Improving water management in European catfish recirculating aquaculture systems through catfish-lettuce aquaponics

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R. Calone[1], G. Pennisi[1,2], R. Morgenstern[3], E. Sanyé-Mengual[1], W. Lorleberg[3], P. Dapprich[3], P. Winkler[3], F. Orsini[1], G. Gianquinto[1]*

[1]Research Centre in Urban Environment for Agriculture and Biodiversity (ResCUE-AB), Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum University of Bologna, Viale Fanin, 44, 40127, Bologna, Italy.

[2]DISAFA, Department of Agricultural, Forest and Food Sciences. Università di Torino, Via Leonardo da Vinci, 44, Largo Braccini 2, 10095, Grugliasco (TO), Italy.

[3]Department of Agriculture, South Westphalia University of Applied Sciences, Lübecker Ring 2, 59494, Soest, Germany.

Abstract

In the context of climate change and population growth, aquaculture plays an important role for food security, employment and economic development. Intensive recirculating aquaculture systems (RAS) allow to treat and recycle fish effluents to reduce waste concentration in outflow water thereby reducing environmental contamination. RAS sustainability may be further improved using aquaponics, a circular productive system in which RAS wastewater is recovered for crop cultivation and recycled back to the fish tanks. In this study, water metabolism of a catfish RAS was assessed and the opportunity to produce lettuce with the RAS effluent was tested. Crop growth and water consumption in aquaponics were compared to those experienced in hydroponics at three nutrient solution concentration (EC of 1.6, 2.0 and 3.0 $dS \cdot m^{-1}$), also considering water- (WUE) and nitrogen- use efficiency (NUE). A scenario for converting the RAS in a catfish-lettuce aquaponic system was, then, proposed.

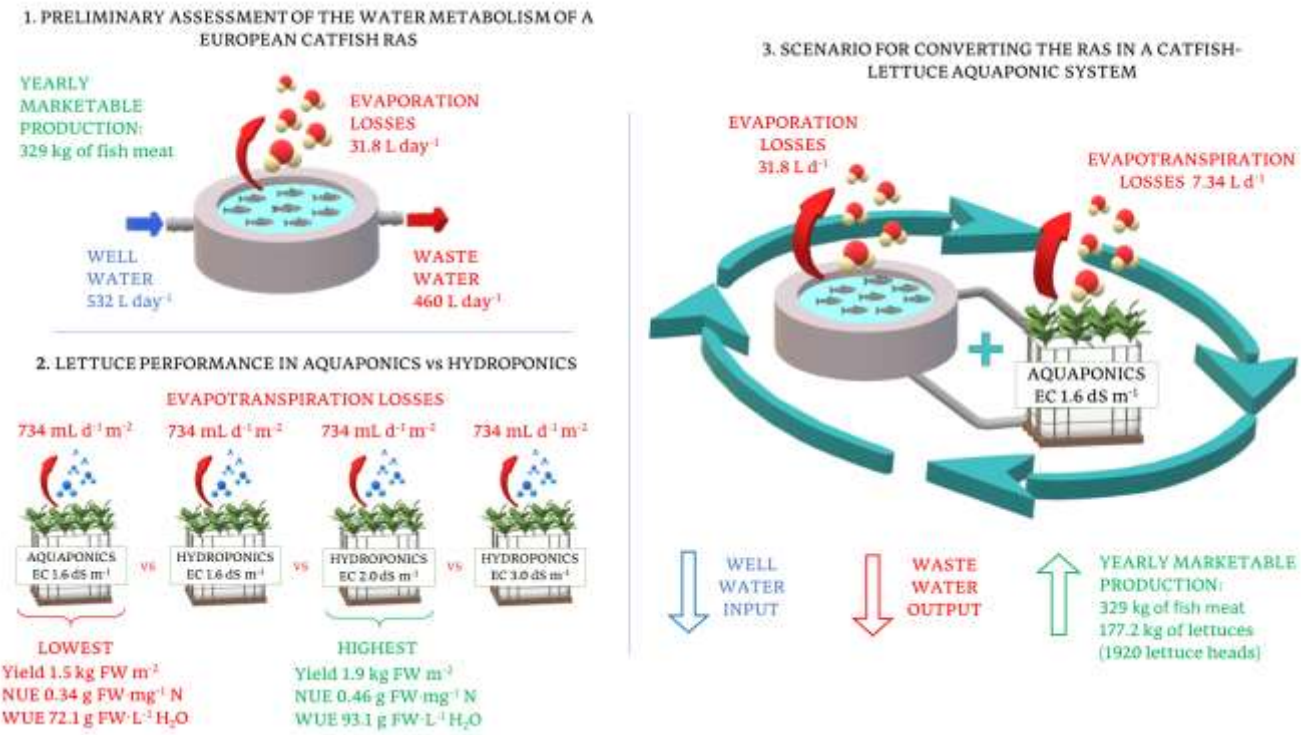
The RAS water balance included an input of 555 $L \cdot day^{-1}$, out of which 32 $L \cdot day^{-1}$ were lost by evaporation from the tubs whereas 460 $L \cdot day^{-1}$ were discarded. The lettuce yield, NUE and WUE in aquaponics were respectively 20.3%, 22.3% and 20.6% lower than those obtained in hydroponics. Best performances in

hydroponics were achieved with EC of 2.0 dS m^{-1}. No difference in term of water consumption arose between the treatments, with average water use of 46 mL·$plant^{-1}$·day^{-1}.

Considering the current RAS productivity of 329 kg $year^{-1}$, a 10 m^2 raft system hosting 160 lettuces would satisfy the nitrogen filtration demand. Once closed the water loop between the two productive sub-units, the current water input of 532 L·day^{-1} could be reduced to the amount needed to replace the water lost by evaporation (50 L·day^{-1}) and the RAS water output would decrease from 555 to 103 L·day^{-1}.

Keywords: Aquaponics, RAS, electrical conductivity, water use efficiency, sustainability

Graphical Abstract



1. Introduction

World population is expected to increase between 20% and 30% by 2050, growing from 7.7 billion people to between 9.2 and 10.2 billion. Accordingly, global food demand is foreseen to increase by 60% by 2025 (Alexandros and Bruinsma, 2012). Concurrently, the global water consumption, already risen by 600% in the last century, will keep growing at a 1% yearly rate Wada et al., (2016) In this framework, the FAO Fisheries and Aquaculture Department (FAO, 2018) highlighted the important role of fisheries and aquaculture in boosting food and nutrition security, job employment and income generation for local communities. Today aquaculture (e.i. the cultivation of aquatic organisms in natural or controlled marine or freshwater environments) accounts for over 50% of the fish destined for human consumption, providing a primary source of protein and essential micronutrients worldwide (FAO, 2018). Current aquaculture systems range from extensive to intensive, depending on the level of inputs, the fish stocking density, and the degree of management. The adoption of intensive land-based systems located close to the markets, i.e. in the areas with high population density, is rapidly growing. Intensive fish farming systems can be categorized in “flow-

through" and "RAS" (Recirculating Aquaculture System). The former are open-systems in which the clean water flows once and is discharged thereafter. They are common in regions with high water availability, but their use is becoming always more limited due to the stringent water regulations regarding wastewater discharges into natural water bodies. The effluents from these open systems, containing residuals of uneaten food and high concentrations of nitrogen and phosphorus, can in fact cause oxygen depletion and eutrophication in the receiving water bodies (Martins et al., 2010). In the RAS, instead, only less than 10% of the total water volume needs to be daily discarded (Timmons and Ebeling, 2017). These systems are provided of mechanical and biological filters and additional water treatment components to depurate water from pollutants and allowing diseases control. Through this filtration process, the same volume of water can be continuously recirculated within the system while only a limited amount needs to be replaced with fresh water to avoid excessive nutrient loading.

Despite the introduction of RAS in aquaculture already contributed to downsizing the wastewater emissions into the environment, the disposing of this water is still a constant concern for aquaculture operators, especially now that environmental measures are evolving toward the concept of zero emission circular economy (EU, 2018). In this context, the set-up of closed-loop productive systems with low- or no-emissions, such as the aquaponics (e.g. a soilless system for crop production integrating aquaculture and hydroponics) concept, is raising growing interest (Endut et al., 2016). A key advantage of aquaponics is the symbiotic relationship between the plants and the fishes of the horticultural and aquaculture systems, respectively, which can be connected through the recirculation of the water flow. The fish dejections in the outflow water from the aquaculture system provide nutrients for the plants growth in the horticultural one, whereas plants, in turn, clean and filter the water that can be reused back to the fish tank (Goddek et al., 2015). This mutual exchange depends on the action of two different groups of bacteria, namely *Nitrosomonas spp.* and *Nitrobacter spp*. These bacteria oxidize the ammonia and nitrites excreted by the fishes to nitrates, which are easier to absorb by the plant roots (Rakocy et al., 2006). When an appropriate balance between the fish waste generation and the plants' nutrient uptake is achieved, the daily water consumption can be reduced to the water required for replacing the losses by evapotranspiration (Timmons and Ebeling, 2017). Accordingly, the discharge of effluents into the environment is reduced. In this sense, aquaponics promotes the minimization of by-product flows from economic activities by employing them as

resources in another activity, thereby contributing to the fulfilment of the goals of circular economy (EU, 2018).

Previous researches positively evaluated the waste-to-input aquaponic technology as a sustainable approach to manage RAS wastewater, assessing the plants potential in utilizing nutrients from fishery effluents and their contribution in maintaining a proper level of water quality (Endut et al., 2016; Espinosa Moya et al., 2017). The goal of the present research is, then, to measure the water consumption of a pre-existing catfish RAS and to make a preliminary evaluation of the potential use of its wastewater for lettuce production in hydroponics, pursuant to the aquaponic principles. The specific objectives are to (a) quantify the water metabolism and losses of the existing aquaculture system, (b) compare the lettuce growth (i.e. yield, Water Use Efficiency - WUE - and Nitrogen Use Efficiency - NUE) in aquaponics vs conventional hydroponics, and (c) propose a design scenario to convert the aquaculture system into a catfish-lettuce aquaponic system. A set of experiments were therefore implemented in Soest (Germany) to address these research questions.

2. Materials and methods

2.1. Case study

Data collection was conducted at the experimental Recirculating Aquaculture System (RAS) of the South Westphalia University of Applied Sciences (SWUAS) in Soest (Germany) between June 2016 and July 2017. This plant was created in 2015 and consisted of three fish tanks of 1100 L each, filled up independently with a water inflow processed in a sedimenter (460 L), a biofilter (nitrification process), and a water heater (800 L). The water outflow from the fish tanks was channeled to the sedimenter for solid removal and, then, re-directed to the biofilter, thereby closing the water cycle. A certain amount of waste-water, however, was discarded by the system and replaced by fresh water in order to dilute contaminant concentration and keeping safe life condition both for fishes and bacteria.

Three different sizes of European Catfish (*Silurus glanis*) fingerlings (50, 300 and 400 g), with a stocking density of 90-100 $kg \cdot m^{-3}$, were separately farmed in each fish tank. Feeding rates were set as a percentage of the total fish biomass, starting from 4% for juveniles to 1.5% for adults. The fish feed used was commercial diet pellet of 2 mm diameter for small-size fishes and 4.5 mm for large-size fishes (Aller Aqua Group, Christiansfeld, Denmark) with 54% protein and 20% fat.

2.2. Experimental setup

The experiment was carried out in two phases. First, an experimental protocol was developed to quantify the water metabolism and the water losses of the aquaculture system. Subsequently, a small scale Deep Water Culture (DWC) hydroponic system was set up in order to evaluate lettuce (*Lactuca sativa* L. cv. Salanova multileaf) yield and water consumption either using the RAS effluent or three hydroponic solutions with different nutrient concentrations.

2.2.1. RAS water balance and efficiency parameters

The daily water balance of the RAS unit was calculated (Eq. 1) including all water inputs and outputs of the water metabolism occurring in the RAS room (Fig. 1). The considered water inputs were the consumption of fresh well water (Q_{ww}) and the water vapour that entered the room through ventilation (Q_{v_in}). The water outputs were the evaporation taking place from the biofilter (Q_b), the sedimenter (Q_s), the fish tanks (Q_{ft}) and the sump tank (Q_{st}), the water assimilated by fishes (Q_f), the water vapour that escaped the room through ventilation (Q_{v_out}), and the water discarded (Q_w).

$$\textit{Water inputs} = \textit{Water outputs}$$

$$\textit{Tap water consumption } (Q_{ww}) + \textit{Ventilation } (Q_{v\_in}) = \textit{Evaporation } (Q_b+Q_s+Q_{ft}+Q_{st}) + \textit{Fish water assimilation } (Q_f) + \textit{Ventilation } (Q_{v\_out}) + \textit{Wastewater } (Q_w) \quad \text{[Eq. 1]}$$

Methods for defining each element of Eq. 1 are described below.

Water consumption. The amount of well water entering the system was measured daily with a water meter.

Ventilation. The water inputs and outputs as water vapour through the ventilation system of the room (expressed as kg H_2O s^{-1}) were calculated according to Eq. 2, based on the absolute humidity (AH, $g \cdot m^{-3}$), the air flow speed (v, $m \cdot s^{-1}$), and the area of the section crossed by the airflow (A_w, m^2).

$$Q_w \left[\frac{kg\ H_2O}{s}\right] = AH \left[\frac{kg}{m^3}\right] \cdot v \left[\frac{m}{s}\right] \cdot A_w [m^2] \quad \text{[Eq. 2]}$$

The absolute humidity was calculated by employing an on-line psychrometric chart (https://www.rotronic.com) based on air pressure, air temperature, and relative humidity of the airflow. These parameters were measured 8 times in one month and the average values were used in the on-line tool. The air flow speed was measured with a propeller anemometer.

Evaporation. The water evaporation produced in the sedimenter, the fish tanks and the sump tank was calculated applying the Eq. 3, based on the evaporation coefficient (θ, $kg \cdot m^{-2} \cdot h^{-1}$), the water surface area (A_s, m^2), and the difference between the saturated air maximum humidity ratio (x_s, kg $H_2O \cdot kg^{-1}$ of dry air) and the actual air humidity ratio (x, kg $H_2O \cdot kg^{-1}$ of dry air) above the water surface.

$$Q_i \left[\frac{kg\ H_2O}{d}\right] = 24 \cdot \theta \left[\frac{kg}{m^2 \cdot h}\right] \cdot A_s \left[m^2\right] \cdot (x_s - x) \left[\frac{kg\ H_2O}{kg\ dry\ air}\right] \quad \text{[Eq. 3]}$$

The evaporation coefficient, θ, results from Eq. 4, where *v* is the velocity of the air above the water surface ($m \cdot s^{-1}$).

$$\theta \left[\frac{kg}{m^2 \cdot h}\right] = 25 + 19v \left[\frac{m}{s}\right] \quad \text{[Eq. 4]}$$

Note that the units of θ do not match with each other as it is a purely empirical formula.

The actual air humidity ratio (x) can be expressed as the ratio between the partial pressure of vapor in moist air (pw) to the atmospheric pressure of moist air (pa), where the factor 0.622 is the ratio between the molecular weight of water vapour (18.015 $g \cdot mol^{-1}$) and the average molecular weight of the other atmospheric gases (28.965 g mol^{-1}) (Shi et al., 2017) (Eq. 5).

$$x = \frac{0.662\ pw\ [Pa]}{pa\ [Pa] - pw\ [Pa]} \quad \text{[Eq. 5]}$$

164 The actual air humidity ratio and the saturated air humidity ratio were calculated based on the air relative
165 humidity, the air temperature, and the atmospheric pressure, using two online calculators of the thermodynamic
166 properties of moist air (https://www.rotronic.com and http://www.tlv.com), according to Hyland and Wexler
167 (1983). The relative humidity and the temperature of the air above the water surfaces of the fish tanks, the
168 sedimenter and the sump were measured with three different sensors, recording data each ten seconds. The air
169 speed at the water surface level was assumed as 0.05 $m \cdot s^{-1}$ (Smith et al., 2014).
170 The water evaporation from the biofilter was calculated considering the difference between the biofilter
171 ventilation outflow humidity when the biofilter is switched on and when it is off (Eq. 6).
172
173 $$Q_b\left[\frac{kg\ H_2O}{s}\right] = Q_{b,on}\left[\frac{kg\ H_2O}{s}\right] - Q_{b,off}\left[\frac{kg\ H_2O}{s}\right] =$$
174 $$\left(AH\left[\frac{kg}{m^3}\right] \cdot v\ \left[\frac{m}{s}\right] \cdot A_w[m^2]\right)_{on} - \left(AH\left[\frac{kg}{m^3}\right] \cdot v\ \left[\frac{m}{s}\right] \cdot A_w[m^2]\right)_{off} \quad \text{[Eq. 6]}$$
175
176 *Fish water assimilation.* The fish water assimilation (L day^{-1}) was calculated by assuming water being 78.11%
177 of the final fresh body weight of European catfish (Żmijewski et al., 2006). The final amount of water stored
178 in the fish biomass was divided by the days of the growing cycle (365).
179
180 *Wastewater*. The amount of discarded water equals the capacity of the sedimenter, which is emptied daily.

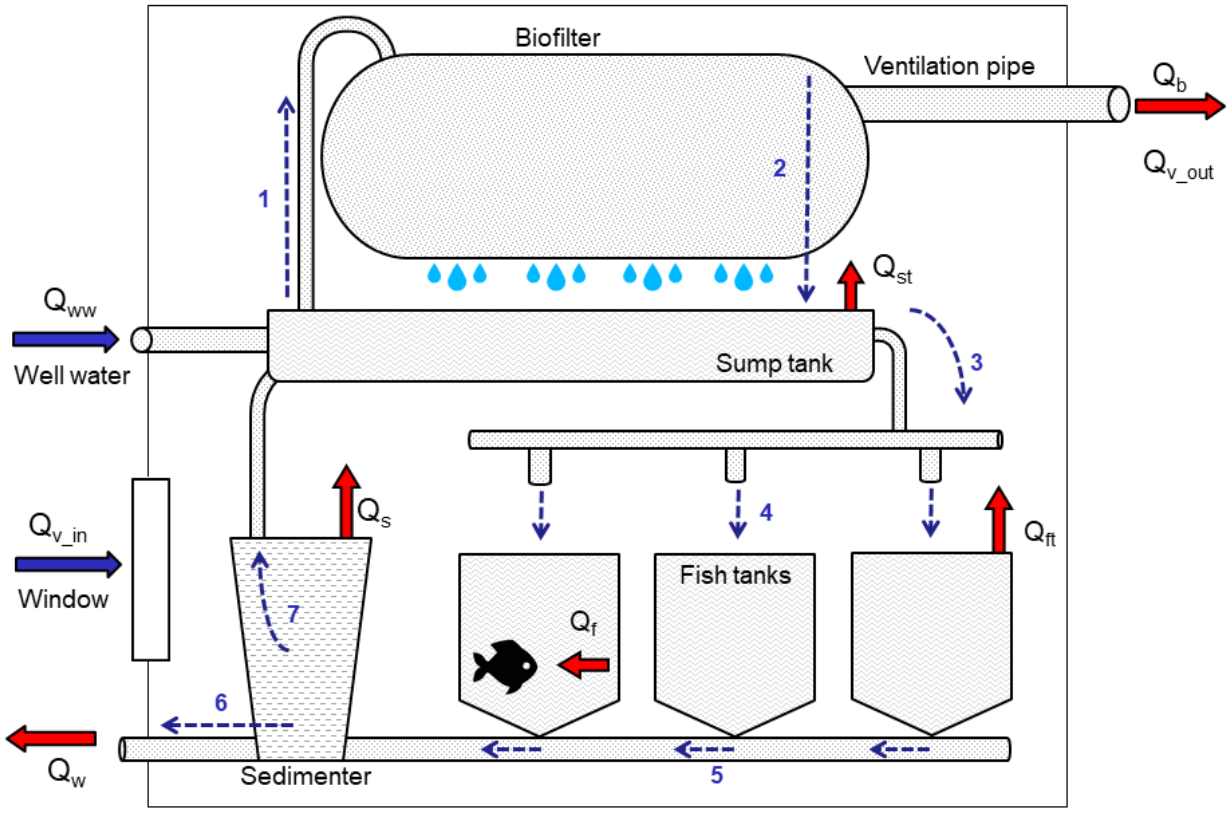


Figure 1. Graphical representation of the water flows across the aquaculture system. Dashed lines indicate the water flow: the well water enters in the sump tank and is raised to the top of the biofilter (1), falls through the biofilter net (2) and drops down again in the sump tank; from here it is distributed in the three fish tanks (3 -4); then, flows towards the sedimenter (5) and, once filtered, cycle back to the sump tank (7), from where restarts its cycle. Once a day, then, the sedimenter is emptied (6). The blue arrows represent the water input ($\mathbf{Q_{ww}}$ Well water consumption, $\mathbf{Q_{v\_in}}$ Vapour flow entering through the ventilation system). The red arrows represent the water output ($\mathbf{Q_{v\_out}}$ Vapour flow exiting through the ventilation system, $\mathbf{Q_f}$ Water assimilated by fishes, $\mathbf{Q_{ft}}$ Water evaporated by the fish tanks, $\mathbf{Q_s}$ Water evaporated by the sedimenter, $\mathbf{Q_{st}}$ Water evaporated by the sump tank, $\mathbf{Q_b}$ Water evaporated from the biofilter, $\mathbf{Q_w}$ Water discarded by the system).

The RAS efficiency was, finally, evaluated in term of water use (m^3 H_2O kg^{-1} FW), by dividing the total annual water inflow of the system (m^3) by the average annual fish fresh yield (kg) (Verdegem et al., 2006).

2.2.2 Evaluation of lettuce cultivation in aquaponics vs traditional hydroponics

Eight boxes of 30 L were employed to grow up the plants, covered by a polystyrene sheet with ten holes to support the seedlings (transplanted at sixth leaf stage). Four water treatments were imposed at different electrical conductivity (EC): Aqua (water from the RAS with EC 1.6 dS m^{-1}), Hydro 1.6 (hydroponic solution with EC 1.6 dS m^{-1}), Hydro 2.0 (hydroponic solution with EC 2.0 dS m^{-1}) and Hydro 3.0 (hydroponic solution with EC 3.0 dS m^{-1}). The initial composition, pH, EC and C:N ratio of the four nutrient solutions are described in Table 1. All the boxes were connected to an air pump to guarantee oxygenation to the nutrient solution. Air humidity and temperature were recorded by a sensor every ten minutes.

Table 1: Composition of the nutrient solutions of the treatments Aqua, Hydro 1.6, 2.0 and 3.0, respectively.

		Aqua	**Hydro 1.6**	**Hydro 2.0**	**Hydro 3.0**
pH		7.3	6.9	6.7	6.5
EC	dS·m^{-1}	1.6	1.6	2	3
NH_4^+	mmol L^{-1}	0.09	0.27	0.33	0.5
NO_3^-	mmol L^{-1}	7.36	6.94	8.73	13.24
K^+	mmol L^{-1}	0.58	6.13	7.67	11.5
Na^+	mmol L^{-1}	4.32	0.9	1.1	1.2
Ca^{2+}	mmol L^{-1}	3.4	2.93	3.67	5.5
Mg^{2+}	mmol L^{-1}	0.43	0.8	1	1.5
Cl^-	mmol L^{-1}	2.77	1.07	1.33	2
S	mmol L^{-1}	1.35	1.07	1.33	2
HCO_3^-	mmol L^{-1}	0.72	0.08	0.1	0.15
P	mmol L^{-1}	0.09	1.07	1.33	2
Fe	µmol L^{-1}	0.28	21.33	26.67	40
Mn	µmol L^{-1}	0.2	2.67	3.33	5
Zn	µmol L^{-1}	2.22	2.67	3.33	5

B	µmol L^{-1}	29.83	26.67	33.33	50
Cu	µmol L^{-1}	0.53	0.53	0.67	1
Mo	µmol L^{-1}	<0.1	0.27	0.33	0.5
N tot	mg L^{-1}	105.05	100.93	126.94	192.44
C:N ratio		0.083	0.010	0.009	0.009

Water consumptions. The evapotranspirative losses (ET_p) were measured by reading the water level decrease inside the boxes through a graduated scale.

Biomass production. Once harvested, plants' shoots were immediately separated from roots and weighted in order to obtain the fresh weight (FW). Dry weight (DW) was determined after oven-drying the sample for three days at 105°C. According to Qiansheng et al. (2018) the lettuce root biomass in hydroponics accounts for only about the 10% of the total fresh biomass, therefore it was considered negligible and not taken into account for the calculation of the below-listed parameters. The dry matter percentage (DM) was calculated by the ratio between the lettuce head dry (g) and fresh (g) weight, and expressed as percentage.

C:N ratio: Total leaf N and C content, were measured on dry samples with a LECO CNS2000 elemental analyzer (LECO Corporation, St. Joseph, MI, USA).

WUE. Total water use efficiency (g FW L^{-1} H_2O) was calculated as the ratio of the FW (g) of the plants of each box to the relative total water consumption (L) (Fallovo et al., 2009).

NUE. Water samples of the nutrient solution were collected at the beginning of exp. 2 and 3. These were analyzed for main macro and microelements by the Eurofins Agro water test laboratories (Binnenhaven 5 NL - 6709 PD, Wageningen, The Netherlands). The NUE (g FW mg^{-1} N) was calculated by the ratio between the average fresh weight of the plants of each box (g) and the total elemental nitrogen available at the beginning of the growing cycle in the nutrient solution (mg) (Benincasa et al., 2011).

2.2.3. Design for a closed water cycle

With the aim of boosting the resources efficiency of the aquaculture system, a DWC hydroponic unit can be introduced in the already existing RAS to recover its water discharge and recycle it as nutrient solution for lettuce cultivation. In such a system, water requirements are only limited to those needed to compensate evaporative water losses. Plants in aquaponics play a key role in water quality control notably as concerns N- and P-containing nutrients (Goddek et al., 2015). For a proper operation of an aquaponics system, therefore, the plant cultivation must be dimensioned to the fish stocking density in order to ensure an efficient control of nutrient load.

Keeping the size of the actual RAS system, a design for an aquaponic system for lettuce-catfish production is here proposed. The proportion of the DWC beds is calculated through a parameter elaborated by Rakocy et al. (2006) called Feeding Rate Ratio (FRR) (Eq. 7), which express the relation between the fish feed amount introduced in the system and the plant growing area.

$$\mathrm{FRR}\ \left[\frac{\mathrm{g}}{\mathrm{m^2 d}}\right] = \frac{\text{fish feed [g]}}{\text{plant area [m}^2\text{]} \cdot \text{day}} \qquad \text{[Eq. 7]}$$

The optimum FRR for raft hydroponics varies from 60 to 100 $g \cdot m^{-2} \cdot day^{-1}$ (Rakocy et al., 2006). Accordingly, for the purpose of this study, a FRR of 80 $g \cdot m^2 \cdot day^{-1}$ for raft hydroponic lettuce production was set. The computations were done assuming that the production should be split and harvested in staggered phases to keep constant the optimal stocking density (Rakocy et al., 2006). A fish productive cycle of 12 months was assumed to allow obtaining adults of 2 kg (with a final stocking density of 100 $kg \cdot m^{-3}$) from fingerlings of 300 g (data established according to the performance of previous cycles and literature data, e.g. Żmijewski et al., 2006). The fish production should be staggered to harvest once a year from each tank, with a time step of about 17 weeks (the three harvests being distributed along the year). Starting from a plant at the sixth unfolded leaf stage, a productive growing cycle of 4 weeks was considered, setting a plant density of 16 plants m^{-2} (Rakocy et al., 2006).

2.3. Statistical analysis

The experimental data were treated statistically using CoStat software package. A two-way analysis of variance (ANOVA) at 95% confidence was performed to test the influence of the growing cycle and the water source on evapotranspiration losses, biomass production, WUE and NUE. Means were compared using the LSD test at $P \leq 0.05$.

3. Results and discussion

3.1. RAS water balance and efficiency parameters

The results of the water balance on the aquaculture unit showed that about 555 L of water flow through the system every day. A description of the water metabolism elements is reported in Table 2.

Table 2. Daily water input and output within the aquaculture system.

AQUACULTURE SYSTEMS			
INPUT		**OUTPUT**	
Air absolute humidity (kg·day $^{-1}$)	22.9 ± 3.0	Air absolute humidity (kg·day^{-1})	52.6 ± 1.41
		Biofilter (kg·day $^{-1}$)	17.7 ± 1.52
		Sump tank (kg·day^{-1})	6.7 ± 0.26
		Fish tanks (kg·day^{-1})	5.8 ± 0.16
Well water (L·day^{-1})	532	Sedimenter (kg·day^{-1})	0.9 ± 0.09
		Fish water assimilation (L·day^{-1})	0.70
		Water discharge (L·day^{-1})	460
		Undetermined losses (L·day^{-1})	10.45
TOTAL INPUT	554.9 ± 3.0	**TOTAL OUTPUT**	554.9 ± 3.44

The biofilter produced the highest evaporation losses (17.7 L·day^{-1}), which resulted 62.5%, 67.2% and 95.2% higher than those generated from the sump, the fish tanks and the sedimenter (6.7, 5.8, and, 0.9 L·day^{-1}), respectively. This could be due to a peculiarity of the biofilter used in this system which exploits the water evaporation to cool down the air temperature.

Water use for catfish in the experimental RAS was 0.59 $m^3 \cdot kg^{-1}$, 2.5 folds higher than that recorded for tilapia production in RAS (0.24 $m^3 \cdot kg^{-1}$) (Eurofish, 2009). Previous experiences also suggest that water consumption values are highly variable in response to the fish productive system adopted: they range from 0.5 to 0.7 m^3 kg^{-}

276 [1] in a super-intensive recirculating system (Verdegem et al., 2006) or average 1 $m^3 \cdot kg^{-1}$ in a RAS system
277 (Bregnballe, 2015) but also increase up to 45 $m^3 \cdot kg^{-1}$ in an extensive pond system (Verdegem et al., 2006).
278 The average fish yield of the studied RAS was 329 $kg \cdot year^{-1}$, which corresponds to a total amount of water
279 assimilated by the fish of 257 L.
280
281 **3.2 Evaluation of lettuce cultivation in aquaponics vs traditional hydroponics**
282
283 ***Climatic condition***. Exp. 1 was performed between July and August 2016. The minimum, average and
284 maximum temperatures were respectively 17.0, 28.4 and 46.0°C in July and 13.7, 23.5 and 34.4°C in August.
285 The mean daily temperature was higher than the average monthly temperature for 13 days. Exp. 2 was
286 performed between the last days of September and October 2016. The minimum, average and maximum
287 temperatures were respectively 14.5, 21.1 and 34.0°C in September and 12.0, 18.0 and 30.5°C in October. The
288 mean daily temperature was higher than the average temperature for 17 days. Exp. 3 was performed between
289 June and July 2017. The minimum, average and maximum temperatures were respectively 16.0, 24.3 and
290 50.0°C in June and 17.0, 23.9 and 39.0°C in July. Mean daily temperature was higher than the average
291 temperature for 20 days. The absence of an automated system for temperature management did not allow to
292 prevent the occurrence of extreme temperatures.
293
294 ***Water and nutrient consumption*.** The water consumptions did not show statistically significant differences
295 (P-value>0.05) among the four treatments (Hydro 1.6, Hydro 2.0, Hydro 3.0 and Aqua), although seasonal
296 variations in total water consumption were observed among the three experiments. The average daily amount
297 of evapotranspiration was 52.0, 27.3 and 58.4 $mL \cdot head^{-1} \cdot day^{-1}$ for the exp. 1, 2 and 3, respectively. Similar
298 results were observed by Ciolkosz et al. (1998) and Conversa et al. (2004), who recorded respectively ET
299 values comprised between 24 and 178 $mL \cdot head^{-1} \cdot day^{-1}$ and between 76 to 214 $mL \cdot head^{-1} \cdot day^{-1}$ in lettuce grown
300 in hydroponics. The lowest ET value were recorded in the exp. 2, which coincided with the lower temperatures
301 experienced during the autumn season. The composition of the nutrient solutions at the end of the experiments
302 is reported in Table 3. In all cycles the EC of the treatments Hydro 1.6 and 2.0 was reduced from the initial
303 value, whereas an increase in EC in both Aqua and Hydro 3.0 treatments was observed during cycles 1 and 3.

A pH increase was observed for all the nutrient solutions in every cycle, with special emphasis for the treatments Hydro 1.6 and 2.0. The ammonium concentration dropped close to zero in all the treatments, while the nitrate concentration increased, especially in hydroponic. The potassium, phosphorus, molybdenum and manganese concentrations in aquaponics, already low at the beginning of the experiment, reached almost zero at the end of the three cycles, while the final potassium and molybdenum concentration in hydroponics was increased from the initial value, possibly as a consequence of water evaporation and root selective uptake (Albornoz and Lieth, 2015). The aquaponics iron concentration, instead, remained almost constant or increased, probably due to both the solution evaporation and the lower iron availability when pH is above 7. Calcium concentration, as well, did not vary largely in aquaponics while it was one forth on average in the other solutions. This might be attributed to the uptake competition played by the sodium ion, since its initial concentration in aquaponics was 4-folds higher than in hydroponics (Albornoz and Lieth, 2015).

Table 3: Composition of the nutrient solutions of the treatments Aqua, Hydro 1.6, 2.0 and 3.0 at the end of the three experiments.

		Aqua			**Hydro 1.6**			**Hydro 2.0**			**Hydro 3.0**		
Cycle		**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
pH		7,52	7,76	7,65	7,55	7,9	7,7	6,9	7,14	7,7	6,4	7,05	6,95
EC	$dS \cdot m^{-1}$	1,85	1,3	1,8	1,5	1,2	1,25	1,8	1,45	1,25	3,25	2,65	3,05
NH_4^+	mmol L^{-1}	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1	<0,1	<0,1	< 0,1
NO_3^-	mmol L^{-1}	9,2	5,7	4,85	1,8	0,4	< 0,1	3,45	1,6	< 0,1	8,7	6,3	4,25
K^+	mmol L^{-1}	0	<0,1	< 0,2	9	6,45	8,4	11,9	8,35	8,4	24,1	17,6	22,6
Na^+	mmol L^{-1}	5,35	3,55	9,65	1,4	1,4	1,95	1,2	1,3	1,95	1,35	1,55	2,2
Ca^{2+}	mmol L^{-1}	4,8	3,5	4,5	0,65	0,65	0,75	0,85	0,85	0,75	2	1,4	1,1
Mg^{2+}	mmol L^{-1}	0,55	0,4	0,5	0,25	0,2	0,2	0,2	0,2	0,2	0,3	0,3	0,2
Cl^-	mmol L^{-1}	3,85	2,55	5,05	2,75	2,55	2,25	3,05	2,8	2,25	5,1	4,75	5,65
S	mmol L^{-1}	2,05	1,5	2,55	2,9	2,35	3,8	3,25	2,75	3,8	5,35	4,35	6,35
HCO_3^-	mmol L^{-1}	0,15	0,8	3,85	0,4	1,5	0,95	0,1	0,9	0,95	<0,1	0,35	0,2
P	mmol L^{-1}	<0,04	<0,04	< 0,04	0,575	0,42	0,81	1,35	0,59	0,81	3,055	1,325	2,32
Fe	µmol L^{-1}	0,35	0,2	0,55	21,5	12	28,5	11,15	16,5	28,5	17,5	23	81,5
Mn	µmol L^{-1}	0,1	< 0,1	< 0,1	0,2	0,15	0,15	0,1	0,15	0,15	1,75	0,8	0,25
Zn	µmol L^{-1}	2,4	2,85	0,15	24	7,4	8,35	48,5	8,65	8,35	75	30,5	14

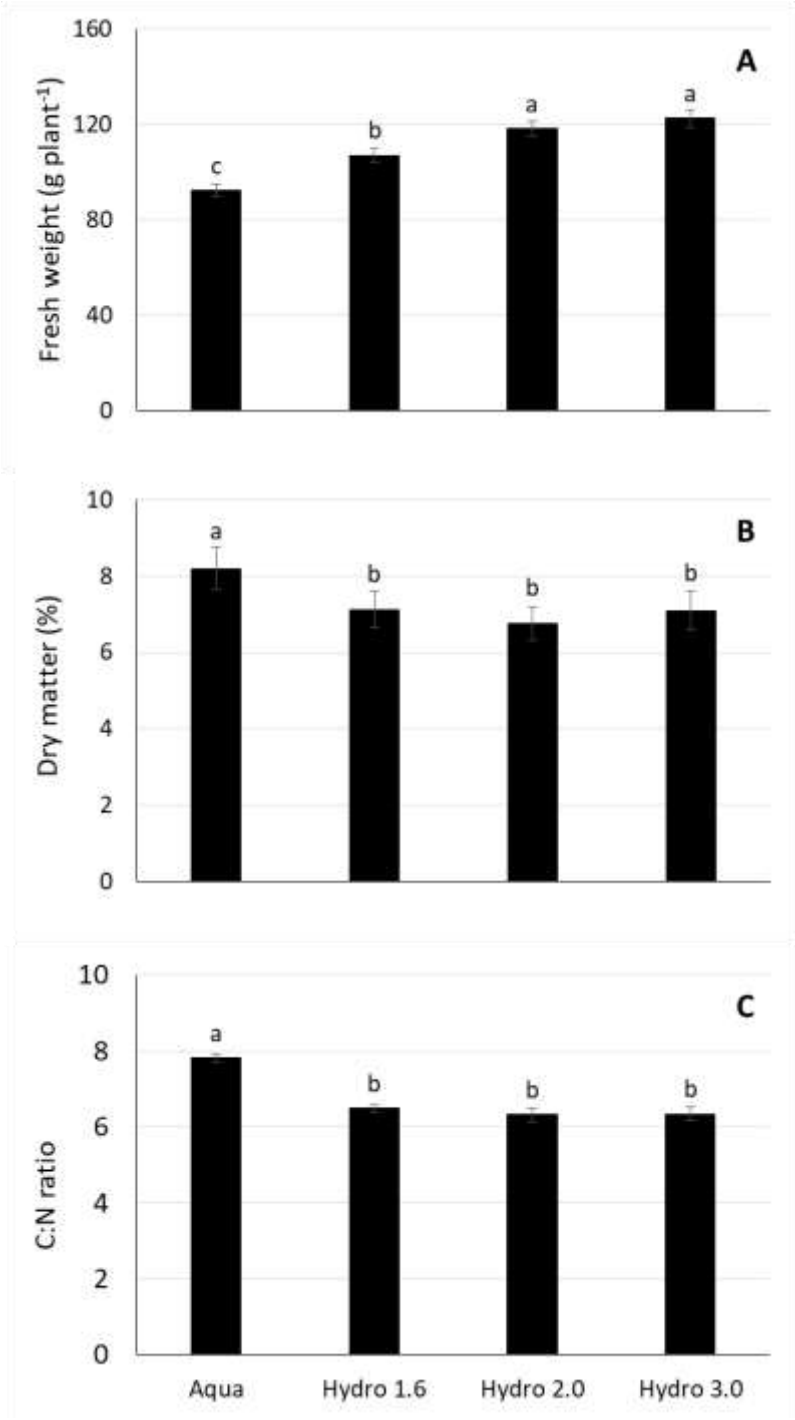
B	µmol L^{-1}	36	21,5	91	53	44	82	65,5	58	82	110	99,5	143
Cu	µmol L^{-1}	0,35	0,45	0,95	1,3	1,4	3,15	1,1	1,7	3,15	2,05	1,7	3,85
Mo	µmol L^{-1}	0	< 0,1	< 0,1	1,7	0,4	0,85	1,35	0,4	0,85	1,55	0,6	1,3
N tot	mg L^{-1}	130,12	81,09	69,19	26,47	6,86	2,52	49,58	23,67	2,52	123,12	89,50	60,79
C:N ratio		0,014	0,118	0,668	0,181	2,625	4,526	0,024	0,457	4,526	0,009	0,047	0,040

Yield. Considering that not statistically significant interactions were detected between the two experimental factors (growing cycle and water source), mean values are used for presenting yield data. Lettuce grew the least in aquaponics (92.3 g·plant^{-1}), whereas higher biomass productions were achieved in the Hydro 2.0 and 3.0 (mean value of 120.3 g·plant^{-1}) similarly to previous studies on aeroponically grown lettuce (Albornoz and Lieth, 2015). The absence of yield differences between Hydro 2.0 and Hydro 3.0 shows that nutrients in the latter resulted in luxury consumption rather than increasing biomass production nor leading to salinity symptoms (Nozzi et al., 2018).

A lower performance in aquaponics versus hydroponics was previously described by El Sayed and Samir (2015), and Johnson et al. (2017), whereas Pantanella et al. (2012) and Delaide et al. (2016) did not observe differences in yield among the two growing systems. The lower yield in aquaponics may be associated to reduced K^+, P^+, Fe^{2+} and Mn^{2+} concentrations in the nutrient solution (Rakocy et al., 2007). The amount of these nutrients in aquaponics is often not adequate to the plant requirements due to their low concentration in most of the commercial fish feeding formulations, and because part of them precipitate and is lost in the form of fish solid excretion (Rakocy et al., 2007; Goddek et al., 2015). Besides, to counteract the pH drops due to the bacterial nitrification process, a bicarbonate buffer was periodically added to the RAS circulating medium. The subsequent higher HCO_3^- concentration in the aquaponics solution, combined with the higher pH (Table 1), may have contributed to reduce the nutrient solubility and absorbability (Pignata et al., 2017), especially for phosphorous, iron, manganese, magnesium and calcium (Trejo-Téllez and Gómez-Merino, 2012). Furthermore, it has also been suggested that the bacterial community from the aquaponics may compromise the nutrient availability by consuming nutrients or by increasing the energetic cost to import them across the root interface (Wielgosz et al., 2017). Goddek et al. (2015) stated that aquaponics can match hydroponics when all the parameters are controlled, and the bacterial community is fully mature. In the current study, the nutrient

imbalance in the aquaponics water solution may indicate that the fish stocking rate and/or their dietary composition need to be adjusted to supply the plants with the proper nutrients amount.

Despite the lowest yield, however, the DM% and C:N ratio in leaf tissues were significantly higher in aquaponics than in hydroponics (Fig. 1). This may depend on the different nutrient composition and availability in the two growing systems. As already mentioned, indeed, the C:N ratio of the nutrient solution in aquaponics was, 8.7-fold higher than the average value in the three hydroponic treatments (Table 1). Moreover, as stated by Nozzi et al. (2018) a P deficiency in the nutrient solution (Table 1) reduces the root N uptake. This, in turn, induces accumulation of non-structural carbohydrates (e.g. organic acids and sugars) for the cellular osmoregulation, resulting in a DM increase and, consequently, also in changes in the C:N ratio. The absence of significant variations in DM among the hydroponic treatments, on the other hand, is in line with the result of Ünlükara et al. (2008) and Scuderi et al. (2009) which reported a nearly constant DM content in plants growing under variable salinities, even over 3 dSm^{-1}.



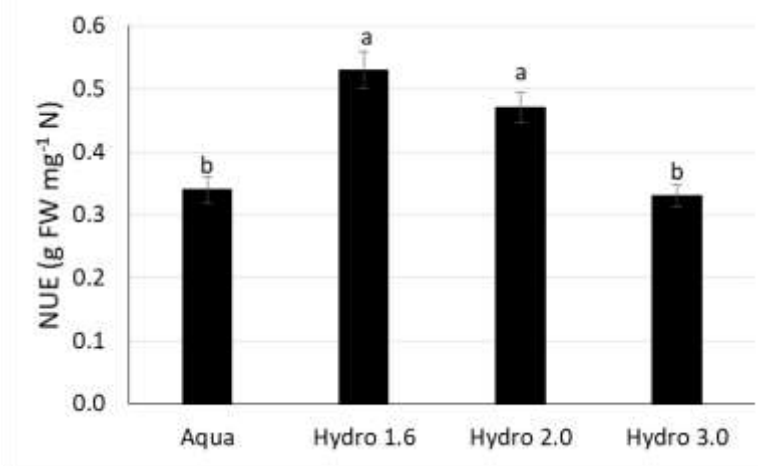
354

355 **Figure 1.** Fresh weight (A), dry matter (B), and C:N ratio (C) of lettuce plants grown under different water
356 regimes. Data referring to exp. 1, 2 and 3 are indicated as mean ± SE. Different letters indicate significant
357 differences at $P \leq 0.05$.

358

359 ***NUE.*** Given that the interaction between the two experimental factors (growing cycle and water source) was
360 not statistically significant, the mean values for NUE were used. In the hereby presented experiments, NUE in
361 aquaponics (0.34 g FW·mg^{-1} N) was significantly lower than in Hydro 1.6 and 2.0 (0.5 g FW·mg^{-1} N on

362 average), but statistically non different from the Hydro 3.0 treatment (0.33 g FW·mg^{-1} N). The lower
363 performance in aquaponics may, again, depend on the different ratio of nitrogen forms in the nutrient solution.
364 Ammonia (NH_4-N) level in aquaponics was, in fact, below 0.1 mmol·L^{-1} (Table 1) due to the nitrification
365 operated by the bacteria of the RAS biofilter. Despite most plants prefer NO_3^- over NH_4^+ as nitrogen source,
366 the NO_3^- acquisition and assimilation is more energy demanding than ammonium and, then, a 1:3 ratio of
367 NH_4^+:NO_3^-, compared with nitrate alone as the sole source of N, showed to be beneficial to plant growth and
368 yield in hydroponics (Savvas et al., 2006).
369 Increasing the nitrogen fertilization and uptake may not necessarily lead to improved crop yield and NUE
370 (M'hamdi et al., 2014). A similar behaviour was observed in the present experiment when, rising the EC up to
371 3 dS·m^{-1}, (N concentration of 243 mg·L^{-1}), the yield did not increase, leading to a decrease in NUE (Fig. 2).
372 This is consistent with the findings of Stefanelli, Winkler and Jones (2011) who reported increased lettuce
373 NUE at N concentration from 40 to 75 mg·L^{-1}, reaching a plateau at 150 mg·L^{-1} and with no subsequent
374 increases between 400 and 2400 mg·L^{-1} N. Similarly, according to Mahlangu et al. (2016), a N concentration
375 of 100-120 mg·L^{-1} is enough to improve growth, yield and quality parameters of hydroponic lettuce. In the
376 present case, the N concentration at 2.0 dS·m^{-1} (162.0 mg·L^{-1} N) enhanced NUE and maximized the
377 production, while the increased nitrogen input in Hydro 3.0 (243 mg·L^{-1} N) resulted in a NUE decline with no
378 further yield increase.
379



380

381 **Figure 2**. Nitrogen use efficiency (NUE) of lettuce plants grown under different water regimes. Data referring
382 to exp. 2 and 3 are indicated as mean ± SE. Different letters indicate significant differences at P≤0.05.

383

***WUE*.** The interaction between the growing season and the water source significantly affected the lettuce WUE. Aquaponics always showed the lowest WUE [69.8 g FW·L^{-1} H_2O for exp. 1, 98.5 g FW·L^{-1} H_2O for exp. 2 and 48.1 g FW·L^{-1} H_2O for exp. 3] (Fig. 3). This is probably due to a decrease in the photosynthetic efficiency rather than in the leaf evaporation as no difference in water consumption arose among aquaponics and hydroponics treatm ents (De Costa and Ariyawansha, 1996). In addition, it is worth to mention that Na^+ concentration in aquaponics was 4-folds higher than in hydroponics (Table 1). As already observed by Tzortzakis (2009), moderate sodium stress is more likely to affect elemental absorption by competition than interfering with the root water uptake, leading to ions imbalance, restricted nutrient uptake and consequent yield reduction. The production of osmolytes to counteract the specific Na^+-related osmotic stress may be another justification of the higher leaf DM encountered in aquaponics. In conclusion, the nutrient imbalance in aquaponics, namely the higher sodium concentration combined with the lack in potassium, phosphorous, iron and manganese, had a more negative impact on plant yield and WUE then the higher water conductivity in the hydroponic treatments.

In hydroponics, different WUE trends were observed in the three cycles. In exp. 1 no WUE differences arose between the three treatments. In exp. 2, the WUE of the Hydro 2.0 and 3.0 treatments were comparable but resulted significantly higher than the Hydro 1.6. In exp. 3, significant differences could only be found between Hydro 1.6 and Hydro 3.0, the latter presenting higher values. According to the obtained results, it may be argued that an EC increase over 1.6 dS·m^{-1} could possibly boost WUE. Moreover, although significant differences could be observed between the treatments Hydro 1.6 and Hydro 3.0, the latter never differed significantly from the intermediate Hydro 2.0. Hence, bearing in mind the objective to contain the productive costs and the chemical input, using an EC of 2.0 in place of 1.6 dS·m^{-1} can boost the crop yield without interfering with the NUE and WUE. The lower WUE observed in the summer cycles (exp. 1 and 3) may be due to the higher solar radiation and temperature which may have increased the atmospheric evaporative demand and the plant respiratory losses (Fallovo et al., 2009) as compared with the autumn cycle (exp. 2). A similar behaviour was observed also by Shaban et al. (2016), who observed a WUE increase when lettuce was subjected to irrigation water cooling and shading. Lettuce, indeed, is a short-day cool season crop with an optimum temperature range of 7-24 °C (Shaban et al., 2016). Under warmer condition the plant increase the transpiration rate to regulate the leaf temperature, through the mechanism of

evaporative cooling. However, although transpiration is positively correlated to biomass accumulation, upon elevate temperature the plant reduces its ability to regulate the water relations and further transpiration losses do not provide extra biomass gain, lowering thereby the WUE (Zhang et al., 2015). The high temperature, combined with the high radiation, may have also resulted in reaching the light saturation point, inducing photoinhibition and stomatal closure (Hunt et al., 1984). Hence, it may be argued that the decreased photosynthesis efficiency and carbon assimilation, associated with an increase in the canopy transpiration, might have negatively affected the plant WUE during the two summer cycles.

The achieved WUE values are, however, consistent with the results of Chabite et al. (2017) that also obtained values among 29.7 and 142.9 $g \cdot L^{-1}$ for lettuce grown using different nutrient solutions.

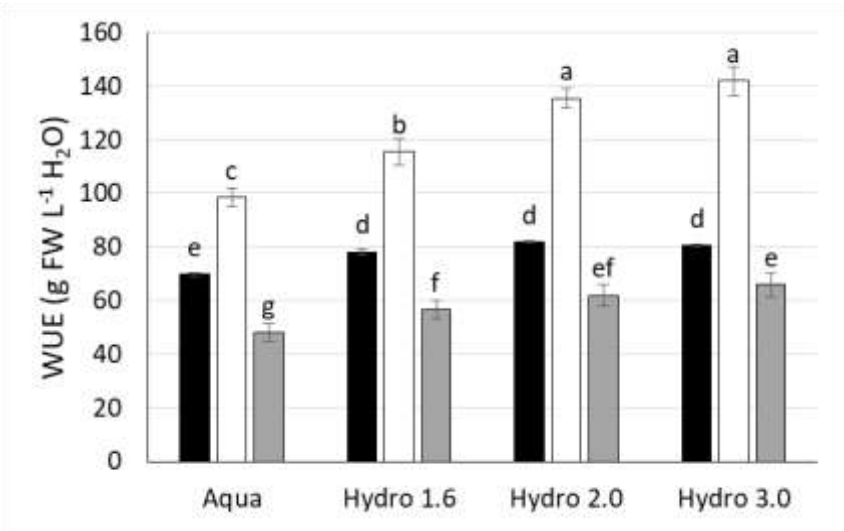


Figure 3. Water Use Efficiency (WUE) of lettuce plants grown under different water regimes. Black bars represent results of exp. 1, white bar represent results of exp. 2, grey bar represent results of exp. 3. Data are expressed as mean ± SE. Different letters indicate significant differences at $P \leq 0.05$.

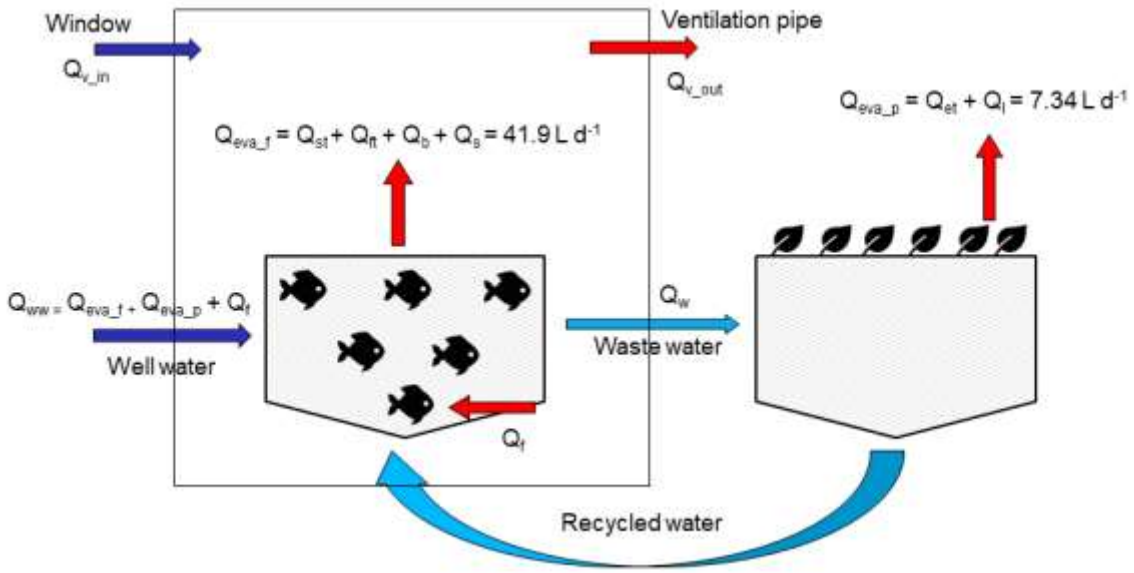
3.2. Design proposal to convert the RAS into a catfish-lettuce aquaponic system

In this section, a design for introducing a lettuce DWC hydroponic unit into the already existing RAS is proposed, with the goal to transform it in a closed productive aquaponic system.

According to Eq. 1 and the data from the Table 2, the total water input and output within the RAS consisted of 554.9 $L \cdot day^{-1}$. The estimated potential catfish production of the current RAS is 329 $kg \cdot year^{-1}$. According to

the calculation procedure elaborated by Rakocy et al. (2006), a lettuce growth bed area of 9.63 m^2 is required to satisfy the nitrogen filtration demand at this productive rate. Keeping the suggested plant density of 16 plants m^{-2}, the projected 10 m^2 DWC plant may host up to 160 lettuces per month. According to the attained results on the lettuce mean water consumption, i.e. 45.9 $mL \cdot head^{-1} \cdot day^{-1}$, an average water consumption (Q_{eva_p}) of 7.3 $L \cdot day^{-1}$ is expected for a raft system hosting 160 plants. The current well water input (Q_{ww} = 532 $L \cdot day^{-1}$) could, therefore, be reduced to the amount needed to replace the water lost by evaporation from the RAS (Q_{eva_f} = 41.6 $L \cdot day^{-1}$), from the hydroponics tubs (Q_{eva_p} = 7.3 $L \cdot day^{-1}$), and the summed water withheld in the fish tissues (Q_f = 0.7 $L \cdot day^{-1}$) (Figure 4). With the introduction of the proposed hydroponic unit, then, the RAS water discharge (Q_w = 460 $L \cdot day^{-1} \cdot m^{-2}$) will be fetched to supply the DWC system and the system water metabolism will improve as the water output will decrease from 554.9 to 102.53 $L \cdot day^{-1}$ (Table 4).

By installing an environment control system in the greenhouse, the lettuce production can be realized continuously over the 365-days cycle of catfish. A staggered crop production system, however, is suggested to keep the water quality relatively constant and allow the lettuce harvest with regular cadency. Assuming a 30-day crop cycle, then, the lettuce production can be staggered so that four growth stages can be simultaneously cultivated in one month and one-fourth of the lettuces can be weekly harvested (40 lettuce heads). For the fish production, as well, three fish ages should be contemporary reared in the three tanks in order to produce an effluent whose composition remains relatively constant. Accordingly, the fish from one tank will be stocked once every four months, with an expected potential production of 82.3 kg $quadrimester^{-1}$. The yearly production, then, will consist of 329 kg of fish meat plus 1920 lettuce heads, which correspond to a biomass production of 17.7 $kg \cdot m^{-2}$, if the average lettuce FW obtained in aquaponics during the hereby study (92.3 $g \cdot plant^{-1}$) is considered.



454

455 **Figure 4.** Graphical representation of the water flows across the proposed catfish-lettuce aquaponic system.
456 The dark-blu arrows represent the water input: $\mathbf{Q_{ww}}$ Well water consumption, $\mathbf{Q_{v\_in}}$ Vapour flow entering
457 through the ventilation system. The red arrows represent the water output: $\mathbf{Q_{eva\_p}}$ = ($\mathbf{Q_{ft}}$ Water evaporated
458 by the fish tanks + $\mathbf{Q_s}$ Water evaporated by the sedimenter + $\mathbf{Q_{st}}$ Water evaporated by the sump tank + $\mathbf{Q_b}$
459 Water evaporated from the biofilter), $\mathbf{Q_f}$ Water assimilated by fishes, $\mathbf{Q_{v\_out}}$ Vapour flow exiting through
460 the ventilation system, $\mathbf{Q_w}$ Water discarded by the system, $\mathbf{Q_{eva\_p}}$ = (Q_{et} Water evaporated by the raft
461 hydroponic system + $\mathbf{Q_l}$ Water assimilated by the plant tissue. The light-blu arrows represent the circular
462 water pathway among the two aquaculture and hydroponic sub-units.

463

464 **Table 4**. Comparison of the water input-output and of the marketable products obtainable from the current
465 RAS system and from the proposed catfish-lettuce aquaponics system. All the water-related items are
466 expressed in L day^{-1}: Q_{ww} Well water consumption, $\mathbf{Q_{v\_in}}$ Vapour flow entering through the ventilation
467 system, $\mathbf{Q_{eva}}$_Water evaporated by the aquaculture tanks and/or the hydroponic tanks, $\mathbf{Q_f}$ Water assimilated
468 by fishes, $\mathbf{Q_{v\_out}}$ Vapour flow exiting through the ventilation system, and $\mathbf{Q_w}$ Water discarded by the system.

Total water input (Q_{v_in} + Q_{ww})	Well water input (Q_{ww})	Total water output (Q_w + Q_{eva} + Q_f + Q_{v_out})	Water discarge (Q_w)	Evaporation losses (Q_{eva})	Yield

Current scenario	554.9	532	554.9	460	41.6	360 kg year^{-1} fish meat
Proposed scenario	72.84	49.64	102.53	/	48.94	360 kg year^{-1} fish meat 1920 lettuce heads year -1

4. Conclusion

The present study contributes to the research in aquaponics, offering innovative figures on the water consumption of a catfish recirculating aquaculture and the potential for wastewater saving associated with lettuce production in aquaponics. The results of this study indicated that the considered RAS aquaculture system has a daily water consumption of 555 L·day^{-1}, out of which the 83% is direct water discharge from the system while evaporation losses from the system' tubs account for 31 L·day^{-1} and humidity detraction from the plant room accounts for 53 L·day^{-1}. With the introduction of a hydroponic component into the system, the discharged water can be recovered and used as nutrient solution for a 10 m^2 raft hydroponic system. Additional researches are, however, needed to lower the component of evaporative losses by the system.

The nutrients requiring supplementation in the hydroponic unit would be ammounium, potassium, phosphorous, iron and manganese, whereas the amount of dissolved sodium and bicarbonate ions may be too elevated to sustain plant productivity. Such nutrient imbalance may be the reason of the lower observed yield, WUE and NUE in aquaponics. Given the already high level of nitrate and sodium, a further increase in the fish stocking density should be avoided while the strategy of integrating the aquaponic solution with synthetic mineral elements, as proposed by Delaide et al. (2016), could be tested to improve the nutrient solution composition. This option would still allow reduced fertilizer costs and environmental impacts compared to traditional hydroponic, but more in-depth investigations would be necessary to determine the nutrient supplementation effects on fish physiology. A change in the fish feeding diets should also be considered. Fish feed contains 0.1 to 0.3% added sodium (Mallick and Rahman, 2005), although such high concentrations is not really necessary to fishes (Rakocy et al., 2007). In order to reduce the levels of Na^+ in the fishery wastewater, then, alternative feed formulations having higher level of potassium and plant-protein, instead of animal-protein, should be tested for combined catfish-lettuce growth. Finally, studies on the role played by

both microorganism and organic compounds on the crop performances could contribute to make this picture more complete.

In conclusion, the aquaponics system overall environmental sustainability builds on the avoided impact associated with both the RAS wastewater released into the environment and the less chemical input needed for lettuce hydroponic production as compared with two systems operating independently.

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