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Full Length Research Paper

Waste production and valorization in an integrated aquaponic system with bester and lettuce

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The purpose of this study was to characterize the wastewater effluent emerging from sturgeon aquaculture and to evaluate its potential for hydroponic lettuce production. Bester sturgeon and *Lactuca sativa* were used to test both nitrogen excretion and uptake in an aquaponic system under two hydraulic regimes. In the end of the experiment, fish growth performance, lettuce yields and nutrient retention were assessed. Higher ammonia excretion for sturgeons held in higher hydraulic retention time (HRT) was found. Similarly, lettuce registered greater amount of both biomass and yield in lower flow treatment. In terms of wastewater treatment efficiency, lower nitrate removal rate and higher total ammonia nitrogen (TAN) removal rate occurred in high flow rate treatments.

Key words: Phytoremediation, recirculating aquaculture, raft hydroponics, *Lactuca sativa*.

INTRODUCTION

Aquaculture has evolved as the fastest growing food-producing sector and developed as an important component in food security. Recirculating aquaculture systems (RAS) offer the great advantage of controlled culture conditions to optimize productivity, therewith to obtain high quality market products. Despite its environmentally friendly characteristics and the increasing number of European countries applying RAS technology, its contribution to production is still small as compared to other production systems (cages, flow-through systems and ponds) (Martins et al., 2010). The main obstacle for the widespread of RAS technology is represented by its first drawback; high initial capital investments. In order to counterbalance the inconvenience and raise RAS's profitability, new approaches were applied. Among them, the easiest is to amplify the productivity by applying higher stocking densities to cover investment costs. Nevertheless, this solution has negative consequence on animal welfare unless it is possible to maintain a constant water quality that will respond to the physiological requirements of cultured species (Roque d'Orbcastel et al., 2009). Another solution could be represented by the re-valorization of the by-products (wastes) from one species in a second crop generated by the co-cultured species. In this case, the profitability is enhanced due to lower refreshment rate and water consumption (Metaxa et al., 2006) or to the crop itself which represent another source of revenue for the farmer. However, in order to manage an environmental friendly and productive recirculating system with minimum impact on fish physiology, the method of bioremediation proved efficient as long as the process is well integrated in a full treatment configuration (Lennard and Leonard, 2006).

Combining aquaculture with the hydroculture technique may serve, as well as double, the purpose of reducing the pollution and increasing the profitability in a way that it consumes less water (McMurtry et al., 1997) and produces additional, saleable crops (Rakocy and Hargreaves, 1993). To recover the high capital cost and operating expenses of aquaponic systems and earn a profit, both fish rearing and hydroponic vegetable components must

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be operated continuously near maximum production capacity (Rakocy et al., 2006). Choosing highly valuable species for coproduction represents another strategy for maximizing incomes, mostly in commercial systems - culture tilapia. However, other species used for aquaponic production include arctic char, trout, perch (Diver, 2006), blue gill, largemouth bass, channel catfish, barramundi, murray cod, jade perch (Nelson, 2008), koi carp, goldfish, pacu and common carp (Rakocy et al., 2006). The hybrid striped bass is one species that reportedly does not perform well in aquaponic systems as it cannot tolerate high potassium levels - a common supplement used for plant growth (Rakocy et al., 2006). No references have been found regarding the adaptation and performance of sturgeon species in integrated aquaponic systems. In Romania, the sturgeon aquaculture expands and the interest for intensive recirculating production increased over the past 10 years. Concomitantly with RAS technology adoption, the interest for profitable solutions which will lead to faster recovery of investment costs also increased. 'Bester', a hybrid between the sterlet *Acipenser ruthenus* (male) which lives in freshwater and the beluga *Huso huso* (female) which grows rapidly, was created in 1952 by Nikoljukin (Chebanov and Billard, 2001) and reared for decades in Eastern Europe. Nowadays, it is cultured also in northern European countries (Bronzi et al., 1999). In Romania, due to its high growth rate under intensive tank rearing conditions, bester is popular among aqua culturists and thus is a good candidate for intensive aquaculture. Previous studies concluded that the quantity of ammonia nitrogen excreted by sturgeons is lower in elasmobranchs, but larger in teleosts (Gershanovich and Pototskij, 1992). For integrated aquaculture such as aquaponic systems, the ammonia represents an important nitrogen source for plants; in this case, the combination of hydroponic production with species that produces ammonia in larger quantities and urea is desirable. Likewise, using tolerant temperature species creates the premise for obtaining constant urea: ammonia excretion ratio as a result of stability of the nitrogen metabolism processes (Gershanovich and Pototskij, 1995).

Regarding nitrogen excretion rates, few studies have been carried out on sturgeons. The trials were conducted on *Acipenser baeri* (Dabrowski et al., 1987; Salin and Williot, 1991), *Acipenser oxyrinchus*, *Acipenser brevirostrom* (Kieffer et al., 2001; Altinok and Grizzle, 2004) and *A. ruthenus* (Gershanovich and Pototskij, 1992). Further, there is a lack of information about ammonia excretion of sturgeon hybrids in general, and bester in particular. Likewise, no references regarding valorization of nitrogenous waste generated by bester hybrid have been found. The purpose of this study was to characterize the wastewater effluent emerging from bester aquaculture and to evaluate its potential for hydroponic lettuce production. As such, the retained nutrients in the fish and vegetable biomass and, indirectly, the efficiency in terms of water quality control were assessed.

MATERIALS AND METHODS

Rearing system facilities

The trials were conducted in the laboratory of the Department of Aquaculture, Environmental Sciences and Cadastre, "Dunarea de Jos", University of Galati. The experiment was carried out in an experimental aquaponic system with a total volume of 1.8 m^3. The aquaponic recirculating system incorporates four fish rearing tanks (268 l) connected to a treatment recycling loop, through which the water circulated an additional hydroponic module for *Lactuca sativa* hydroponic production. However, a general diagram of the systems is presented in Figure 1.

Water treatment units had the mission to control and maintain, in optimal range, the main water quality parameters as: oxygen concentration, total ammonia nitrogen concentration, total suspended solids concentration, pH and carbon dioxide. Thus, for total solids (TS) control, the recirculating system has been provided with a backwash sand filter where wastewater from the storage tank was continuously pumped with a constant flow of 48 l/min. Water, free of solids, is pumped to the top of the biofilter via a 'spray bar', then trickled across the biological filter medium (0.4 m^3, Bactoballs, 200 m^2/m^3). Biologically filtered water was pumped to hydroponic modules that consist of four troughs (60 x 90 x 30 cm), independently alimented and provided with valves which permitted flow control and adjustments. The flow rate through hydroponic troughs was 8 l/m for two units and 16 l/m for the other two units. The water from each hydroponic trough was gravitationally conducted to one fish rearing unit. Before returning to the fish, tanks treated water was passed through a degassing column for CO_2 striping. The sterilization and disinfection process was realized with a UV filter (TETRA POND, Type UV-C 35000 and 36 Watt). For the oxygen concentration supply dictated by the stocking intensification degree, the recirculation system was provided also with an oxygenation unit formed by one compressor Resun Air-Pump, Model: ACO-018 A with a flow of 260 l/min. Water distribution installation was done in pipelines made of polyvinyl chloride and three pumps, Grundfos, type UPbasic 25-6 180 max.10bar. Lighting (for plant growth in the hydroponic beds) consisted of 3×100 W fluorescent lamps situated above the hydroponic beds at a height of 700 mm from the surface, with one lighting unit located at the interface between two hydroponic troughs. The lighting regime was controlled by a digitally timed electrical switch (timer).

Experimental design

The experiment was designed to compare the effect of the hydraulic regime on waste production, water quality parameters, lettuce performance and fish growth in an integrated aquaponic system. The experimental design was elaborated taking into account the recommendations for simple designs with single factor, avoiding interferences of the filtration system's influence as the second, uncounted factor (Tlusty, 2010). The trial ran for 21 days from planting to harvesting. Before starting up the experiment, the biofilter was activated for developing a healthy population of nitrifying bacteria capable of removing the ammonium and nitrite. During the preconditioning period (21 days), ammonium chloride (NH_4Cl) was added daily at a rate of 15 mg/L. Daily ammonia, nitrite and nitrate levels were taken to determine the degree of oxidation of ammonia to nitrate, showing evidence that the biological filter was working and the steady state of bacterial biomass in the biofilter was obtained.

After three weeks, the system was populated with bester sturgeons which were kept in the system for two weeks. This time was sufficient for the nutrient to be accumulated to the appropriate concentrations requested by plants. At the initiation of the experiment, nutrient concentrations were recorded (55.6 mg/l $N\text{-}NO_3$,

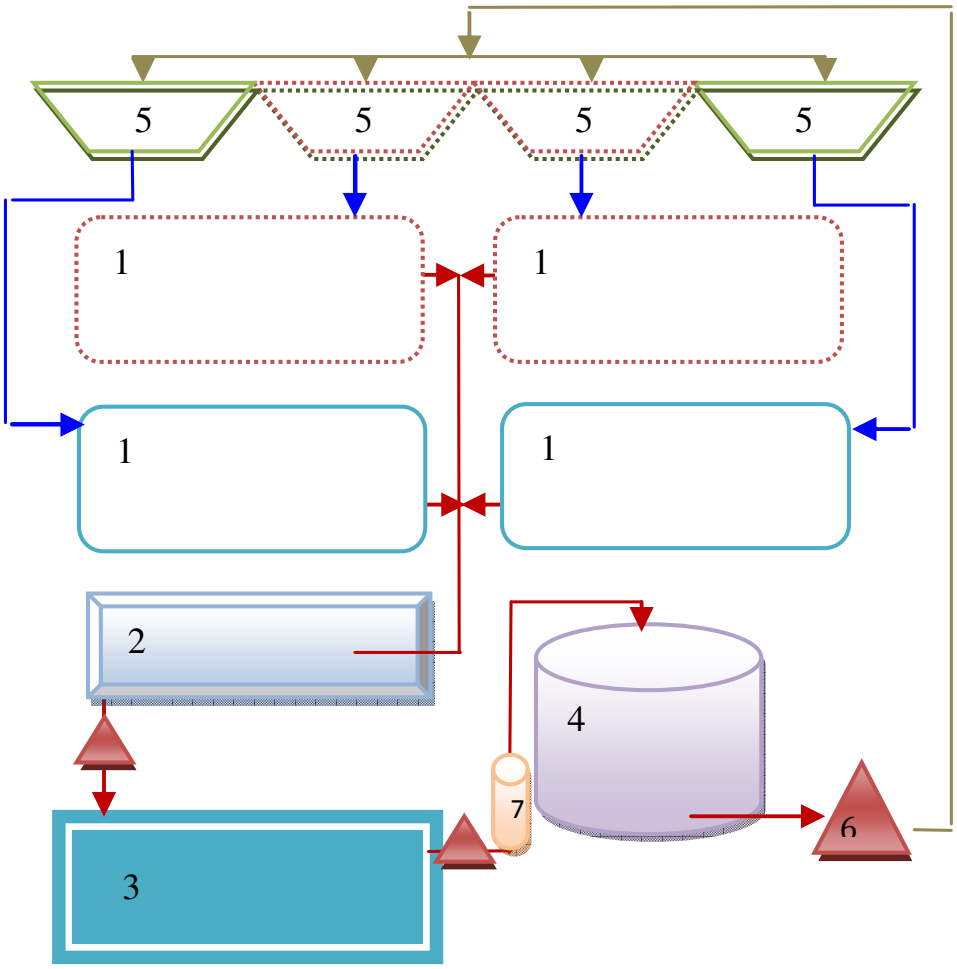

Figure 1. Schematic representation of the recirculation aquaponic system: 1, Rearing tank; 2, water distribution storage tank; 3, sand filter; 4, biofilter; 5, hydroponic units; 6, pumps; 7, UV reactor; dashed line ... low retention time (LFR); continuous line –-- high retention time (HFR).

6.85 mg/l P-PO_4 and 1.31 mg/l N-TAN) and all the fish were weighted and randomly distributed in experimental tanks (each tank was populated with 22 fish amid a mean individual weight of 113.67 ± 5.21g). The initial density was 7.56 kg/m^3. During the trial, the fish (2% body weight/day [BW/d]) were fed with commercial trout pellets (3 mm), containing; 46% protein, 16% fat, 1.5% fiber, 9.5% ash and 13% phosphorus. Lettuce plantlets (four to six leafs) were planted in a density of 44 plants/m^2 (24 plants for each raft). The individual initial weight (biomass) of the plantlets was recorded (weight with attached soil plug). Initial leaf weight was estimated by recording the weights of 15 plantlets with and without attached soil plugs because plantlets had attached soil plugs. These weights were used to establish a mean ratio of leaf only to leaf + plug weight (Lennard and Leonard, 2006). The water flow rate through the treatment units (sand filter and biological filter) was fairly constant during experiment (48 l/m). The flow rate through two hydroponic units and two fish rearing units was 8 and 16 l/m, respectively. The two tested hydraulic regimes are hereby noted with low flow rate (LFR) and high flow rate (HFR) corresponding to a hydraulic retention time (HRT) of 5.4 and 2.7 min, respectively. The lighting regime for plant growth was 10 h on and 14 h off, with lights coming on at approximately 09:00 h and going off at 19:00 h. Water level in each trough was kept at 15 cm deep. Lettuce was grown in small pots placed in floating Styrofoam sheets suspended at the water surface in such a manner to permit that the pots have only the lower portion flooded. In this case, only small portions of the substrate at the bottom of the pots are saturated, while the upper parts are moist and contain plenty of air.

During the experiment, no water was discharged or displaced except for replacing water lost through evaporation, transpiration and sludge removal. The Fe^{+2} derived from fish feed is insufficient for hydroponic vegetable production (Rakocy, 2006). In the present study, the wastewater derived from fish production has been supplemented with iron in the concentration of 2.0 mg/L. In order to prevent the precipitation of iron and make it unavailable to plants, chelated Fe^{2+} has been used.

Water sampling and analysis

In order to evaluate the total production of ammonia nitrogen, nitrification efficiency and nutrient retention at the hydroponic modules and water samples (50 ml) were collected from the outlet of the fish rearing tanks, at the inlet and outlet of the biofilter (considered as the inlet of the aquaponic units) and at the outlet of the hydroponic modules (considered as the inlet of the fish tanks). On weekdays, at

the points of nutrient sampling, dissolved oxygen (DO), conductivity and pH levels were determined with hand-held devices (WTW Oxi 315 i, Conductimeter WTW and WTW pH meter 340 respectively) that were calibrated daily according to their respective manuals. Determination of NH_4^-, NO_2^-, NO_3^- and PO_4^{3-} was carried out using the spectrophotometric method (Specol UV-VIS).

The fish and vegetable biomass was quantified and biochemically evaluated in order to estimate the retained nutrients. Crude protein content (Nx6.25) was calculated using the Kjeldahl method (mineralization with sulfuric acid and catalyst, after which proceeded to alkalizing with sodium hydroxide solution, ammonia distillation and distillate collection in a determined quantity of sulfuric acid solution followed by titration with sodium hydroxide solution). Lipid (fat) content was determined according to the Soxhlet method (extracted with ethyl ether), while dry matter and ash were determined according to standard methods.

The released quantities of inorganic dissolved nitrogen were estimated through "hydrobiological" approach (Roque d'Orbcastel, 2008). Thus, the ammonia nitrogen excretion rate (P_{TAN}, mg/g fish weight/h) was calculated with formula:

$$P_{TAN} = [(Ce-Ci)xQ]/W$$

where, Ce, Ci = ammonia nitrogen concentrations in the effluent (Ce) and influent (Ci), respectively (mg/l); W = weight of fish in a tank (g); Q = water flow rate (l/h).

The TAN removal rate in hyroponic units was calculated with the following formula:

$$TAN_{retained}\,(g/m^2/day) = ((Q/V * (C_{in}-C_{out}) - dC_{out}/dt)*d$$

where, Q = the flow rate (m^3/day), V = system volume (m^3), C = concentration of TAN (g/m^3), d = depth (m), t = time (d).
The biofilter performance (volumetric TAN conversion rate; ΔTAN) expressed as grams of TAN oxidized per media volume per day was calculated from Franco-Nava .et al, 2004):

$$\Delta TAN\,(g/m^3/day) = [(TAN_0 - TAN_1) X Q]/V$$

where, TAN_0 = total ammonia nitrogen concentration in biofilter inflow ($N\text{-}NH_4^+$ + N-NH3; g/m^3); TAN_1 = total ammonia nitrogen concentration in biofilter outflow (NNH_4^+ + $N\text{-}NH_3$; g/m^3); Q = water flow rate (m^3/ day); and V = volume of media in biofilter (m^3).

Measurement of growth and yield

At the end of the experiment, fish biomass and plant (leaf only) biomass were determined by wet weight. Fish growth performance was assessed using the following equations:

Weight gain (W) = Final weight (Wt) - Initial weight (W0) (g)

Food conversion ratio (FCR) = Total feed (F) / Total weight gain (W) (g/g)
Specific growth rate (SGR) = 100 x (ln Wt to ln W0) / t (% BW/d)

Relative growth rate (RGR) = (Wt -W0)/ t /BW) (g/ kg/.d)

Protein efficiency ratio (PER) = Total weight gain (W) / Amount of protein fed (g)
Relative Weight Gain (RWG %) = (Wt – W0) x 100 / Wt

Statistical methods

Statistical analysis was performed using the SPSS 15.0 for Windows. Statistical differences between treatments were tested using T test (α = 0.05) after a normality test (Kolmogorov-Smirnov). When the condition of normality was not satisfied, the Mann-Whitney test, for two independent populations, non-parametric analysis was used. Comparisons between sampling points for all parameters were assessed using analysis of variance (ANOVA) and least significant difference (LSD) post-hoc analysis where appropriate (an equal variance test for homoscedasticity was applied before ANOVA). The coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean in order to have a measure of dispersion in fish population.

RESULTS

Plant growth

In the end of the experiment, the lettuce biomass from all hydroponic components was assessed. The individual final weight averaged 84.87 ± 3.12 g and 84.51 ± 2.11 g for LFR treatments and 77.74 ± 2.12 g and 74.29 ± 3.01 g for HFR treatments respectively. Final wet weight of the plants differed ($p>0.05$) between hydroponic troughs, where two distinct groups associated to treatments were shown by Duncan test. Similarly, mean relative growth rate (RGR) was 10.33%/d for higher hydraulic retention time, and 9.66% /d for lower hydraulic retention time. In terms of productivity expressed as fresh weight (FW) per surface unit in HFR treatment, lower yields (3.45 kg and 3.30 kg FW/m^2) were achieved comparatively with LFR treatments (3.77 and 3.76 kg FW/m^2). In the end of the experiment, nitrogen retention in lettuce tissue was assessed. No significant differences were found between treatments with respect to nitrogen content in leafs, but due to higher yield in LFR treatment, the overall nitrogen retention was higher in these troughs (Table 1).

Fish growth

The overall production performance of bester sturgeon is shown in Table 2. The observed growth rate was different between treatments. The mean specific growth rate for the LFR treatment (8 L/min) and HFR treatment (16 L/min) was 1.39 and 1.64% BW/d, respectively. FCR values were in the range of 1.01 to 1.25 with smaller values for the lower hydraulic retention time. After 21 days, differences between treatments with respect to all growth efficiency indices were observed, and also significant differences were detected ($p<0.05$, T-test) when compared with the individual final weight.

Water quality

Temperature

Water temperature in the aquaponic recirculating system reached values that are between 17.83 and 20.58°C; although, the average for the experimental period was

Table 1. *Lactuca sativa* growth performance.

Growth indicator	Flow rate			
	LFR	LFR	HFR	HFR
Nr. Plants	24	24	24	24
Initial biomass (g)	225.12	240	236.16	243.84
Final biomass (g fresh weight)	2036.88	2028.2	1865.68	1782.9
Weight gain (g fresh weight)	75.49	74.51	67.90	64.13
Weight gain (g dry weight/plant)	9.55	9.44	8.72	8.21
RGR (%/d)	10.49	10.16	9.84	9.47
Productivity (kg fresh weight/m^2)	3.77	3.76	3.45	3.30
Retained N (g)	12.78	12.79	11.88	11.30
Retained N (g/m^2/d)	1.13	1.13	1.05	1.00

The relative growth rates were estimated by formula: RGR = ln (W_t/W_0) / t× 100 = %/d, where W_0 is the initial biomass and W_t is the biomass at last day t.

Table 2. Technological performance of bester sturgeon under different hydraulic regimes.

Growth performance	Flow rate			
	LFR	LFR	HFR	HFR
Initial biomass- IB (g)	1914.00	1995.00	2101.00	2092.20
Mean initial weight –MIW (g/piece)	95.70	95.00	95.50	95.10
Final biomass FB (g)	2576.00	2664.90	2954.60	2963.40
Mean final weight –MFW (g/piece)	128.80	126.90	134.30	134.70
Individual weight gain - IWG (g/piece)	33.10	31.90	38.80	39.60
Total weight gain - TWG(g)	662.00	669.90	853.60	871.20
Specific growth rate -SGR (% BW/day)	1.41	1.38	1.62	1.66
Daily growth rate – DGR (g/kg/day)	1.58	1.52	1.85	1.89
Food conversion ratio - FCR (g/g)	1.21	1.25	1.03	1.01
Protein efficiency ratio - PER	1.79	1.74	2.10	2.16

Regarding the protein content of fish in the beginning and the end of the trial, significant differences were not found between treatments. Thus, the protein content in the beginning was 11.54±0.4% and in the end, it was 12.27±0.7%.

19.49 ± 0.56°C. The optimum temperature for sturgeon growth appears to be closer to 23°C than to 26°C, and the optimum feeding rates at 23 and 26°C are 2.0 to 2.5% and 2.5 to 3.0% BW/d, respectively (Hung et al., 1993). Our own observations confirm that bester grows better at 22 to 23°C when compared with 19°C (Dediu et al., 2011). The pH registered values comprised between 6.98 and 7.28, with the average for the experimental period of 7.18 ± 0.76 (Table 2). Comparison between treatments did not show significant differences regarding pH values ($p>0.05$).

Dissolved oxygen (*DO*)

Concentration varied between 4.60 mg/l (min) and 7.21 mg/l (max), with average of 6.25 ± 0.85 mg/l. Regarding the dynamic of dissolved oxygen concentration, the T Student test emphasized significant differences ($p< 0.05$) between mean values registered for both fish tanks and hydroponic troughs under different hydraulic regimes (Table 3).

The average total ammonia nitrogen (TAN) concentration within the system ranged from 0.21 to 1.31 mg/l (the highest value was measured before adding plants in hydroponic troughs). Statistically, there were significant differences in TAN concentration between treatments ($p < 0.05$). Thus, the mean TAN concentration at the outlet of the hydroponic troughs was 0.43 ± 0.18 mg/l for LFR treatment and 0.39 ± 0.28 mg/l for HFR treatment. For fish tanks, the mean of TAN concentration measured to the outlet was 0.51 ± 0.11 for LFR treatment and 0.47 ± 0.09 mg/l for HFR treatment. Nitrite concentration registered an average of 0.05 ± 0.12 mg/l, without differences (ANOVA, $p>0.05$) between mean values registered for different sampling points. Over a production cycle period, the nitrate concentration evolution has a tendency to accumulate and rise above the mentioned values; although, the only possibility to control nitrates in this case is represented by water replacement/refreshing rate. In aquaponic recirculating systems, the nitrates represent the main source of nutrients for plants development and, for this reason, the accumulation rate should be null, when an appropriate operational management is

Table 3. Oxygen concentration, pH and temperature (± standard deviation, STD) for both systems and all sampling points.

Water parameter	Sampling point	LFR		HFR	
		Mean	STD	Mean	STD
Oxygen (mg/l O_2)	Hydroponic outlet/ Fish tank inlet	6.26*	0.17	6.37*	0.15
	Fish tank outlet	5.52*	0.23	6.27*	0.11
pH	Hydroponic outlet/ Fish tank inlet	7.23	0.70	7.15	0.04
	Fish tank outlet	7.14	0.57	7.04	0.09
Conductivity (µS/cm)	Hydroponic outlet/ Fish tank inlet	574.4	53.8	573.5	52.6
	Fish tank outlet	577.6	53.8	574.7	51.9
TAN	Hydroponic outlet/ Fish tank inlet	0.43	0.18	0.39	0.28
	Fish tank outlet	0.51	0.11	0.47	0.09
NO_3 - N	Hydroponic outlet/ Fish tank inlet	32.52	7.06	34.52	6.26
	Fish tank outlet	37.59	9.22	39.93	7.09

*Significant differences ($p<0.05$).

applied. During the present trial, nitrate nitrogen (NO_3-N) concentration declined from 55.6 to 8.63 mg/l at the end of the experiment. The average concentration of nitrates (NO_3-N) at outlet of hydroponic troughs was 34.52 ± 6.26 mg/l for HFR treatment and 32.25 ± 7.06 mg/l for LFR treatment.

DISCUSSION

Fish growth and waste production

In terms of growth rate (% body weight/d), the results from the present study show similar values with other reports for different sturgeon species of the same size class. Our results were similar to those of the study of Prokes et al. (1997) which, for Siberian sturgeon (180 g), found a specific growth rate of 1.4% body weight/d. Also, it was similar to the study of Hung et al. (1989) which reported 1.5% BW/d for 250 g white sturgeon fed with 2% BW/d. More so, Jahnichen and Rennert (1999) found a growth rate of 1.1% BW/d for sterlet *A. ruthenus* (20 g) fed with 3.5% BW/d, while Hassani et al. (2011) found a SGR of 0.9% BW/d body for *A. persicus* (10.2 to 103.5 g) fed with 45% protein. For beluga held in different light conditions, Ghomi et al. (2010) reported specific growth rate values of 1.08 and below 1% BW/d. Other previous studies regarding the influence of stocking density on the growth performance of beluga weighing 45 to 67 g showed that SGR values of 4.7% BW/d can be possible (Vasilean and Cristea, 2009). The same author reported SGR values of 1.06 to 1.2% BW/d for beluga weighing 156 to 203 g (Vasilean et al., 2009). For our study, bester exemplars were selected from a highly heterogenic population where the exemplars showed distinct phenotypic features inherited from beluga or sterlet. The dominance of "beluga-bester" exemplars in experimental populations could explain the higher growth rate when compared with other studies when smaller sturgeon species were used. FCR values varied between 1.01 and 1.25 (Table 2) with notable differences among treatments. The highest conversion efficiency was obtained for HFR treatments where FCR values were 17.14% lower than LFR treatment. These findings could be explained by the significant higher oxygen concentration regime in HFR treatment and lower TAN concentration during experiment. TAN excretion rates are directly related to dietary nitrogen and protein intake in fish (Liao and Mayo, 1974; Rychly, 1980). In the end of the experiment, the nitrogen content of the fish permitted the quantification of the total retained nitrogen reported to feed nitrogen (73.6 gN/kg feed) for each treatment: 25.56% for LFR treatment versus 30.14% for HFR treatment. These findings are in accordance with values reported by different authors for other species. Thus, the trout sizing of 24 to 55 g and 156 to 238 g was fed *ad libitum* to fish with a feed containing 67 gN/kg, and it was observed that the feed retained was 23 gN/kg (34.32%) and 21 gN/kg (31.34%) respectively (Kim et al., 1998). For marine species such as sea bream (50 to g) fed with 1 to 4%BW/d feed containing 64 gN/kg, it was observed that approximately 26.56% of the feed was retained from ingested nitrogen (Shpigel et al., 1993).

For most of the fish, nitrogen excretion represents 50 to 70% of the nitrogen intake. Previous studies describe high correlation between TAN production and water temperature (Kelly et al., 1994). Likewise, the rearing species, stage of development (Dosdat et al., 1996) or feed quality (Ballestrazzi et al., 1994) directly influence the ammonia excretion rate. Other authors also support the importance of protein input as the major factor influencing ammonia production rates (Gershanovich and Pototskij, 1992), a series of studies being conducted to demonstrate that the rate of ammonium released by fish increased with dietary protein content (Rychly, 1980;

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Conductivity (µS/cm)	Hydroponic outlet/ Fish tank inlet	574.4	53.8	573.5	52.6
	Fish tank outlet	577.6	53.8	574.7	51.9
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	Fish tank outlet	0.51	0.11	0.47	0.09
NO_3 - N	Hydroponic outlet/ Fish tank inlet	32.52	7.06	34.52	6.26
	Fish tank outlet	37.59	9.22	39.93	7.09

*Significant differences ($p<0.05$).

Buttle et al., 1995). During the present trial, high protein content feed (46%) was used. It was stated that for sturgeons, efficient protein level for the experimented size class was around 38 to 42% depending on the species. This could explain the higher production of nitrogenous waste in the present trial, where protein level exceeded the requirements. The total residual N (accounted from the difference from intake nitrogen and retained nitrogen) reported for nitrogen intake represented 69.86% for HFR treatment and 74.44% for LFR treatment. These results are in agreement with those of other findings, which reported that the non faecal nitrogen loss recorded for a large number of species was approximately 30 to 65% feed N, while faecal loss was 10 to 30% feed N (Schneider et al., 2005). For the present experiment, average TAN represented 54.93 and 62.80% from total nitrogenous excretions for HFR and LFR treatment respectively, while a higher TAN excretion represented 71 to 81% for different sizes of lake sturgeon fed Artemia (Bharadwaj et al., 2008). In terms of TAN production rate, significant differences ($p<0.05$) were registered between treatments, in which the average values for high flow and low flow treatment was 23.54 ± 0.36 and 28.67 ± 0.09 mg/kg/h respectively. These findings are in accordance with other reports for sturgeons. The ammonia production rates measured for *A. transmontanus* ranged from 1.51 to 27.6 mg/kg/h for sturgeon ranging from 0.09 to 3.8 kg (Thomas and Piedrahita, 1998). In a laboratory study on sterlet sturgeon fingerlings, ammonia-nitrogen excretion rates were measured as low as 8.8 mg TAN /kg fish/h in 38 g fish starved for 24 h, and as high as 27.7 mg TAN/kg fish/h for fish fed *Chironomus* sp. at a ratio of 8.4% BW/d (Gershanovich and Pototskij, 1992) (Table 4).

The higher production of ammonia nitrogen in lower flow rate regime could be explained by the chronic exposure of fish biomass to a higher concentration of TAN which showed, as a direct consequence, lower retention and higher excretion. Wood (2004), in a small scale experiment with rainbow trout exposed for 72 days to a concentration as low as 225·µmol//l TAN, observed that fish consumed more food and excreted more ammonia, achieving the same mass and protein content as the controls. Other authors, however, support the theory that ammonia excretion declines when blood ammonia level increases; although, exposure of fish to increased ammonia concentrations is a factor that raises blood ammonia concentration (Randal and Right, 1987 and references therein). For European sea bass, reared in different water hydraulic regimes, Franco-Nava et al. (2004) reported a higher nitrogen excretion for fish held in higher flow rate as a consequence of increased swimming activity. In our trail, no different behavior related to treatments was observed. The significant ($p<0.05$) higher mean oxygen concentration registered for HFR treatment could, however, induce a lower nitrogen excretion (Gordon, 1970). Measurements of ammonia-nitrogen production rates in this study were done in a dynamic system where microbial organisms coexisting with fish population may produce or consume a portion of the total ammonia produced. As in other trails (Thomas and Piedrahita, 1998), the measured TAN included bacterially produced ammonia and the rates of ammonia consumption.

Lettuce production and waste valorization

In terms of lettuce productivity, in the present study, higher values were obtained in the hydroponic units operating under higher hydraulic retention time (LFR): 3.76 ± 0.14, versus 3.38 ± 0.10 kg/m^2 obtained for the treatment under lower hydraulic retention time (HFR). These results are below the values reported by Lennard and Leonard (2006) for lettuce grown in floating raft

aquaponics which yielded 4.47 kg/m^2, with a reduced amount of 0.58 kg/m^2 in comparison with gravel aquaponic system, but with a higher amount of 0.34 kg/m^2 in comparison with NFT technique. In a previous study regarding the comparison of the reciprocating flow versus constant flow in an integrated gravel bed aquaponic system, the same authors (Lennard and Leonard, 2004) found that lettuce production within the constant flow was significantly higher than lettuce production within the reciprocating control replicates (4.97 ± 0.10 kg/m^2 versus 4.34 ± 0.20 kg/m^2). Other papers reveal similar or smaller yields than the present trial: 3.3 to 4.5 kg/m^2 (Burgoon and Baum, 1984) and 2 kg/m^2 (Seawright et al., 1998). In terms of growth rate, lettuce productivity can reach 12 g/m^2 per day (Adler and Summerfelt, 2003). For the present study, the daily growth rate for lettuce grown in low and high flow rate was 3.57 ± 0.37 and 3.14 ± 0.17 g/m^2/d respectively. Similar results were given in a more recent study of Trang et al. (2010), where the lettuce productivity was 1150 gFW/m^2 after 60 days cultivation period. Nitrogen content in lettuce tissue was 49.67 ± 0.05 gN/kg DW for LFR treatment and 49.51 ± 0.11 gN/kg DW for HFR treatment. Fallovo et al. (2009) in an experiment concerning the nutrient content of lettuce in different seasons and nutrient supplementation in a floating raft hydroponic system, in greenhouse, found that the nitrogen content in lettuce (var. acephala) can take values from 47.1 g/Kg DW, in spring, and 51.2 g/Kg DW, in summer. The retained nitrogen after 21 experimental days was 1.13 ± 0.3 and 1.02 ± 0.12 g/m^2/d for LFR and HFR treatments respectively. Other authors reported higher nitrogen retention up to 2.2 g/m^2 (Trang et al., 2010). When the total nitrogen input introduced in the system through feed (250.46 g) was reported, it was observed that lettuce removed 48.74 g which represented 19.46%. Dry matter content of lettuce plants measured in the end of the experiment was 12.66 ± 0.9 and 12.83 ± 0.3% for LFR and HFR treatments respectively. Without significant differences ($p>0.05$) between treatments or replicates, similar results were obtained in an experiment of hydroponic lettuce production using catfish pond wastewater (Sikawa and Yakupitiyage, 2010). However, the normal range of dry matter content of lettuce is 4 to 6%, but in the case of N-stressed plants, the DM content could reach values that are 3 to 4 times higher than those of normal range (Seginer et al., 2004).

If we consider the recommendation of Soundry et al. (2005) of maintaining 60 to 90 mg N/l in hydroponic solution for lettuce optimum root and shoot growth, it could be considered that plants were under nitrogen privation as in the present study, the dissolved inorganic nitrogen concentration was under the recommended range. Nevertheless, given the obtained yields and growth rates which are comparable with other findings, it could be considered that cellular nutrient concentrations could sustain growth even though nutrients within the flow are becoming limiting (Adler and Summerfelt, 2003).

Water treatment and recycling

Aquaculture waste nutrients should ideally meet the requirements of plants co-cultured in aquaponic systems (Trang et al., 2010). In order to have a well design treatment configuration, a good knowledge of nutrients that are dynamic for each compartment under the operating hydraulic regime should be acquired. A recent research (Cockx and Simonne, 2003) demonstrated that if ammonium is the predominant form of nitrogen, plant growth is slowed. The aforementioned authors have also shown that the optimum nitrate/ammonium ratio to develop biomass production and obtaining appropriate horticultural crop in aquaponic systems is 3:1. Therefore, the nitrification and oxidation of ammonia to nitrite and then to nitrate is essential for development in plant biomass. In our recirculating system, the maximum TAN removal rate (measured after feeding) in biofilter was 0.26 g/m^2/d. For trickling filters, TAN removal rate should be in the range of 0.24 to 0.64 g/m^2/d as demonstrated by different authors (Eding et al., 2006; Lyssenko and Wheaton, 2006). TAN removal rate in hydroponic units reached values of 0.32 g/m^2/d for HFR and 0.27 g/m^2/d for LFR, while nitrate removal rate was oscillated between 0.44 and 0.80 g/m^2/d with a mean value of 0.64 ± 0.11 g/m^2/d for HFR treatment and 0.47 and 0.89 g/m^2/d with a mean value of 0.72 ± 0.17 g/m^2/d for LFR treatment.

Lower nitrate removal rate and higher TAN removal rate which were accounted for in HFR treatments were in accordance with the findings of Endut et al. (2009) in a study of aquaculture effluent treatments under different hydraulic loading rates using *Ipomoea aquatica*. In the present trial, it was estimated that TAN retention in hydroponic modules was approximately 14.5 and 10.8% for HFR and LFR treatments respectively, from the total TAN produced daily by bester sturgeon. Comparing this study with other studies (Endut et al., 2010) where the percentage removal values of TAN and NO_3-N exceeded the threshold of 50%, the lower values found in the present study were due to the higher nitrogen load (8.6 g waste N/m^2/d generated by 75 g/m^2/d feed). For production of 3.5 kg/m^2 lettuce, a ratio of 1.09 plants/fish (1.84 g feed/day/plant) was used. At this ratio, residual nutrients were not accumulated in the system. Lower nitrate removal rate measured for hydroponic troughs under HFR treatment could be explained through the nitrification process that took place in biofilms developed on the plant roots. Most probably, the rhizoplane development which was more evident here was enhanced by the higher oxygen concentration and lower retention time in these units. Likewise, the nitrification process that took place in all hydroponic units concur to ammonia depletion and, thus, to nitrogen dynamics within the system. However, further investigations are necessary in order

to separate the process of nitrification from active uptake.

The remaining unexplained residual nitrogen is considered as part of the amount of solid waste accumulated in the sand filter from where they were removed by backwashing of the filter layers.

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