Effects of fish size and biofiltration techniques on water quality and nitrogen removal efficiency in recirculating aquaculture systems

[1,2]Tareq Irhayyim, [1]Ravi Fotedar

[1] Curtin Aquaculture Research Laboratory, Department of Environment and Agriculture, Curtin University, 01 Turner Avenue, Bentley, Western Australia, 6102, Australia; [2] Georgikon Aquatic Research Laboratory, Georgikon Faculty, University of Pannonia, Keszthely, 8360, Hungary. Corresponding author: T. Irhayyim, tareqsaad72@yahoo.com

Abstract. The study aimed to evaluate and compare the effect of the initial size of fish and biofiltration types on the nitrogen cycling efficiencies in recirculating aquaculture systems. Two sizes (9.4±0.4 g and 107.4±9.0 g) of koi carp (*Cyprinus carpio haematopterus*) were tested with two most commonly used biological filters; an aquatic plant (*Hydrocotyle rotundifolia*) and bacterial biofilm of a trickle-down filter in 12 recirculating systems for 8 weeks. The efficiencies were evaluated by comparing fish growth and survival, water quality parameters, and the removal capacities of dissolved inorganic nitrogen between biofilters. The results showed that the increase in the initial stocking size of fish significantly decreased the specific growth rate and increased the feed conversion ratio. The fish size did not affect the removal efficiencies of total ammonia nitrogen, nitrite nitrogen and nitrate nitrogen; and both biofilters were independent of the fish size. However, the bacterial biofilm filter showed higher removal rates of total ammonia nitrogen and nitrite nitrogen; while, *H. rotundifolia* had higher nitrate nitrogen removal rates. Despite, both biofilters were efficient in maintaining water quality parameters, and providing an acceptable environment for growth and survival of koi; an inverse relationship was observed between the initial fish size and the total ammonia nitrogen excreted into the fish tank.
Key Words: aquatic plant, biofilters, fish body weight, koi carp.

Introduction. Recirculating aquaculture systems (RASs) offer many advantages in terms of reducing water consumption, better diseases 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 study 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 the 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 technique are critical to the technical and economic success of the RAS. A 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 aquatic plants (Redding et al 1997; Lekang & Kleppe 2000; Ridha & Cruz 2001; Jo et al 2002; Tseng & Wu 2004; Guerdat et al 2010; Velichkova & 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 study 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 most commonly used biological filters; an aquatic plant (*H. rotundifolia)* and bacterial biofilm of a trickle-down filter in recirculating koi rearing systems.

Material and Method

***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 prior to the experiment.

***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 rate 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.

***Experimental systems and rearing conditions**.* The trial was conducted from 17 June 2012 to 12 August 2012 and 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 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 70 L 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.

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 at 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 = $[(TAN_I - TAN_E) / TAN_I] \times 100\%$; where, TAN_I and TAN_E are the total ammonia nitrogen in the influent and effluent waters of biofilter respectively (Tseng & 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 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.

Results

Water quality parameters in fish tanks. Temperature, DO and pH among all treatments were not significantly different ($p > 0.05$) during the study period (Table 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 1). Small fish tanks which connected with *H. rotundifolia* filter had significantly higher ($p < 0.05$) means of TAN than the other treatments over the entire period of the study (Figure 1A). 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 1). The mean NO_2-N in both tanks of small and large fish which connected with *H. rotundifolia* remained constant below 0.80±0.021 mg L^{-1} (Figure 1C). The mean NO_3-N in both tanks of small and large fish which connected with bacterial biofilm filters were significantly higher ($p < 0.05$) than those connected with *H. rotundifolia* filters (Table 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 1). Similarly, both tanks of small and large fish had the same mean of NO_3-N ($p > 0.05$) when they connected with bacterial biofilm filter (Figure 1D).

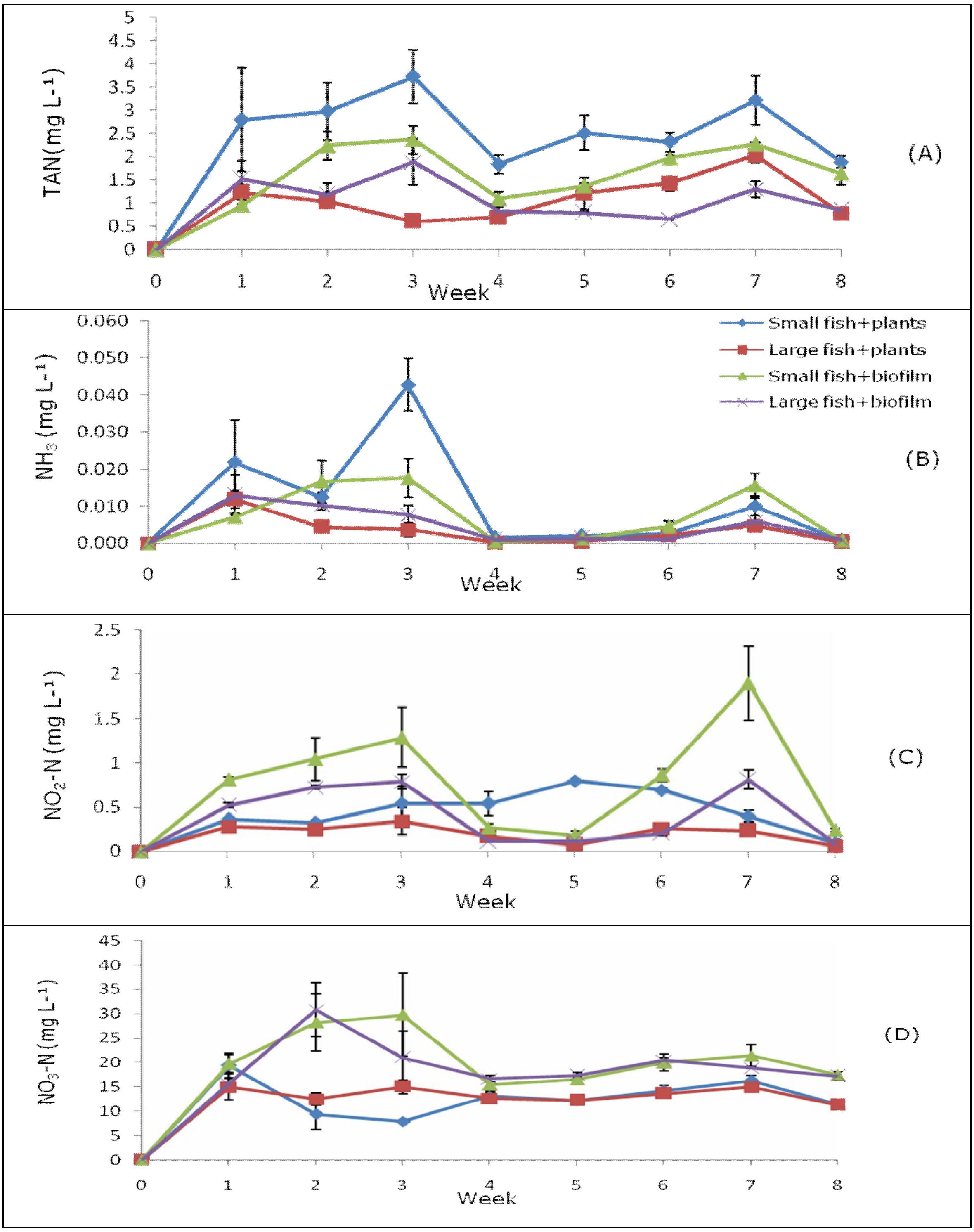

Figure 1. Weekly mean concentrations of (A) total ammonia nitrogen, (B) unionized ammonia, (C) nitrite-nitrogen, and (D) nitrate-nitrogen in tanks of *C. carpio haematopterus* reared in RAS for 8 weeks trial (error bars indicate the standard error).

Table 1

Overall mean water quality parameters of tanks used for culturing different size of kio 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 (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.

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 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 2). The mean TAN removal rate of all filters stocked with *H. rotundifolia* decreased as the trial progressed (Figure 2A); while the mean NO_2-N removal rates increased over time (Figure 2B). All filters stocked with bacterial biofilm showed a decreasing trend in the NO_3-N removal rates with the progression of the trial (Figure 2C).

Table 2

Overall mean removal rates of *H. rotundifolia* and bacterial biofilm filtration used with different size of kio 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 (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.

Growth and survival rates of koi. 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). The mean FCR of small fish was significantly lower ($p < 0.05$) than large fish (Table 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 which connected with bacterial biofilm filters (Table 3). After eight weeks of culture, there was no significant difference ($p > 0.05$) 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).

Table 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.

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. Helfrich & Libey (1990) reported that the capacity of the biofilter is influenced by the surface area of the biofilter, hydraulic loading and the turnover time. In this study, both biofilters were designed and sized hypothetically based on the total ammonia production rates, 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 & Cruz (2001) and Lekang & 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 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 study (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 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 2B and 2C) 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).

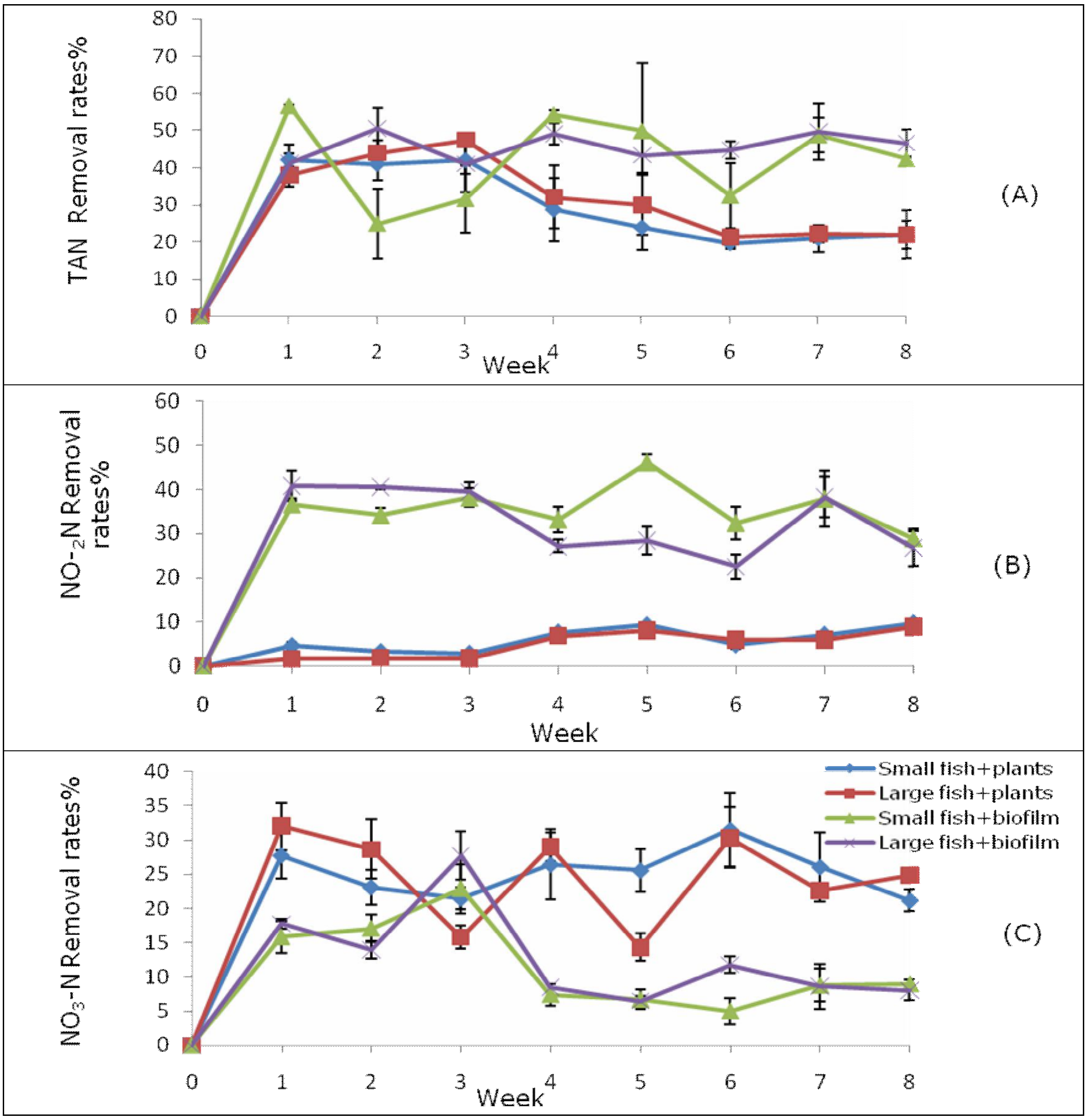

Figure 2. Mean removal rates of *H. rotundifolia* and bacterial biofilm for (A) total ammonia nitrogen, (B) nitrite-nitrogen and (C) nitrate-nitrogen in recirculating koi systems during the 8 weeks trial (error bars indicate the standard error).

Fish excrete various nitrogenous wastes through gill diffusion, gill exchange, urine and faeces excretion. In our study, 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. This 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 of respiratory organs (Post & Lee 1996).

In the current study, 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 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 1B) was much less than the lethal levels of 1.23 mg L^{-1} reported by Hasan & 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 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 & Barat 2005; Colt 2006) as well as fish size, stocking density, access to food, and water exchange (Jobling 1993). In our study, 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 at 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 current study (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 & 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 & Cruz (2001) for (62 g) Nile tilapia (*Oreochromis niloticus* L.). The higher SGR achieved with small fish in this study 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 current study, no mortality was found between large koi with both biofilters. The large koi 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 on survival rates. However, the survival rates of small koi (94.0-95.5%) were higher than the 92% reported by Knaus & Palm (2017) for (36.3 g) common carp and the 62-93% obtained by Jha & Barat (2005) for (0.14 g) koi carp.

Conclusions. Based on the findings of the present study, 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 size of fish 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.

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Conflict of interest: the authors declare that they have no conflict of interest.
Ethical approval: fish were maintained according to the animal ethics standards (approval by animal ethics committee).

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Authors:
Tareq Irhayyim, Curtin Aquaculture Research Laboratory (CARL), Department of Environment and Agriculture, Curtin University, 1 Turner Avenue, Bentley, WA 6102, Australia; Georgikon Aquatic Research Laboratory, Georgikon Faculty, University of Pannonia, 16 Deák Ferenc street, Keszthely 8360, Hungary, e-mail: tareqsaad72@ yahoo.com
Ravi Fotedar, Curtin Aquaculture Research Laboratory (CARL), Department of Environment and Agriculture, Curtin University, 1 Turner Avenue, Bentley, WA 6102, Australia, e-mail: r.fotedar@curtin.edu.au

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