more cost-effective to be used.

5. NEW SCIENTIFIC RESULTS

Based on the results obtained from the four experiments conducted in the present research, the following points highlight the new scientific results and confirm the objectives of the research.

1. The use of plant based biofilter technique can be beneficial in decreasing nutrient overload, adding harvestable products and providing good conditions for common carp growth and survival. Among the tested plant species in the present research, *Hygroryza aristata* is a more suitable plant for removing nutrients, followed by *Lemna minor*. While from a technical point of view, the bacterial biofilm filter is the strongest biofilter to reduce high concentrations of NH_4-N and NO_2-N.

2. The size of fish did not affect the removal efficiencies of TAN, NO_2-N, and NO_3-N, and both the bacterial biofilm and plant based biofilters (*H. rotundifolia*) were independent of the fish size. The research shows that small size fish (initial size of 33-46 g) has better performance in the IRAS. This size of fish should be stocked into the IRASs at the beginning of the rearing season to achieve better performance in fish.

3. The biweekly harvesting of less than 25% of the growing area of watercress plants is recommended for improving nutrient removal efficiency and sustaining the growth of both fish and plants in the IRASs (Aquaponics).

4. Regarding the potential of using magnetized treated water in the IRASs, the research results show that the use of magnetized water in the IRASs can improve the growth performance of both fish and plants. However, the magnetized water had no significant effects on the concentrations of NH_4-N, NO_2-N, and NO_3-N in the IRASs.

6. REFERENCES

Abbas, H.H. 2006. Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. *Pakistan Journal of Biological Sciences*, 9, 2215-2221.

Ahmed, A.R., Moody, A.J., Fisher, A., Davies, S.J. 2013. Growth performance and starch utilization in common carp (*Cyprinus carpio* L) in response to dietary chromium chloride supplementation. *Journal of Trace Elements in Medicine and Biology*, 27, 45-51.

Ahn, O., Petrell R.J., Harrison P.J. 1998. Ammonium and nitrate uptake by *Laminaria saccharina* and *Nereocystis luetkeana* originating from a salmon sea cage farm. *Journal of Applied Phycology* 10,333-340.

Akinbile, C.O., Yusoff, M.S. 2011. Water hyacinth (*Eichhornia crassipes*) and lettuce (*Pistia stratiotes*) effectiveness in aquaculture wastewater treatment. *International Journal of phytoremediation,* 14, 201–211.

Ali, Y., Samaneh, R., Zohre, R., Mostafa, J. 2014. Magnetic Water Treatment in Environmental Management, A Review of the Recent Advances and Future Perspectives. *Current World Environment*, 9, 1008-1016.

Álvarez, J.A., Bécares, E. 2008. The effect of vegetation harvest on the operation of a surface flow constructed wetland. *Water SA, 34*, 645–650.

Ardiansyah, A., Fotedar, R. 2016. Water quality, growth and stress responses of juvenile barramundi (*Lates calcarifer* Bloch), reared at four different densities in integrated recirculating aquaculture systems. *Aquaculture*, 458, 113-120.

Atwood, H.L., Fontenot, Q.C., Tomasso, J.R., Isely, J.J. 2001. Toxicity of nitrite to Nile Tilapia: Effect of fish size and environmental chloride. *North American Journal of Aquaculture*, 63, 49-51.

Aure, J., Stigebrandt, A. 1990. Quantitative estimates of the eutrophication effects of fish farming on fjords. *Aquaculture*, 90, 135-156.

Badiola, M., Mendiola, D., Bostock, J. 2012. Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges. *Aquacultural Engineering*, 51, 26–35.

Bartlett, F., Neumann, D. 1998. Sensitivity of brown trout alevins (*Salmo trutta* L.) to nitrite at different chloride concentrations. *Bulletin of Environmental Contamination and Toxicology*, 60, 340-346.

Bartodziej, W.M., Blood, S.L., Pilgrim, K. 2017. Aquatic plant harvesting: An economical phosphorus removal tool in an urban shallow lake. *J. Aquat. Plant Manag.* 2017, *55*, 26–34.

Billard, R. 1999. *Carp: biology and culture*, Springer-Praxis, Chichester, UK. 335p.

Biswas, J.K., Sarkar, D., Chakraborty, P., Bhakta, J.N., Jana, B.B. 2006. Density dependent ambient ammonium as the key factor for optimization of stocking density of common carp in small holding tanks. *Aquaculture*, 261, 952-959.

Boyd, C. 1985. Chemical budgets for channel catfish ponds. *Transactions of the American Fisheries Society*, 114, 291–298.

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

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

Brizhik, L. 2014. Biological effects of pulsating magnetic fields: role of solitons. arXiv:1411.6576 [Physics, q-Bio]. (2014). https://arxiv.org/abs/1411.6576

Bucking, C. 2017. A broader look at ammonia production, excretion, and transport in fish: a review of impacts of feeding and the environment. *Journal of Comparative Physiology,* B 187,1-18.

Bunce, J.T., Ndam, E., Ofiteru, I.D., Moore, A., Graham, D.W. 2018. A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. *Front. Environ. Sci., 6*, 1–15.

Buzby, K.M., Lin, L.S. 2014. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering, 63*, 39-44.

Cahill, P., Hurd, C., Lokman, L. 2010. Keeping the water clean — Seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. *Aquaculture*, 306, 153-159.

Cai, R., Yang, H., He, J., Zhu, W. 2009. The effects of magnetic fields on water molecular hydrogen bonds. *Journal of Molecular Structure*, 938 (1), 15-19.

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

Carbonell, M., Martinez, E., Amaya, J. 2000. Stimulation of germination in rice (*Oryza sativa* L.) by a static magnetic field. *Electro-and Magnetobiology*, 19 (1), 121-128.

Chen, H., Hu, B., Charles, A. 1995. Chinese integrated fish farming: a comparative bio-economic analysis. *Aquaculture Research*, 26, 81–94.

Chen, S., Stechey, D., Malone, R. 1994. Suspended solids control in recirculating aquaculture systems. *In*: Timmons, M., Losordo, T., ed. *Aquaculture Water Reuse Systems: Engineering Design and Management*. Elsevier, Amsterdam, 61–100.

Cheng, X.Y., Chen, W.Y., Gu, B., Liu, X., Chen, F., Chen, Z., Zhou, X., Li, Y., Huang, H., Chen, Y. 2009. Morphology, ecology, and contaminant removal efficiency of eight wetland plants with differing root systems. *Hydrobiologia*, 623, 77-85.

Chien, Y.H., Tsai, W.S. 1985. Integrated pond culture: a type of spatially sequential polyculture. *Journal of the World Mariculture Society*, 16, 429-436.

Chin, K., Gng, S., Foo, S. 1993. A water treatment and recycling system for intensive fish farming. *Water Science and Technology*, 27, 141-148.

Chuntapa, B., Powtongsook, S., Menasveta, P. 2001. Control of water quality in high density prawn culture by integrating with the microalga, *Spirulina platensis*. In 27 th Congress on Science and Technology of Thailand, Technical Information Services (TIS)/KMUTT, Songkla, Thailand.

Colt, J. 2006. Water quality requirements for reuse systems. *Aquacultural Engineering*, 34,143-156.

Corpron, K.E., Armstrong, D.A. 1983. Removal of nitrogen by an aquatic plant, *Elodea densa*, in recirculating *Macrobrachium* culture systems. *Aquaculture*, 32, 347-360.

Crab, R. 2010. Bio-flocs technology: an integrated system for the removal of nutrients and simultaneous production of feed in aquaculture. PhD thesis, Ghent University, Belgium.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W. 2007. Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*, 270, 1–14.

Crab, R., Kochva, M., Verstrate, W. Avnimelech, Y. 2009. Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, 40, 105–112.

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

Delaide, B., Goddek, S., Gott, J., Soyeurt, H., Jijakli, M. 2016. Lettuce (*Lactuca sativa* L. var. Sucrine) growth performance in complemented aquaponic solution outperforms hydroponics. *Water,* 8,467.

Driever, S.M., van Nes, E.H., Roijackers, R.M.M. 2005. Growth limitation of *Lemna minor* due to high plant density. *Aquatic Botany*, 81, 245-251.

DUAN, P., CHANG, Z., XU, Y., BAI, X., WANG, F., ZHANG, L. 2013. Hydrothermal processing of duckweed: Effect of reaction conditions on product distribution and composition. *Bioresource Technology,* 135**,** 710-719.

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

Eding, E., Klapwijk, A., Verreth, J. 2003. Design and performance of an upflow sludge blanket reactor in a ‘‘zero’’ discharge recirculation system. *In*: Chopin, T., Reinertsen, H., ed. *EBT Beyond Monoculture*. Trondheim, Norway, 172–173 (Special Publication).

Enache, I., Cristea, V., Ionescu, T., Ion, S. 2011. The influence of stocking density on the growth of common carp, *Cyprinus carpio,* in a recirculating aquaculture system. *AACL Bioflux*, 4,146-153.

Endut, A., Jusoh, A., Nora'aini, A. 2011. Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. *Desalination and Water Treatment,* 32, 422–430.

Engle, C. 2015. Economics of Aquaponics. *Southern Regional Aquaculture Center*. SRAC Publication No. 5006.

Estim, A., Saufie, S., Mustafa, S. 2019. Water quality remediation using aquaponics sub-systems as biological and mechanical filters in aquaculture. *J. Water Process. Eng.*, 30, 100566.

Fang, Y. Y., Babourina, O., Rengel, Z., Yang, X. E., Pu, P. M. 2007. Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. *Annals of Botany,* 99, 365-370.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Feeley, M.W., Benetti D.D., Ault J.S. 2007. Elevated oxygen uptake and high rates of nitrogen excretion in early life stages of the cobia *Rachycentron canadum* (L.), a fast-growing subtropical fish. *Journal Fish Biology*, 71, 1662-1678.

Ferdoushi, Z., Haque, F., Khan, S. and Haque, M. 2008. The Effects of two Aquatic Floating Macrophytes (*Lemna* and *Azolla*) as Biofilters of Nitrogen and Phosphate in Fish Ponds. *Turkish Journal of Fisheries and Aquatic Sciences*, 8, 253-258.

Folke, C., Kautsky, N. 1989. The role of ecosystems for a sustainable development of aquaculture. *Ambio*, 18, 234–243.

Foy, R., Rosell, R. 1991. Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. *Aquaculture*, 96, 17–30.

Franco-Nava, M.A., Blancheton, J.P., Deviller, G., Charrier, A., Le-gall, J.Y. 2004. Effect of fish size and hydraulic regime on particulate organic matter dynamics in a recirculating aquaculture system elemental carbon and nitrogen approach. *Aquaculture*, 239,179-198.

Gabrielli, C., Jaouhari, R., Maurin, G., Keddam, M. 2001. Magnetic water treatment for scale prevention. *Water Research*, 35(13), 3249-3259.

Gichana, Z., Meulenbroek, P., Ogello, E., Drexler, S., Zollitsch, W., Liti, D., Akoll, P. Waidbacher, H. 2019. Growth and Nutrient Removal Efficiency of Sweet Wormwood (*Artemisia annua*) in a Recirculating Aquaculture System for Nile Tilapia (*Oreochromis niloticus*). *Water, 11*, 923.

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

Goddek, S., Joyce, A., Kotzen, B., Burnell, G.M. 2019. Aquaponics Food Production Systems (p. 619). Springer Nature.

Groeneweg, J., Sellner, B., Tappe, W. 1994. Ammonia oxidation in *nitrosomonas* at NH_3 concentrations near km: Effects of pH and temperature. *Water Research*, 28, 2561-2566.

Guan, B., Hu, W., Zhang, T., Duan, M., Li, D., Wang, Y., Zhu, Z. 2010. Acute and chronic un-ionized ammonia toxicity to 'all-fish' growth hormone transgenic common carp (*Cyprinus carpio* L.). *Chinese Science Bulletin*, 55, 4032-4032.

Guerdat, T.C., Losordo, T.M., Classen, J.J., Osborne, J.A., Delong, D.P. 2010. An evaluation of commercially available biological filters for recirculating aquaculture systems. *Aquacultural Engineering*, 42, 38-49.

Hach, 2009. DR/890 Colorimeter Procedures Manual. Ninth edition, Hach Co., Loveland, Colorado, USA, 614 pp.

Hach, 2019. DR/3900 *Spectrophotometer Procedure Manual*, HACH Company: Loveland, CO, USA.

Han, P., Vijayaraghavan, K., Reuben, S., Estrada, E.S., Joshi, U.M. 2013. Reduction of nutrient contaminants into shallow eutrophic waters through vegetated treatment beds. *Water Science Technology*, 68, 1280–1287.

Hao, O.J., Huang, J. 1996. Alternating aerobic-anoxic process for nitrogen removal: process evaluation. *Water Environment Research*, 68, 83-93.

Hargreaves, J.A. 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering*, 34, 344-363.

Hasan, M.R., Macintosh, D.J. 1986. Acute toxicity of ammonia to common carp fry. *Aquaculture*, 54, 97-107.

Hassan, S., Rahman, R. 2016. Effect of exposure to magnetic field on water properties and hatchability of *Artemia salina*. *ARPN Journal of Agricultural & Biological Sciences*, 11, 416-423. Available at: http://www.arpnjournals.org/jabs/research_papers/rp_2016/jabs_1116_829.pdf

Hassan, S., Rahman, R., Kamaruddin, R., Madlul, N. 2018b. Effect of exposure of African catfish (*Clarias batrachus*) to magnetic field on water properties and egg hatching. *Borneo Journal of Marine Science and Aquaculture*, 2, 54-59.

Hassan, S., Sulaiman, M., Abdul Rahman, R., Kamaruddin, R. 2018a. Effects of long term and continuous magnetic field exposure on the water properties, growth performance, plasma biochemistry and body composition of tilapia in a recirculating aquaculture system. *Aquacultural Engineering*, 83, 76-84.

Hassan, S., Sulaiman, M., Madlul, N., Fadel, A., Abdul Rahman, R. 2019. Influence of continuous magnetic field exposure on water properties and subsequent effects on the growth performance, plasma biochemistry, nutritive value and liver histopathology of Jade Perch *Scortum barcoo* in a recirculating system. *Aquaculture Research*, 00, 1-11.

Hastie, B.A. 1992. *The use of aquatic plants in wastewater treatment: a literature review*. TEXAS UNIVERSITY AT AUSTIN.

Helfrich, L.A., Libey, G. 1990. *Fish farming in recirculating aquaculture systems (RAS)*. Department of Fisheries and Wildlife Sciences, Virginia Tech, New York, 19 pp.

Horváth, L., Tamas, G., Seagrave, C. 2002. *Carp and pond fish culture. 2nd edition*. Oxford, UK, Blackwell science Ltd., 185 pp.

Houlihan D., Boujard T., Jobling M. 2001. *Food intake in fish*. Blackwell, Carlton, Victoria, Australia, 418 pp.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A., Khanal, S. 2015. Effect of plants pieces on nitrogen recovery in aquaponics. *Bioresource Technology*, 188, 92–98.

Huisman, E.A. 1976. Food conversion efficiencies at maintenance and production levels for carp, *Cyprinus carpio* L, and rainbow trout, *Salmo gairdneri* Richardson. *Aquaculture*, 9, 259-273.

Hulata, G. 1995. A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture*, 129, 143-155.

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 Aquac. 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.

Ibraheim, M.H., Khater, Z.Z.K. 2013. The Effect of Electromagnetic Field on Water and Fish *Clarias Garpienus*, Zagazig, Egypt. *Life Science Journal*, 10(4), 3310-3324.

Iguchi, K., Ogawa, K., Nagae, M., Ito, F. 2003. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture*, 220, 515–523.

Ikuta, K., Yamaguchi, M. 2005. The present state of carp fisheries and aquaculture in Japan. *Bulletin of Fisheries Research Agency*, 2, 55-58.

Iqbal, F., Qureshi, I.Z., Ali, M. 2004. Histopathological changes in kidney of common carp, *Cyprinus carpio*, following nitrate exposure. *Journal of Research Science*, 15, 411-418.

Irhayyim, T., Fotedar, R. 2019. Effects of fish size and biofiltration techniques on water quality and nitrogen removal efficiency in recirculating aquaculture systems. *AACL Bioflux*, 12(5),1606-1616.

Jeke, N.N., Zvomuya, F., Cicek, N., Ross, L., Badiou, P. 2019. Nitrogen and phosphorus phytoextraction by Cattail (*Typha spp.*) during wetland-based phytoremediation of an end-of-life municipal lagoon. *J. Environ. Qual., 48*, 24–31.

Jha, P., Barat, S. 2005. The effect of stocking density on growth, survival rate, and number of marketable fish produced of Koi carps, *Cyprinus carpio* vr. *koi* in concrete tanks. *Journal of Applied Aquaculture*, 17, 89-102.

Jhingran, V.G., Pullin, R.S.V. 1985. *A hatchery manual for the common, Chinese and Indian major carps*. Asian Development Bank, Manila, Philippines, 191p.

Jianbo, L., Zhihui, F., Zhaozheng, Y. 2008. Performance of a water hyacinth (*Eichhornia crassipes*) system in the treatment of wastewater from a duck farm and the effects of using water hyacinth as duck feed. *Journal of Environmental Sciences*, 20, 513-519.

Jinadasa, K., Tanaka, N., Sasikala, S., Werellagama, D., Mowjood, M., Ng, W. 2008. Impact of harvesting on constructed wetlands performance—A comparison between *Scirpus grossus* and *Typha angustifolia*. *J. Environ. Sci. Health A, 43*, 664–671.

Jo, J.Y., Ma, J.S., Kim, I.B. 2002. Comparisons of four commonly used aquatic plants for removing nitrogen nutrients in the intensive bioproduction Korean (IBK) recirculating aquaculture system. In Proceeding of the 3rd International Conference on Recirculating Aquaculture, Roanoke, Virginia, 25-29 pp.

Jobling, M. 1993. Bioenergetics: feed intake and energy partitioning. *In*: *Fish ecophysiology.* Rankin J. C., Jensen F. B. (eds), pp. 1-44. London, Chapman and Hall, 421 pp.

Jones, A., Preston, N., Dennison, W. 2002. The efficiency and condition of oysters and macroalgae used as biological filters of shrimp pond effluent. *Aquaculture Research*, 33 (1), 1-19.

Jones, C., Olson-rutz, K., Dinkins, C. 2015. *Nutrient Uptake Timing by Crops, to Assist with Fertilizing Decisions*, Montana State University: Bozeman, MT, USA, p. 8.

Jørgensen, E., Christiansen, J., Jobling, M. 1993 Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture*, 110, 191-204.

Kamstra, A., Blom, E., Terjesen, B.F. 2017. Mixing and scale affect moving bed biofilm reactor (MBBR) performance. *Aquacultural Engineering*, 78, 9-17.

Karakatsouli, N., Papoutsoglou, E., Sotiropoulos, N., Mourtikas, D., Stigen-Martinsen, T., Papoutsoglou, S. 2010. Effects of light spectrum, rearing density and light intensity on growth performance of scaled and mirror Common Carp *Cyprinus carpio* reared under recirculating system conditions. *Aquacultural Engineering*, 42, 121-127.

Karnai, L., Szűcs, I. 2018. Outlooks and perspectives of the common carp production. *Roczniki Naukowe* , 2018 (1230-2019-3607), 64-72.

Kerepeczki, A., Gal, D., Szabo, P., Pekar, F. 2003. Preliminary investigations on the nutrient removal efficiency of a wetland-type ecosystem. *Hydrobiologia*, 506, 665-670.

Kestemont, P. 1995. Different systems of carp production and their impacts on the environment. *Aquaculture*, 129, 347-372.

Kim, E., Yoo, S., Ro, H.Y., Han, H.J., Baek, Y.W., Eom, I.C., Kim, H.M., Kim, P. and Choi, K. 2013. Aquatic toxicity assessment of phosphate compounds. *Environmental health and toxicology*, 28, 1-7.

Kim, S.Y., Geary, P.M. 2001. The impact of biomass harvesting on phosphorus uptake by wetland plants. *Water Sci. Technol.*, *44*, 61–67.

Knaus, U., Palm, H. W. 2017. Effects of the fish species choice on vegetables in aquaponics under spring-summer conditions in northern Germany (Mecklenburg Western Pomerania). *Aquaculture*, 473, 62-73.

Kroupova, H., Prokes, M., Macova, S., Penaz, M., Barus, V., Novotny, L., Machova, J. 2010. Effect of nitrite on early-life stages of common carp (*Cyprinus carpio* L.). *Environmental Toxicology and Chemistry*, 29, 535-540.

Kroupova, H., Machova, J., Svobodova, Z. 2005. Nitrite influence on fish: a review. *Veterinární medicína*, 50, 461-471.

Krzemieniewski, M., Debowski, M., Janczukowicz, W., Pesta, J. 2003. Changes of tap water and fish-pond water properties induced by magnetic treatment. *Polish Journal of Natural Science*, 14, 459-474.

Krzemieniewski, M., Teodorowicz, M., Debowski, M., Pesta, J. 2004. Effect of a constant magnetic field on water quality and rearing of European sheatfish *Silurus glanis* L. larvae. *Aquaculture Research*, 35(6), 568-573.

Lam, S., Ma, N., Jusoh, A., Ambak, M. 2015. Biological nutrient removal by recirculating aquaponic system: optimization of the dimension ratio between the hydroponic & rearing tank components. Int. *Biodeterioration & Biodegradation*, 102, 107–115.

Lankin, K.F., Peck, M.A., Buckley, L.J., Bengtson, D.A. 2008. The effects of temperature, body size and growth rate on energy losses due to metabolism in early life stages of haddock (*Melanogrammus aeglefinus*). *Marine Biology*, 155, 461-472.

Lee, H., Shoda, M. 2008. Removal of COD and colour from livestock wastewater by the Fenton method. *Journal of Hazardous Materials*, 153, 1314–1319.

Lekang, O.I., Kleppe, H. 2000. Efficiency of nitrification in trickling filters using different filter media. *Aquacultural Engineering*, 21, 181-199.

Lewis, W.M., Morris, D.P. 1986. Toxicity of nitrite to fish: A review. *Transactions of the American Fisheries Society*, 115, 183-195.

Li, X., Deng, Y., Li, X., Ma, X., Wang, J., Li, J. 2020. Integration of Marine Macroalgae (*Chaetomorpha maxima*) with a Moving Bed Bioreactor for Nutrient Removal from Maricultural Wastewater. *Archaea*, *2020*.

Lin, C., Jaijen, K., Muthuwan, V. 1993. Integration of intensive and semi-intensive aquaculture: concept and example. *CRSP Research Reports*, 93-54.

Lin, Y., Jing, S., Lee, D. 2003. The potential use of constructed wetlands in a recirculating aquaculture system for shrimp culture. *Environmental Pollution*, 123, 107–113.

Lin, Y., Jing, S., Lee, D., Wang, T. 2002. Nutrient removal from aquaculture wastewater using a constructed wetlands system. *Aquaculture*, 209, 169–184.

Ling, J., Chen, S. 2005. Impact of organic carbon on nitrification performance of different biofilters. *Aquacultural Engineering*, 33, 150-162.

Liu, S., Yang, F., Meng, F., Chen, H., Gong, Z. 2008. Enhanced anammox consortium activity for nitrogen removal: Impacts of static magnetic field. *Journal of Biotechnology*, 138, 96-102.

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

Lyssenko, C., Wheaton, F. 2006. Impact of positive ramp short-term operating disturbances on ammonia removal by trickling and submerged-upflow biofilters for intensive recirculating aquaculture. *Aquacultural Engineering*, 35, 26-37.

Malik, V., Mohammad, I., Pranita, P. 2014. Ecology and conservation of Rare *Hygroryza aristata* (Retz.) Nees ex Wight and Arn. *Biological Forum – An International Journal*, 6, 57-58.

Malone, R., Pfeiffer, T. 2006. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquacultural Engineering*, 34, 389-402.

Martinez-Porchas, M., Martinez-Cordova, L., Porchas-Cornejo, M., Lopez-Elias, J. 2010. Shrimp polyculture: a potentially profitable, sustainable, but uncommon aquaculturual practice. *Reviews in Aquaculture*, 2, 73–85.

Martins, C., Eding, E., Verdegem, M., Heinsbroek, L., Schneider, O., Blancheton, J., D'orbcastel, E., Verreth, J. 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, 43, 83-93.

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* 2019, *14*.

Mcchesney, C. 1994. Literature review of the genus *Hydrocotyle* L. (Apiaceae), with particular emphasis on *Hydrocotyle ranunculoides* L.f. Report No. 18, Swan River Trust, Perth, Australia, pp. 1-39.

Menasveta, P., Panritdam, T., Sihanonth, P., Powtongsook, S., Chuntapa, B., Lee, P. 2001. Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock. *Aquacultural Engineering*, 25 (1), 35-49.

Midlen, A., Redding, T. 1998. *Environmental Management for Aquaculture*. London, UK: Chapman and Hall.

Msuya, F., Kyewalyanga, M., Salum, D. 2006. The performance of the seaweed *Ulva reticulata* as a biofilter in a low-tech, low-cost, gravity generated water flow regime in Zanzibar, Tanzania. *Aquaculture*, 254 (1-4), 284-292.

Mukherjee, A., Kalita, P., Unni, B., Wann, S., Saikia, D., Mukhopadhyay, P. 2010. Fatty acid composition of four potential aquatic weeds and their possible use as fish feed nutraceuticals. *Food Chemistry*, 123, 1252-1254.

Nakphet, S., Ritchie, R., Kiriratnikom S. 2017. Aquatic plants for bioremediation in red hybrid tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*) recirculating aquaculture. *Aquaculture International*, 25, 619-633.

Neori, A., Chopin, T., Troell, M., Buschmann, A., Kraemer, G., Halling, C., Shpigel, M., Yarish, C. 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, 231 (1-4), 361-391.

Neori, A., Krom, M. 1991. Nitrogen and phosphorous budgets in an intensive marine fishpond: the importance of microplankton. *In*: Cowey, C., Cho, C., eds. *Nutritional Strategies and Aquaculture Waste*. University of Guelph. Guelph, Ontario, Canada, 223–230.

Neori, A., Msuya, F., Shauli, L., Schuenhoff, A., Kopel, F., Shpigel, M. 2003. A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. *Journal of Applied Phycology*, 15, 543-553.

Nhan, H.T., Tai, N.T., Liem, P.T., Ut, V.N., Ako, H. 2019. Effects of different stocking densities on growth performance of Asian swamp eel *Monopterus albus*, water quality and plant growth of watercress *Nasturtium officinale* in an aquaponic recirculating system. *Aquaculture*, *503*, 96–104.

Nofouzi, K., Sheikhzadeh, N., Jassur, D., Ashrafi-Helan, J., Shabazfar, A., Tukmechi, A., Nagshara, H., Baradaran, B., Mahmoudi, R. 2017. Effects of low frequency electromagnetic fields on growth, total antioxidant activity and morphology of the intestine in rainbow trout (*Oncorhynchus mykiss*). *Iranian Journal of Veterinary Science and Technology*, 8, 18-24.

Ormandy, E., Dale, J., Griffin, G. 2011. Genetic engineering of animals: ethical issues, including welfare concerns. *The Canadian Veterinary Journal*, 52, 544–550.

Oyugi, D., Cucherousset, J., Baker, D., Britton, J. 2012. Effects of temperature on the foraging and growth rate of juvenile common carp, *Cyprinus carpio*. *Journal of Thermal Biology*, 37(1), 89-94.

Pa´ez-Osuna, F. 2001. The environmental impact of shrimp aquaculture: causes, effects and mitigating alternatives. *Environmental Management*, 28, 131-140.

Pagand, P., Blancheton, J., Lemoalle, J., Casellas, C. 2000. The use of high rate algal ponds for the treatment of marine effluent from a recirculating fish rearing system. *Aquaculture Research*, 31, 729–736.

Palachek, R.M., Tomasso, J.R. 1984. Nitrite toxicity to fathead minnows: Effect of fish weight. *Bulletin of Environmental Contamination and Toxicology*, 32, 238-242.

Papoutsoglou, S.E., Mylonakis, G., Miliou, H., Karakatsouli, N.P., Chadio, S. 2000. Effects of background color on growth performances and physiological responses of scaled carp (*Cyprinus carpio* L.) reared in a closed circulated system. *Aquacultural Engineering*, 22, 309-318.

Piedrahita, R. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture*, 226, 35–44.

Pillay, T.V.R., Kutty, M.N. 2005. *Aquaculture Principles and Practices*, 2nd Ed, Blackwell, Oxford, UK. 624p.

Popp, J., Váradi, L., Békefi , E., Péteri , A., Gyalog, G., Lakner, Z., Oláh, J. 2018. Evolution of Integrated Open Aquaculture Systems in Hungary: Results from a Case Study. *Sustainability*, 10, 1-19.

Porath, D., Pollock, J. 1982. Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquatic Botany*, 13, 125-131.

Porter, C., Krom, M., Robbins, M., Bricknell, L., Davidson, A. 1987. Ammonia excretion and total N budget for gilthead seabream *Sparus aurata* in marine fish-ponds. *Aquaculture*, 59, 299– 315.

Post, J.R., Lee, J.A. 1996. Metabolic ontogeny of teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, 53, 910-923.

Rahman, M.M. 2015. Role of common carp (*Cyprinus carpio*) in aquaculture production systems. *Frontiers in Life Science*, 8, 399–410.

Rakocy, J. 1999. the status of aquaponics: Part 2. *Aquaculture Magazine*, 25, 64–70.

Rakocy, J. 2007. Aquaponics: integrated fish and plant culture. *In*: Timmons, M., Ebeling, J., eds. *Recirculating Aquaculture*. NRAC Publ. no. 01-007. Cayuga Aqua Ventures, Ithaca, NY, pp. 767–822, 975.

Rakocy, J., Allison, R. 1981. Evaluation of a closed recirculating system for the culture of tilapia and aquatic macrophytes. Bio-Engineering Symposium for Fish Culture. Fish Culture Section, American Fisheries Society, Publ. 1, pp. 296-307.

Rakocy, J., Shultz, R., Bailey, D., Thoman, E. 2003. Aquaponic production of tilapia and basil: comparing a batch and staggered cropping system. *South Pacific Soil*, 63–69.

Rakocy, J.E., Bailey, D.S., Shultz, R.C., Thoman, E.S. 2004. Update on tilapia and vegetable production in the UVI aquaponic system. *In*: *New Dimensions on Farmed Tilapia, Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, Manila, Philippines, September 12–16*, Remedios, B., Graham, M., Kevin, F., Center Roxas Boulevard: Manila, Philippines.

Rakocy, J.E., Masser, M.P., Losordo, T.M. 2016. Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture; *Southern Regional Aquaculture Center*: Stoneville, MS, USA, pp. 1–16.

Redding T., Todd S., Midlen A. 1997. The treatment of aquaculture wastewaters - a botanical approach. *Journal of Environmental Management*, 50, 283-299.

Reina, F., Pascual, L. 2001. Influence of a stationary magnetic field on water relations in lettuce seeds. Part I: theoretical considerations. *Bio Electro Magnetics*, 22, 589-595.

Ridha, M.T., Cruz, E.M. 2001. Effect of biofilter media on water quality and biological performance of the Nile tilapia *Oreochromis niloticus* L. reared in a simple recirculating system. *Aquacultural Engineerig*, 24, 157-166.

Robb, T., Abrahams, M.V. 2003. Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? *Journal of Fish Biology*, 62, 1067-1081.

Rodriguez, M., Petitclerc, D., Nguyen, D., Block, E., Burchard, J. 2002. Effect of electric and magnetic fields (60 Hz) on production, and levels of growth hormone and insulin-like growth factor 1 in lactating, pregnant cows subjected to short days. *Journal of Dairy Science*, 85, 2843-2849.

Rodrigueza, M., Montaño, M. 2007. Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms. *Journal of Applied Phycology*, 19 (6), 755-762.

Roongtanakiat, N., Tangruangkiat, S., Meesat, R. 2007. Utilization of vetiver grass (*Vetiveria zizanioides*) for removal of heavy metals from industrial wastewaters. *Science Asia*, 33, 397-403.

Roque d'Orbcastel, E., Blancheton, J.P., Aubin, J. 2009. To-wards environmentally sustainable aquaculture: Comparison between two trout farming systems using Life Cycle Assessment. *Aquacultural Engineering*, 40, 113–119.

Roy, K., Vrba, J., Kaushik, S. J., and Mraz, J. 2019. Feed-based common carp farming and eutrophication: is there a reason for concern?. *Reviews in Aquaculture*, 1-23.

Ruane, N., Carballo, E., Komen, J. 2002. Increased stocking density influences the acute physiological stress response of common carp *Cyprinus carpio* (L.). *Aquaculture Research*, 33, 777–784.

Saeed, T., Sun, G. A. 2012. review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.*, 112, 429–448.

Santos, L.O., Deamici, K.M., Menestrino, B.C., Garda-Buffon, J., Costa, J.A. 2017. Magnetic treatment of microalgae for enhanced product formation. *World Journal of Microbiology and Biotechnology*, 33(9), 169.

Schneider O., Sereti V., Eding E. H., Verreth J. A. 2005. Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquacultural Engineering*, 32, 379-401.

Shete, A.P., Verma, A.K., Chadha, N.K., Prakash, C., Chandrakant, M.H., Nuwansi, K.K.T. 2017. Evaluation of different hydroponic media for mint (*Mentha arvensis*) with common carp (*Cyprinus carpio*) juveniles in an aquaponic system. *Aquac. Int., 25*, 1291–1301.

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*, 53–57.

Siddiqui, A., Al-Harbi, A. 1999. Nutrient budgets in tanks with different stocking densities of hybrid tilapia. *Aquaculture* 170, 245– 252.

Simplício, N., Muniz, D., Rocha, F., Martins, D., Dias, Z., Farias, B., Oliveira-Filho, E.C. 2017. Comparative analysis between ecotoxicity of nitrogen-, phosphorus-, and potassium-based fertilizers and their active ingredients. *Toxics*, *5*, 2.

Singh, O., Labana, S., Pandey, G., Budhiraja, R., Jain, R. 2003. Phytoremediation: An overview of metallic ion decontamination from soil. *Applied Microbiology and Biotechnology*, 61, 405-412.

Singhal, V., Rai, J. 2003. Biogas production from water hyacinth and channel grass used for phytoremediation of industrial effluents. *Bioresource Technology*, 86,221–225.

Smith, E.N. 2007. Watercress (*Nasturtium officinale*) Production Utilizing Brook Trout (*Salvelinus fontinalis*) Flow-Through Aquaculture Effluent. Master's Thesis, West Virginia University, Morgantown, WV, USA.

Snow, A., Ghaly, A. 2008. A Comparative Study of the Purification of Aquaculture Wastewater Using Water Hyacinth, Water Lettuce and Parrot's Feather. *American Journal of Applied Sciences*, 5 (4), 440-45.

Solbé, J.F., Cooper, V.A., Willis, C.A. and Mallett, M.J. 1985. Effects of pollutants in fresh waters on European non-salmonid fish I: Non-metals. *Journal of Fish Biology*, 27,197-207.

Sooknah, R. 2000. Review of the mechanisms of pollutant removal in water hyacinth systems. *Science and Technology Research Journal*, 6, 50-57.

Spasić, M.M., Poleksić, V.D., Stanković, M.I., Dulić, Z.P., Rašković, B.S., Živić, I.M., Ćirić, M.D., Relić, R.R., Vukojević, D.B., Bošković, D.D., Marković, Z.Z. 2010. Selective breeding programme of common carp (*Cyprinus carpio* L.) in Serbia-preliminary results. *Journal of Agricultural Sciences*, 55, 243-251.

Stowell, R., Ludwig, R., Colt, J., Tchobanoglous, T. 1981. Concepts in aquatic treatment design. *Journal of Environment Engineering*, 112, 885-894.

Summerfelt, S. 2006. Design and management of conventional fluidized-sand biofilters. *Aquacultural Engineering*, 34, 275-302.

Sun, S., Gao, L., He, S., Huang, J., Zhou, W. 2019. Nitrogen removal in response to plants harvesting in two kinds of enhanced hydroponic root mats treating secondary effluent. *Sci. Total Environ.*, 670, 200–209.

Suzuki, Y., Maruyama, T., Numata, H., Sato, H., Asakawa, M. 2003. Performance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel: Towards zero emission. *Aquac. Eng.*, *29*, 165–182.

Talbot, C. 1993. Some aspects of the biology of feeding and growth in fish. *The Proceedings of the Nutrition Society*, 52, 403-416.

Tan, S.N., Ge, L., Tow, T.S., Wong, W.S., Suresh, A., Chua, J.C. and Yong, J.W. 2014. Developing suitable phytofiltration systems for water cleansing in the tropics using suitable species including plant hyperaccumulators. Paper presented at the 8th International Conference for Serpentine Ecology, Kota Kinabalu, Sabah, Malaysia.

Tang, J., Zhao, W., Chi, J., Liu, G., Yu, X., Bian, L. 2015. Effects of magnetic treatment on growth and immune and digestive enzyme activity in the juvenile sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture*, 435, 437-441.

Tarazona, J.V., Muńoz, M., Ortiz, J.A., Nunéz, M., Camargo, J.A. 1987. Fish mortality due to acute ammonia exposure. *Aquaculture Research*, 18, 167-172.

Thurston, R.V., Russo, R.C. 1983. Acute toxicity of ammonia to Rainbow trout. *Transactions of the American Fisheries Society*, 112, 696-704.

Thurston, R.V., Russo, R.C., Phillips, G.R. 1983. Acute toxicity of ammonia to Fathead minnows. *Transactions of the American Fisheries Society*, 112, 705-711.

Tiaz, L., Zeiger, E. 2002. *Plant Physiology*. 3th ed. Sinauer Associates Inc., Massachusetts.

Tilak, K.S., Lakshmi, S.J., Susan, T.A. 2002. The toxicity of ammonia, nitrite and nitrate to the fish, *Catla catla*. *Journal of Environmental Biology*, 23, 147-149.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J. 2002. *Recirculating aquaculture systems.* Second edition, Cayuga Aqua Ventures, Ithaca, USA, 800 pp.

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

Tkalec, M., Malarić, K., Pevalek-Kozlina, B. 2005. Influence of 400, 900, and 1900 MHz electromagnetic fields on *Lemna minor* growth and peroxidase activity. *Bio Electro Magnetics*, 26, 185-193.

Troell, M. 2009. Integrated marine and brackishwater aquaculture in tropical regions. *In*: Soto, D., ed. Rome, *Integrated Mariculture: A global review*. FAO Fisheries and Aquaculture Technical Paper. No. 529.

Tseng, K.F., Wu, K.L. 2004. The ammonia removal cycle for a submerged biofilter used in a recirculating eel culture system. *Aquacultural Engineering*, 31, 17-30.

Van der Steen, P., Brenner, A., Oron, G. 1998. An integrated duckweed and algae pond system for nitrogen removal and renovation. *Water Science and Technology*, 38, 335-343.

Van Khoi L., Fotedar R. 2011. Integration of western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) and green seaweed (*Ulva lactuca* Linnaeus, 1753) in a closed recirculating aquaculture system. *Aquaculture*, 322–323, 201–209.

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

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

van Rijn, J., Shnel, N. 2001. Tilapia recirculating systems: design and performance of a zero-discharge system. *In*: AES Special Session: *International Recirculating Systems*, Orlando, FL, USA.

Vandeputte, M. 2003. Selective breeding of quantitative traits in the common carp (*Cyprinus carpio*): A review. *Aquatic Living Resources*, 16, 399-407.

Velichkova, K.N., Sirakov, I.N. 2013. The usage of aquatic floating macrophytes (*Lemna* and *Wolffia*) as biofilter in recirculation aquaculture system (RAS). *Turkish Journal of Fisheries and Aquatic Sciences*, 13, 87-96.

Verhofstad, M., Poelen, M., Van Kempen, M., Bakker, E., Smolders, A. 2017. Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants. *Aquacultural Engineering*, 106, 423–430

Vian, A., Davies, E., Gendraud, M., Bonnet, P. 2016. Plant responses to high frequency electromagnetic fields. *BioMed Research International*, 2016, 1-13.

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, 380, 48-65.

Vymazal, J., Kröpfelová, L., Švehla, J., Štíchová, J. 2010. Can multiple harvest of aboveground biomass enhance removal of trace elements in constructed wetlands receiving municipal sewage? *Ecol. Eng.*, 36, 939–945.

Wang, Q., Xie, H., Zhang, J., Liang, S., Ngo, H.H., Guo, W., Liu, C., Zhao, C., Li, H. 2014. Effect of plant harvesting on the performance of constructed wetlands during winter: Radial oxygen loss and microbial characteristics. *Environ. Sci. Pollut. Res.*, 22, 7476–7484.

Watson, C.A., Hill, J.E., Pouder, D.B. 2004. Species profile: koi and goldfish. *Southern Regional Aquaculture Center*, SRAC Publication No, 7201, 1-6 pp.

Wei B., Yu X., Zhang S., Gu L. 2011. Comparison of the community structures of ammonia-oxidizing bacteria and archaea in rhizoplanes of floating aquatic macrophytes. *Microbiological Research*, 166, 468-474.

Wersal, R.M., Madsen, J.D. 2012. Aquatic plants their uses and risks: A review of the global status of aquatic plants. FAO, Rome, Italy, 94 pp.

Wongkiew, S., Hu, Z., Chandran, K., Lee, J.W., Khanal, S.K. 2017. Nitrogen transformations in aquaponic systems: A review. *Aquac. Eng.*, *76*, 9–19.

Woynarovich, A., Bueno, P.B., Altan, Ö., Jeney, Z., Reantaso, M., Xinhua, Y., Van Anrooy, R. 2011. Better Management Practices for Carp Production in Central and Eastern Europe, the Caucasus and Central Asia. FAO, Fisheries and Aquaculture Technical Paper: Ankara, Turkey, p. 153.

Xia, H., Ma, X. 2006. Phytoremediation of ethion by water hyacinth (*Eichhornia crassipes*) from water. *Bioresource Technology*, 97, 1050-1054.

Xie, D., Yu, D., You, W., Wang, L. 2013. Morphological and physiological responses to sediment nutrients in the submerged macrophyte *myriophyllum spicatum*. *Wetlands*, 33, 1095–1102.

Yang, Z., Wang, Q., Zhang, J., Xie, H., Feng, S. 2016. Effect of plant harvesting on the performance of constructed wetlands during summer. *Water*, 8, 24.

Yaycili, O., Alikamanoglu, S. 2005. The effect of magnetic field on *Paulownia* tissue cultures. *Plant Cell Tissue and Organ Culture*, 83(1), 109-114.

Zachritz, W., Hanson, A., Sauceda, J., Fitzsimmons, K. 2008. Evaluation of submerged surface flow (SSF) constructed wetlands for recirculating tilapia production systems. *Aquacultural Engineering*, 39, 16–23.

Zheng, Y., Dzakpasu, M., Wang, X., Zhang, L., Ngo, H.H., Guo, W., Zhao, Y. 2018. Molecular characterization of long-term impacts of macrophytes harvest management in constructed wetlands. *Bioresour. Technol.*, 268, 514–522.

Zheng, Y., Wang, X.C., Ge, Y., Dzakpasu, M., Zhao, Y., Xiong, J. 2015. Effects of annual harvesting on plants growth and nutrients removal in surface-flow constructed wetlands in northwestern China. *Ecol. Eng.*, 83, 268–275.

Zhu, S. and Chen, S. 1999. An experimental study on nitrification biofilm performance using a series of reactor systems. *Aquacultural Engineering*, 20, 245-259.

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

7. SCIENTIFIC PUBLICATIONS OF THE AUTHOR

7.1. Articles in Peer-reviewed Journals

Irhayyim, T. 2019. Techniques of Nutrient Removal in Recirculating Aquaculture Systems: A review. *Hungarian Journal of Aquaculture and Fisheries*, 5/1, 7-14. http://www.agrarlapok.hu/sites/default/files/Hal%C3%A1szat%20diigatlis_2019_1.pdf

Irhayyim, T., Beliczky, G., Bercsényi, M. **(Accepted).** Nutrient bioremediation efficiency of bacterial biofilms and plant based biofilters in a recirculating common carp (*Cyprinus carpio*) culture system**.** *Iranian Journal of Fisheries Science*. In press (**IF: 0.711**)

Irhayyim, T., Fotedar, R. 2019. Effects of fish size and biofiltration techniques on water quality and nitrogen removal efficiency in recirculating aquaculture systems. *AACL Bioflux*, 12(5):1606-1616. http://www.bioflux.com.ro/docs/2019.1606-1616.pdf. **(Scopus)**

Irhayyim, T., Fehér, M., Lelesz, J., Bercsényi, M., Bársony, 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(5), 1419. https://doi.org/10.3390/w12051419. (**IF: 2.524**)

Irhayyim, T., Beliczky, G., Havasi, M., Bercsényi, M. 2020. Impacts of magnetic water treatment on water quality, feeding efficiency, and growth performance of common carp in integrated recirculating aquaculture systems. *Journal of Central European Agriculture*, 21(2), 246-255. https://doi.org/10.5513/jcea01/21.2.2672. **(Scopus)**

7.2. Full Articles in Conferences

Irhayyim, T., Beliczky, G., Havasi, M., Bercsényi, M. (2019). Water quality and growth performance of fish under the exposure effect of electromagnetic fields. XLIII. Halászati Tudományos Tanácskozás. 2019, 58-61. **ISSN: 0230-8312.**

Irhayyim, T., Bercsényi, M. (2019). Integration of Aquatic Plant (*Lemna minor*) and Bacterial Biofilms as Biofilter Media in a Recirculating Aquaculture System. The Eleventh Conference of Agronomy Students with international participation,14 -16 August 2019, University of Kragujevac, Faculty of Agronomy, Čačak, Serbia. The proceeding book, Vol (11), Issue (11), 397- 405. **ISSN: 2334-9883.**

7.3. Abstract in Conferences

Irhayyim, T., Beliczky, G. (2018). Comparison of four biofiltration types for water quality and growth performance of fish reared in recirculating aquaculture systems. 60thGeorgikon International Scientific Conference 2018, October 4-5, University of Pannonia, Georgikon Faculty, Keszthely. Abstract Volume, p. 70. **ISBN: 978-963-9639-91-1**.

Irhayyim, T., Beliczky, G., Havasi, M., Bercsényi, M. (2019). The influence of using magnetized treated water in an integrated recirculating aquaculture system. 61th Georgikon International Scientific Conference 2019, October 3-4, University of Pannonia, Georgikon Faculty, Keszthely. Abstract Volume, p. 96. **ISBN: 978-963-396-129-2**.

Irhayyim, T., Bercsényi M. (2019). Influences of initial body weight and biofilter type on inorganic nitrogen removal rates in recirculating aquaculture systems. Scientific Conference of Ph.D. Students of FAFR, FBFS and FHLE SUA in Nitra, 7th November 2019, Slovak University of Agriculture, Nitra, Slovakia, Proceedings of Abstracts, 2019, p. 14. **ISBN: 978-80-552-2083-3.**

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Tareq Irhayyim Saad Sulaimawi

Keszthely, Hungary,

9. APPENDICES

Appendix 1: Pictures of the First Experiment

Integrated Recirculating Aquaculture Systems designed to investigate different plant based biofilters with bacterial biofilm filter

Plant based biofilter with Duckweed *(Lemna minor)*

Plant based biofilter with Asian Watergrass plants *(Hygrorysa aristata)*

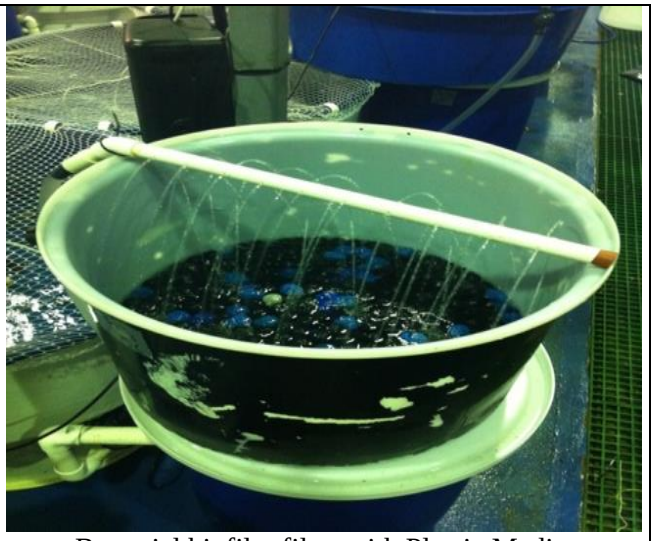
Plant based biofilter with Red Root Floater *(Phyllanthus fluitans)*

Bacterial biofilm filter with Plastic Media

Appendix 2: Pictures of the Second Experiment

Integrated Recirculating Aquaculture Systems designed to investigate different Size of Fish and Bio-filtration Types

Plant based biofilter with Pennywort Plants (*Hydrocotyle rotundifolia*)



Bacterial biofilm filter with Plastic Media

Large Size Fish used in the Second Experiment

Small Size Fish used in the Second Experiment

Appendix 3: Pictures of the Third Experiment

Start of the third Experiment

End of the third Experiment

Integrated Recirculating Aquaponic Systems designed in the third Experiment to test the harvesting of

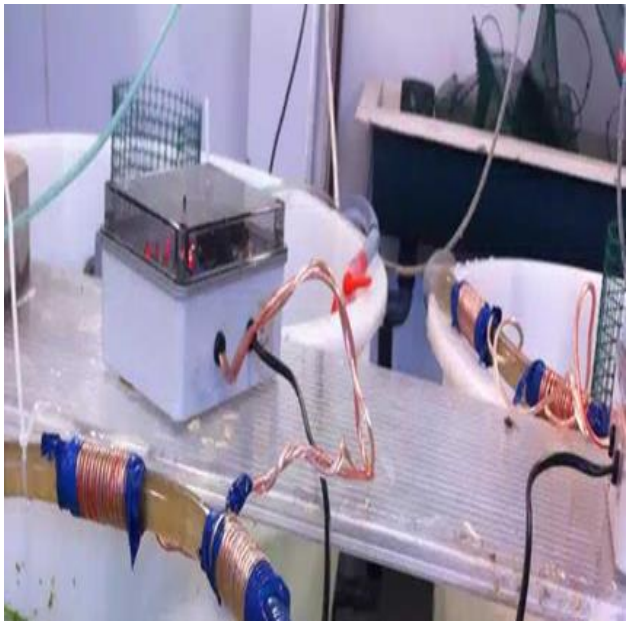
different biomasses of plants

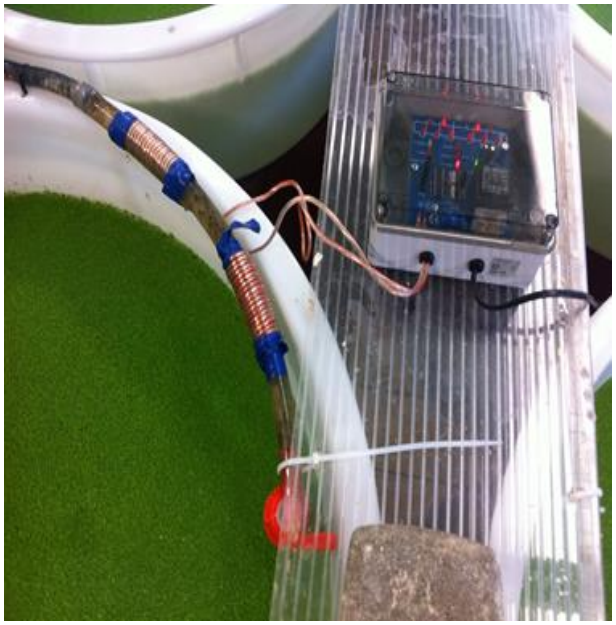
Watercress Plants (*Nasturtium officinale*) used in the Third Experiment

Appendix 4: Pictures of the Fourth Experiment

Integrated Recirculating Aquaculture Systems designed with the Electromagnetic Field Generator

Duckweed plants (*Lemna minor*) with the Electromagnetic Field Generator in the Fourth Experiment





Electromagnetic Field Generator Multi Plus used in the Fourth Experiment