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Nutrition and Quality of Aquaponic Systems

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Dedicated to my beloved family,
Alessandro, Ana, Kae, Louise
and all my friends

Declaration

I hereby declare that this thesis has been composed entirely by myself and has not been previously submitted to any other degree or qualification.

The work of which it is a record has been carried out by me. The nature and extent of any work carried out by, or in conjunction with, others has been specially acknowledged by reference.

Edoardo Pantanella

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Abstract in English

The future of the agriculture is in the development of sustainable production systems that make better use of inputs and by-products as well as improve quality standards under competitive costs. Integration is seen one of the key aspects to optimize improve sustainability. In the case of agriculture sustainability can be achieved by closing the loop between crop and animal productions. The combination of plants with fish thus appears an optimal strategy to enhance productivity and to reduce footprint to minimal terms. Aquaponics is a vegetable production technique that integrates soilless cultivation with closed recirculating aquaculture systems. In aquaponics plants grow on aquaculture wastes and reclaim water back to fish through phytodepuration. The zero-discharge and fertilizer-free management makes aquaponics an environmental-friendly and profitable production system. Aquaponics infact overtakes all the pollution and input management issues that agriculture and aquaculture would have if taken alone. Nutrient concentrations in aquaponics are sensitively lower than those used in hydroponic systems due to the storage of nutrients into the recirculating water and the continuous supply of minerals by fish. Although aquaponic systems have been studied for decades, vegetable quality and productivity of aquaponics against hydroponics still needs to be fully developed. The expansion of aquaponics to large scale commercial systems is possible providing that production maintain same standards and quality of marketed products.

The research, carried out at the Experimental Farm of the University of Tuscia assessed the production and quality of aquaponic productions. Several vegetables and fish species were tested across three years of experiments to determine system efficiency and identify best farming practices.

In the case of lettuce (*Lactuca sativa* L cv Integral) cropped with tilapia (*Oreochromis niloticus* L.) similar productions were obtained between aquaponics and hydroponics despite the very low levels of nitrogen (from 15 mg L^{-1}) and electrical conductivity (0.6 dS m-1). Quality assessment showed that chlorophyll in plants is similar if not higher to hydroponics and production does not have harmful levels of nitrates in leaves.

The quality of basil and the influence of fish diet on water nutrients was studied in two consecutive summer trials. For sweet basil (*Ocimum basilicum* L. cv superbo) African catfish (*Clarias gariepinus* B.) was stocked under same densities but different protein diets (31 and 40% protein). Productivity in aquaponics was not different from hydroponics and comparable to literature data. The qualitative traits of plants in terms of chlorophyll and leaf/stem ratio were not different among treatments. Nevertheless plants obtained higher leaf biomass par rapport to literature data. Fish performance was similar to traditional recirculating systems, which suggested

that aquaponics is not a limiting environment for fish. Nevertheless nitrogen levels grew with double pace with higher proteins in diet. System assessment suggested that optimal nutrient uptake efficiency occurrs when water nutrient concentrations are low.

Production and quality of fruity plants was tested in aquaponics with the aim to assess system performance under lower potassium concentrations than hydroponics. Cucumber (*Cucumis sativus* L. cv Ekron) grew on largemouth bass (*Micropterus salmoides* L.) wastewater in a summer crop under greenhouse environment. Yields, marketable product (fruit weight above 180 g), pH, brix degree and tritatable acidity were similar to hydroponics. Likewise nitrogen uptake efficiency appeared similar despite higher nitrogen losses in aquaponics due to ecosystem's metabolism.

Aquaponics could also integrate marine farming through salt-tolerant species. Impact of marine aquaculture can be attenuated by using plants that are widely used in horticulture as specialty salads. Three *Salsola soda* trials were carried out with mullet (*Mugil cephalus* L) under raising salt concentrations: 5 g L^{-1}, 10 g L^{-1}, 20 g L^{-1}, 30 g L^{-1}. Production and nutrient uptake were assessed to determine the stripping capacity and the salt accumulation of the plants. Salinity at 10 g L^{-1} resulted the most productive, but interesting growth was observed till 20 g L^{-1} of salt. Aquaponics proved to be more performing than hydroponics even though nitrogen levels were 3-5 times lower thus confirming the good potential of cropping high value vegetables as a phytoremediation strategy.

The choice of growing method is a critical factor in aquaponics. Not only is growth affected by nutrient concentrations, but also by delivery systems. Growth performance in lettuce (*Lactuca sativa* L. cv Verde degli Ortolani) was assessed on floating system, NFT and substrate system for three consecutive crops under growing nutrient concentrations. Results showed high productions from floating systems, which was the most constant system in terms of output. However NFT resulted outperforming (+18%) whenever proper nutrient concentrations, flow rate and root/water volume was guaranteed. Substrate aquaponics resulted always underperforming by 30-40% due to inadequate nutrient exchange at root level. Results suggested NFT as the most performing method to strip nutrients from water, providing that optimal nutrient flow is guaranteed at root level.

Research assessed food safety aspects and the compliance to national or international regulations on microbial loads for irrigation water. Although products appear safe due to the water delivery methods used (subirrigation instead of aspersion) presence of bacteria in water above set levels may be a limiting factor that may exclude aquaponics from HAACP driven productions. An assessment of UV-treated aquaponics against untreated systems showed more than 99% total coliform abatement and the compliance to EU regulations for irrigation water. Moreover

productivity of both lettuce (*Lactuca sativa* L. cv Verde degli Ortolani) and tilapia (*Oreochromis niloticus* L.) was not affected by sterilization.

Keywords: Soilless systems, integrated aquaculture, phytoremediation, hydroponics, sustainable agriculture

Abstract in italiano

Il futuro dell'agricoltura sta nello sviluppo di produzioni sostenibili che possano meglio impiegare input produttivi ovvero ottimizzare il riciclo di prodotti di altre filiere. A livello agricolo una maggiore sostenibilità può essere ottenuta attraverso l'uso di sinergie produttive tra produzione vegetale e animale. Nel caso dei sistemi di agricoltura-acquacoltura integrata esistono indubbi vantaggi legati alla riduzione del *footprint* ecologico, vista l'elevata produttività raggiungibile dai pesci. L'aquaponica è un sistema fuori suolo che integra la produzione vegetale al recupero di acque di impianti ittici a ricircolo. Con l'acquaponica esistono vantaggi non solo a livello di produzione, ma anche nella riduzione/eliminazione dell'uso di fertilizzanti e nella completa fitodepurazione dell'acqua. In tal senso l'integrazione di due filiere permette il completo abbattimento di tutte quelle problematiche ambientali prima esistenti a livello di ciascuna filiera

Sebbene l'aquaponica sia stata studiata per anni, le sue potenzialità produttive non sono quasi mai paragonate all'idroponica. Risulta inoltre pressochè inesistente l'analisi qualitativa dei prodotti, un fattore chiave per dare all'acquaponica il suo ruolo a livello di produzione commerciale.

La ricerca, effettuata presso L'azienda Agraria Didattico Sperimentale dell'Università della Tuscia, ha avuto come obiettivo quello di studiare la produzione e la qualità dei sistemi acquaponici per diverse tipologie di vegetali e di pesci. La ricerca ha valutato altresì l'efficienza produttiva di questi sistemi identificando le migliori metodologie produttive.

Nel caso della lattuga (*Lactuca sativa* L cv Integral) cresciuta su reflui di tilapia (*Oreochromis niloticus* L.) sono state osservate medesime rese tra acquaponica e idroponica per valori di azoto superiori a 15 mg L^{-1} ovvero di conducibilità elettrica di 0,6 dS m^{-1}. L'analisi qualitativa ha mostrato che i livelli di clorofilla sono simili se non superiori all'idroponica per i medesimi valori di azoto. I livelli di nitrati nelle foglie sono risultati inferiori ai limiti di legge, e comunque non difformi dall'idroponica.

La qualità del basilico e l'influenza della dieta a livello di bilancio di nutrienti sono state oggetto di un'altra serie di prove. Queste hanno visto l'uso di basilico genovese (*Ocimum basilicum* L. cv superbo) e pesce gatto africano (*Clarias gariepinus* B.) alimentato con 2 differenti diete contenenti rispettivamente 31% e 40% di proteine. Cosi come per la lattuga anche per il basilico non sono state rilevate differenze a livello di produzione ed le risposte produttive sono state in linea con i dati forniti in letteratura. I valori di clorofilla così come quelli del rapporto foglie/steli tra aquaponica e idroponica sono stati simili. Tuttavia per le prove effettuate sono stati ottenuti valori di biomassa fogliare superiori a quelli raggiunti con densità di impianto superiori. I parametri di crescita dei pesci sono apparsi simili a quelli ottenibili da sistemi di acquacoltura intensiva. Ciò ha confermato che i sistemi acquaponici non limitano lo sviluppo ottimale degli animali. Differenze sono invece

state notate nei valori di azoto nell'acqua, con livelli di crescita doppi nella dieta al 40% di proteine rispetto a quella al 31%.L'analisi delle performance di sistema ha mostrato che i migliori valori di efficienza nutritiva delle piante si hanno per basse concentrazioni di nutrienti nell'acqua.

La produzione e la qualità delle ortive da frutto è stata studiata in acquaponica con l'obiettivo di valutare le risposte produttive a più basse concentrazioni di potassio rispetto a quelle usate in idroponica. La produzione di cetriolo (*Cucumis sativus* L. cv Ekron) fatto crescere su di un sistema stoccato con persico trota (*Micropterus salmoides* L.) non ha evidenziato differenze significative per quanto riguarda la produzione totale e quella commerciale (pezzatura superiore a 180g). Non sono state rilevate differenze significative nemmeno per l'acidità totale, livello di zuccheri (°brix) ed acidità titolabile. I valori di efficienza di uso dell'azoto sono apparsi simili nonostante l'acquaponica abbia avuto delle perdite di sistema superiori.

L'acquaponica può altresì integrare l'acquacoltura marina attraverso l'uso di alofite. L'impatto delle produzioni animali sul mare può essere infatti attenuato grazie alla coltivazioni di specie ortive resistenti alla salinità. Per le tre prove effettuate è stata usata la *Salsola soda*, coltivata su reflui di cefalo (*Mugil cephalus* L.) e fatta crescere a livelli di salinità di: 5 g L^{-1}, 10 g L^{-1}, 20 g L^{-1}, 30 g L^{-1}. La produzione ottimale è risultata essere a 10 g L^{-1}, ma valori di biomassa interessanti sono stati ottenuti anche a 20 g L^{-1}. L'acquaponica è risultata più performante rispetto all'idroponica nonostante livelli di azoto 3-5 volte inferiori. I valori di azoto nell'acquaponica salata, del tutto simili a quelli che normalmente sono misurabili in impianti di acquacoltura tradizionale, mostrano le grandi potenzialità di questo sistema produttivo anche coi pesci marini.

In acquaponica la produttività non dipende soltanto dalle concentrazioni dei nutrienti ma anche dalle modalità di distribuzione degli stessi. Nel caso specicifco sono stati testati il floating system, l'NFT e il fuori suolo a substrato in subirrigazione per valutare le risposte produttive di lattuga (*Lactuca sativa* L. cv verde degli ortolani) a valori crescenti di azoto. I risultati hanno evidenziato che le rese più costanti sono quelle ottenute dal floating system, mentre per L'NFT valori superiori di produzione (+18%) si sono avuti solo dietro determinati flussi di nutrienti e di bagnatura radicale. I sistemi a sub-irrigazione a substrato sono invece risultati di produttività inferiore rispetto al floating system (-30-40%).

Per quanto riguarda la qualità microbiologica degli alimenti prove di sterilizzazione a raggi UV hanno dimostrato che è possible raggiungere un grado di sterilizzazione superiore al 99% ed una riduzione della carica microbica (coliformi) nei limiti dei parametri di legge. Simili performance a livello produttivo delle piante e dei pesci hanno evidenziato l'assenza di fenomeni di inibizione dell'attività batterica nitrificante ovvero di inibizione della crescita delle piante ed animali.

Parole chiave: fuori suolo, fitodepurazione, agricoltura-acquacoltura integrata, idroponica

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GENERAL PART

Introduction

Sustainable agriculture

Sustainability is the pillar for future agriculture development. The assertion widens the idea that long-lasting development is the mere result of ecological thinking. Although several definitions of sustainability have been given in the last decades, one of the most significant ones was given by the Brutland commission (WECD, 1987): *Sustainability is a form of development that meets present needs without compromising the ability of future generations to meet their needs*. This anthropocentric vision outlines the dualism between humankind and nature. Since its dawn men shaped their surrounding environment to secure future. Agriculture in that sense summarizes the shift from ecosystem into agroecosystem, where nature and people coexist under a symbiotic relationship. Therefore agroecosystems not only include natural environment, but also the social and the economical aspect of human existence. It is just with the equilibrium and the integration among the three components that optimal conditions are set for long-lasting development.

System sustainability is the result of several factors:

- Environmental sustainability - which includes wiser uses of inputs, energy efficiency, cross-integration and ecological services
- Economic sustainability – which is linked to profitability, cost-opportunity and competition with alternative development choices
- Social sustainability – which includes culture and heritage, health and safety, quality of life, quality of productions.

In modern world the question if the equilibrium between social environmental and economical aspect of sustainability has been achieved up to present is still unanswered. Undoubtedly current systems analysis suggests that in many cases such equilibrium has been disrupted to the detriment of environment or society. In addition the exponential demand due to growing world population and the cultural changes following globalization has weakened the liaisons between men and their surroundings. An important concept that arose in the ’80 was *carrying capacity* , which refers to the number of beings that a defined territory can sustain (WRI/IIED, 1986). Several attempts in identifying the thresholds between *optimal* and *maximum* carrying capacity were tried (Odum, 1983, Ophuls. 1977) with the aim to increase system resiliency. Although the optimal carrying limits are close to 50% of the maxim allowable capacity (Ophuls, 1977), the threshold can be moved forward by means of technology or investments (Brown, 1987). Nevertheless more recent debates arose the question whether higher outputs from agriculture are possible by technical or scientific breakthroughs (Brown and Kane 1994; Seckler, 1994; Waggoner, 1994). Despite

technology there are robust evidences that natural resources, such as water and fossil energy, are overutilized (UN-Water 2012) and cannot guarantee for further increases.

Although technology-driven progress still has wide frontiers that need to be unveiled, it is questionable if new advances are at the expense of other resources. Sustainability should firstly promote systems integration as a way to reduce inefficiencies and market failures. One of the major problems in modern societies is pollution, which is a system loss, both in terms of resources and energy. Pollution is mainly a consequence of deficient approaches in accounting for externalities in traditional production systems. Pollution issues have never been addressed due to the common belief that wastes are uniquely considered a cost rather than a resource. Although not any waste can be a resource and cannot be completely converted into a benefit according to thermodynamic laws, it is possible to have a high degree of recycle at agroecosystem levels. In the case of agriculture in fact system integration may increase land productivity and save resources with limited efforts in terms of costs and reorganization.

Wiser uses of inputs in agriculture specifically focus to the maintenance of fertility and carbon stocks into soil as well as the avoidance of negative balances between energy employed and energy obtained. Undoubtedly this approach should take into account drastic reductions in the use of oil-driven operations to comply with carrying capacity. According to Franks and Hadingham (2012) almost half of the greenhouse emissions are derived from chemical fertilizer productions. It is obvious that the simplest solution to abate negative CO_2 and energy balances can be found in the reduction of fertilization uses through optimal plant nutrition and limited loss of nutrients. However such option still looks not sufficient to reduce fertilization budgets. Although modern research aims at improving GMOs with autonomous capacity of uptaking nitrogen from atmosphere, there is still need of targeted research to achieve results whose impact has to be verified. Furthermore, adoption of innovative techniques, as in the case of GMOs, is not widely accepted and may cut expectations of rapid shifts towards the use of more sustainable plants. The objective to increase food and energy outputs following increasing world population demand is a holistic challenge. The reasons stand behind the difficulties to achieve very high advances in productivity with technology alone and in the limited availability of land suitable for agriculture. Undoubtedly the shift towards integration would wisely allocate resources and reduce the impact that each production system has, if considered as a standalone unit. System stability conforms to the theories of resilience (Elton, 1958), where increased level of complexity improves ecosystem stability either on ecological and social level (Walker et al., 2006). Therefore the acknowledgement of the ecological services provided by each level of complexity would help agroecosystems to benefit from newly available synergies and optimized resources.

Success in agriculture sustainability is essentially an economy-driven result. A less polluting product may costs less for externalities and thus be more competitive on the markets. Better uses of resources imply optimization of production systems and increased productivity.

A more ecological-driven reflection brings our attention to the competition for land, water and energy by industrial or societal activities. Competition should indeed lead agroecosystems to rethink their renewed role for modern sustainable development. Agriculture may thus become the main supplier of ecosystem services and be the key actor for added value benefits to societies. One example in that sense comes from Germany, where farmers were granted for not using chemical fertilization next to drinkable water reservoirs (Sachs et al., 1998). Nevertheless progressive competition for land and water in urban areas pushes towards a progressive intensification of farming systems and the development of agroecosystems in areas traditionally not devoted to agriculture. Water and land competition forces societies to think at new solutions to save, or better use, limited resources. This research activity would undoubtedly bring societies to a new awareness for less impacting production systems, as well as changes into consumption habits.

Quality and sustainability

There is an inverse proportion between growth and pollution, which is described by the environmental Kuznets curve (Kuznets, 1966). Increased development brings to increased awareness of health and pollution hazards. As a results advanced societies are more attentive to sustainable production systems. Not only should sustainable agriculture be the effect of virtuous managements, but also the trademark for enhanced food quality, food safety and ethical productions. Quality is thus the "ID" of sustainable production systems that make consumers aware of their role in deciding which sustainable choice need to be taken by simply buying a product rather than another.

According to ISO standards (ISO 8402, 1986) quality is t*he totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs.* The definition outlines two key words: characteristics and compliance. *Characteristic* is the sum of all the attributes that are distinctive and relevant for a product or service, which are unequivocally set and assessed as a benchmark. Given the specific subjectivity in identifying the parameters, the assessing criteria for each product is extremely variable and depends on local contexts and needs. *Compliance* is the degree to which a specific product conforms to a design or specification (Gilmore, 1974) Although products' characteristics are easily identifiable, *compliance* to specific requirements should meet consumers' expectations and purchasing criteria. Compliance also includes the full

sensorial expectations of the consumers, who eventually decides which production and distribution chain favour with their value-for-money criteria. The concept is well synthesized by Carruba et al (1975): *Product integrity consists of a predetermined optimum balance of performance; aesthetic appeal; reliability; ease, economy, and safety of operation; ease, economy, and safety of maintenance; and consistency — all at a given cost*.

Several aspects determine the quality of a product:

- *Origin*. It univocally identifies the facility/ies where the production or transformation occurred and provides the traceability for each single input used to produce the merchandise.
- *Production method.* It explicitly shows the use of specific methods adopted for the production, as well as the input used. It also states the compliance to regulations on environmental impact, pollution, use of xenobiotics/biologic control as well as the respect of specific laws on workers' safety or animal wellbeing. More advanced information refer also on the production footprint or carbon emissions.
- *The characteristics of the product* which can be further divided in
    - Safety. It analytically specifies the risks of pathogens, residues, heavy metals, addictives or drugs. In the case of processed food the standards should comply to specific protocols, such as HACCP.
    - Nutritional/organolectic quality. It analytically informs on the chemical composition of the product, the nutraceutical characteristics and the possible risks factors for intolerant consumers.
    - Intrinsic characteristics. Refer to the specific sensorial characteristics of the product in terms of shape, colour, taste, flavour/off-flavour, texture as well as the degree of the processing.
    - Value added characteristics. Refer to the specific identification of a product in terms of equity, heritage, indirect health benefits derived from its consumption.
    - Packaging and marketing. It refers more on the post-production part of the chain. Most of the added quality is targeted to the packaging method or in the materials used, in the product availability, delivery and constant supply through the year. Quality also covers labelling through additional information on the product origin (e.g. farmer's name) customer care, fidelity program, as well as cooking instruction or additional recipes.

For quality there is thus a plurality of hidden values and characteristics that are uniquely recognized by the consumer, who is the unique and principal actor in the purchasing process. A survey carried out in the USA (Tronstad, 1995) analyzed the degree of characteristics that consumers saw behind the term quality. In synthesis quality was associated with: Appearance/condition (97%), taste/flavor

(95%), freshness/ripeness (96%), price (70%), certified safe by residue testing (68%), nutritional value (66%), Shelf life (60), convenient to eat/prepare (51%), size (45%), in season (41%), displayed loose (37%), calorie content (26%), Organically grown (22%), growing area of origin (19%), prepackaged (11%), brand name (10%)

Clearly alternative ways to produce sustainable food should firstly address sensorial/intrinsic characteristics of the product, secondly the price and thirdly the food safety profile. The production method, although important, is not at the top of priorities, as witnessed by the 22% of organically grown and the 19% of area of origin. Nevertheless production method and food miles are important determinants in the final price, which is eventually at the top of consumers' wish. What arises is that whatever the sustainable method any farmers must prioritize sensorial and safety aspects of the products.

Sustainable scenarios

There is a big debate on how future generations will produce more in a sustainable way. According to Tillman et al. (2002) the biggest environmental impacts in agriculture are found in the conversion of natural land to agriculture, in nutrient leaching and in the use of chemicals. Use of chemical fertilization for example has augmented by 20 times the amount of nitrogen present in the ocean (Downing et al., 1999) or created severe problems in river areas due to eutrophication (NRC, 1999). One suggested way to abate such impact is closing the crop and animal loop, improve water and nutrient efficiency and favour integrated management to control pest and diseases or get rid of wastes. However the increase in productivity that agriculture obtained in the last decades following the Green Revolution are far to be further achieved. The reason stands in the proximity to the standing crop capacity in already fertile areas as well as the difficulty to find new land for agriculture. From this scenario it appears clear that increased pressure on production outputs makes it harsh for organic agriculture to withstand intensive farm practices.

In the case of horticulture the expansion of soilless agriculture would undoubtedly be a point of advantage, both in terms of nutrient efficiency and production quality.

Soilless systems show some advantages over conventional production systems (Resh, 2004; Leoni, 2003), they can be summarized in:

- Increased yields due to protected environment uses, in which it is easier to control climatic parameters
- Lack of competition for nutrients from weeds
- Control of nutrients according to the growth stage of the crops.

- Increase in quality of productions, due to optimal plant nutrient balances.
- Reduction in residues and pesticide due to increased climatic control and integrated/biologic management
- Better organoleptic and nutraceutical characteristics due to optimal nutrient management
- Lack of crop rotation to avoid "soil tiredness"
- Better control of soil-borne disease through physical avoidance of micro-organisms and biological management.

Soilless cultivation can be developed in urban and suburban areas, which sensibly reduce transport costs. Furthemore hydroponics can be developed in areas not suitable for traditional agriculture due to adverse soil conditions or water quality. The development of micro-scale agriculture, as in the case of soilless productions, allow small households to improve their livelihoods. In the envision of promoting environmental, economic and social sustainability the adoption of simple farming would promote huge impacts in terms of social change and food security. Well-known examples of urban agriculture were found in Cuban cities under the economic blockade in the nineties, Eastern Europe after the collapse of the former USSR, and more recently, cities in Eastern Congo or Gaza Strip (FAO, 2007). In Brazil the PROVE programme, which was designed to fight urban poverty by promoting small-scale agricultural production, processing and trade in marginalized households, not only has shown changes in productive practices, but also paved the way for a gradual change in people mentality. Urban agriculture, as witnessed by PROVE, raised social awareness of the producers who no longer perceived themselves as passive elements of the society but rural entrepreneurs and individuals more concerned of their role (Homem de Carvalho, 2001) .
Beyond any strategy the future of agroecosystem passes through use optimization of limited resources, such as water and energy. Undoubtedly expected scenarios will see urban areas more and more vocated to production by using wastes and sewage nutrients for plants production (fertirrigation) energy (biomass, microalgal biofuel) or even animal feed (microalgae, biofloc)

Case study – the integration of aquaculture with agriculture

Integrated aquaculture

Integration with aquaculture lay down in the history of agriculture. There are many examples from history of the intensification of agriculture output through fish. In Mesoamerica terrains in shape of strips were made with decaying organic matter in swampy areas. (Netting, 1993; Sutton and Anderson, 2004) This integrated system, called *chinampas*, was one of the most intensive forms of

agriculture and was said to provide food for 18 people ha^{-1} (Adams, 2005), a productivity higher than modern agriculture. Ecosystem complexity was also developed in China under the form of polyculture (Beveridge and Little, 2007), in which fish with different feeding habits used to colonize ponds and synergically used all the available sources of food (Milstein, 1992). Traditional indigenous system in Asia consisted in pond cultures where plants were cropped on banks to take up water nutrients. Another technique largely used was the integration of rice with fish, where staples not only benefited by fish wastes but also from the biological control of weeds and pests operated by fish. The increase in production of such integration could raise paddy productivity by 10-15%. (Lightfoot, et al, 1992 ; Halwart, 1994 ; Frei and Becker, 2005)

There are several advantages in running aquaculture instead of traditional animal husbandry. Being fish a cold blooded animal it does not consume most of the energy for body heating, as it happens instead for mammals, with almost 70%. This saving strategy, if on one side makes aquatic animals more prone to climatic conditions, on the other side it improves their feed conversion efficiency (FCR) up to 1 (1 kg of feed for 1 kg increase of body weight) or even below 1. On the contrary, FCR in warm-blooded animals is 2-4 (2-4 kg of feed for 1 kg increase of body weight) for chicken and monogastric animals or 6-7 of ruminants (Verdegem et al., 2006). Beyond the considerable savings in water for the reduced feed footprint, aquatic animals have excellent nutritional characteristics in terms of poly-unsatured fatty acids (PUFA) or highly-unsaturated fatty acids (HUFA). PUFA and HUFA are essential elements in brain development, both for enhancing mental capacity in offsprings and against aging (Uauy and Dangour, 2006; Innis, 2007) In addition they stimulate immune response (Fritsche et al 1992; Wander et al., 1997) and reduce the incidence of cholesterol-derived pathologies.

Aquaculture covers more than one third of the world fish production (FAO 2010). FAO projections state that aquaculture will raise up to 50% of world fish production by 2030 (Tidwell and Allen, 2001). At the present state fish supply approximately 15% of animal protein intake to 3 billions of people worldwide. The major share of aquaculture production is owned by freshwater species (FAO, 2010), which can easily be integrated in agricultural management.

Up to present aquaculture has operated in most cases with little control on environmental impact. If in western countries national environmental protection agencies (EPA) have ruled waste discharges limits for fresh and seawater farming, the same could not be said for most developing countries. The outlook on environmental impact, in prevision of future aquaculture expansions, raises many concerns on sustainability, pollution control and in waste disposal.

Fish production strictly depends on the degree of farming intensification, which is a trade-off between land occupancy, resource use (feed, energy, water) and waste emissions. In *extensive*

systems fish rely uniquely on water primary production. If on one side this is a sustainable approach because the whole food chain and pollution control is managed within the system, on the other side it implies a huge surface in lieu of very little production (few hundreds of kilos per hectare per year). On the contrary *semi-intensive systems* rely on progressive management intensification operated in ponds by means of: water fertilization and supplementary feeding (Barnabé, 1990). Fertilization in semi-intensive management enhances pond primary production (phytoplankton) which directly supports fish nutrition. Supplementary feeding integrates pond primary production and support animal growth beyond pond plankton feeding capacity.

Progressive level of intensification brings to *intensive systems* where complete feeding, aeration and water exchange are operated. In intensive management the percentage of nutrients from primary production is minimal, and most of the fish nutritional needs are supplied by complete feeds. Aeration aims at supplementing fish with adequate levels of oxygen in water. Given the high level of metabolic discharge intensive fish management requires a continuous water exchange to limit the build-up of toxic substances for fish (Barnabé, 1990; Diana et al., 1997). Nevertheless intensive farming systems, if on one side can be highly productive with more than 100 tons of fish per hectare per year (Edwards, 1993; Verdegem et al., 2006), on the other side would cause significant pollution (Piedrahita, 2003; Verdegem et al., 1999; Verdegem et al., 2006) and raise concerns about unsustainability (Chamberlaine and Rosenthal, 1995; Costa Pierce, 1996)

In the last decades advances in technology brought fish farming to recirculating aquaculture systems (RAS). In RAS water is not discharged but reclaimed and recirculated back to fish. There are several advantages with RAS:

- Fish grow in a controlled environment with stable water parameters (temperature, oxygenation) that favour optimal growth conditions
- Very limited water is wasted
- High/total control of wasted pollutants
- High savings in terms of water heating (in cold climates) to optimize production.

RAS has higher productivity than traditional farming and at the same time allows for quite big water savings, compared to extensive or semi intensive land managements (Verdegem et al., 2006).

The core functioning of RAS stands behind water reclamation (Fig. 1), which is operated by solid removal (uneated feed, fish solids, dead fish) and bacterial dissimilation operated by biological filtration (van Rijn, 1996). Fish release ammonia in water. However ammonia is a toxic product of fish metabolism, whose accumulation in water causes massive deaths (0-2 mg L^{-1} or more, depending on the species). It is thus fundamental that aerobic bacteria convert ammonia into nitrate

to avoid risks of toxicity. A successive stage in RAS passes through anaerobic treatments, where nitrates are reduced and released in the atmosphere as nitrogen gas. Nevertheless depending on RAS efficiency a moderate water exchange (100-1000 L kg feed^{-1} or more) is necessary to maintain minerals and nitrate within safe limits (Piedrahita, 2003; Martins et al., 2010; Verdegem et al., 2006).

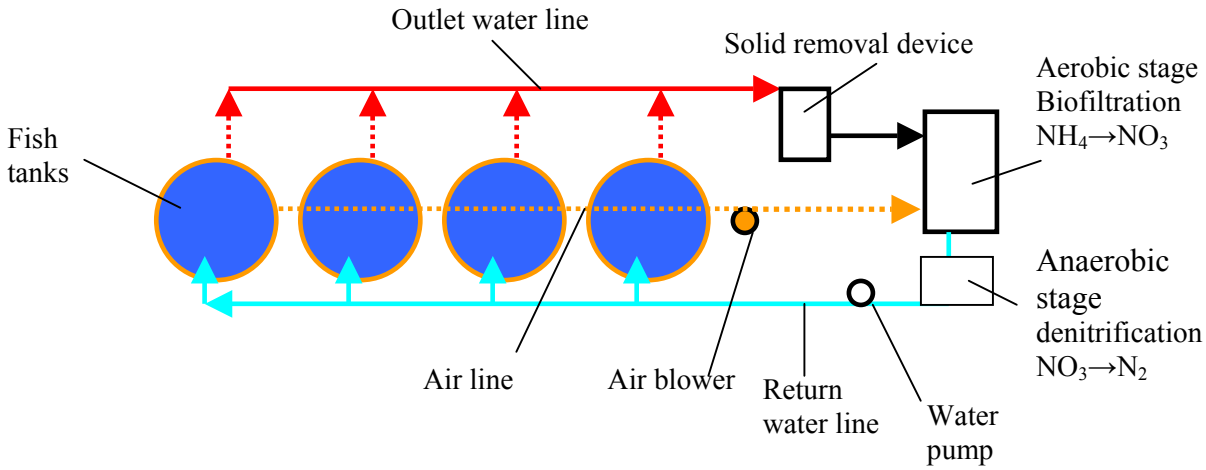

Figure 1. Recirculating aquaculture system - RAS

An alternative strategy to bacteria dissimilation in recirculating systems makes use of plants to absorb excess of nitrogen and minerals. Plant assimilation can be performed by seaweed (Neori et al., 2004), aquatic macrophites (Brix and Schierup, 1989) or vegetables (Edwards, 1987). An evolution of RAS is aquaponics, the soilless plant production (hydroponics) integrated with aquaculture. Plants grow on aquaculture effluents and take up nutrients dissolved in wasted water. In aquaponics nutrients reach significant levels that are compatible with plant growth. On the other side plants remove continuously nutrients and avoid any toxic nutrient build up (Rakocy and Hargreaves, 1993).

Aquaponics

Aquaponics has been developed mainly within recirculating aquaculture systems (RAS) (Rakocy and Hargreaves, 1993; Lennard, 2004). Water in aquaponics shows higher nutrient concentrations than in open aquaculture systems (flow-through). Plants clean water from fish excreta and dissolved minerals and at the same time keep nutrients dissolved in water to minimal concentrations.

Aquaponics includes a microscopic ecosystem constituted by nitrifying bacteria, rhizobacteria, beneficial fungi, plankton. This complex ecosystem makes aquaponics a more resilient habitat where all food niches are occupied and prevent plant pathogens to grow.
In an aquaponic ecosystem many processes are performed:

- fish growth
- conversion of ammonia to nitrate
- absorption of nutrients by plants (phytodepuration)
- absorption of nutrients by microrganisms (bacteria, rizobacteria, fungi, algae, plankton)
- mineralization of solid microparticles
- denitrification
- plant growth

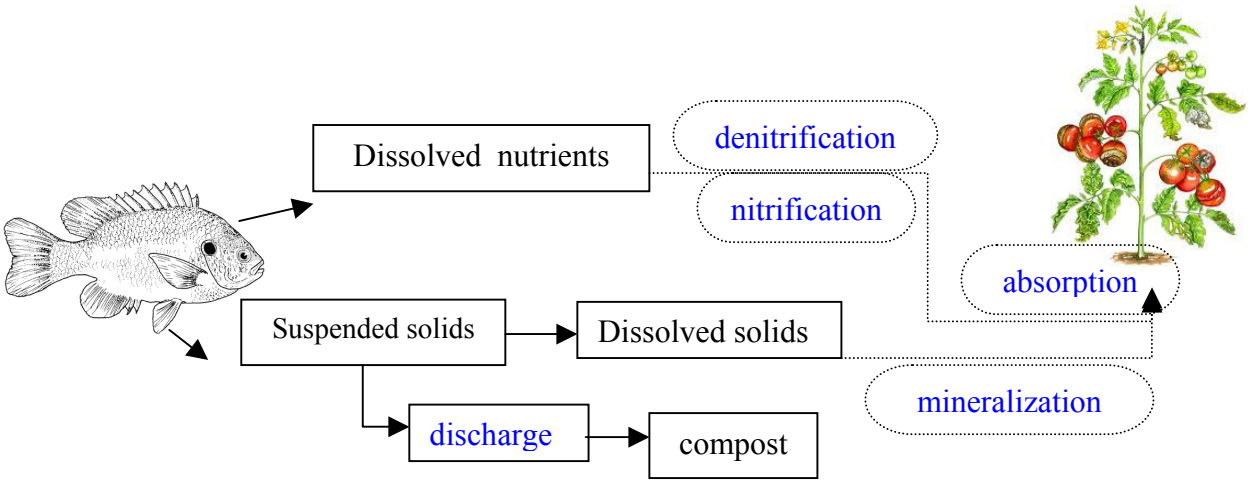

Figure 2. Scheme of a fish -bacteria- plant aquaponic system.

An aquaponic system uses the same concept of RAS, but does not have well identifiable units for biofiltration and denitrification. The oxidation of ammonia to nitrate or its elimination occurs in fact in the water column, on system's surfaces, at plant root level or through direct plant absorption (Fig.2). Aquaponics also differs in the solid management efficiency, since presence of dissolved particulate matter contributes to raise the nutrients' pool through mineralization
An aquaponic system is made of several components (Fig.3)

- Fish tank
- Clarifier (solid removal unit)
- Filtering tank

- Degassing tank
- Plant trough
- Sump
- Blower (air line)

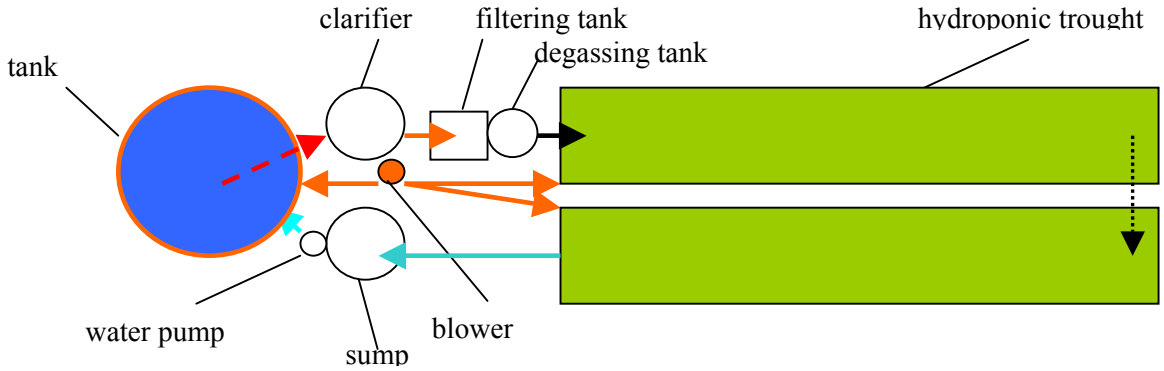

Figure 3. Aquaponic system

Fish tanks can vary in stocking densities, depending on the species reared and on the oxygen delivery technology. In general 80-90% oxygen saturation can be supported by standard aerators up to a density at harvest of 60-70 kg m^{-3}. Higher densities or higher saturation is possible providing that pure oxygen is supplied. Standard biomass at harvest is 70-80 kg m^{-3} but densities up to 140-200 kg m^{-3} are possible. Fish are in general stocked at preadult stage (20-50g) and left grown till harvest. In commercial aquaponic systems fish are managed with a staggered production, which means that each tank has fish of the same size, and tanks are stocked cyclically during the year soon after harvest. Staggered management allows to harvest one tank of single-sized fish at the time and to have a continuous supply of fish depending on the number of tanks available. Staggered management maintains a fairly constant fish biomass (average between preadult and adult fish), which eventually keeps costant nutrient concentrations for plants. Depending on the fish growth stage different diets are delivered to animals. Diets for preadult fish are in fact richer in protein (40-50 % protein), while those at maturity stage vary between 30 and 40%. The higher the protein content and the amount of feed given to fish the higher is the nitrogen supplied to plants. Diet choice thus influence the type of crop (leafy vegetables for higher nitrogen levels) or the cultivable area used as nutrient sink.

Intense aeration occurs in fish tanks with the multiple purpose to keep water oxygenated for fish breathing, improve solid mineralization and nitrification, and keep oxygen high at root level. Fish tanks have an hydraulic retention time of 1-2 hours to avoid ammonia build ups after animal feeding.

Clarifiers are conical-bottomed tanks where solid settle under a water retention time of 20 minutes (Rakocy, 2007). In general the removal performance is 59%. Suspended solids need to be removed since their presence clog plant roots and increase solid accumulation into the system. Presence of excessive solids is negative because of the risks of anaerobic spots, which are the causes of toxic hydrogen sulphide production. On the other hand solid mineralization allows more nutrients to be released into water.

Filtering tanks are filled with orchard nets. At this stage nearly all the suspended solids settle on the mesh and mineralize or are digested by bacteria to produce biofilms. In addition anaerobic conditions occur wherever thick layers of solids settle on nets (Rakocy, 2008, personal communication). Anaerobiosis and biofilm growth are key processes in aquaponic nutrient management, firstly because lack of oxygen in nets increases denitrification and secondly because carbon-based residues trapped on net surfaces are an ideal energy substrate for biofilm bacteria. Bacteria management is an easy biological tool to adjust the N:K ratio because micro organisms grow by taking-up nitrogen out of water. In aquaponic management high N:K ratios favour leaf vegetables growth, while fruits prefer higher levels of potassium. N:K control is performed through biofilm removal, since reduced bacteria biomass on nets heighten the nitrogen content in water.

In **degassing tanks** water is intensely aerated to eliminate hydrogen sulphide and carbon dioxide after the anaerobic phase. The former gas is extremely toxic for fish and must be removed efficiently.

Plant troughs are the tanks where vegetable grow and where suspended solids are reduced further up to 4.2 mg L^{-1} (Rakocy, 2007). They vary in size and type. In the University of the Virgin Island Agricultural Experiment Station (UVI-AES) the surface ratio between fish tanks and plant area was 1:7.3.

Sump Downstream plant trough there is a collecting tank where base addition occurs. Addition of bases is needed to correct pH decrease due to nitrification. Alkali are added in this separated area to prevent direct exposition to plants or fish.

Blower (air line) Oxygen for plants is mainly supplied through aerators in water culture or through timely distribution of water for NFT and gravel/sand systems (Resh, 2004). Aeration has two advantages: the first is that it enhances water turbulence, which favourably helps roots to absorb nutrients, the second is that it keeps solids suspended for an easy removal.

Type of systems

Three main growing methods are the most diffused around the world (Diver, 2007; Nelson, 2007): Floating system, NFT and gravel/substrate systems. Similarly to hydroponics in *floating systems* plants grow on rafts by means of polystyrene sheets. The layer of water is 20-30 cm deep. Floating systems show some advantages in terms of big water volume, which keeps oxygen and heat stored into the water. Oxygen storage reduces the risks of anaerobic conditions in case of black outs or blower failure. On the other hand heat storage allow the system to avoid excessive temperature fluctuations. In addition the big amount of water available dilutes ammonia peaks that occur after fish feeding,

In *nutrient film technique* (NFT) water flows in a thin layer in pipes. Irrigation can be continuous or intermittent, thus leaving roots under wet and dry cycles. NFT uses less water than any other system, but is prone to water delivery failures, as plants may wilt very quickly in case of power failure.

In *gravel/substrate* systems growing media is contained in a 20-30 cm tanks where water is supplied through trickling irrigation or "ebb and flow" delivery. Gravel/substrate systems, similarly to floating systems, have a consistent water volume that prevent plants from any water stress due to system failures. Gravel/substrate systems benefit from the big surface-to-volume ratio of the media, which enhances bacteria biofiltration. Growing media also helps to buffer water through the carbonates contained in the minerals. There are however some disadvantages from the higher costs of settings and the difficult maintenance of the system in case of media clogging. Excess of suspended solid may in fact create anaerobic phases into the media, which favours hydrogen sulphide release.

Aquaponics vs traditional aquaculture systems

Closed recirculating systems have the considerable advantage that do not waste pollutants into the environment. All the products from fish metabolism are in fact kept into the system and processed and disposed accordingly. In the case of RAS a certain amount of water is daily wasted to dilute the build up in nutrients, however this exchange is minimal compared to traditional land-based intensive systems where no biofiltration is performed to abate ammonia levels. However, contrarily to RAS, aquaponics does not have any water loss, with the exception of plant evapotranspiration and solid flush out. In aquaponics in fact all the nutrients are absorbed by plants.

RAS and aquaponic systems are a valuable strategy to culture fish with scarce water resources or as a way to avoid heat loss from fish tanks in cold environments (Singh, 1996). Constant water temperatures towards the optimal ones for fish are an ideal condition for animals to avoid stress and

to have excellent growth. Water consumption in RAS/aquaponics is hundreds of times lower than traditional aquaculture (Vergegen et al., 2006). This makes intensive closed aquaculture systems more sustainable in terms of pollution control and productivity. On a qualitative point of view RAS/aquaponics appear more competitive on food safety. Fish containment prevents in fact any risks of parasites or pollution from external chemicals sources. Potential risks of heavy metal accumulation in closed systems has been proven to be inconsistent, as fish show similar concentrations to traditional systems (Martins et al., 2011). However the extent of the operations in closed (recycling) environments implies higher levels of expertise and management, since solutes within the system must not reach toxic levels for plants (Singh, 1996).

Aquaponics vs hydroponics

Aquaponics has some different characteristics from hydroponics. The levels of nutrients supplied to plants are in fact sensitively lower, this because fish continuously supply nutrients into water. The nutrients measured at the aquaponic facility of the University of Virgin Islands (Tab.1) are consistently lower than hydroponics, which suggests for safer nitrate-free productions.

Table 1 - Aquaponics vs hydroponics (Shultz, 2008). values in mg L^{-1}

Nutrient*	**Aquaponics**	**Hydroponics**
TDS	62-779	1200
Ca	10-82	150
Mg	0.7-12.9	50
K	0.3-192	150
NO_3	0.4-82	115
PO_4	0.4-15	50
SO_4	0-23	113
Fe	0.03-4.3	5.0
Mn	0.01-0.19	0.5
Cu	0.01-0.11	0.3
Zn	0.11-0.8	0.05
B	0.01-0.23	0.5
Mo	0-0.17	0.05

On microbiological level hydroponics strategy is to keep sterility on the highest to avoid plant pathogens. In aquaponics the common approach is to have the maximum microorganisms occupancy in water. The prevention from diseases in aquaponics is an indirect result of bacteria presence due to the high microbial competition, which prevents any exogenous pathogen to colonize the system. In aquaponics bacteria are essential elements for mineralization and

nitrification. In addition, presence of rhyzobacteria that grow in a "liquid humus" gives additional benefits to the systems because of stimulation on plants.

Another characteristics that makes aquaponics distinctive is the organic management. No chemical is in fact allowed. This to prevent any risks of plant and fish-cross-toxicity. Aquaponics is mostly run with integrated pest/disease management and the use of biological treatments.

State of the art of aquaponics

Several aquaponic systems have been tested with different fish species: tilapia (Sarotherodon aurea, Steindachner; Oreochromis niloticus, Linnaeus; Tilapia rendalli, Boulenger) (Watten & Butsh, 1984, Zweig, 1986; Rakocy et al., 1993, 1999a, 1999b; Seawright et al, 1998), Murray cod (Maccullochella peeli peeli, Mitchell) (Lennard, 2004), rainbow trout (Oncorhynchus mykiss, Walbaum) (Adler et al, 2003), common carp (Cyprinus carpio, Linnaeus) (Naegel, 1977) Asian Barramundi (Lates calcarifer, Bloch 1790) (Rakocy, 2007). For some fish species, a feed/plant ratio was developed as an important issue in aquaponics design, since the amount of plants nutrients is proportional to the feed digested and excreted by fish. In addition greater content of protein in fish diet increases the level of nitrogen available: in University of Virgin Island (UVI) Rakocy et al. (1992) determined that 2.4 g day^{-1} of feed with 36% protein to tilapia could supply nutrients to one plant of lettuce grown on rafts within a recirculation system, a further assessment for the same location set the feed/plant ratio at 60-100 g $m^{-2}day^{-1}$ (Rakocy, personal communication, 2008). Lennard (2004) on the other hand determined in 0.6 m^2 of green oak lettuce (20-25 plants) the appropriate nitrate sink for 1 kg of Murray cod fed at 2% of body weight (BW) with 43% of protein. Plant uptake changes between and within species and environmental conditions: Gloger (1995, cited by Van Anrooy, 2002) determined 0.56 g m^{-2} day^{-1} of ammonia, 0.83 g $m^{-2}day^{-1}$ of total nitrogen and 0.17 g m^{-2} day^{-1} of phosphorus the nutrient removal of romaine lettuce in Virgin Island in closed tilapia system, while Alder (2003) estimated that 0.94 g m^{-2} of nitrogen and 0.1 g m^{-2} of phosphorus was the absorption rate by lettuce in a rainbow trout recirculation system. The priority to assess optimal fish/plant ratio was raised by Rakocy (1997).

Plant uptake however can be increased by system design (Lennard and Leonard, 2006), since deeper contact of roots in solution ease plant absorption. Floating and gravel systems thus allow plants for increased water contact and nutrient absorption.

The Freshwater Institute (1998) outlined plants' limiting nutrients, which are not sufficiently supplied by the fish diet. Main deficiencies were found in chelated iron, potassium and calcium. Rakocy et al. (1993) while reviewing a wide range of aquaponics systems made an extensive list of

nutrients supplied, but he did not stress massive deficiencies, apart for the three elements mentioned above.

Resh (2004), Leoni (2003) and Sonneveld and Straver (1989) assessed optimal nutrient concentrations for plants. Interestingly, Alder (2003) demostrated the possibility of growing lettuce below limiting concentrations of phosphorus, (up to 1 μg L^{-1}) by taking advantage of plant 'luxury consumption'. Plants tend to store nutrients in excess in rich media and to continue their growth and nutrient stripping in limiting conditions by using their internal reserves.

Nevertheless, nutrient balance may vary according to growth stage (Leoni, 2003). Van Anrooy (2002) noted that high concentration of nitrates favours vegetative growth in leaf vegetables but fruiting plants require different system managements during growing season (i.e. increased denitrification during ripening).

Tyson et al. (2004) explored the NO_3:NH_4 ratio and suggested 3:1 as a good proportion between plant nitrate uptake and ammonia levels for fishes. On the other hand, Rakocy et al. (1992) used NH_4-N concentrations for lettuce equal to 10-20% of the whole nitrogen pool. Losordo et al. (1998) stated that fishes can tolerate NO_3 levels at 200 mg L^{-1} but concentrations above 300 mg L^{-1} were toxic (Masser et al., 1999).

As far as water quality is concerned, variability in plant nutrient uptake is also determined by pH (Rakocy et al., 2006; Losordo et al., 1998, Tyson et al, 2004), since pH levels at 7-8.5 favour nitrifying bacteria but might affect solutes availability outside the optimal range of pH 5.5-6.

Further relevant water quality parameters are: electrical conductivity, which should range between 2.00-4.00 dS m^{-1} or less to avoid phytotoxicity (Resh, 2004; Rakocy et al., 1992, 2006); alkalinity above 100 mg L^{-1} for optimal nitrification buffering (Rakocy, 1997); BOD below 20 mg L^{-1} (Rakocy, 1997) to avoid anaerobiosis; dissolved oxygen (DO) above 5 mg L^{-1} both for optimal plant and nitrifying bacteria development (Rakocy, 1997)

Sub-system design could help in modifying environmental conditions and plant growth. Lennard (2004) stated that a gravel system acts as a nitrification filter, carrying out at the same time buffering tasks with limited mineral release. Rakocy et al. (2006) confirmed that sand and gravel are optimal media; although they outlined some clogging occurrences in intensively fed systems or with unprocessed waste water. Gravel however could damage stems in outdoor windy areas and could increase transplanting and maintenance tasks when plants are removed at the end of the growing season (Rakocy et al., 2006). As far as other sub-systems are concerned, floating-rafts are cheaper and less labour-intensive than gravel, but they could limit water heating in temperate regions, since sunrays cannot directly 'hit' the water (Rakocy et al., 2006). On the contrary nutrient film technique (NFT) could avoid low water temperature problems in Nordic countries, due to the

limited amount of liquid used and the heat exchange occurring alongside the troughs. Nevertheless NFT is riskier in terms of clogging and system failures (Rakocy et al., 2006).

Aquaponic/hydroponic plant production show higher yields if compared to conventional soil crops. Resh (2004) stated that soilless cultivation can at least double the harvest of conventional horticulture (from 1 kg m^{-2} in soil to 2.3 kg m^{-2} in soilless for lettuce; from 1.2-2.4 kg m^{-2} in soil to 14-74 kg m^{-2} in soilless for tomato). Rakocy et al. (1992) showed a yield of 4.35 kg m^{-2} from lettuce grown at 25 plants m^{-2} in 21 days. Goulden (2005) achieved 2.25 kg m^{-2} of biomass with bib lettuce over a similar time period, while Lennard (personal communication 2005, cited by Goulden, 2005) harvested 3.3-4.5 kg m^{-2}

Qualitative and quantitative issues in aquaponic productions

Research has proved that aquaponics can produce as much as hydroponics or traditional agriculture (McMurty et al., 1990; Savidov, 2005). However, if on leafy vegetables productivity seems to be optimal, the nutrient management for fruity plant still need to be fully investigated. One of the three critical elements in aquaponics is in fact potassium, which is an important component in fruiting and ripening. Potassium is added as KOH to enhance pH in tanks, however its level of inclusion may not be sufficient to compensate for plant uptake. N:K ratio is undoubtedly an important factor in fruit production. Savidov (2005) obtained cucumber and tomato yields comparable to hydroponics when potassium levels exceeded those of nitrogen. On the contrary Graber and Junge (2011) measured lower fruit biomass for low levels of potassium, which suggests that aquaponics might not be competitive on fruity productions under certain levels of minerals. According to the quality survey from Tronstad (1995) the highest rank in quality is obtained by the appearance and taste of the product, however not much knowledge at present is available on the qualitative parameters of leaf vegetables in terms of appearance, biomass, dry matter and colour or in fruit characteristics such as acidity, dry matter % and sugar content.

One issue raised everywhere is about food safety of aquaponic productions. Being aquaponic a complex system it appears obvious the presence of bacteria in water. Microbiological contamination could be in fact a limiting factor if products are assessed for their safety and may be prone to cross contamination. Although cold blooded animals have different bacteria from warm blooded animals and do not have *Escherichia coli,* the presence of coliforms may be a possible risk, especially for high quality productions such as the ready-to-eat vegetables. Aquaponics could benefit from water sterilization, however it is not clear whether drastic elimination of bacteria may harm nitrifying bacteria activity and thus be a risk factor for fish.

Research objectives

The objective of the PhD program was to assess the nutrition and quality of aquaponic productions. The present manuscript reassumes the outcomes of 12 trials out of 22 carried out for three years at the University of Tuscia aquaponics experimental facility.

Six chapters, developed in form of articles, reassume the principal results on

- Production and quality of leafy vegetables
- Production and quality of fruity vegetables
- Plant/fish ratio with different fish species
- Subsystem performances (floating system, NFT and substrate soilless systems)
- Food safety
- Saline agriculture

The first article, assessed the production and quality of romaine lettuce (*Lactuva sativa*) growing on tilapia (*Oreochromis niloticus*) wastewater. The experiment, which lasted for two crops, measured the yields and the nutrient patterns in aquaponics and hydroponics. For all treatments the assessment of leaf nitrates determined the safety of aquaponic productions.

The second article assessed the food safety aspects of aquaponic water by monitoring the levels of coliforms and *Escherichia coli* with and without UV sterilization. Production and quality traits of lettuces (*Lactuca sativa*) and tilapia (*Oreochromis niloticus*) were assessed to determine the influence of sterilization and the overall aquaponic system performance against hydroponics.

The third article assessed the production and quality of Genovese basil (*Ocimum basilicum*) grown on African catfish (*Clarias gariepinus*) water for two consecutive crops. Beyond the qualitative and quantitative traits compared against hydroponics the scope of the trials was to investigate the nutrient uptake efficiency and determine system performances through nutrient budgets.

The forth article compared the performances of three soilless systems for three consecutive crops floating system, NFT and substrate. Scope of the research was to compare growth performances and nitrogen uptake of romaine lettuce (*Lactuca sativa*) grown under different water delivery systems and increasing levels of nutrients and to identify the optimal subsystem choice for aquaponics.

The fifth article assessed the production and quality of cucumber (*Cucumis sativus*) grown on largemouth bass (*Micropterus salmoides*) water. Quality traits consisted in the measure of marketable yields, fruit dry matter, pH, tritratable acidity and degree brix in fruits. A nitrogen uptake assessement measured system efficiencies between hydroponics and aquaponics.

The sixth article investigated the potentials of saline aquaponics by cropping salt-resistant varieties. The scope of the trials was to explore the potentials for developing aquaponics with marine fish, or

in saline areas, where traditional agriculture cannot be developed. Specifically the three trials assessed the growth and nutrient uptake of agretti salad (*Salsola soda*) grown under raising salt concentrations. The fish species used for the trials was mullet (*Mugil cephalus*)

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EXPERIMENTAL PART

Chapter 1

Aquaponics vs Hydroponics: Production and Quality of Lettuce Crop

Abstract

Aquaponics is a vegetable production system that integrates soilless cultivation and aquaculture. Plants strip nutrients from fish waste water and convert metabolites toxic to fish. Aquaponics is an environmental-friendly production system due to its full reuse of waste and nutrients. The research, carried out at the Experimental Farm of the University of Tuscia, compared summer yields of two romaine lettuce crops (*Lactuca sativa* L. cv Integral) grown on aquaponic and hydroponic floating systems. For the hydroponic treatment a nutritive solution of 1.7 dS m^{-1} and pH 5.5 supported plant growth. For the aquaponic system two treatments under different fish densities supplied nutrients at different concentrations. Every aquaponic treatment consisted of 3 independent 250-litre tanks stocked with Nile tilapia (*Oreochromis niloticus* L.). Each fish tank fed a 1.5 m^2 floating system under a 20 plant m^{-2} density. For the first crop 110 g and 24 g tilapia were stocked at system setup respectively under a low (5 kg m^{-3}) and high (8 kg m^{-3}) density and supplied nutrients with an electrical conductivity (EC) of 0.4 and 0.6 dS m^{-1}. For the second crop 168 g and 90 g tilapia respectively stocked under a low (6 kg m^{-3}) and high (20 kg m^{-3}) stocking density raised EC levels to 0.5 and 1.0 dS m^{-1}. Production of 2.8 kg m^{-2} from the first hydroponic crop was similar to the 2.7 kg m^{-2} assessed in the high density aquaponic treatment. Conversely the 2.3 kg m^{-2} measured in the low density treatment was smaller. For the second trial no differences were noticed between the 6.0 kg m^{-2} measured in the hydroponic system and the 5.7 kg m^{-2} and 5.6 kg m^{-2} assessed in the high and low-density aquaponic treatments, respectively. Nevertheless different nutrient concentrations in water affected plant mineral composition. Aquaponic leaves were poorer in phosphorus but richer in calcium, potassium magnesium and sodium.

Keywords: integrated aquaculture, soilless agriculture, tilapia, *Oreochromis niloticus*, floating system

Introduction

Aquaponics is a vegetable production system that integrates soilless cultivation and aquaculture. The almost no discharge of pollutants, the reuse of water and nutrients from fish wastes makes aquaponics a profitable and environmental-friendly production system that can be developed in integrated agriculture-aquaculture systems (IAAS). In aquaponics plants grow in a closed system on

nutrients released by fish and clean up water from ammonia and other metabolites noxious to fish (Rakocy and Hargreaves, 1993; Lennard, 2004).

Main components of an aquaponic system are (Fig.1): fish rearing tanks, a solid removal device (clarifier) to reduce suspended solids (fish waste, uneaten feed, biofloc), a filtering tank to minimize remaining suspended solids. Following the filtering stage the hydroponic troughs serve for the biological conversion of fish excreted ammonia to nitrate, which is less toxic to fish (Rakocy et al., 2006), and to strip water nutrients.

Concentration in nutrients in aquaponics are usually lower than those found in hydroponic systems. At the University of the Virgin Island Experimental Station (UVI) (Rakocy et al., 1992, 2004a, 2004b, 2006) aquaponic vegetables were cropped under the following nutrient concentration ranges: 1.8-3.0 mmol L^{-1} NO_3-N, 0.1-0.2 mmol L^{-1} NH_4-N, 0.3-0.5 mmol L^{-1} P, 1.1-1.6 mmol L^{-1} K, 0.3-0.6 mmol L^{-1} Ca, 0.2-0.3 mmol L^{-1} Mg, 54-104 μmol L^{-1} Fe, 1-14 μmol L^{-1} Mn, 6-8 μmol L^{-1} Zn, 0.5-0.8 μmol L^{-1}Cu, and 8-17 μmol L^{-1} B.

Although aquaponic systems have been studied for decades, most of the research refers to plant-fish optimization and to the water/nutrient balance in aquaculture systems (Endut, et al., 2010; Graber and Junge, 2009, Rakocy et al., 2004b; Seawright et al., 1998; Tyson et al., 2004) or to the system design or management (Adler, 1998; Lennard and Leonard, 2004, 2006; Rakocy et al. 2004a).

Vegetable quality and production from aquaponics needs to be fully acknowledged to provide clear production and quality standards for future commercial expansion. Recent research on growth and yield of leafy vegetables outlined that productivity in aquaponics is often similar or even higher than hydroponics systems (Graber and Junge, 2009; Licamele, 2009; Savidov, 2005). Savidov (2005), in trials carried out under greenhouse conditions, stated that mature aquaponic systems gave accelerated growth to several vegetable varieties and higher yields than standard hydroponics for tomato and cucumber. High yields in leaf vegetables were assessed by Lennard and Leonard (2004) who harvested 4.96 kg m^{-2} of green oak lettuce planted at 40 plant m^{-2} in a three-week trial. Similarly, lettuce yields of 4.7 kg m^{-2} with same levels of biomass and chlorophyll to hydroponics ($p<0.05$) were obtained by Licamele (2009) in a 35-day aquaponic trial. Different environmental conditions from hydroponic systems do not affect quality of lettuce productions, Rico-Garcia et al. (2009) assessed that nitrate in leaves from two summer lettuce crops grown on aquaponics were below 2400 mg kg^{-1} and within the EU limits set for summer greenhouse productions (European Commission, 2002).

The objective of the present study was to assess production and quality of summer crops of lettuce grown on two aquaponic treatments and a standard floating system. Minimal nitrogen concentration for quality lettuce in aquaponic system was also investigated.

Material and Methods

The aquaponic trials took place between August and October 2009 in a greenhouse at the Experimental Farm of Tuscia University, central Italy (latitude 42°25′N, longitude 12°08′E, altitude 310 m). Two aquaponic treatments were compared against an hydroponic control under mineral fertilization. Every treatment was replicated three times.

The aquaponic plant consisted on six independent closed systems (Fig.1), each supplied by one 250 L fish tank. Each system was equipped with a 100 L clarifier and a 25 L filtering tank to keep suspended solids to the lowest values. After solid removal water supplied a 1.5 m^2 floating system unit before being sent back to fish tank. The total water volume in each aquaponic system was 850 L under a flow rate of 250 L h^{-1}, giving a water retention time of 50 minutes in fish tanks and 95 minutes in floating troughs.

The aquaponic systems were filled with osmotized water and stocked on 21st July with two Nile tilapia strains (*Oreochromis niloticus* L.) to raise plant nutrients for 17 days prior trial commencement. The nutrient pool in the aquaponic systems was supported by addition of small quantities of potassium (1.7 mmol L^{-1}), phosphorus (0.4 mmol L^{-1}), sulphur (0.6 mmol L^{-1}), magnesium (0.6 mmol L^{-1}) and iron (36 µmol L^{-1}) with the aim of raising the initial electrical conductivity (EC) from 0.01 up to 0.4 dS m^{-1}. The pH of the systems was maintained at 6.5-7.0 by means of $CaCO_3$, KOH, $Ca(OH)_2$ and H_3PO_4.

The hydroponic control consisted of three 0.5 m^2 floating systems of 100 L volume. The nutrient solution was made with NO_3-N 14.0 mmol L^{-1}, NH_4-N 1.5 mmol L^{-1}, P 1.2 mmol L^{-1}, K 5.0 mmol L^{-1}, Ca 4.5 mmol L^{-1}, S 1.9 mmol L^{-1}, Mg 1.8 mmol L^{-1}, Mn 7.3 µmol L^{-1}, Fe 35.7 µmol L^{-1}, B 44.7 µmol L^{-1}, Zn 1.5 µmol L^{-1}, Cu 0.8 µmol L^{-1}, Mo 0.2 µmol L^{-1}. The EC was 1.7 dS m^{-1} and pH was maintained at 5.5.

The first 4-week trial starting on August 8th assessed the production and quality of lettuce growing on two different aquaponics treatments: a low density (LD) fish treatment stocked at 4.8 kg m^{-3} (mean fish weight 110 g) under an EC of 0.4 dS m^{-1} and a high density (HD) fish treatment stocked at 8 kg m^{-3} (mean fish weight 24 g) under an EC of 0.6 dS m^{-1}. For the second trial starting on September 4th the aquaponic systems had a LD fish treatment stocked at 5.2 kg m^{-3} (mean fish weight 168 g) under an EC of 0.5 dS m^{-1} and a HD stocked at 20 kg m^{-3} (mean weight 90 g) under an EC of 1.0 dS m^{-1}. For both trials the LD treatments were supplied with a 31% protein fish diet (Skretting, Classic K 3P, Hendrix S.p.A., Mozzecane, Verona, Italy). For the first HD trial the feed protein content was 43% (Skretting, Classic K 1P) while for the second experiment the protein content was 40% (Skretting, Classic K 2P).

For both trials a 3-week old romaine lettuce seedlings (*Lactuca sativa* cv. Integral, Syngenta, Mariano Comense, Italy) were planted on floating rafts at a density of 20 plants m^{-2}. For the first crop plants were left grown under a 50% shading.

Lettuce was harvested and shoot fresh weight was recorded. Roots and shoots were dried in a forced-air oven at 80 °C for 72 h for dry biomass determination. Leaf area was measured with an electronic area meter (Delta-T Devices Ltd, Cambridge, UK). Specific leaf area was calculated by dividing leaf area by leaf dry weight of each plant.

Dissolved oxygen, EC and pH were measured twice a week with Hanna HI9147-04 DO meter and HI98130. Ammonia and nitrate in water and plants were measured with Thermo Helios Beta spectrophotometer following the ammonia nitrogen method by Anderson and Ingram (1989) and the salicil-solphoric acid method for nitrate nitrogen by Cataldo et al. (1975). Leaf chlorophyll content was measured with Minolta SPAD 502 two weeks after transplant and at harvest. Plant macro and micronutrients were analyzed by ICP.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Results and Discussion

Nitrate nitrogen concentrations in water at the beginning of the first crop were 0.6 mmol L^{-1} in the low fish density treatment (LD) and 1.1 mmol L^{-1} in the high fish density treatment (HD) while final values were 1.1 mmol L^{-1} (LD) and 5.0 mmol L^{-1} (HD), respectively. In the second lettuce crop NO_3-N concentrations at the end of the trial were 1.4 mmol L^{-1} (LD) and 9.3 mmol L^{-1} (HD). Conversely hydroponic treatment showed a NO_3-N concentration settled at 11.4-12.1 mmol L^{-1}. Ammonia nitrogen levels in all aquaponic crops were well below 0.1 mmol L^{-1} due to nitrification. EC levels in the LD treatment were almost settled at 0.4-0.5 dS m^{-1} in both crops. HD showed a raising trend from 0.4 to 0.8 dS m^{-1} in the first crop and 0.8 to 1.2 dS m^{-1} in the second crop.

The first lettuce crop (Table 1) gave 2.8 kg m^{-2} biomass from the hydroponic treatment, which was similar to the 2.7 kg m^{-2} obtained from the HD system but higher ($p<0.05$) than the 2.3 kg m^{-2} measured in the LD treatment. For the second crop no differences were noticed among the three treatments, with yields of 6.0 kg m^{-2}, 5.7 kg m^{-2} and 5.6 kg m^{-2} respectively for the hydroponic, HD and LD aquaponic treatments.

Water nutrients (Table 2) showed the highest concentrations in the hydroponic treatment. However, sodium turned out to be higher in aquaponics due to supply from the fish feed. Phosphorus was more concentrated in the LD (0.9 mmol L^{-1}) than HD (0.3-0.4 mmol L^{-1}) system due to pH correction with

H_3PO_4. Conversely potassium and calcium levels were higher in the HD system due to pH correction with $Ca(OH)_2$ and KOH.

Aquaponic lettuces followed the similar water trend for sodium, with higher levels in leaf tissues than hydroponics (Table 3). Although potassium and calcium in aquaponic water was lower than hydroponics (Table 2), plants resulted richer of both these elements in the first crop ($p<0.05$) (Table 3). Higher levels of phosphorus in LD water resulted in similar leaf concentrations to hydroponics in the first crop ($p>0.05$). Nevertheless LD system was richer in magnesium than the other two treatments. No differences ($p>0.05$) in plant tissues were noticed for the second crop among treatments.

Chlorophyll assessment (Table 4) showed similar SPAD readings at first crop harvest ($P>0.001$) in both HD system and hydroponics. However, HD had higher values than hydroponics in the second crop ($P<0.001$), while LD was similar to hydroponics ($P>0.001$). Nitrate content in leaves (Table 5) were similar in all treatments ($p>0.05$) and well below the EU limit of 3500 mg kg^{-1} set for summer greenhouse lettuce production (European Commission, 2002).

Conclusions

The results showed that there was no difference in yields between hydroponic and aquaponic treatments whenever nitrogen concentrations were above 1.4 mmol L^{-1}. From the trials constant EC values and nitrate concentrations measured in LD treatments suggested in 1 kg of fish for 20 lettuce heads an optimal fish/plant ratio for romaine lettuce. Quality assessment of lettuce showed no differences between aquaponics and hydroponics for chlorophyll content in leaves. The low levels of nitrates found in aquaponic lettuce, not different from those found in the hydroponic controls, suggested aquaponics to be a valid production system for healthy vegetables. The good yields and quality traits assessed in the trials confirm the big potential of aquaponics to be developed as a more sustainable and integrated production system for quality horticulture .

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Tables

Table 1. Yield, mean head weight, shoot dry biomass, leaf area and specific leaf area (SLA) of lettuce grown in hydroponics and aquaponic systems.

Treatment	Yields ($kg\ m^{-2}$)	Plant fresh weight ($g\ plant^{-1}$)	Plant dry weight ($g\ plant^{-1}$)	Leaf Area ($cm^2\ plant^{-1}$)	SLA ($cm^2\ g^{-1}$)
			1st crop		
aquaponics LD[3]	2.37 b[1]	118.6 a	6.30 b	3366.7	535.9
aquaponics HD	2.71 a	135.3 b	6.96 b	3631.0	525.7
hydroponics	2.84 a	142.2 b	8.19 a	4625.1	562.5
Significance[2]	*	***	**	ns	ns
			2nd crop		
aquaponics LD	5.67	283.3	13.31	5686.9	427.5 b
aquaponics HD	5.70	285.2	13.71	5356.4	390.5 a
hydroponics	6.02	300.9	15.05	6073.9	403.7 a
Significance	ns	ns	ns	ns	**

[1] Means within columns separated using Duncan's multiple range test, p = 0.05.
[2] ns = not significant. *, **, ***, equal significance level of P<0.05, P<0.01, and P<0.001, respectively.
[3] LD = Low density fish biomass, HD = high density fish biomass

Table 2. Nutrient concentrations in hydroponic and aquaponic systems ($mmol\ L^{-1}$).

Treatment	$N\text{-}NO_3$	$N\text{-}NH_4$	P	K	Ca	Mg	Na
				Start 1st crop			
Aquaponics LD[3]	0.5 a[1]	0.0 a	0.0 a	0.3 a	0.9 a	0.3 a	0.32 b
Aquaponics HD	1.0 a	0.1 b	0.1 a	0.4 a	0.9 a	0.3 b	0.29 a
Hydroponics	11.2 b	1.9 c	1.2 b	4.7 b	4.8 b	3.1 c	0.32 b
Significance[2]	***	***	***	***	***	***	*
				End 1st crop			
Aquaponics LD	0.8 a	0.0 a	1.0 ab	1.8 a	1.8 a	1.5 a	0.51 c
Aquaponics HD	4.6 b	0.1 a	0.4 a	2.1 a	2.9 a	1.7 b	0.50 b
Hydroponics	11.4 c	0.4 b	1.2 b	5.2 b	5.7 b	3.5 c	0.33 a
Significance	***	***	*	***	**	***	**
				End 2nd crop			
Aquaponics LD	1.1 a	0.0	0.9 c	0.9 a	1.4 a	1.3 a	0.84 c
Aquaponics HD	9.8 b	0.1	0.3 a	2.7 b	4.5 b	1.8 b	0.76 b
Hydroponics	11.6 b	0.1	0.8 b	3.6 c	5.6 c	3.2 c	0.47 a
Significance	***	ns	***	***	***	***	***

[1] Means within columns separated using Duncan's multiple range test, p = 0.05.
[2] ns = not significant. *, **, ***, significance level of P<0.05, P<0.01, and P<0.001, respectively.
[3] LD = Low density fish biomass, HD = high density fish biomass

Table 3. Mineral composition of leaf lettuce grown in hydroponic and aquaponic systems (g kg^{-1} d.wt).

Treatments	N	P	K	Ca	Mg	Fe	Na
				1st crop			
Aquaponics LD[3]	33.7	8.2 b[1]	75.3 b	12.2 b	7.7 b	0.12 a	1.9 c
Aquaponics HD	26.1	6.9 a	74.9 b	12.1 b	6.4 a	0.16 a	1.2 b
Hydroponics	31.8	8.1 b	65.7 a	10.7 a	6.5 a	0.23 b	0.8 a
Significance[2]	ns	*	*	*	*	*	***
				2nd crop			
Aquaponics LD	28.9	7.0	70.5	10.7	5.9	0.06	1.2 b
Aquaponics HD	30.0	5.8	59.0	8.7	4.4	0.06	0.6 a
Hydroponics	25.9	7.5	70.5	9.4	5.1	0.06	0.5 a
Significance	ns	ns	ns	ns	ns	ns	*

[1] Means within columns separated using Duncan's multiple range test, p = 0.05.
[2] ns = not significant. *, **, ***, equal significance level of P<0.05, P<0.01, and P<0.001, respectively.
[3] LD = Low density fish biomass, HD = high density fish biomass

Table 4. Chlorophyll concentrations in leaves two weeks after transplant (mid) and at harvest (end). Values are in SPAD units.

	1st crop		**2nd crop**	
Treatment	mid	end	mid	end
Aquaponics LD	28.0 a[1]	28.8 a	29.7 a	30.9 a
Aquaponics HD	32.4 b	34.1 b	31.8 b	34.0 b
Hydroponics	36.2 c	34.7 b	33.6 c	30.2 a
Significance[2]	***	***	***	***

[1] Means within columns separated using Duncan's multiple range test, p = 0.05.
[2] *** significance level at P<0.001
[3] LD = Low density fish biomass, HD = high density fish biomass

Table 5. Nitrate content in leaves.

	1st crop	**2nd crop**
Treatment	leaf nitrate (mg kg^{-1} f.wt.)	
Aquaponics LD[2]	545.3	1501.3
Aquaponics HD	285.6	1181.9
Hydroponics	143.3	1591.5
Significance[1]	Ns	ns

[1] ns = not significant
[2] LD = Low density fish biomass, HD = high density fish biomass

Figures

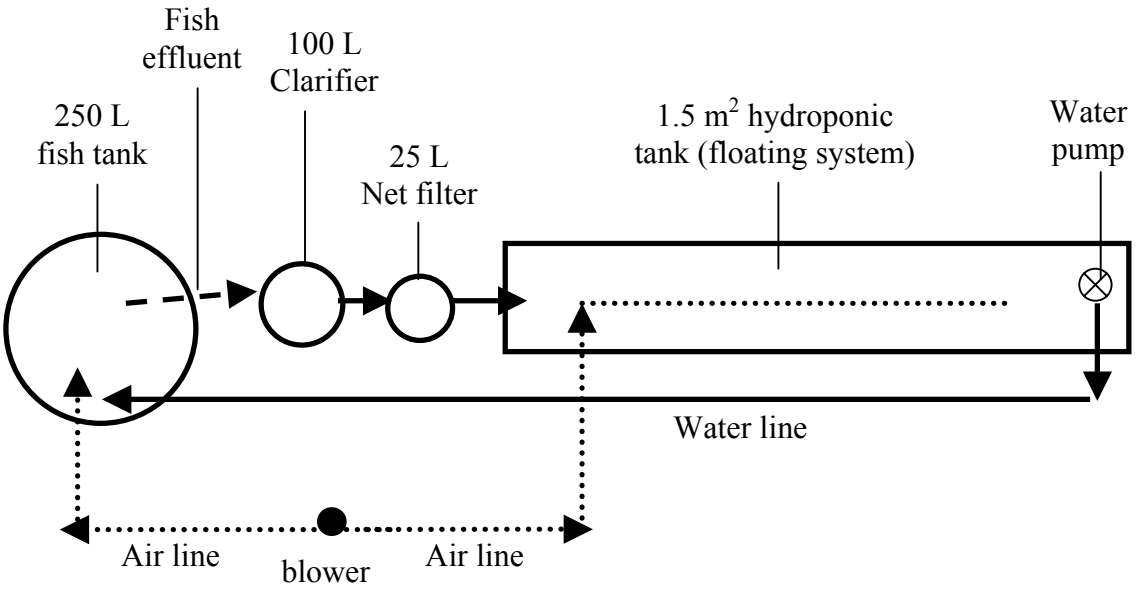

Figure 1. Design of aquaponic systems used in the experimental trials.

Chapter 2

Aquaponics and Food Safety: Effects of UV Sterilization on Total Coliforms and Lettuce Production

Abstract

Aquaponics is an integrated production system where plants grow in a soil-less medium of aquaculture waste. This kind of production is seen favourably nowadays since waste utilization could increase farm productivity and reduce environmental impacts. This research compared the microbiological quality of aquaponic water under ultraviolet (UV) sterilization and its compliance to international directives on irrigation water uses. An assessment of crop productivity was also carried out to outline differences in productive traits of lettuce (*Lactuca sativa* L. cv Verde degli Ortolani) grown in sterilized and not-sterilized aquaponic systems against a hydroponics floating system supplied with a nutrient solution of 1.6 dS cm^{-1}. Total coliform under UV disinfection showed counts well below 1 CFU ml^{-1} and a reduction in microbial loads higher than 99%. No significant differences were recorded for productive traits (yield, mean shoot weight, shoot and root dry biomass, leaf area, specific leaf dry weight), suggesting that aquaponics is a valid method to produce vegetables with high hygienic standards.

Keywords: Hydroponics, integrated aquaculture, lettuce, food safety, ultraviolet, water disinfection

Introduction

Food production, is nowadays looking to system integration as a strategy to reduce the impact on environment and to increase productivity. Integrated agriculture-aquaculture systems, by simply using mutual resources, can be seen as a valid strategy to produce food with low ecological footprint. On this matter aquaponics, the soiless production of plants on aquaculture wastes, is progressively growing interest among farmers for its almost complete reuse of water and nutrients within a recirculating system: plants take advantage of minerals released by fish and at the same time act as biofiltration units by reducing levels of ammonia (Rakocy and Hargreaves, 1993; Lennard, 2004; Goulden, 2005; Singh, 1996).

Main components of an aquaponic system are: fish rearing tanks, a solid removal device (clarifier) to reduce suspended solids and BOD (fish waste, uneaten feed, biofloc), a filtering tank unit to mineralize remaining suspended solids, and hydroponic troughs used as biofilters to transform fish excreted ammonia to less toxic nitrate (Rakocy et al, 2006). Aquaponics shows a sensitive lower

nutrient balance compared to hydroponics, nutrients are stored into the recirculating water and are continuously supplied by fish (Shultz, pers. commun., 2008). Nutrient concentrations in the aquaponic systems are usually lower than those used in hydroponic systems. This is due to the fact that fishes can tolerate $N\text{-}NO_3$ levels of 200 mg L^{-1} (Losordo et al., 1998) and concentrations above 300 mg L^{-1} appear to be toxic (Masser et al., 1999). At the University of the Virgin Island Experimental Station (UVI) Rakocy et al. (1992, 2004a, 2004b, 2006) grew vegetable crops in aquaponic systems with nutrient concentrations (mg L^{-1}) in the following ranges: (26-42) $N\text{-}NO_3$, (0.95-2.2) $N\text{-}NH_4$, (8-16.4) P, (44-63) K, (11.9-24.2) Ca, (6.0-6.5) Mg, (1.3-2.5) Fe, (0.06–0.8) Mn, (0.34-0.44) Zn, (0.03–0.05) Cu, and (0.09-0.19) B.

Growth and yield of leafy vegetables are often similar or even higher in aquaponics than in hydroponics systems. Savidov (2005) in trials carried out under greenhouse conditions stated that mature aquaponic systems, with higher nutrient concentrations than those used in UVI, can give higher yields than standard hydroponics.

One important issue in aquaponics that has not yet been studied is the assessment of microbiological water quality and the reduction of microbial loads to comply with food safety standards. Presence of fish and excreta on this matter may give the perception that aquaponics is not completely safe. Reuse of wastewater for irrigation purposes is however ruled differently from country to country. World Health Organization (WHO) wastewater reuse guidelines (1989) rules in 1,000 fecal coliform 100 mL^{-1} the limit for unrestricted irrigation water, although a more stringent normative with 200 CFU 100 mL^{-1} is set by WHO for lawn irrigation (EPA, 2004). A strict regulation is found in the State of California Wastewater Reclamation Criteria (1978) where the limit for total coliform is set to 2.2 CFU 100 mL^{-1}.

In European countries there is not a common guideline for all the member states. In Italy the law sets in 10 CFU 100 mL^{-1} the limit for *Escherichia coli* in irrigation water from 80% of the total samplings and rules that the maximum tolerated count must not be higher than 100 CFU 100 mL^{-1}. However if the water is originated by phytoremediation systems the norm sets in 50 CFU 100 mL^{-1} the average value from 80% of the total samples and 200 CFU 100 mL^{-1} the maximum accepted value for single measures (DM n. 185 of 12 June 2003). Other European countries such as France and Spain (Andalusia) are less stringent for fecal coliform with limits set at <1000 CFU 100 mL^{-1} (Brissaud, 2008). However recommendations are given in France to avoid direct contact of edible parts with water. In addition a new Spanish draft poses to <200 CFU 100 mL^{-1} the limit for fecal coliform (Brissaud, 2008).

Abatement of microbial loads is an important process in effluent disinfection. Use of ultraviolet light (UV) sterilizers has proven to be effective in reducing coliform, however depending on the water quality different UV intensities are needed. Andreadakis et al. (1999) stated that fecal coliform concentration of less than 10 CFU 100 mL^{-1} can be achieved with a UV dose of 40-50 mW sec cm^{-2} in tertiary effluents.

Such value was also confirmed by Antonelli et al. (2008) with a dose of 30 mW sec cm^{-2} to achieve *E. coli* concentration of 10 CFU 100 mL^{-1} under average suspended solid concentrations of 2.5 mg L^{-1}.

The objective of the present study was to carry out a preliminary assessment on bacterial loads in aquaponic systems with and without UV sterilization and to determine if there was difference in productive traits of lettuce between the two aquaponic treatments (with or without UV sterilization in recirculating water) and a standard hydroponic system.

Material and Methods

The UV sterilization trial took place in December 2009 in a heated greenhouse at the Experimental Farm of Tuscia University, central Italy (latitude 42°25′N, longitude 12°08′E, altitude 310 m). The minimum air temperature was set at 17°C ±1.

The aquaponic plant consisted of 6 independent systems, each supplied by one 250 L fish tank. Fish tanks were kept at a temperature of 21°C ±1 by means of water heaters under a 12 hour daylight photoperiod. Each system was equipped with a 100 L clarifier and a 25 L filtering tank. The filtering tank was made by a two-layer netting of 1.2 mesh (inlet) and 25 mesh (outlet) to keep suspended solids to the lowest values. After solid removal water supplied a 1.5 m^2 floating system unit before being sent back to fish tank. For three of the six systems a UV sterilizer was positioned at the inlet of the plant production unit with the aim to control microbial contamination from fish to plant tanks. The UV sterilizer consisted in two commercial 25W lamps used for pond disinfection joint in series. Each UV lamp had a standard TL mercury low pressure bulb housed in a quartz tube of 372.4 cm^2. Flow rate through the lamps was set at 3 L min^{-1} with a water retention time of 10 seconds. The average total suspended solids (TSS) in water was 4 mg L^{-1}.

Microbiological assessment was carried out both in the sterilized and not-sterilized treatments. For each system samples were taken three times during the trial (9, 15 and 20 December) in fish tanks, at the outlet of the plant production unit and, only for the sterilized treatments, at the outlet of the UV unit. Water sampling procedure consisted in a 4-hour water settling into a falcon tube to reduce any presence of particulate matter before plate inoculation. A 1:10 dilution was carried out for all those samples that showed high number of colonies. Plates were incubated in a thermostatic chamber at 35°C for 24 hours. For total coliform assessment chromogenic Compact Dry plates (Nissui Pharmaceutical co., Ltd., Tokyo, Japan) were used. The plates, which are AOAC certified and whose performance was also tested and validated by ARPA water quality laboratory in Viterbo (Luiso, pers. commun., 2009), can differentiate coliform from *Escherichia coli* colonies due to two chromogenic enzyme substrates present in the nutritive media: Magenta-GAL and X-GLUC.

Seedlings of lettuce (*Lactuca sativa* L. - cv. Verde degli Ortolani, SAIS Sementi S.p.A., Cesena, Italy) were transplanted on December 7 at a density of 200 plant m^{-2}. The sterilized and not sterilized aquaponic treatments were compared; an hydroponic floating system was also included as control. Treatments were replicated three times. The nutrient solution of the hydroponic system was prepared by blending commercial fertilizers (Table 1) and had an EC of 1.6 dS cm^{-1}. In the acquaponic systems, fertilizers were added on November 2nd at system start-up (Table 1) giving an EC of 0.6 dS cm^{-1}. Nutrients were supplied with the aim to reach the standard concentrations of a mature aquaponic system. In the present trial, 5 kg of Nile tilapia (*Oreochromis niloticus* L.) with an average body weight of 0.22 kg were stocked in each tank. Fish were fed at 0.8% of body weight with pelletted feed of 31% crude protein (Skretting, Classic K 3P, Hendrix S.p.A., Mozzecane - Verona, Italy). During the trial, nitrogen concentration as nitrate in aquaponic water was between 110-130 mg L^{-1} while ammonia levels was in the range of 0.27 - 2.5 mg L^{-1}. Water pH in the aquaponic systems was kept at 6.5 by addition of KOH and $Ca(OH)_2$ to compensate acidifying effect by nitrifying bacteria.

Lettuce was harvested after two weeks on December 21 and shoot fresh weight was recorded. Roots and shoots were dried in a forced-air oven at 80 °C for 72 h for dry biomass determination. Leaf area was measured with an electronic area meter (Delta-T Devices Ltd, Cambridge, UK). Specific leaf area was calculated by dividing leaf area by leaf dry weight of each plant.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Results and Discussion

Trends in total coliform showed a good sterilization effect at the UV lamp outlet with almost zero CFU mL^{-1} in all the samples (Table 2). Plate counts revealed no *E. coli* presence in any samples for both sterilized and no-sterilized treatments. Comparison of UV outlet against culturable fraction of coliforms in corresponding fish tanks showed almost a 3 log reduction of bacteria from the system due to UV disinfection. Although health guidelines refer to volumes of 100 mL, data suggested adequate sterilization effect and thus a UV intensity in line with the values of 30-50 mW sec cm^{-2} outlined by Andreadakis et al. (1999), Antonelli et al. (2008) and Hijnen et al. (2006). Presence of coliform in UV treated water found in just one sterilizer on December 15 could be also justified by presence of suspended particles or flock that shielded bacteria from UV rays. On this matter Jolis et al. (2001) stated that a minimum dose of 100 mW sec cm^{-2} was required for a log 3 inactivation with particles bigger than 7-8 μm.

In the vegetable production tanks total coliform followed the same trend found at the UV outlet with values settled almost to zero (Table 2). Interestingly comparison with culturable fraction of coliforms measured before the beginning of the experiment (2 December) showed a negative trend from 23.3 CFU mL^{-1} to 0-2 CFU mL^{-1} in the vegetable production tanks presumably due to the continuous flow of disinfected water from the UV lamps. On the contrary an opposite trend was noticed in the fish tanks where average culturable fraction of coliforms settled to values of 700 CFU mL^{-1} at the end of the trial.

The not-sterilized treatment showed in the fish tanks an average value of 1000 CFU mL^{-1}, higher than the corresponding count found in the sterilized tanks (Table 2). In the lettuce tanks the trend was similar to that found in fish tanks but bacteria counts settled at about 400 CFU mL^{-1}. Data comparison between fish tanks and plant production units suggested a bioremediation effect carried out by plants, with a 50-60% abatement of culturable fraction of coliforms from the level measured in the fish tanks. However, loads in not-sterilized lettuce tanks were still high and seemed not to comply with less stringent health guidelines for irrigation water set by WHO (1989).

No significant differences were recorded for yield, mean plant weight, shoot and root dry biomass, leaf area and specific leaf area of lettuce grown in the different systems (Table 3). Despite the lower nutrient concentrations found in the aquaponic systems, growth and yield of lettuce observed in aquaponic systems were similar to those recorded in hydroponic system. The results are in agreement with those observed in previous trials conducted at University of Tuscia where no difference in lettuce productivity was noticed between hydroponics and aquaponics treatments with nitrate nitrogen concentrations from 25 mg L^{-1} (unpublished data). The high growth and yield of lettuce observed in aquaponic systems could be the result of the presence in the recirculating water of organic compounds and plant beneficial bacteria (e.g. plant growth promoting rhyzobacteria) which could have promoted the nutrient uptake and assimilation. Similar conclusions were reported by Savidov (2005).

Conclusions

The preliminary assessment on the UV sterilization in aquaponic systems gave interesting results both in terms of production and bacteria control. Absence of culturable fraction of coliforms from UV sterilizers and similar yields achieved in all treatments suggested the absence of any negative interaction of UV sterilization to plant and fish growth. UV disinfection with 3 log abatement confirmed the idea that safe vegetable production can be achieved in aquaponic systems with limited investments.

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Tables

Table 1. Fertilizer rates (mg L^{-1}) used in the hydroponic and aquaponic systems

Fertilizer	Hydroponics	Aquaponics
Calcium nitrate (15,5% N; 28% CaO)	812.9	316.1
Monopotassium phosphate (52% P_2O_5; 34% K_2O)	163.9	81.9
Potassium sulfate (50% K_2O; 45% SO_3)	346.7	
Magnesium sulphate (16% MgO; 32% SO_3)		147.9
Potassium bicarbonate (46% K_2O)		100.1
Potassium nitrate (13% N; 46% K_2O)		50.8
Magnesium nitrate (11% N; 16% MgO)	448.1	
Ammonium nitrate (27% N)	121.5	
Mikron (4% Fe; 4% Mn; 1% Zn; 0.5% B; 0.5% Cu; 0.2% Mo)	10.0	
Myr Ferro (5% Fe)	42.6	53.2
Boric acid (17% B)	2.6	

Table 2. Bacteria count in sterilized and not-sterilized aquaponic systems 2, 8 and 13 days after transplanting (DAT)

Treatment	Sampling position	Total bacteria (CFU mL^{-1})		
		2 DAT	8 DAT	13 DAT
Aquaponics	Fish basin	-	1053.3 b	956.7 c
	Lettuce floating bed	-	450.0 ab	420.0 b
Aquaponics + UV	Fish basin	373.7 b	1250.0 b	693.3 bc
	UV lamp outlet	0.0 a	0.7 a	0.0 a
	Lettuce floating bed	-	0.0 a	2.0 a
Significance		***	*	***

Means within columns separated using Duncan's multiple range test, $p = 0.05$.

$^{NS, *, ***}$Nonsignificant or significant at $p < 0.05$, or 0.001 respectively.

Table 3. Yield, mean head weight, shoot and root dry biomass, leaf area and specific leaf area (SLA) of lettuce grown in hydroponics, aquaponics and aquaponics+UV systems

Treatment	Yield ($kg\ m^{-2}$)	Mean shoot weight ($g\ plant^{-1}$)	Shoot dry biomass ($g\ plant^{-1}$)	Root dry biomass ($g\ plant^{-1}$)	Leaf area ($cm^2\ plant^{-1}$)	SLA ($cm^2\ g^{-1}$)
Aquaponics	2.0	10.1	0.63	0.10	894	548
Aquaponics + UV	1.9	9.6	0.59	0.12	1001	592
Hydroponics	1.9	9.7	0.53	0.12	1178	549
Significance	ns	ns	ns	ns	ns	ns

ns = Non significant

Chapter 3

Aquaponics and Sustainability: Production, Quality and Nutrient Efficiency in Sweet Basil and African Catfish

Abstract

Increasing world population and food demand have in recent years raised concerns about agroecosystems sustainability. While it is not certain if past trends in agriculture productivity can continue with the same pace, there is a raising awareness that food production should look into system integration. Aquaculture is seen as one of the solutions available, since fish can be more productive than warm blooded animals. Nevertheless integration of aquaculture wastes with agriculture can reduce sensitively the use of production inputs. In the case of aquaponics, the soilless plant production (hydroponics) on aquaculture water, there is a symbiotic association between fish and plants. Wastes from animals are nutrients for plants, which grow with no use of additional fertilization and serve to reclaim water into the system. Given optimal fish/plant ratio the system could be as performing as high-input agriculture. Two consecutive aquaponics trials with sweet basil (*Ocimum basilicum* L. cv superbo) and African catfish (*Clarias gariepinus* B.) were carried out at the experimental farm of the university of Tuscia in summer 2010. Two treatments, consisting of fish feeding a low protein (LP) and high protein diet (HP) under same stocking densities, supplied nutrients to sweet basil plants growing on floating rafts. Vegetable production was compared against a standard hydroponic control. No significant differences were noticed between aquaponics and hydroponics for both production and quality of plants, however vegetables differed in mineral composition, following the nutrient patterns present in water. Likewise catfish growth and feed conversion rate was similar in both diets. A nutrient budget was developed to determine nutrient uptake efficiency and to optimize fish/plant ratio. For the first trial uptake efficiency for both nitrogen, phosphorus and potassium was similar in all treatments, however in the second trial the increased fish biomass and the consequent increases in nutrient wastes lowered plant uptake efficiency in aquaponics. The optimal feed/plant ratio resulted in 27.2 g feed plant^{-1} (LP) and 18.8 g feed plant^{-1} (HP).

Introduction

Agriculture fed humanity since its dawn. Every advance in agricultural practices brought huge impacts to people and favoured the development of more complex societies. In the last decades science and technology supported food production through intensification of agricultural systems,

however debates on the agroecosystems' carrying capacity are still seeking for reliable evidence on the possibility to produce more food in future (Brown and Kane 1994; Seckler, 1994; Waggoner, 1994). Progressive growth of population poses new challenges in developing sustainable agriculture systems that can address the needs for increased yields and guarantee for an efficient and long-lasting use of natural resources (Harris, 1996, Tilman et al., 2002).

According to Franks and Hadingham (2012) nearly half of greenhouse gases is produced by fertilizers, a percentage that raises up severe issues on agriculture sustainability. A key factor is undoubtedly the increase of nutrient use efficiency (Tillman et al., 2002) as well as the closing of the nitrogen and phosphorus cycles in agriculture.

Sustainable intensification is a term used by Godfray et al. (2010) as a way to describe any increase in land output through the adoption of integrated-driven managements. The integration with agriculture and aquaculture appears an interesting challenge as it could preserve biodiversity and raise productivity (Godfray et al., 2010).

Aquaculture is one of the most sustainable production systems, since fish can convert feed into body biomass 2-4 times more efficiently than warm-blooded animals (Verdegem et al., 2006).

Aquaculture accounts for more than one third of the world fish production (FAO 2010). Fish supply approximately 15% of animal protein intake to 3.0 billions of people worldwide. The major share of aquaculture production is owned by freshwater species (FAO, 2010), which can easily be integrated in agricultural management. Nevertheless traditional fish farming systems, if on one side can achieve up to 100 tons of fish per hectare per year (Edwards, 1993; Verdegem et al., 2006) depending on farming intensification and availability of natural and supplementary feeds (Barnabé, 1990; Diana et al., 1997), on the other side would cause significant environmental impacts due to polluting wastes released in water bodies (Piedrahita, 2003; Verdegem et al., 1999; Verdegem et al., 2006).

More advanced farm practices have been developed with recirculating aquaculture systems (RAS). The continuous recycle of water allows for highly-intensive productions with minimum environmental impact (Martins et al., 2010). Water in RAS can be fully reused if solid removal (fish faeces and uneaten feed) and biological filtration transforms toxic ammonia excreted by fish into nitrate. The efficiency of recirculating systems depends on RAS design (Martins et al., 2010), however if on one side fish productivity and water use is maximized, on the other side aquaculture by-products, in the form of nitrogen, phosphorus and other minerals, are not used or wasted (Piedrahita, 2003; Martins et al., 2010; Verdegem et al., 2006).

An evolution of RAS is aquaponics, a production system that integrates soilless plant production (hydroponics) and aquaculture. Plants growing on aquaculture effluents take up nutrients dissolved

in wasted water and provide tools for bioremediation. In aquaponics nutrients reach significant levels compatible with plant growth through continuous recycle of water. On the other side plants remove continuously nutrients and avoid any toxic build up (Rakocy and Hargreaves, 1993).

Aquaponic systems are made of different components (Fig. 1). Fish waste water flow through a removal unit (clarifier, swirl separator) where suspended solids settle and are removed. A net filter follows the sedimentation stage where fine particles are sequestered to obtain clean water. In the successive stage different troughs host plants that take nutrients out of the system and return clean water back to fish.

Three are the main aquaponic systems adopted worldwide: floating systems, where plants grow on polystyrene rafts floating on water; nutrient film technique (NFT) where plant growth by stripping nutrients from a thin layer of water flowing at the bottom of plastic pipes; gravel/sand where growth is secured by micro-irrigation or 'ebb and flow' systems. Oxygen for plant roots is mainly supplied through aerators (floating systems) or through timely distribution of water (NFT and gravel/sand systems), which allow roots to be in direct contact with air (Resh, 2004). Aquaponics has nutrient levels well below those of conventional hydroponics, but has the advantage that water is never wasted and continuously enriched by fish.

Beyond high fish productivity and improved water use efficiency, which can be tens or hundreds of times lower than traditional aquaculture systems (Piedrahita, 2003; Verdegem et al., 2006), the integration with agriculture is seen as an easy strategy to reduce fertilizers use or not renewable inputs (Franks and Hadingham, 2012; Tilman et al., 2002).

Some studies have assessed optimal fish/plant ratio for some aquaculture species. Gloger (1995) determined 0.56 g m^{-2} day^{-1} of ammonia, 0.83 g $m^{-2}day^{-1}$ of total nitrogen and 0.17 g m^{-2} day^{-1} of phosphorus the nutrient removal of romaine lettuce in Virgin Island in closed tilapia system, while Rakocy et al. (1992) determined in 2.4 g day^{-1} of feed with 36% protein the amount of nutrients for one plant of lettuce growing on floating system. Likewise Adler et al. (2003) estimated that 0.94 g m^{-2} of nitrogen and 0.1 g m^{-2} of phosphorus was the absorption rate of lettuce growing in a rainbow trout recirculation system. Nevertheless there is not an extensive knowledge on the potential of aquaponics against traditional highly intensive agriculture, both in terms of production, quality and nutrient uptake efficiency.

The objective of the present study was to assess the production and quality of aquaponics production against hydroponics, to determine the aquaponic nutrient uptake performance and to develop a nutrient model to optimize nutrient use and fish/plant ratio.

Materials and Methods

Two consecutive aquaponic trials were carried out in a greenhouse between July and September 2010 at the Experimental Farm of the University of Tuscia, central Italy (latitude 42°25′N, longitude 12°08′E, altitude 310 m).

The aquaponic facility consisted of 6 independent recirculating systems, each supplied by one 250 L fish tank. Every system was equipped with a 100 L clarifier and a 25 L filtering tank tightly filled with a 1.2 mesh net to take suspended solid out from circulating water. Downstream a 2 m^2 trough hosted plants growing on two 0.75 m^2 floating rafts. Plant troughs were 0.20 m deep. The hydraulic retention time in the fish tanks was 40 minutes and 1 hour in the plant troughs

The aquaponic systems were stocked on 4th July with African catfish (*Clarias gariepinus* B.) (Fleuren & Nooijen Viskwekerij BV, Someren, The Netherlands) to build a sufficient pool on nutrients prior trial commencement. Each aquaponic system was enriched with small quantities of potassium (65 mg L^{-1}), phosphorus (12.4 mg L^{-1}), sulphur (20.2 mg L^{-1}), magnesium (14.2 mg L^{-1}) and iron (3 mg L^{-1}) to raise the electrical conductivity (EC) by 0.15 dS m^{-1} and meet the nutrient concentrations of mature aquaponic systems. Additional iron (0.15 mg L^{-1}) was dissolved in aquaponic tanks at the beginning of the second trial. Systems pH was maintained at 6.5-7.0 by addition of $CaCO_3$ and KOH in both aquaponic and hydroponic tanks. The amount of calcium added through $CaCO_3$ in tanks accounted for 52 mg L^{-1} (aquaponics LP, HP) and 39 mg L^{-1} (hydroponics) in the first trial and 52 mg L^{-1} (LP), 94 mg L^{-1} (HP) and 78 mg L^{-1} (hydroponics) in the second trial. The amount of potassium added as KOH in the second trial was 14 mg L^{-1} (LP), 22 mg L^{-1} (HP) and 4 mg L^{-1} (hydroponics)

For both trials two aquaponic treatments under same fish densities but different protein diets were compared against a chemically fertilized hydroponic control containing NO_3-N 196 mg L^{-1}, NH_4-N 21 mg L^{-1}, phosphorus 37.2 mg L^{-1}, potassium 195 mg L^{-1}, calcium 180 mg L^{-1}, sulphur 60 mg L^{-1}, magnesium 42 mg L^{-1}, manganese 0.2 mg L^{-1}, iron 0.7 mg L^{-1}, boron 0.5 mg L^{-1}, zinc 50 μg L^{-1}, copper 20 μg L^{-1} and molybdenum 3.2 μg L^{-1}. The EC of the solution was 1.9 dS m^{-1} and pH 5.5. Every treatment was replicated three times.

For the first trial every fish tank was stocked under a density of 9.2 kg m^{-3} and average body weight of 81.3 ± 12.3 g. For the second trial stocking density was 20.9 kg m^{-3} and average body weight was 188.1 ± 25.6 g.

Fish were fed a low protein diet (LP) (Skretting, Classic K 3P, Hendrix S.p.A., Mozzecane, Verona, Italy) and a high protein diet (HP) (Skretting, Classic K 2P) containing respectively 31% and 40% proteins.

For both trials a 3-week old Genovese basil seedlings (*Ocimum basilicum* cv Superbo, SAIS Sementi S.p.A., Cesena, Italy) were planted on floating rafts at a 36 plant m^{-2} density. Plants were left grown for 4 weeks before harvest. In both aquaponic and hydroponic systems water was kept aerated to reach dissolved oxygen concentrations of 6-7 mg L^{-1}.

Plants were assessed for fresh weight for both leaves and stems to determine leaf/stem ratio. Roots and shoots were dried in a forced-air oven at 80 °C for 72 h for dry biomass determination. Leaf area was measured with an electronic area meter (Delta-T Devices Ltd, Cambridge, UK). Specific leaf area was calculated by dividing leaf area by leaf dry weight of each plant.

Fish growth performance was determined as body weight gain and specific growth rate (SGR), which measures growth performance as percentage of daily body weight gain according to the formula:

$$SGR = [(LnWf - LnWi)/days]x100$$

Where Ln is the natural logarithm, Wf is the final weight of the fish, Wi is the initial weight and days is the time of the experiment. Feed conversion ratio (FCR) was also assessed to measure the animal ability to convert feed into body biomass. It was calculated by dividing the feed consumed (dry weight) by the fish body weight gain.

Electrical conductivity, temperatures pH and dissolved oxygen were measured twice a week with Hanna HI98130 and Hanna HI9147-04 DO meter. Nitrogen levels in water were analyzed by spectrophotometric quantification of ammonia an nitrate following the ammonia nitrogen method by Anderson and Ingram (1989) and the salicil-solphoric acid method for nitrate nitrogen by Cataldo et al. (1975). Leaf chlorophyll content was measured with Minolta SPAD 502 two weeks after transplant and at harvest. Plants, fish, feed and system water were analyzed for their mineral profile with ICP-MS.

Water loss due to evapotranspiration or system management was accounted twice a week. Osmotized water was used for any water refill to initial system levels. Incoming water was measured with a water meter. Plants net water consumption was determined from the volume used to compensate losses, from which the fraction used for solid removal was detracted.

A nitrogen budget was accounted by using a mass balance approach for each of the inputs and outputs measured in their dry matter and total Kjeldhal nitrogen content:

$$[N_{feed}] + [N_{fertilization}] + [N_{initial\ water}] = [N_{fish}] + [N_{plants}] + [N_{roots}] + [N_{final\ water}] + [N_{lost}]$$

where the incoming nitrogen amount for each crop cycle was supplied by feed, fertilization (hydroponics) or the nutrient pool in water (aquaponics). The outgoing nitrogen was the result of fish, plant and root uptake. From the mass balance the amount of nitrogen lost by the system was determined as the result of denitrification, fish metabolism, solid/feed discharge, bacterial/plankton sequestration and settled organic matter into the system.

System efficiency was determined by measuring the nutrient uptake efficiency (NUE) (Van Eerd, 2007). NUE was calculated by dividing the mass of nutrient in plant tissue by the net supplied nutrients, according to the equation :

$$NUE = [Nutr_{plants}] / [Nutr_{supplied}]$$

Net supplied nutrients accounted for the amount of mineral already stocked into the water or given by feed after fish uptake.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Percentages values were analyzed upon square root or arc sine transformation. Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Results

Water quality

Differences in protein content from the two fish diets determined different water nitrogen patterns between the two treatments. The nitrate concentrations (Fig. 2) in the LP aquaponic tanks raised from 25 to 36 mg L^{-1} in the first trial and climbed up to 60 mg L^{-1} at the end of the second trial. Conversely the higher protein content in the HP diet affected the nitrate trend with a two-fold growth in concentrations, which brought nitrate levels from 31 to 62 mg L^{-1} in the first trial up to 120 mg L^{-1} at the end of the second trial. In both experiments aquaponic nitrate were well below the concentrations observed in hydroponics, where readings ranged from 200 mg L^{-1} up to 130-150 mg L^{-1}.

Water levels of ammonia in LP treatment appeared constantly close to 0 mg L^{-1} (Fig. 3), due to the efficient nitrification operated by bacteria. On the contrary higher ammonia values were observed in the second crop in HP tanks. The ammonia trend noticed in HP is similar to the temperature patterns (Fig. 5), which suggests for suboptimal activity of nitrifying bacteria.

Electrical conductivity (Fig. 4) in LP tanks resulted flat in the first crop (0.75 to 0.76 dS m^{-1}), which suggested for an equilibrium between nutrient supply and plant uptake. However, values in LP raised up to 0.97 dS m^{-1} in the first half of the second crop due to reduced plant uptake, but settled to constant values towards plant maximum growth. EC readings in HP tanks raised by 19% and 29% in the first and second crop respectively, thus suggesting higher supply of nutrients.

Plant production and quality

Sweet basil yields, plant biomass and dry matter content in aquaponics showed no significant differences against hydroponics (Tab. 1). The second trial showed an overall reduction in yields of

27-35% due to suboptimal ambient temperatures. Nevertheless the dry matter content was similar among treatments and almost constant in both trials. Root biomass (Tab. 1) in aquaponics was bigger than hydroponics in the first trial ($p<0.05$), but no differences were noticed successively.
Leaf productivity (Tab. 2) was similar in all treatments. Differences noticed in production between the first and second trial were explained by the reduced leaf biomass production per plant in both LP aquaponics (-20%), HP aquaponics (-29%) and hydroponics (-29%). Leaf/stem ratio and specific leaf area (SLA) values were similar in both trials. Nevertheless, some qualitative issues were noticed in the chlorophyll content of leaves in LP during the second trial (Tab.3), although differences were higher at mid crop (-11%) than at harvest (-7%).

Fish production

Fish biomass gain in the first trial resulted similar in both treatments ($p>0.05$) with 2.65 kg ± 0.14 (LP) and 3.10 kg ± 0.42 (HP), under a feeding regime of 1.9-2.0 % of body weight day^{-1} (equivalent to 2.9 kg of feed). Biomass gain in the second trial was 2.14 kg ± 0.16 (LP) and 2.12 kg ± 0.14 (HP) under a feeding regime of 1.5% of body weight day^{-1} (equivalent to 2.7 kg feed). Fish mortality accounted for an average of 2.4% (LP) and 3.6% (HP) in the first trial and 2.3% (LP) and 4.0% (HP) in the second trial.
No differences were noticed between LP and HP treatment for Specific Growth Rate (Tab.4), however SGR was lower in the second trial due to reduced fish metabolism as a consequence of lower water temperatures (Fig. 4). Similarly feed conversion ratio did not show differences in each trial between LP and HP ($p<0.05$) (Tab. 4), although values were lower in the second trial (-14% LP, -34% HP) due to reduced fish metabolism.

Water macro-micronutrients patterns

The mineral concentrations observed in hydroponics were significantly higher ($p<0.05$) than aquaponics, with the exception of sodium that was lower ($p<0.05$). Raising trends for magnesium, sodium and calcium were observed in aquaponics, although the concentrations of the first two minerals settled to constant values by the end of the first trial.
Increasing levels of calcium in all treatments were due to pH correction with calcium carbonate to counterbalance water acidification operated by nitrifying bacteria.
Potassium levels appeared constant in the two trials, although the addition of 14 mg L^{-1} (LP) and 22 mg L^{-1} (HP) as KOH to level pH up in aquaponic tanks compensated for plant uptake in the second trial. On the contrary phosphorus showed a negative trend with nearly 60% reduction observed in both treatments in the first trial and a further 77% decrease occurred in LP tanks in the second trial.

Micronutrients concentrations were consistently higher in hydroponics ($p<0.05$) for all the samples with the exception of zinc, where no significant differences were noticed from the end of the first trial. For copper, manganese, and boron concentrations in aquaponics remained constant from the end of the first crop. Iron addition into aquaponics occurred after first sampling and accounted for 3 mg L^{-1}, which was sequestered by plants for nearly 97% by the end of the first trial. Successive addition of iron in the second trial resulted in a 67% uptake by aquaponic systems.

Plant and root macro-micronutrient patterns

No significant differences were noticed for plant nitrogen, potassium and calcium uptake in both aquaponics and hydroponics ($p>0.05$) (Tab. 7) despite the lower concentrations of nutrients measured in aquaponic water (Tab. 5). A 10% increase in plant nitrogen levels was observed in the second crop. A similar trend was assessed in roots (Tab. 8) where nitrogen concentrations were similar among treatments, but raised by 8-10% in the second trial. Potassium in roots (Tab. 8) had similar concentrations with those observed in plants (Tab. 7), but values between treatments were different in the first crop ($p<0.05$). Raising levels of calcium in water brought equal concentrations in roots in the second crop, however values were four times lower than those observed in plants.

Phosphorus in leaves and roots (Tab. 7, Tab. 8) showed a nutritional gradient among treatments, which followed water concentrations (Tab 5). However phosphorus plant/root distribution differed considerably between the first (higher concentrations in root) and the second trial (higher concentrations in leaves). Sodium concentrations patterns were similar in both plants and roots with higher values observed in LP aquaponics ($p<0.05$), which followed water concentrations (Tab. 5).

Iron, copper, manganese and boron (2^{nd} trial) were higher in hydroponic plants ($p<0.05$) than aquaponics (Tab. 9), following similar water trends (Tab. 6). Conversely zinc was richer in HP systems ($p<0.05$) than LP and hydroponics, although water concentrations were similar among treatments. Root micronutrients (Tab. 10) did not differ significantly among treatments for both copper, manganese, boron and zinc (2^{nd} crop), but higher concentrations of iron were observed in hydroponics ($p<0.05$). Apparently roots had a higher sink capacity for iron, copper and zinc than the aerial parts. Values in roots were in fact 3-5 time higher for iron and twice as big for copper.

Water use

Total water consumption from aquaponic tanks accounted for an average of 334 litres in the first trial and 320 litres in the second trial. Water for solid removal resulted in 24 litres per system in the first trial and 17 litres for the second trial. Flush out water was not included in the plant water balance as it solely regarded the standard solid disposal occurring in aquaculture management.

Water consumption per kilogram of fresh biomass varied sensitively among the systems in both trials (Tab. 11). For the first crop LP and HP aquaponics accounted for +60% and 40% more water than hydroponics. In the second trial aquaponic treatments increased net water consumption by 2.8 (LP) and 2.1 (HP) times the volume of water used in hydroponics. However, higher water consumption in aquaponics was also due to the intense aeration occurred in fish tanks as well as the splash effects of the incoming water into the plant troughs.

System performance

The nitrogen budget developed for aquaponics (Fig. 6) considered the net amount of nitrogen available for plants as the sum of the nitrogen coming from fish metabolism and the nitrogen already stocked into the water (nitrate contained in the well water at initial fish stocking or the nutrient pool originated from the first trial). The budget for the first trial included the initial 14-day water enrichment period, when fish were stocked with no plants in order to build up nutrients.

In the first trial 44-45% of the feed nitrogen was retained by fish while the remaining quota served to build the nutrient pool for plants. Plant sink (leaf and roots) accounted for nearly 1/3 of the plant available nitrogen. Root uptake quota was always close to 30% of the aerial part. Although the percentage of nitrogen sequestrated in LP appeared higher than HP due to lower nitrogen concentrations in LP water, plants in HP treatment accounted for a higher sinking capacity in both first and second trial. The nitrogen stripped by each plant (leaves, stems and roots) during 28-day cycles accounted for 0.5 g (LP), 0.53 g (HP) and 0.45 g (hydroponics) in the first trial and 0.35 g (LP), 0.42 g (HP) and 0.33 g (hydroponics) in the second trial.

The amount of nitrogen lost accounted for a very high percentage, however these values included losses due to fish metabolism, wasted feed and solids taken out of the systems, bacterial floc, micro organisms growth (plankton, fungi) and the natural nitrogen loss due to denitrification.

A similar pattern was noticed for the second trial (Fig. 6), however reduced fish performance affected the level of nutrients released for plant growth (59.9% LP and 67.6% HP). Higher end water concentrations (19% higher than first trial) were also due to unfavourable plant growth conditions, which limited plant sinking capacity.

Increase in nitrogen losses observed in the second trial (+8% LP and +15% HP) could be justified by an augmented fraction of wasted feed taken out from the system consequent to reduced fish intake, which can be hypothesized by the higher FCR (Tab. 4) observed in fish.

Hydroponics (Fig. 7) was apparently a better nitrogen sink for plants than aquaponics (Fig. 6) despite higher wasted levels in end water. However differences in plant nitrogen uptake were not significant

in the first trial ($p>0.05$) (Tab. 12). The 12-19% nitrogen loss observed in hydroponics (Fig. 7) suggested for denitrification effect.

Nutrient uptake efficiency (Tab. 12) measured as the ratio between plant uptake and plant available nutrients did not show significant differences in the first trial for nitrogen, phosphorus, potassium and magnesium, but not for calcium which was higher in hydroponics ($P<0.05$).

Uptake efficiency in the second trial was higher in hydroponics than aquaponics for both nitrogen, potassium, calcium and magnesium ($p<0.05$), but not for phosphorus. A reason for reduced aquaponic performance was due to the increased nutrient pool available for plants as a result of the remaining water nutrients from the first trial and the increased biomass.

Discussion

Aquaponic system performance

Sweet basil production from aquaponics was similar to hydroponics (Tab. 1), but higher yields were obtained in the first trial (4.1-4.4 kg m^{-2}) than the second one (2.7-3.0 kg m^{-2}). Although there is not extensive literature on basil yields for similar planting densities, plant achieved higher biomass production per square meter than Rakocy et al. (2004) who harvested 2 kg m^{-2} Genovese basil under a planting density of 8 plant m^{-2} in a 28-day aquaponic crop. On the contrary yields from 35-day trials carried out on hydroponics (Raimondi et al., 2006) resulted in 7.4 kg m^{-2} and 5.6 kg m^{-2} under a density of 66 and 100 plant m^{-2} respectively. The biomass production appeared higher in soilless production than traditional soil-based agriculture. Sifola et al. (2006) in fact obtained 2.5 kg m^{-2} of basil in a 50-day crop under a planting density of 12.5 plant m^{-2}.

Heavier root dry weight observed in the first crop (Tab. 1) in both aquaponic treatments (3.7 g $plant^{-1}$ against 2.4 g plant-1 of hydroponics) seemed to confirm the observations of Savidov (2005) on the higher vegetative growth achievable in aquaponic systems due to synergic support of the aquatic ecosystem.

As far as quality of production is concerned leaf area in both trials (Tab. 2) was higher than values obtained by Raimondi et al. (2006) who measured 984 cm^2 $plant^{-1}$ from hydroponic basil, but with higher planting densities. Data on leaf biomass (Tab. 2) showed a decreasing trend from the first (2.4 kg m^{-2} in LP, 3.0 kg m^{-2} in HP) to the second crop (1.9 kg m^{-2} for LP, 2.1 kg m^{-2} for HP). Nevertheless the level of leaf biomass appeared similar, if not higher, to those observed by Raimondi et al. (2006) who measured 2.0-2.3 kg m^{-2} of fresh leaves under higher planting densities.

Likewise leaf/stem ratio, which is an important value to determine the amount of marketable leafs against discarded stems, showed values of 1.15-1.28 in both aquaponics and hydroponics. The

ratios observed were definitely higher than Raimondi et al. (2006) and Sifola et al (2006) who obtained respectively values of 0.56 and 0.68.

Fish performance for specific growth rate did not differ from literature. The daily increment in percentage of fish body weight, which was 1.96% (LP) and 2.13% (HP) in the first trial and 1.36% for both LP and HP in the second trial (Tab. 4) was comparable to the experimental results obtained by Pantazis and Neofitou (2002) and Ahmad et al. (2008), who observed respectively a specific growth rate of 1.24% for subadults of 102 g and 1.94% for 10 g fish fed with 35% protein diets. The higher performance noticed for the first trial was also confirmed by the conclusions of Hogendoorn et al. (1983) who stated that optimal temperatures for juvenile and preadult fish were 30°C and 25°C respectively. The values of food conversion ratio obtained (Tab. 4) were similar to the results of Degani et al (1988) who observed FCR values of 0.94 and 1.29 in juvenile fish fed a 45% protein diet at 27°C. According to literature feed conversion values are generally close to 1 (Hogendoorn et al., 1983), although better performances were observed by Pantazis and Neofitou (2002) and Verdegem et al (2006) respectively with 0.72 and 0.85. Higher conversion rates observed during the second trial are connected to lower water temperatures (Fig. 5), which suggested for optimal aquatic ecosystem management to meet fish demand or choice of cold tolerant fish during cold seasons.

Water budget for aquaponics gave a total water consumption per system of 345 L (LP) and 324 (HP). Given the fish biomass increase (2.6-3.1 kg in the first trial, 2.12-2.14 kg in the second trial) and the feed consumed (2.9 kg of feed in the first trial, 2.7 kg in the second trial) the efficiency level of aquaponic systems appeared very high, since conventional recirculation systems account for 100-1,000 litres of water replacement per kg feed supplemented, while the most innovative systems stand below 100 L kg $feed^{-1}$ (Martins et al., 2010) or equivalent to 105 L kg $fish^{-1}$ (Piedrahita, 2003). Verdegem et al. (2006) stated that African catfish water consumption is around 100 L kg $feed^{-1}$ in intensive RAS, which makes aquaponics plant production highly competitive in terms of water consumption, given the nearly 3 kg of feed consumed per tank in the trials. The increase of fish productivity per unit of land compared to traditional aquaculture systems (Verdegem et al., 2006) finds further advantages in the full use of nitrogen for plant production, which is not wasted away as it happens in RAS (Martins et al, 2010), and in the conversion of a costly treatment system (biofiltration and denitrification in RAS) into a profit (aquaponic plant production).

Aquaponic system optimization

The nitrogen budged (Fig. 6) revealed a suboptimal aquaponic system balance between inputs and outputs, which was also visible in the raising nitrate (Fig. 2) and EC (Fig. 4) levels.

For the first aquaponic crop the budget included the initial 14 days of nutrients enrichment when fish were left growing with no plants. From this account the amount of nitrogen left after fish ingestion, plant/root uptake and natural loss was 27.2 g (LP) and 50.1 g (HP), equivalent to a further sink of 54 and 94 plants to be added respectively to every LP and HP system.

However, in practical terms, if the initial enrichment period had not been accounted in order to maintain a stock of nutrients, the nitrogen break even would have been reached with just 8.4 g (LP) and 26.4 g (HP) more nitrogen to be stripped by plants. Such surplus was equivalent to 17 more plants for LP (+31%) and 50 more plants for HP aquaponics (+92%). The updated number of plants adjusted to the new nitrogen balance would raise NUE values for plants up to 30% for LP and 38% for HP aquaponics.

Given a constant growth rate and fish feeding regime the amount of feed required for each single plant under same growth conditions would be 27.2 g $plant^{-1}$ (LP) and 18.8 g $plant^{-1}$(HP).

For the second crop the break even, calculated only on the nitrogen balance supplied by feed (but not from water stock), was equal to additional 50 plants for the LP treatment (+93%) and 97 plants (+180%) for HP treatment. Given the feed consumed by fish the amount of feed required for each single plant under same growth conditions would have been 25.6 g $plant^{-1}$ (LP) and 18.2 g $plant^{-1}$(HP).

The sensitive increase of the number of plants in the second crop was due to the increased fish biomass into the system and reduced plant absorption.

Conclusions

Trials showed that there were no differences in yields between aquaponics and hydroponics for any nitrogen concentrations. Quality assessment did not show significant differences both in terms of leaf biomass, leaf/stem ratio, although some differences were noticed in the mineral composition of leaves due to the different levels of nutrients between hydroponics and aquaponics. Comparisons against other sweet basil productions from literature outlined the good performance obtained by aquaponics with higher leaf/stem ratio than soil-based agriculture and hydroponics.

As far as African catfish is concerned no differences were noticed in both growth and feed conversion ratio between the HP and LP diets. Fish performance from aquaponics were similar to those obtainable in standard RAS. Nevertheless HP differed from LP for nitrate levels, which grew with a double pace in HP treatments. On the contrary the nitrogen assimilation of LP fish seemed higher in both trials, which suggested a better growth performance at lower protein diets.

The nutrient uptake efficiency in aquaponics was affected by the fish/plant ratio, although uptake rates were not significantly different among treatments in the first trial. Given the nitrogen budget, constant

environmental conditions and growth, the optimal amount of feed per plant resulted in 27.2 g plant^{-1} (LP) and 18.8 g plant^{-1} (HP) in a 28-day cycle.

Given the considerable savings in terms of fertilizers and water, aquaponics would be a competitive and more sustainable agricultural systems than traditional (and disjoined) agriculture and aquaculture farming systems.

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Tables

Table 1 Sweet basil production in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

treatment	Yields (kg m^{-2})	Plant fresh weight (g $plant^{-1}$)	Plant dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	plant height (cm)	% dry matter
			1st crop			
aquaponics LP	4.1 ± 0.1	106.0 ± 47.4	11.2 ± 1.4	3.7 ± 0.7a	59.7 ± 8.7	8.7 ± 0.2
aquaponics HP	4.4 ± 0.2	134.1 ± 82.8	13.8 ± 2.3	3.7 ± 0.3a	62.9 ± 10.4	8.5 ± 0.1
hydroponics	4.2 ± 0.5	114.5 ± 41.4	10.1 ± 0.8	2.4 ± 0.3b	60.6 ± 6.6	8.3 ± 0.5
significance	ns	ns	ns	*	ns	ns
			2nd crop			
aquaponics LP	2.7 ± 0.2	80.4 ± 30.4	8.6 ± 2.2	2.7 ± 0.8	52.0 ± 7.7	8.6 ± 0.3
aquaponics HP	3.2 ± 0.1	95.0 ± 28.7	9.4 ± 1.3	2.8 ± 0.4	53.7 ± 7.6	8.5 ± 0.5
hydroponics	2.7 ± 0.4	77.8 ± 23.5	6.8 ± 0.3	2.3 ± 0.2	50.9 ± 6.1	8.0 ± 0.3
significance	ns	ns	ns	ns	ns	ns

Table 2 Leaf area, specific leaf area (SLA) and leaf productivity in low protein (LP) and high protein (HP) aquaponics and hydroponic (mean ± SD)

Treatment	Leaf Area (cm^2 $plant^{-1}$)	SLA (cm^2 g^{-1})	Leaf weight (g $plant^{-1}$)	Leaf/Stem ratio
		1st crop		
Aquaponics LP	2089.4 ± 384.8	316.5 ± 17.1	68.2 ± 25.1	1.28 ± 0.27
Aquaponics HP	2692.8 ± 633.2	333.9 ± 25.8	83.6 ± 23.3	1.19 ± 0.26
Hydroponics	2042.4 ± 279.7	339.5 ± 25.2	64.8 ± 20.5	1.25 ± 0.20
significance	ns	ns	ns	ns
		2nd crop		
Aquaponics LP	1835.2 ± 352.3	338.5 ± 20.6	54.0 ± 15.3	1.26 ± 0.15
Aquaponics HP	1945.4 ± 284.6	321.6 ± 9.2	59.5 ± 18.5	1.25 ± 0.24
Hydroponics	1537.7 ± 140.8	363.5 ± 29.8	46.2 ± 12.3	1.15 ± 0.14
significance	ns	ns	ns	ns

Table 3 SPAD readings 14 days after transplant and at harvest in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

	1st crop		2nd crop	
treatment	**mid**	**end**	**mid**	**end**
Aquaponics LP	35.2 ± 3.0	33.7 ± 3.2	28.7 ± 3.5a	30.0 ± 3.0a
Aquaponics HP	34.4 ± 2.9	34.1 ± 2.5	32.3 ± 3.6b	32.4 ± 3.4b
Hydroponics	35.4 ± 2.6	33.4 ± 3.1	32.9 ± 3.5b	32.5 ± 3.3b
significance	ns	ns	***	***

Table 4 Feed conversion ratio (FCR) and specific growth rate (SGR) in African catfish under low protein (LP) and high protein (HP) diets (mean ± SD)

	1st trial		2nd trial	
	FCR	**SGR**	**FCR**	**SGR**
LP	1.11 ± 0.05	1.96 ± 0.09	1.25 ± 0.11	1.36 ± 0.08
HP	0.97 ± 0.12	2.13 ± 0.19	1.30 ± 0.07	1.36 ± 0.11
Sign.	ns	ns	ns	ns

Table 5 Water macronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatment	P	K	Ca	Mg	Na
	---------- mg L^{-1} ----------				
	start 1st crop				
Aquaponics LP	11.5±2.3a	22.8±2.3a	44.2±4.6a	16.7±2.1	16.4±1.5b
Aquaponics HP	15.7±2.7b	25.2±5.9a	53.9±6.6b	18.9±2.5	16.4±3.2b
Hydroponics	30.0±0.0c	66.1±0.0b	100.4±0.0c	19.4±1.1	3.2±0.0a
significance	***	***	***	ns	***
	end 1st crop				
Aquaponics LP	4.5±1.8	20.8±1.5a	68.6±3.4a	28.7±2.45a	42.7±2.7c
Aquaponics HP	6.7±0.8	19.8±1.3a	100.4±2.6b	30.8±0.4a	31.5±0.9b
Hydroponics	14.2±13.7	68.0±9.1b	166.4±15.4c	42.0±5.3b	10.1±2.1a
significance	ns	***	***	***	***
	end 2nd crop				
Aquaponics LP	1.1±0.3a	20.2±1.4a	94.0±6.0a	26.7±2.5a	45.3±3.9c
Aquaponics HP	6.7±1.8a	21.0±10.1a	161.9±29.5b	29.7±7.7a	28.1±8.4b
Hydroponics	42.4±5.9b	124.3±11.9b	199.4±4.2c	47.2±3.8b	10.8±0.6a
significance	***	***	***	***	***

Table 6 Water micronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatment	Fe	Cu	Mn	B	Zn
	---------- µg L-1----------				
	start 1st crop				
aquaponics LP	3.6±0.4a	6.2±0.7a	5.7±1.8a	141.3±7.7	17.7±4.5a
aquaponics HP	4.6±3.4a	6.9±0.2a	6.9±1.5a	148.1±16.0	13.4±2.0a
hydroponics	198.6±97.3b	17.6±0.0b	125.4±0.0b	160.6±0.0	57.5±0.0b
significance	***	***	***	ns	***
	end 1st crop				
aquaponics LP	98.9±12.9a	19.0±0.6a	7.0±2.2a	244.6±11.6a	32.4±6.6
aquaponics HP	82.3±10.9a	19.2±2.1a	51.0±15.8a	240.7±12.4a	54.3±4.5
hydroponics	515.9±74.5b	42.3±7.7b	144.5±57.2b	477.2±77.1b	50.0±16.9
significance	***	***	**	***	ns
	end 2nd crop				
aquaponics LP	80.5±3.7a	20.1±0.8a	7.8±0.1a	220.0±20.5a	27.3±1.1
aquaponics HP	82.9±19.1a	18.0±3.0a	39.5±40.1a	233.6±60.4a	52.7±32.8
hydroponics	663.8±75.3b	57.9±2.6b	198.8±76.7b	513.5±36.1b	63.3±16.2
significance	***	***	**	***	ns

Table 7 Plants macronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatments	N	P	K	Ca	Mg	Na
	---------- g kg^{-1} d.w.----------					
	1st crop					
Aquaponics LP	37.1 ± 3.0	7.3 ± 0.7a	45.8 ± 3.7	18.2 ± 0.9	4.3 ± 0.3b	0.49 ± 0.09b
Aquaponics HP	37.9 ± 1.8	9.6 ± 1.3b	45.0 ± 1.0	20.5 ± 0.8	3.8 ± 0.3b	0.31 ± 0.04a
Hydroponics	37.8 ± 1.8	10.5 ± 0.5b	42.3 ± 6.6	18.4 ± 1.5	2.7 ± 0.4a	0.26 ± 0.02a
significance	ns	*	ns	ns	**	**
	2nd crop					
Aquaponics LP	40.7 ± 5.6	9.8 ± 3.8a	44.4 ± 1.1	19.1 ± 0.8	3.7 ± 0.2b	0.46 ± 0.06b
Aquaponics HP	40.3 ± 4.9	13.0 ± 0.5ab	42.8 ± 4.2	20.1 ± 1.0	2.7 ± 1.3a	0.33 ± 0.04a
Hydroponics	41.1 ± 3.1	17.0 ± 1.4b	47.0 ± 2.9	20.5 ± 0.9	2.9 ± 0.3a	0.32 ± 0.03a
significance	ns	*	ns	ns	**	*

Table 8 Root macronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatments	N	P	K	Ca	Mg	Na
	---------- g kg^{-1} d.w.----------					
	1st crop					
Aquaponics LP	35.5 ± 3.9	11.6 ± 1.3a	41.2 ± 1.6a	4.7 ± 0.2b	10.1 ± 0.9b	2.4 ± 0.3c
Aquaponics HP	37.7 ± 1.8	13.4 ± 0.7ab	40.8 ± 1.9a	3.3 ± 0.2a	6.5 ± 1.1a	1.9 ± 0.2b
Hydroponics	34.8 ± 2.3	14.8 ± 1.2b	45.5 ± 1.6b	3.7 ± 0.2a	5.0 ± 1.0a	0.9 ± 0.0a
significance	ns	*	*	***	*	***
	2nd crop					
Aquaponics LP	39.8 ± 2.0	5.7 ± 0.3a	39.3 ± 2.2	4.9 ± 0.8	9.0 ± 1.3b	2.3 ± 0.2c
Aquaponics HP	41.0 ± 3.4	6.7 ± 0.9a	37.4 ± 10.7	4.1 ± 0.7	5.9 ± 0.9a	1.6 ± 0.4b
Hydroponics	37.2 ± 2.9	9.1 ± 0.9b	52.1 ± 5.9	4.9 ± 0.2	6.4 ± 0.2a	0.8 ± 0.2a
significance	ns	**	ns	ns	*	**

Table 9 Plants micronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatments	Fe	Cu	Mn	B	Zn
	---------- mg kg^{-1} d.w.----------				
	1st crop				
Aquaponics LP	36.7 ± 0.3a	6.1 ± 0.3a	14.8 ± 3.2a	19.6 ± 0.8	36.0 ± 3.8a
Aquaponics HP	42.1 ± 1.8a	9.5 ± 0.6b	39.4 ± 5.3b	16.7 ± 0.6	53.4 ± 1.8b
Hydroponics	59.0 ± 8.5b	8.4 ± 1.4b	62.3 ± 5.4c	17.0 ± 2.6	36.1 ± 1.9a
significance	**	**	***	ns	***
	2nd crop				
Aquaponics LP	30.3 ± 1.8a	8.3 ± 0.7a	12.0 ± 3.5a	14.9 ± 0.6a	44.1 ± 7.4a
Aquaponics HP	34.4 ± 8.6a	8.8 ± 0.8a	70.2 ± 10.4b	14.4 ± 0.7a	62.6 ± 5.3b
Hydroponics	57.8 ± 2.4b	10.9 ± 0.7b	64.6 ± 9.5b	18.2 ± 1.3b	35.6 ± 1.3a
significance	***	*	***	**	**

Table 10 Root micronutrients in low protein (LP) and high protein (HP) aquaponics and hydroponics (mean ± SD)

Treatments	Fe	Cu	Mn	B	Zn
	---------- mg kg^{-1} d.w.----------				
			1st crop		
Aquaponics LP	131.0 ± 15.6a	13.0 ± 0.6	39.9 ± 5.6	14.9 ± 0.8	67.1 ± 10.2b
Aquaponics HP	112.2 ± 41.6a	15.6 ± 3.1	39.4 ± 9.2	12.7 ± 2.1	86.6 ± 16.5b
Hydroponics	302.1 ± 60.1b	13.5 ± 0.9	59.8 ± 14.8	14.4 ± 0.3	39.0 ± 6.0a
significance	**	ns	ns	ns	**
			2nd crop		
Aquaponics LP	110.9 ± 36.1a	15.2 ± 2.8	40.5 ± 7.0	14.8 ± 1.7	71.3 ± 13.1
Aquaponics HP	137.8 ± 63.0a	18.2 ± 5.0	85.5 ± 30.6	12.0 ± 1.6	107.7 ± 54.8
Hydroponics	360.3 ± 106.1b	19.7 ± 3.1	87.2 ± 47.8	14.7 ± 2.3	60.0 ± 5.0
significance	*	ns	ns	ns	ns

Table 11 Net water use per kg of fresh product in low protein (LP), high protein (HP) aquaponics and hydroponics (mean ± SD)

	water use (L kg^{-1} f.w.)	
Treatment	**1st crop**	**2nd crop**
Aquaponics LP	51.8 ± 1.5 c	79.7 ± 10.1 c
Aquaponics HP	45.5 ± 0.6 b	59.4 ± 2.1 b
Hydroponics	32.3 ± 3.0 a	28.1 ± 2.1 a
significance	***	***

Table 12 Plant nutrient uptake efficiency in low protein (LP), high protein (HP) aquaponics and hydroponics. Values are in % (mean ± SD)

Treatments	N	P	K	Ca	Mg
			1st crop		
Aquaponics LP	22.8 ± 3.2	20.5 ± 7.3	28.6 ± 2.4	8.3 ± 0.6 a	7.9 ± 0.7
Aquaponics HP	19.8 ± 2.9	39.5 ± 24.8	30.9 ± 2.1	10.1 ± 0.6 a	7.5 ± 0.6
Hydroponics	27.9 ± 3.6	45.5 ± 7.5	35.2 ± 9.2	13.8 ± 2.6 b	10.4 ± 2.7
significance	ns	ns	ns	*	ns
			2nd crop		
Aquaponics LP	12.0 ± 0.4 a	29.0 ± 20.0	7.2 ± 0.6 a	5.7 ± 0.2 b	4.8 ± 0.3 b
Aquaponics HP	10.1 ± 0.7 a	43.4 ± 16.4	6.1 ± 2.5 a	4.8 ± 0.5 a	3.9 ± 0.3 a
Hydroponics	17.9 ± 2.6 b	43.1 ± 4.4	17.1 ± 1.9 b	7.7 ± 0.4 c	6.5 ± 0.4 c
significance	**	ns	**	***	***

Figures

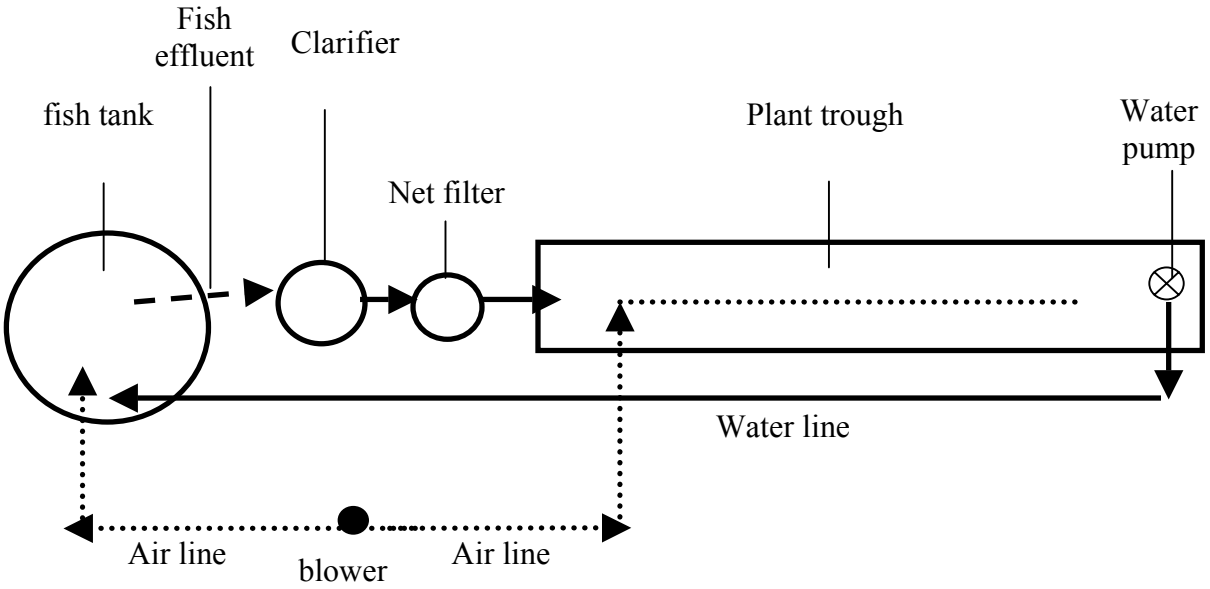

Figure 1 Schematic design of aquaponic system

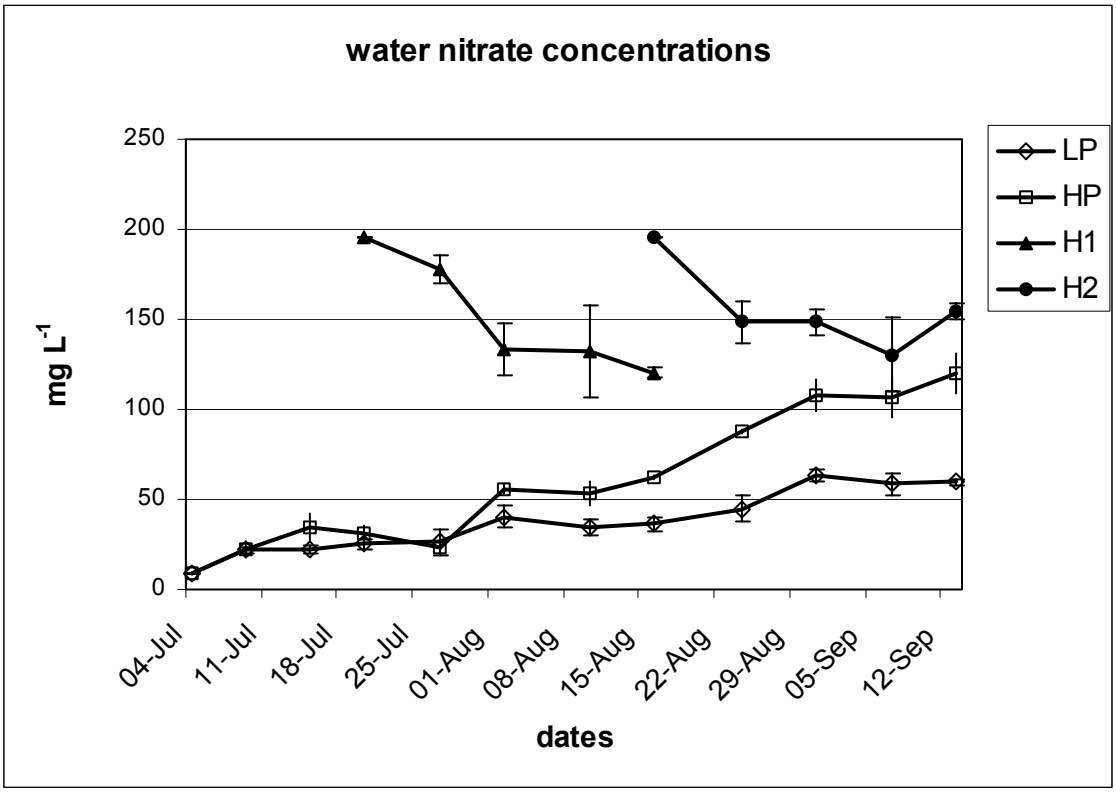

Figure 2 Nitrate (NO_3-N) concentrations in low protein (LP) and high protein (HP) aquaponic systems and hydroponic first crop (H1) and second crop (H2). Means ± standard error

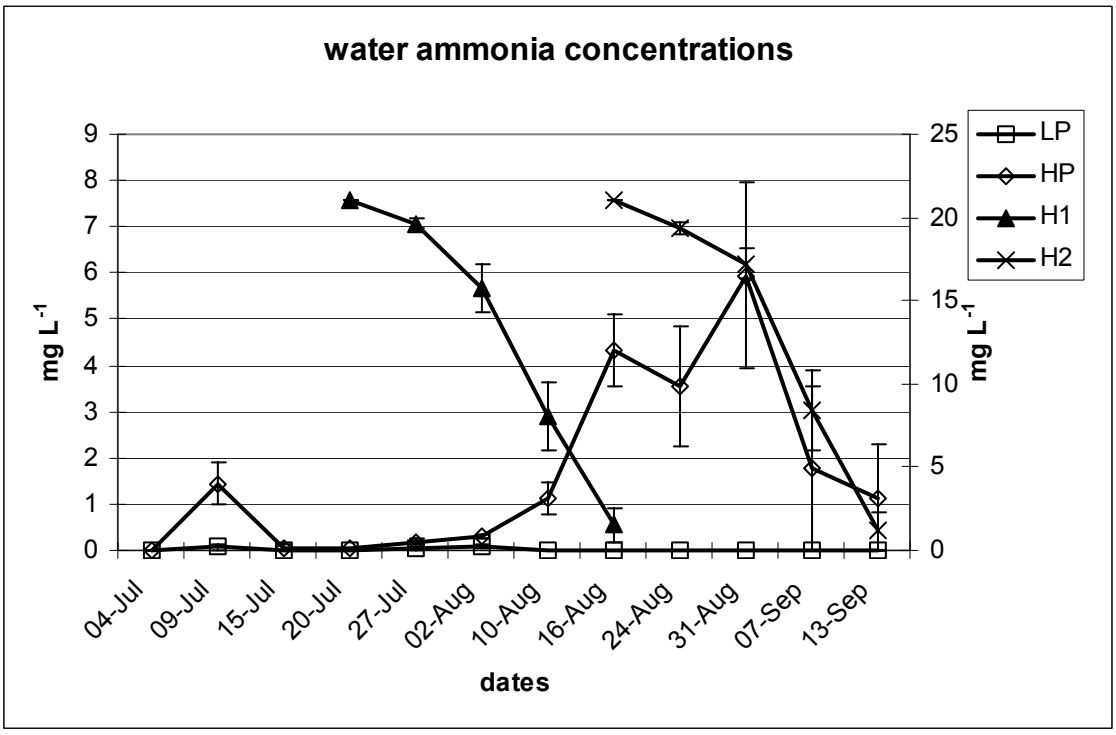

Figure 3 Ammonia (NH_4-N) concentrations in low protein (LP) and high protein (HP) aquaponic systems and hydroponic first crop (H1) and second crop (H2). Means ± standard error

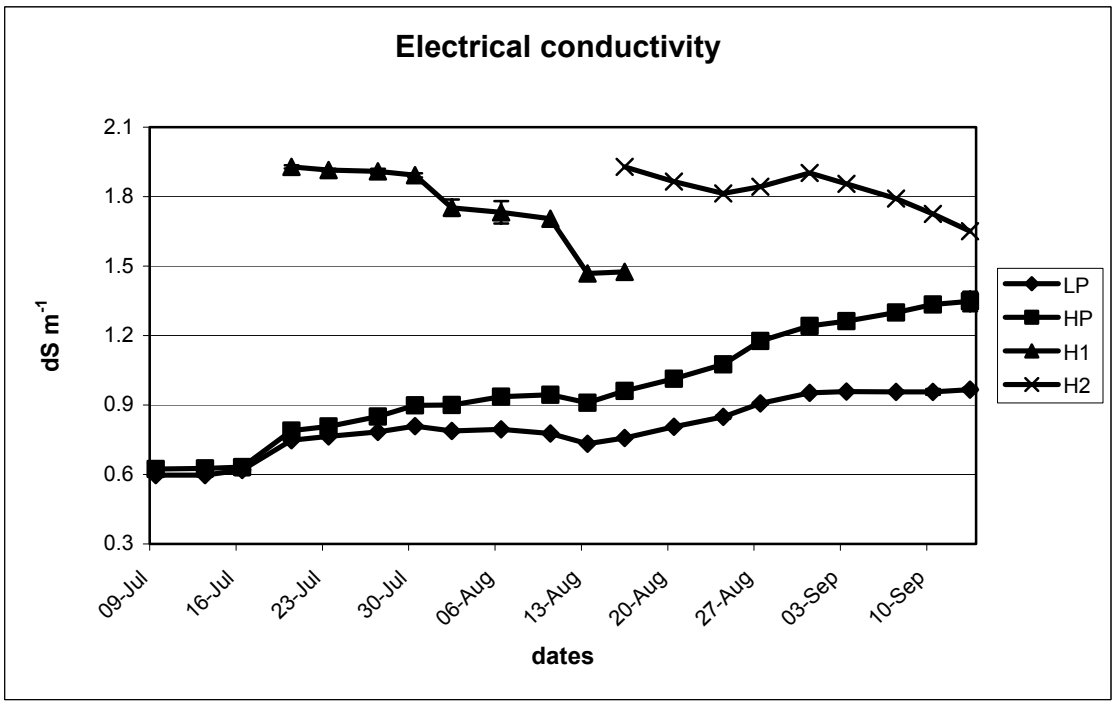

Figure 4 Electrical conductivity in low protein (LP) and high protein (HP) aquaponics and hydroponic first crop (H1) and second crop (H2). Means ± standard error

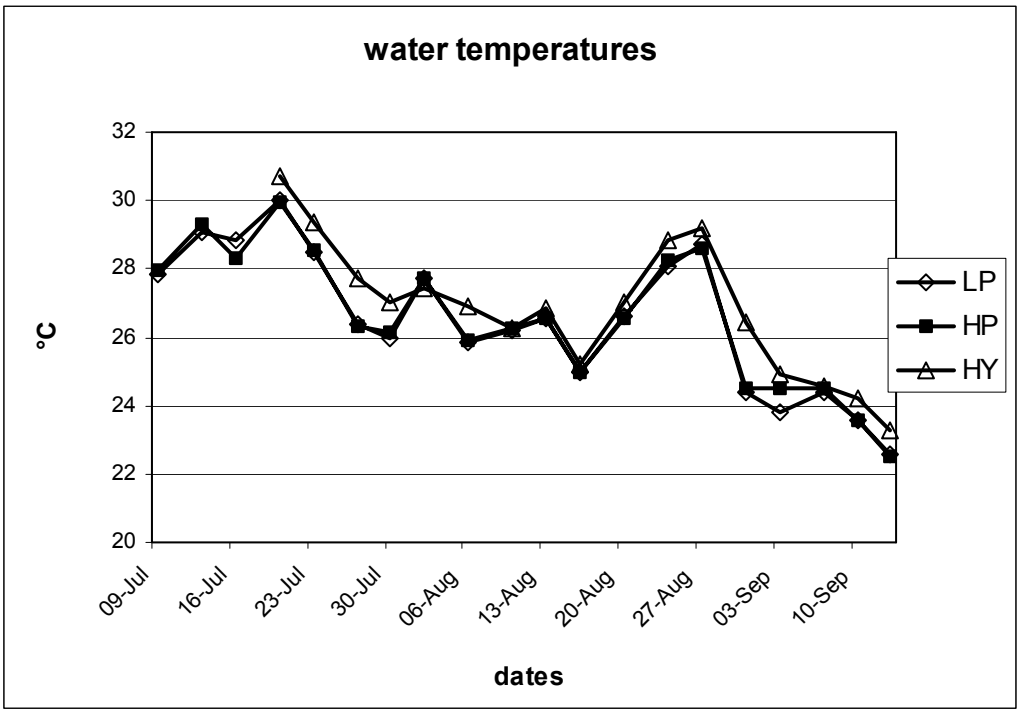

Figure 5 Water temperatures in low protein (LP) and high protein (HP) aquaponics and hydroponics Measurements at 9:00 AM.

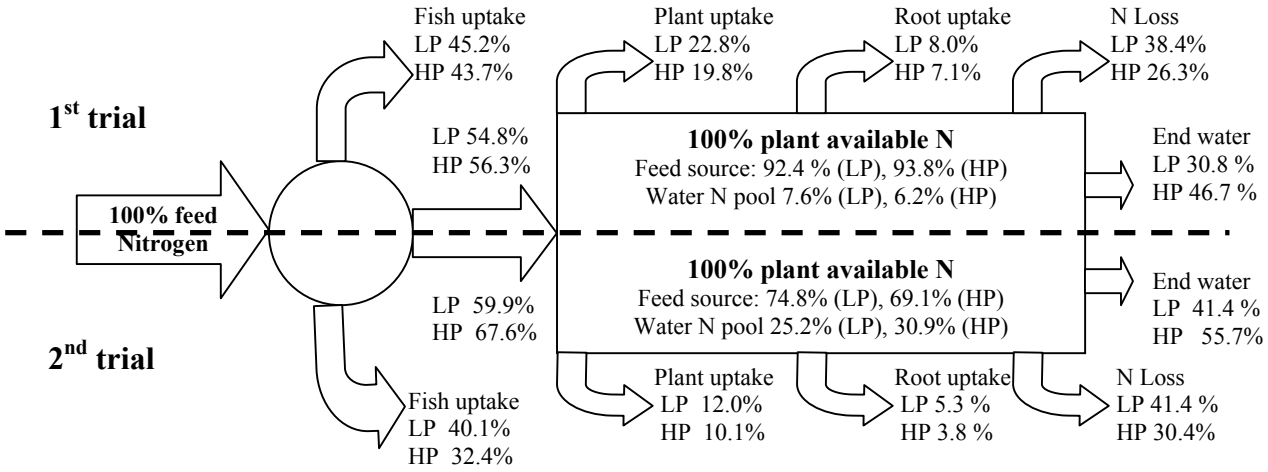

Figure 6 Nitrogen budget for first (upper half) and second trial (lower half) in low protein (LP) and high protein (HP) aquaponics

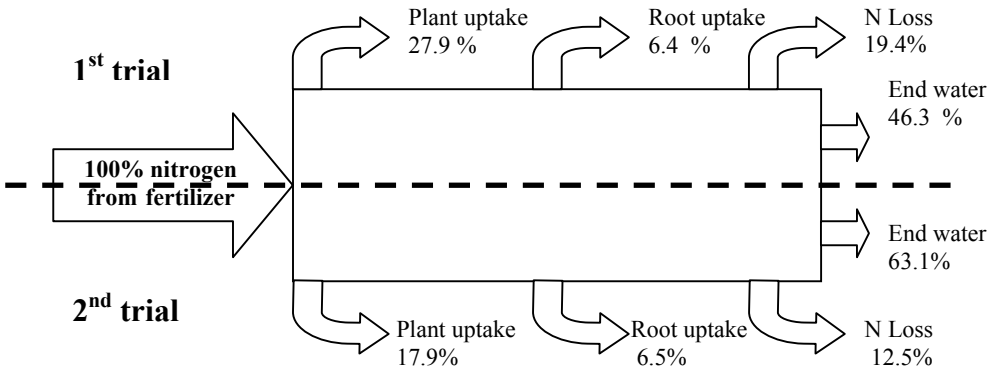

Figure 7 Nitrogen budget for first and second trial in hydroponics

Chapter 4

Yields, Quality and Nitrogen Uptake of Aquaponic Lettuce Growing on Floating Systems, NFT and Substrate Culture

Abstract

Aquaponics integrates recirculating aquaculture systems with plant production. Since its development as an alternative aquaculture biofiltration system, aquaponics captured interest of researchers and industry for its horticultural potentials. Aquaponics is mostly developed with floating or gravel systems, however big expansion may be achieved by tuning aquaponics management to industrial-scale hydroponic practices. The scope of the present study was to compare the performances of three different aquaponic sub-systems: Floating system, nutrient film technique (NFT) and substrate systems under sub-irrigation. Production and quality traits of aquaponic romaine lettuce (*Lactuca sativa* cv Verde degli ortolani) under raising nutrient concentrations were assessed against an hydroponic control (floating system) for three consecutive crops. Result showed constant productions from floating systems, however NFT resulted outperforming (+18%) whenever proper nutrient concentrations, flow rate and root/water volume was guaranteed. Substrate aquaponics resulted underperforming by 30-40% due to not adequate nutrient exchange at root level. Lettuce quality was similar in all treatments. Nitrogen concentrations in plant tissues was constant above water concentrations of 50 mg L^{-1}., which suggested that nitrogen sink was rather biomass-dependent. Results suggested NFT as the most performing method to strip nutrients from water, providing that optimal nutrient flow is guaranteed at root level.

Keywords: Integrated aquaculture, hydroponics, soilless cultivation, Oreochromis niloticus

Introduction

Recirculating aquaculture systems (RAS) have been used for intensive fish production to limit environmental impact. Reuse of reclaimed water in closed systems shows several advantages in terms of reduced impact of pollution, minimal water use (Verdegem et al., 2006), limited health risks (Summerfelt et al., 2009) and optimized fish growth through all-year-round environmental control (Timmons and Ebeling, 2007). However the degree of water reuse depends on the quality of feed and on the level of system engineering, both for solid waste treatment (Bergheim and

Asgard, 1996; Chen et al., 1994; Davidson and Summerfelt, 2005) and aerobic or anaerobic biofiltration to reduce ammonia from fish water (Rijn and Rivera, 1990; Timmons and Ebeling, 2007). Despite high performances RAS are not very diffused due to their higher costs or lower profitability with less valuable fish species (Piedrahita, 2003). In addition a certain amount of nutrients are discarded through daily water exchange, which can be 5 or 10% of total volume (Timmons and Ebeling, 2007) or equivalent to 0.1-1.0 m^3 of water per kg feed (Martins et al, 2010). Use of plants to reduce water nutrients has been widely acknowledged in constructed wetlands (Kandlec and Knight, 1996) where organic matter and nitrate reduction rates are 80-85% (Thomas et al. 1995) or up to 90% (Luederitz et al., 2001). However the extensive treatment areas required and low plant marketability reduce wetlands development into aquaculture operations. One valid RAS evolution is aquaponics, a production system that integrates hydroponics with recirculating aquaculture. In aquaponics plants strip nutrient from recirculating water and at the same time provide tools for phytoremediation. With its full waste and water reuse aquaponics achieves the same goals of RAS but at the same time it increases farm revenues by producing high-quality vegetables (Rakocy and Hargreaves, 1993; Timmons and Ebeling, 2007).

Different aquaponic systems have been used since the seventies. Substrate aquaponics, where plants grow in gravel, are widely diffused in Australia (Nelson, 2007) or backyard aquaponic systems. Many researches focused on substrates with the aim to improve biofiltration in recirculating systems as well as solid removal (Lennard and Leonard, 2006). However, some concerns arose about excessive substrate weight and fish solid clogging into media with consequent risks of anaerobic conditions (Rakocy, 2007). Watten and Busch (1984) cropped tomato on gravel beds under continuous trickling irrigation in tilapia recirculating systems and obtained higher yields than soil-based agriculture. Likewise McMurtry et al. (1990) in sand bed trials under furrow irrigation in tilapia recirculating systems obtained higher yields than soil for beans and cucumbers, but not for tomato. Tyson et al. (2008) in trials with tilapia and cucumber on perlite noticed optimum ammonia removal at pH 8, but lower marketable yields in early fruits with raising pH. Graber and Junge (2009) in trials with expanded clay (Leca) used both for plant and biofiltration observed similar yields in tomato but lower potassium levels affected fruit quality. In gravel systems water distribution plays a key role, since increased water-roots nutrient exchange is obtained under increased surface or flow rate. Lennard and Leonard (2004) compared flood-and-drain and constant flow gravel systems and noticed higher lettuce growth and better nitrate assimilation in constant flow conditions.

Floating system is perhaps the most diffused aquaponic system around the world (Nelson, 2007). Like every hydroponic floating systems using a significant volume of water the UVI system is made

with floating polystyrene sheets supporting plants on 30-40 cm deep water, which allow roots to have maximum exposition to water (Rakocy, 2007). Floating systems have consistent nutrient stocks, bigger buffering capacity to smooth peaks of ammonia and settle water temperatures to constant ranges. Trials carried out by Rakocy et al. (2004) on basil outlined yields three times higher than soil agriculture, although batch culture showed excessive nutrient uptake due to intense grow of plants of the same age. Savidov (2005) in trials carried out under greenhouse conditions on floating rafts observed that mature aquaponic systems could give accelerated growth to several vegetables varieties and higher yields than standard hydroponics for both tomato and cucumber.

In nutrient film technique (NFT) plant growth is supported by a shallow stream of nutritive solution that flows at the bottom of a narrow gully under minimal slope. Although NFT is one of the most used hydroponic technique in the world (Hassall & Associates, 2001), it is not widely adopted in aquaponics. Lennard and Leonard (2006) tested three aquaponic sub-systems and obtained lower biomass in NFT than gravel and floating system.

Plant nitrogen uptake is an important factor in designing aquaponic systems. Rakocy et al. (1992) determined in 2.4 g day^{-1} of 36% protein feed the amount of nutrients sufficient for one plant of lettuce growing on floating systems. Likewise Gloger (1995) determined that romaine lettuce uptake was 0.56 g m^{-2} day^{-1} of ammonia, 0.83 g m^{-2} day^{-1} of total nitrogen and 0.17 g m^{-2} day^{-1} of phosphorus. Similarly Alder et al. (2003) estimated that 0.94 g m^{-2} of nitrogen and 0.06 g m^{-2} of phosphorus was the daily absorption rate of lettuce in a rainbow trout recirculation system. Plant uptake however can be increased by system design (Lennard and Leonard, 2006), since deeper contact of roots in solution ease plant absorption.

Aquaponics market success not only stands behind investment and management costs, but also in the use of systems that show advantages both in terms of yields, disease management (Tognoni and Pardossi, 2000) and flexibility to obtain quality productions that meet market demand (Schnitzler and Gruda, 2003). Given the wide diffusion of NFT, substrate and rockwool hydroponics around the world (Hassall & Associates, 2001) aquaponics should not miss the need to assess its full potential with the most common industrial-scale hydroponic practices.

The objective of the research was to identify differences in both production and quality in plants growing on three different aquaponic susbsystems: floating, NFT and perlite under subirrigation. Aquaponic plant nitrogen uptake was observed under raising nutrient concentrations in order to identify optimal subsystems management and nutrient stripping potential within recirculating systems.

Materials and Methods

The aquaponic trials were carried out in a heated greenhouse at the experimental farm of the University of Tuscia, central Italy, between November 2010 and March 2011. Six independent recirculating systems of 750 L each were used for three consecutive trials in a heated greenhouse. Each system consisted of a 250 L fish tank, a 100 L clarifier and a 25 L net tank filled with a 1.2 mesh net to abate suspended solids. Behind clarification each system hosted a plant growing area with three aquaponic subsystems: 1) A floating system (Floating) made with 4 cm polystirene rafts housed on a 25 cm deep tank; 2) a NFT (NFT) channel made with 6.3 cm round PVC pipe under a 1% slope containing a 1 cm (1st and 2nd trial) or 2 cm water film (3rd trial); 3) a 40cm wide trough containing 1L perlite vases (Substrate) sub-irrigated by a shallow film of water (2 cm). The hydraulic retention time in fish tanks was 40 minutes and 60 minutes in the plant area. For both NFT and substrate aquaponics water flow was set at 0.7 L min^{-1}. Planting density for each sub-system was 20 plant m^{-2}

The aquaponic systems were stocked on 21st November with monosex Tilapia (*Oreochromis niloticus* L.) (Manila strain, Tilaqua, the Netherlands) to raise plant nutrients for 10 days prior trial commencement. Each aquaponic system was enriched at system start up with small quantities of potassium (18.2 mg L^{-1}), phosphorus (12.4 mg L^{-1}), sulphur (20.3 mg L^{-1}), magnesium (14.2 mg L^{-1}) and iron (0.4 mg L^{-1}) to raise the electrical conductivity (EC) by 0.15 dS m^{-1} and meet the nutrient concentrations of mature aquaponic systems. Systems pH was maintained at 6.5-7.0 by addition of $CaCO_3$ and $Ca(OH)_2$ in both aquaponic and hydroponic tanks.

For the trials two aquaponic treatments under same fish densities but different protein diets were compared against a chemically fertilized hydroponic control containing NO_3-N 196 mg L^{-1}, NH_4-N 21 mg L^{-1}, phosphorus 37.2 mg L^{-1}, potassium 195 mg L^{-1}, calcium 180 mg L^{-1}, sulphur 60 mg L^{-1}, magnesium 42 mg L^{-1}, manganese 0.2 mg L^{-1}, iron 0.7 mg L^{-1}, boron 0.5 mg L^{-1}, zinc 50 µg L^{-1}, copper 20 µg L^{-1} and molybdenum 3.2 µg L^{-1}. The EC of the nutritive solution was 1.9 dS m^{-1} and pH 5.5. Every treatment was replicated three times.

The two protein diets consisted in a commercial feed with 31% of crude protein (LP) (Skretting, Classic K 3P, Hendrix S.p.A., Mozzecane, Verona, Italy) and 40% crude protein (HP) (Skretting, Classic K 2P).

For the first trial every fish tank was stocked under a density of 12.0 ± 0.2 kg m^{-3}. Fish had average body weight of 88.0 ± 5.4 g (LP) and 54.0 ± 0.9 (HP) and daily feeding regime was 1.7% of body weight (BW). For the second trial stocking density was 16.2 ± 0.2 kg m^{-3} with fish average body weight of 122.6 ± 5.3 (LP) and 81.6 ± 6.5 (HP) under a feeding regime of 1.2% BW. Stocking

density in the third trial was 19.5 ± 0.3 kg m^{-3} with fish average body weight of 146.4 ± 7.4 (LP) and 99.3 ± 11.4 (HP) under feeding regime of 1.8% BW.

For all trials a 3-week old romaine lettuce seedlings (*Lactuca sativa* L. cv verde degli ortolani, SAIS Sementi S.p.A., Cesena, Italy) were transplanted on hydroponic floating rafts and aquaponic subsystems. Plants were left grown for 31 days (1st and 2nd trial) and 28 days (3rd trial) before harvest.

In both aquaponic and hydroponic systems water was kept aerated to maintain a dissolved oxygen concentration above 6 mg L^{-1}. Measurements were done twice a week with Hanna HI9147-04 DO meter. Water temperatures were maintained at an average temperature of 23°C ± 2 by means of electric heaters.

Plant were assessed for fresh weight. Roots and shoots were dried in a forced-air oven at 80 °C for 72 h for dry biomass determination. Leaf area was measured with an electronic area meter (Delta-T Devices Ltd, Cambridge, UK). Specific leaf area was calculated by dividing leaf area by leaf dry weight of each plant. Chlorophyll content of leaves was measured with Minolta SPAD 502 two weeks after transplant and at harvest.

Fish growth performance was determined as body weight gain and specific growth rate (SGR), which measures growth performance as percentage of daily biomass gain according to the formula:

$$SGR = [(LnWf - LnWi)/days]x100$$

Where Ln is the natural logarithm, Wf is the final weight of the fish, Wi is the initial weight and days is the time of the experiment. Feed conversion ratio (FCR) was measured to assess animal ability to convert feed into body biomass. It was calculated by dividing the feed consumed (dry weight) by the fish body weight gain.

Electrical conductivity and pH were measured twice a week with Hanna HI98130 meter.

Nitrogen levels in water were analyzed with a spectrophotometer following the ammonia nitrogen method by Anderson and Ingram (1989) and the salicil-solphoric acid method for nitrate nitrogen by Cataldo et al. (1975). Nitrogen levels in plant tissues were measure with kjeldhal method.

Data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Percentages values were analyzed upon square root or arc sine transformation. Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Results

Nitrate in aquaponic tanks followed different trends in LP and HP treatments (Fig. 1). Lower protein content in LP diet determined a reduction in nitrate levels by 20-30% compared to HP systems. Nitrate levels in LP grew from 23.7 mg L^{-1} to 57.7 mg L^{-1} (1st crop), 128.9 mg L^{-1} (end 2nd

crop) up to 135.0 mg L^{-1} (end of 3rd crop). Nitrate levels in HP tanks raised from 28.4 mg L^{-1} to 83.6 mg L^{-1} (1st crop) up to 147.9 mg L^{-1} (end of 2nd crop) and 194.8 mg L^{-1} (end of 3rd crop). Nitrate concentrations in HP at the end of the third trial were similar to the hydroponics.

Electrical conductivity (Fig. 2) in HP followed the same nitrate patterns (Fig.1) with values higher than LP by 14% at the end of the first trial (LP 1.08 dS m^{-1}, HP 1.23 dS m^{-1}), 18% at the end of the second trial (LP 1.38 dS m^{-1}, HP 1.62 dS m^{-1}) and 25% at the end of the third trial (LP 1.54 dS m^{-1}, HP 1.92 dS m^{-1}). EC values in HP at the end of the last trial were similar to those observed in hydroponics (Fig. 2)

Fish growth did not show significant differences ($p>0.05$) between the LP and HP treatments. Specific growth rate was 0.73 ± 0.05 (LP) and 0.88 ± 0.13 (HP) in the first trial, 0.57 ± 0.12 (LP) and 0.63 ± 0.13 (HP) in the second trial and 0.60 ± 0.01 (LP) and 0.62 ± 0.08 (HP) in the third trial. Feed conversion ratio did not show significant differences ($p>0.05$) between treatments. FCR values were in fact 2.17 ± 0.17 (LP) and 1.98 ± 0.26 (HP) in the first trial, 2.10 ± 0.48 (LP) and 1.90 ± 0.27 (HP) in the second trial and 1.83 ± 0.11 (LP) and 1.82 ± 0.19 (HP) in the third trial. Higher water temperatures measured during the third trial determined better feed conversion rates in fish.

Highest plant production (Tab. 1) in the first trial was in the HP floating system ($p<0.05$) followed by floating LP and NFT HP, which were 8.5% and 4.2% higher than hydroponics ($p<0.05$). The lowest biomass was found in substrate systems, respectively 33% (LP) and 45% (HP) lower than floating HP, and in NFT LP (-35%). Root weight in the first trial (Tab.1) showed the highest biomass in the LP substrate ($p<0.05$), followed by floating HP (-15%), floating LP and NFT HP (-24%) ($p<0.05$). Hydroponics showed the smallest root biomass, with a reduction in dry weight of 48% ($p<0.05$). Root data suggested the presence of an inverse correlation between biomass and water nutrient availability.

The second trial showed the same trend observed in the first trial, with highest biomass in floating HP ($p<0.05$) followed by floating LP (-15%). Hydroponics performance was similar to NFT HP. Lowest biomass was found in substrate systems (-42% for LP, -33% for HP) and NFT LP (-43%) Biomass values were sensitively smaller in LP treatments ($p<0.05$). Contrarily to first trial root biomass did not show differences in biomass.

In the third trial NFT had the water layer increased by 1cm (from 1 to 2 cm) to avoid wilt risks due to power shortages and increased seasonal temperatures. The highest yield was measured in NFT HP followed by floating systems (-16% for LP, -18% for HP) and NFT LP (-18%) ($p<0.05$). Substrate systems showed the lowest biomass (-35% for LP, -40% for HP) and were similar to hydroponics (-40%) ($p<0.05$). Root biomass showed opposite behaviour, with heavier roots observed in substrate systems ($p<0.05$) and NFT HP.

Leaf area (Tab. 2) did not show significant differences in all three trials with the exception of the NFT systems and floating LP in the third crop ($p<0.05$). Chlorophyll measures (Tab. 2) showed highest values in hydroponics ($p<0.05$), but not in the third crop where values were minimal. Despite differences among aquaponic treatments it appeared that there was not a clear correlation between SPAD readings and nitrogen concentrations in water.

Nitrogen content in leaves (Tab. 3) showed strong correlations with water nutrient concentration in the first trial. The lowest values were in fact measured in all LP subsystems ($p<0.05$), while the highest concentrations were measured in hydroponics followed by floating HP ($p<0.05$). Increase in water nitrogen concentrations in the second and third trials did not determine significant variations in leaf nitrogen.

Nitrogen concentration in roots seemed to follow plant biomass patterns (Tab. 3). In all three trials the lowest values were found in substrate systems ($p<0.05$) while the highest were in both floating and NFT HP (2^{nd} and 3^{rd} crop).

Discussion

Floating systems was the most constant aquaponic production system, regardless the water nutrient concentrations. The wide surface between roots and recirculating water could have justified for optimal plant nutrition. NFT appeared a competitive system, although performances were sensitively affected by water nutrients or root/water exchange surface. This behaviour was clearly visible in the first two trials where lettuce biomass in NFT HP was similar to floating LP (Tab. 1), which was growing with 20% less nitrogen (Fig. 1). Nevertheless a different performance was observed in the third trial, as a consequence of an increase in NFT water level by 1 cm. NFT lettuce biomass within each aquaponic system was similar (LP) or higher (HP) than floating system. On the contrary the lower NFT performance observed in the LP treatments in the first and second trial was likely due to inconstant water flow as a consequence of tap clogging.

Substrate aquaponics was always underperforming compared to the other two aquaponic sub-systems. The reason was due to an inadequate nutrient distribution through subirrigation. Despite the small substrate volume (1 litre) and the high media porosity sub-surface irrigation did not appear effective in granting optimal root/water exchange.

Results from trials were different from those observed by Lennard and Leonard (2006), for which NFT resulted the most underperforming sub-system. Nevertheless root wetting played an important role in plant growth efficiency, according to their conclusions.

Quality traits in lettuce were constant both for biomass (Tab.1) and leaf parameters (Tab. 2), irrespectively for the subsystem used. Therefore values of nitrogen in tissue were determined by

water nutrient concentrations only for nitrate levels below 50 mg L^{-1} (1^{st} trial). Plant nitrogen uptake was strongly correlated to biomass production rather than aquaponic sub-system choice. In the third trial, with lettuce yielding 2.8-3.5 kg m^{-2} plant nitrogen uptake corresponded to 0.25-0.3 g m^{-2} day^{-1}. Such values were lower to the 0.83 g m^{-2} day^{-1} measured by Gloger et al. (1995) and the 0.94 g m^{-2} day^{-1} assessed by Alder et al (2003). However winter trials at the experimental farm were not meant to achieve absolute yields, since plants did not benefit from any artificial light, higher temperatures and enhanced evapotranspiration. Nevertheless summer production from romaine lettuce obtained at the experimental farm was almost two times the yields obtained from the current trial (unpublished data), which eventually would have met the nitrogen sink values observed in literature.

Conclusions

Floating system looks the most constant aquaponic sub-system due to the large root/water exchange area. However NFT outperformed whenever adequate water flow and root/water surface was granted. Any water film increment in NFT appeared beneficial for plant growth. Substrate aquaponics through subirrigation did not achieve similar yields than the other subsystems, thus suggesting in the adoption of different watering strategies (surface irrigation) to supply nutrients to roots.

Quality traits in lettuce were not different among aquaponic subsystems and against hydroponics. Nitrogen uptake in leaves did not show nutritional patterns for water nitrate concentrations above 50 mg L^{-1}, which suggested that nitrogen uptake was rather biomass-dependent. The smaller water volume needed in NFT par rapport to floating systems would undoubtedly give plants some advantages in terms in reduced dilution of nutrients and in the raise of nutrient uptake efficiency in plants.

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Tables

Table 1 Plant production. Means ± standard deviation

Treatment	Leaf fresh weight (g plant^{-1})	Leaf % dry matter	Root dry weight (g plant^{-1})	shoot root ratio
Aquaponics NFT LP	19.88 ± 6.97 ab	5.48 ± 0.31	0.14 ± 0.02 ab	7.44 ± 0.24 ab
Aquaponics Floating LP	25.55 ± 7.51 c	5.01 ± 0.44	0.16 ± 0.01 abc	7.90 ± 1.55 b
Aquaponics Substrate LP	20.18 ± 8.24 ab	5.37 ± 0.66	0.21 ± 0.06 c	5.31 ± 0.97 a
Aquaponics NFT HP	24.63 ± 7.43 c	5.02 ± 0.80	0.16 ± 0.02 abc	7.72 ± 1.38 b
Aquaponics Floating HP	30.31 ± 5.84 d	4.59 ± 0.21	0.18 ± 0.01 bc	7.68 ± 0.07 b
Aquaponics Substrate HP	16.65 ± 6.70 a	6.21 ± 0.54	0.15 ± 0.01 ab	6.66 ± 1.31 ab
Hydroponics	23.62 ± 7.88 bc	5.14 ± 0.65	0.11 ± 0.02 a	10.64 ± 1.89 c
significance	***	ns	*	**
Aquaponics NFT LP	33.52 ± 11.29 a	5.14 ± 0.62	0.29 ± 0.10	6.18 ± 0.43 a
Aquaponics Floating LP	51.79 ± 15.34 c	5.13 ± 0.94	0.36 ± 0.04	7.46 ± 1.66 a
Aquaponics Substrate LP	34.98 ± 10.96 a	5.85 ± 0.92	0.37 ± 0.09	5.59 ± 1.01 a
Aquaponics NFT HP	48.90 ± 14.25 bc	5.18 ± 1.05	0.35 ± 0.06	7.15 ± 1.32 a
Aquaponics Floating HP	60.57 ± 15.67 d	4.73 ± 0.60	0.40 ± 0.02	7.18 ± 0.29 a
Aquaponics Substrate HP	40.16 ± 15.49 ab	5.56 ± 0.47	0.38 ± 0.09	5.98 ± 0.78 a
Hydroponics	48.76 ± 23.10 bc	6.61 ± 0.25	0.33 ± 0.08	9.77 ± 0.22 b
significance	***	ns	ns	**
Aquaponics NFT LP	144.25 ± 59.24 b	4.96 ± 0.34	0.96 ± 0.20 a	7.32 ± 0.41 b
Aquaponics Floating LP	149.83 ± 26.15 b	4.70 ± 0.21	0.86 ± 0.06 a	8.10 ± 0.19 b
Aquaponics Substrate LP	114.16 ± 32.65 a	5.95 ± 1.30	1.42 ± 0.09 c	4.81 ± 1.31 a
Aquaponics NFT HP	176.38 ± 49.35 c	4.73 ± 0.24	1.18 ± 0.14 b	7.02 ± 0.52 b
Aquaponics Floating HP	145.42 ± 35.20 b	4.82 ± 0.33	0.88 ± 0.04 a	7.98 ± 0.43 b
Aquaponics Substrate HP	106.54 ± 42.50 a	5.51 ± 0.45	1.56 ± 0.04 c	3.75 ± 0.93 a
Hydroponics	114.69 ± 23.91 a	5.41 ± 0.73	0.85 ± 0.06 a	7.26 ± 1.10 b
significance	***	ns	***	***

Table 2 Leaf quality and chlorophyll. Means ± standard deviation

Treatment	Leaf Area (cm^2 $plant^{-1}$)	SLA (m^2 kg^{-1})	SPAD
		1st crop cycle	
Aquaponics NFT LP	724.1 ± 75.1	67.5 ± 3.0	29.0 ± 3.1 a
Aquaponics Floating LP	847.7 ± 164.8	65.9 ± 1.5	30.7 ± 2.9 abc
Aquaponics Substrate LP	739.3 ± 79.9	69.2 ± 5.6	31.0 ± 2.5 bc
Aquaponics NFT HP	793.9 ± 38.2	65.1 ± 5.6	29.5 ± 3.0 ab
Aquaponics Floating HP	944.4 ± 44.5	68.2 ± 2.3	32.3 ± 2.7 c
Aquaponics Substrate HP	756.6 ± 227.4	74.0 ± 9.2	30.3 ± 3.6 ab
Hydroponics	721.8 ± 92.2	60.8 ± 5.3	34.1 ± 4.2 d
significance	ns	ns	***
		2nd crop cycle	
Aquaponics NFT LP	892.5 ± 338.5	49.8 ± 1.9	28.5 ± 3.2 a
Aquaponics Floating LP	1288.4 ± 400.2	47.8 ± 1.8	29.8 ± 3.6 ab
Aquaponics Substrate LP	947.8 ± 96.1	47.5 ± 5.8	30.8 ± 3.1 bc
Aquaponics NFT HP	1215.0 ± 39.6	49.1 ± 1.2	31.0 ± 3.0 bc
Aquaponics Floating HP	1377.9 ± 68.6	48.4 ± 3.5	32.4 ± 2.5 bd
Aquaponics Substrate HP	1086.4 ± 148.8	48.7 ± 2.4	31.2 ± 2.5 bc
Hydroponics	1352.8 ± 255.5	42.8 ± 5.7	33.9 ± 3.9 d
significance	ns	ns	***
		3rd crop cycle	
Aquaponics NFT LP	3735.6 ± 526.3 ab	53.8 ± 5.5	34.4 ± 3.7 bc
Aquaponics Floating LP	3528.8 ± 170.3 ab	50.6 ± 3.3	33.9 ± 3.4 ab
Aquaponics Substrate LP	3340.0 ± 785.2 a	49.1 ± 1.1	36.2 ± 2.5 bc
Aquaponics NFT HP	4421.8 ± 708.1 b	53.1 ± 1.6	34.9 ± 4.4 bc
Aquaponics Floating HP	3356.6 ± 156.2 a	48.0 ± 3.1	35.1 ± 5.0 bc
Aquaponics Substrate HP	2889.4 ± 518.3 a	49.8 ± 3.2	36.5 ± 3.7 c
Hydroponics	2850.1 ± 476.8 a	46.4 ± 6.9	32.0 ± 3.3 a
significance	*	ns	**

Table 3. Nitrogen content in leaf and roots. Means ± standard deviation

Treatment	Leaf mg total N g^{-1} dry matter		
	1st crop cycle	2nd crop cycle	3rd crop cycle
Aquaponics NFT LP	43.3 ± 1.3 a	45.3 ± 2.8	43.5 ± 5.4
Aquaponics Floating LP	46.6 ± 1.9 ab	43.9 ± 2.0	44.1 ± 2.5
Aquaponics Substrate LP	44.8 ± 1.5 ab	43.3 ± 4.6	46.4 ± 2.1
Aquaponics NFT HP	43.6 ± 0.7 ab	44.5 ± 2.4	46.4 ± 1.1
Aquaponics Floating HP	47.1 ± 2.7 b	43.7 ± 0.4	45.9 ± 3.6
Aquaponics Substrate HP	45.6 ± 2.2 ab	46.6 ± 4.2	43.9 ± 0.2
Hydroponics	51.1 ± 2.5 c	47.5 ± 2.1	43.1 ± 2.1
significance	**	ns	ns

Treatment	Root mg total N g^{-1} dry matter		
	1st crop cycle	2nd crop cycle	3rd crop cycle
Aquaponics NFT LP	38.0 ± 5.0 b	41.2 ± 1.7 b	38.1 ± 0.9 c
Aquaponics Floating LP	40.4 ± 2.3 b	41.5 ± 2.7 b	42.4 ± 1.5 d
Aquaponics Substrate LP	22.1 ± 1.9 a	30.0 ± 2.9 a	20.0 ± 2.0 a
Aquaponics NFT HP	40.6 ± 2.6 b	46.0 ± 3.0 bc	41.7 ± 2.4 d
Aquaponics Floating HP	41.6 ± 2.6 b	47.8 ± 1.3 c	42.1 ± 0.4 d
Aquaponics Substrate HP	23.2 ± 5.4 a	30.7 ± 4.6 a	18.0 ± 1.1 a
Hydroponics	37.7 ± 6.7 b	42.4 ± 2.0 b	35.1 ± 2.0 b
significance	***	***	***

Figures

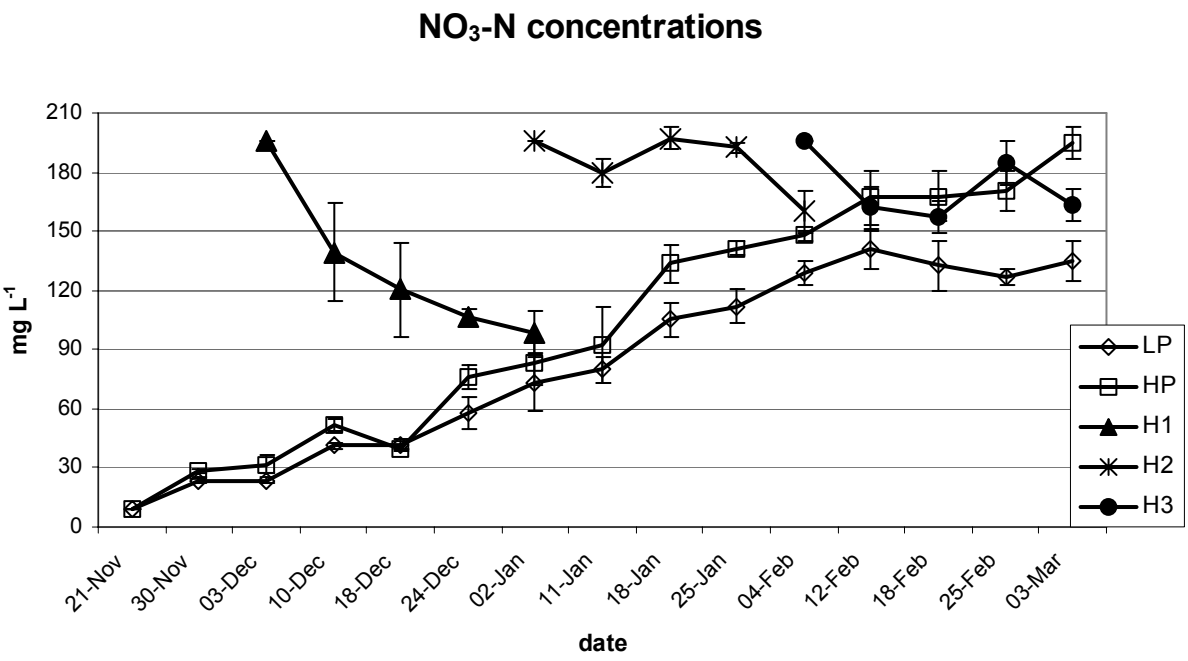

Figure 1. Water nitrate concentrations. Mean and S.E.

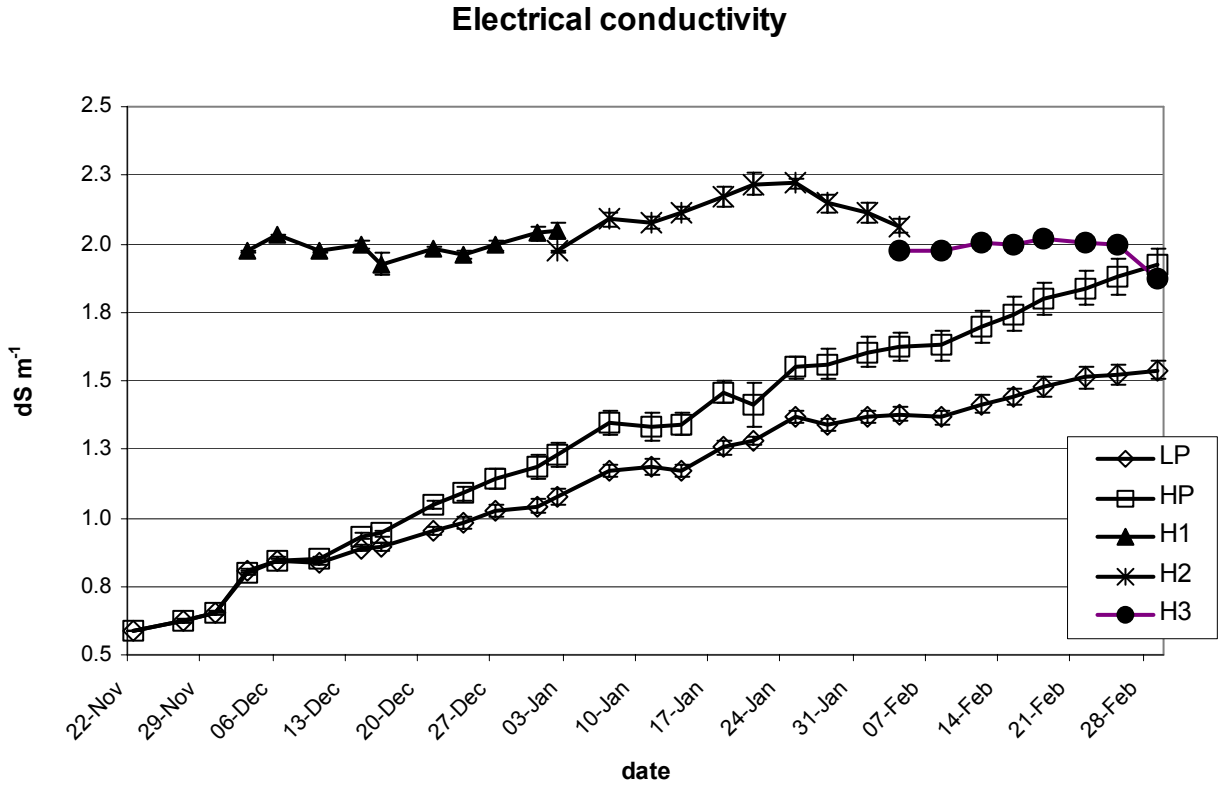

Figure 2 Water electrical conductivity. Mean and S.E.

Chapter 5

Qualitative Traits and Crop Performance of Aquaponic Cucumber

Abstract

Sustainability seek to reduced footprint and increased integration to promote long lasting development. Aquaponics, a production system that integrates aquaculture with soilless agriculture, has huge potentials, providing that it meets the same productivity, profitability and quality standards of hydroponics. While leaf vegetables have already shown optimal results in aquaponics, wider assessments are needed for fruity plants due to their different nutrient requirements. Research carried at the University of Tuscia assessed production, quality and system performance of cucumber (*Cucumis sativus* L. cv Ekron) grown on largemouth bass (*Micropterus salmoides* L.) water. Yields in aquaponics (2.55 ± 0.62 kg plant^{-1}) were similar to hydroponics (2.50 ± 0.59 kg plant^{-1}). Likewise fruit quality did not differ in both sugar content (2.9 ± 0.1 °brix vs 3.0 ± 0.0 °brix), total acidity (pH 5.4 ± 0.0 vs pH 5.6 ± 0.1), tritatable acidity (5.8% ± 0.5 vs 5.2% ± 0.6), but fruits showed lower nitrogen content (31.13 ± 0.51 g Kg^{-1} DW vs 36.62 ± 3.15 g kg^{-1} DW). Nitrogen uptake efficiency, harvest index and apparent N recovery did not differ significantly, but the percentage of nitrogen loss by aquaponics was higher than natural denitrification occurring in hydroponics. Optimal fish to plant ratio was found in 330 g plant^{-1}.

Keywords: *Cucumis sativus*, largemouth bass, *Micropterus salmoides*, hydroponics, floating system

Introduction

Aquaponics is a growing system that integrates fish with soilless plant production. Born as an evolution of recirculating aquaculture system (RAS) aquaponics uses plants to clean up water from ammonia, which is toxic to fish. The presence of plants not only allows for the complete reuse of water and nutrients but also opens up opportunities for more sustainable food productions.

Despite differences in designs (Nelson, 2007) aquaponics is mainly constituted by fish rearing tanks, suspended solid removal units and plant troughs (Diver, 2006; Losordo et al., 1998; Losordo et al., 1999). Plant units are similar to those used in hydroponics, in which floating systems, NFT and gravel systems are the most represented.

Aquaponics has proven to be a valid agricultural system with various vegetables and under different designs (Lennard and Leonard, 2004; McMurty et al 1990; Nelson, 2007; Rakocy et al., 1992; Savidov, 2005). Furthermore aquaponics can reach nutrient concentrations close to hydroponics

(Rakocy, 1997). Rakocy et al. (2004) proved that sweet basil from floating aquaponics was three times higher than soil productions. Similarly McMurty et al. (1990) found that yields of beans and cucumber on sand substrates were respectively three and two times higher than soil controls, but not for tomato, which resulted 25% lower. Comparisons between aquaponics and hydroponics showed higher yields in mature aquaponic floating systems both for tomato and cucumber (Savidov, 2005) providing that optimal management and significant nutrient levels were maintained in water. On the contrary Graber and Junge (2009) from trials carried out with tomato and cucumber grown on expanded clay obtained respectively 9% and 36% lower yields than hydroponics, which was presumably due to low potassium concentrations.

Optimal nutrition is a determinant key to increase productivity. Geometrical increases in cucumber yields were in fact observed from raising nitrogen concentrations up to 225 mg L^{-1} with perlite substrates (Jasso-Chaverria et al., 2005) and 275 mg L^{-1} of nitrogen with 75 mg L^{-1} of phosphorus were suggested to avoid nutrient depletion by cucumber grown on rockwool (Schon and Peggy-Compton, 1997). The NO_3-NH_4 ratio in the nutritive solution also affects biomass and yields, since a 3:1 ratio can achieve the highest productivity and leaf area in hydroponically grown cucumber under total nitrogen concentrations of 200 mg L^{-1} (Azarmi and Esmaeilpour, 2010).

Growing conditions determine different plant behaviours, Tyson et al. (2008) noticed that early production of cucumber is proportionally reduced with increased pH values in aquaponic water, however total yields were not affected by pH levels.

Fruit quality is affected by system choice. In the case of Gomez-Lopez et al. (2006) lower acidity was noticed in cucumbers juices grown on NFT than perlite substrate, presumably due to 1% of higher nitrogen uptake occurring on NFT.

The objective of the present research was to assess the production and quality traits of aquaponic cucumber against hydroponics and to develop a nitrogen budget to determine aquaponic system performance and optimal fish/plant ratio.

Materials and methods

Three aquaponic floating systems were set up in spring 2011 in a greenhouse at the experimental farm of the University of Tuscia, Italy. Each independent system consisted of a 250 L fish tank serving a 100 L solid removal unit and a 20 L net filter downstream. The filtration system served a 2 m^2 tank containing floating polystyrene rafts. The total volume of each system was 750 L. Largemouth bass (*Micropterus salmoides* L.) were stocked on 28 May 2011 in the system filled with well water. Fish were left grown for five days prior trial commencement to enrich water with plant nutrients. Stocking biomass was 2.77 ± 0.03 kg and fish average body biomass was 33.6 ± 1.0

g. The resulting stocking density was 11.06 ± 0.31 kg m^{-3}. Fish were fed a 44% protein diet (Optiline P1, Skretting, Mozzecane, Verona, Italy) under a daily feeding regime equal to 0.9% of body weight.

Each aquaponic system was enriched with small quantities of nutrients to level concentrations of those of a mature aquaponic system: potassium (18.2 mg L^{-1}), phosphorus (12.4 mg L^{-1}), sulphur (20.3 mg L^{-1}), magnesium (14.2 mg L^{-1}) and iron (0.4 mg L^{-1}). Upon fertilization electrical conductivity (EC) was raised by 0.15 dS m^{-1}, up to 0.75 dS m^{-1}.

The three aquaponic systems were compared against three hydroponic controls at 1.9 dS m^{-1} EC. Hydroponic tanks were filled with osmotized water and fertilized with NO_3-N 196 mg L^{-1}, NH_4-N 14 mg L^{-1}, phosphorus 46.5 mg L^{-1}, potassium 195 mg L^{-1}, calcium 170 mg L^{-1}, sulphur 99 mg L^{-1}, magnesium 30 mg L^{-1}, manganese 0.96 µg L^{-1}, iron 0.48 µg L^{-1}, boron 0.12 µg L^{-1}, zinc 0.72 µg L^{-1}, copper 0.24 µg L^{-1} and molybdenum 0.012 µg L^{-1}.

Water pH in aquaponic and hydroponic tanks was maintained respectively at 6.7-7.5 and 5.5-6.0 by adding $CaCO_3$ and KOH. Dissolved oxygen was kept above 90% saturation at 6-7 mg L^{-1}. Water temperatures were at 26 ± 3°C for the whole crop cycle in both hydroponic and aquaponic systems. Any water loss due to evapotranspiration was compensated by addition of osmotized water and measured. For hydroponics supplement of fertilized water occurred whenever electrical conductivity was below 1.6 dS m^{-1}.

Three weeks old cucumber seedlings (*Cucumis sativus* L. cv Ekron, Enza Zaden, Tarquinia, Italy) were transplanted on June 2nd on floating rafts at a density of 3 plant m^{-2} (40 cm on the line) five days after fish stocking. Plants were trained with double-stem method for a 48-day crop length.

A 30 L $plant^{-1}$ water volume was set in hydroponics, in aquaponics the volume of nutritive solution available for each plant was 50 L $plant^{-1}$.

Electrical conductivity (EC) and pH were measured twice a week with Hanna HI98130 meter. Ammonia and nitrate nitrogen levels in water were measured with a spectrophotometer following the ammonia nitrogen method by Anderson and Ingram (1989) and the salicil-solphoric acid method for nitrate nitrogen by Cataldo et al. (1975).

Fish growth performance was determined as body weight gain and feed conversion ratio, calculated as the amount of feed used per kg of fresh biomass. Specific growth rate (SGR) was determined according to the formula:

$$SGR = [(LnWf - LnWi)/days]x100$$

Where Ln is the natural logarithm, Wf is the final weight of the fish, Wi is the initial weight and days is the time of the experiment.

Plants fruits were harvested every 4-5 days and weighted for fresh and dry weight. Fruits were assessed for brix degree with an optical refractometer. Juice was dried at 80 °C for 72 hours and dry weight was determined. Total acidity and tritatable acidity were measured with Crison GLP 21 pH meter. Tritation was carried out with NaOH 0.1 M.

Plants leaves and stems were determined for their fresh and dry weight. Leaf area was measured with an electronic area meter (Delta-T Devices Ltd, Cambridge, UK). Specific leaf area was calculated by dividing leaf area by leaf dry weight of each plant. Chlorophyll content of leaves was measured with Minolta SPAD 502.

A nutrient budget was accounted from each of the inputs and outputs, measured in their dry matter and total Kjeldahl nitrogen content:

$$[N_{feed}] + [N_{fertilization}] + [N_{initial\ water}] = [N_{fish}] + [N_{leaf}] + [N_{stem}] + [N_{fruit}] + [N_{roots}] + [N_{final\ water}] + [N_{lost}]$$

The incoming nitrogen amount for each crop cycle was supplied by feed, fertilization (hydroponics) and the nutrient pool in well water (for aquaponics). The outgoing nitrogen was accounted from fish biomass gain, leaves, stems, fruits and roots uptake. From the budget the amount of nitrogen lost by the system was determined as the result of denitrification and, just for the aquaponic systems, by fish metabolism, solid/feed discharge, bacterial/plankton sequestration and settled organic matter into the system.

System efficiency was determined by measuring the nitrogen uptake efficiency (NUE), apparent nitrogen recovery, harvest index, N harvest index and standardized nitrogen removal (Van Eerd, 2007). NUE was calculated by dividing plant nitrogen uptake by the net supplied nutrients according to the equation:

$$NUE = [N_{plants}] / [N_{supplied}]$$

where supplied nutrients accounted for the amount of mineral already stocked into the water or given by feed after fish uptake. Apparent nitrogen recovery was determined as the ratio between the nitrogen removed by crop yield and the available nutrients into the systems. Harvest index was calculated as the weight ratio between crop yield and full plant production while N harvest index was the nitrogen ratio between yield and full plant. Standardized nitrogen removal was the ratio between fruit nitrogen and fruit dry weight.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Percentages values were analyzed upon square root or arc sine transformation.

Results

Water nitrate (Fig. 1) in hydroponics settled at 150 mg L^{-1} for the whole crop due to 2-3 addition of nutritive solution in each tank. Aquaponics showed nitrate concentrations 3-4 times lower than

hydroponics. Nitrate trend in aquaponic tanks was raising up to fruiting start, then a successive falling trend was observed due to more intense nitrogen absorption by plants. Water ammonia nitrogen in hydroponics was seen decreasing and settled to 0 mg L^{-1} at fruiting due to nitrification occurring in tanks. Hydroponic ammonia values ranged between 0 mg L^{-1} and 5 mg L^{-1} afterwards following successive fertilizations. Ammonia in aquaponic tanks was always next to 0 mg L^{-1}.

Electrical conductivity (Fig. 2) in aquaponics settled at 1.7 dS m^{-1} during the whole crop cycle. Aquaponics showed EC values approximately 60% lower than hydroponics. However, constant values observed during vegetative growth were followed by a decreasing trend during fruit harvest.

Total yield in aquaponics (2.55 ± 0.62 kg) was not significantly different from hydroponics (2.50 ± 0.59 kg). The same occurred for the number of fruits, which were 10.4 ± 2.7 in aquaponics and 10.5 ± 2.6 in hydroponics. Marketable yields, which accounted for fruits heavier than 180 g, was similar in both treatments, with discarded weight accounting for 5.1% in aquaponics (2.42 ± 0.63 kg) and 2% in hydroponics (2.45 ± 0.55 kg). Plants behaved similarly in both systems with cumulative production showing similar trends (Fig. 3). However hydroponics appeared to have a slight advantage at early production due to higher nutrient concentrations.

Plant biomass values (Tab. 1) were similar in both treatments ($p>0.05$) both for fresh weight and % of dry matter. Leaf area values were similar ($p>0.05$) in both systems (0.94 m^2 ± 0.10 aquaponics, 1.01 m^2 ± 0.20 hydroponics) as well as specific leaf area which accounted for 30.2 m^2 kg^{-1} ± 2.3 in aquaponics and 21.3 m^2 kg^{-1} ± 2.5 in hydroponics. On the contrary leaf chlorophyll, measured in SPAD units, was significantly different ($p<0.01$) between aquaponics (48.1 ± 7.9) and hydroponics (51.6 ± 7.0).

Fruit quality assessment did not show differences between aquaponics and hydroponics, both for dry matter content (3.1% ± 0.3 aquaponics, 3.0% ± 0.4 hydroponics), brix degrees (2.9 ± 0.1 aquaponics, 3.0 ± 0.0 hydroponics), pulp pH (5.4 ± 0.0 aquaponics, 5.6 ± 0.1 hydroponics) and % of acidity (5.8% ± 0.5 aquaponics, 5.2% ± 0.6 hydroponics)

Nitrogen concentration in leaves (Tab. 2) did not differ between aquaponics and hydroponics, despite differences in chlorophyll content. Nevertheless the higher nutrients in the nutritive solution in hydroponics determined a higher nitrogen content in stems (+27%, $p<0.001$) and in fuits (+18%, $p<0.05$). On the contrary no significant differences were noticed in roots.

Fish consumed 1583g ± 16.5 of feed with a feed conversion ratio (FCR) of 1.50 ± 0.16. Fish biomass increase was 1068g ± 122 under a specific growth rate of 0.77% ± 0.03. Mortality was 7.7% among replicates.

The amount of water consumed for evapotranspiration in aquaponics was 730.0 L ± 75.5. The loss for fish solid removal accounted for just 0.7% of the missing water. Every plant consumed an

average of 104.3L ± 10.8 in aquaponics and 88.5L ± 8.8 in hydroponics, a volume which resulted to be similar in both treatments. Likewise average water consumption per kg of fruit harvested resulted similar in both systems, with 42.8 ± 6.2 litres in aquaponics and 35.6 ± 0.3 litres in hydroponics.

The nutrient budget (Fig. 4) was calculated after kjeldahl and spectrophotometer analysis of inputs and outputs. In the aquaponics the assimilation by fish was 28% of the total nitrogen supplied from feed. The nitrogen left after fish served to increase the nutrient stock for plants, which also accounted for the quantity dissolved in well water (13.6 mg L^{-1} equivalent to 12.1%). Conversely the hydroponic nitrogen (210 mg L^{-1}, as ammonia and nitrate) was uniquely supplied by fertilizer. From the total stock the biggest sink was observed in leaves and fruits (Fig. 4). Although nitrogen levels in fruits was different between aquaponics and hydroponics, the uptake rate measured as apparent N recovery reselted similar (Tab. 3). Nevertheless differences were seen in stems (Fig. 4, Tab. 2), which suggested for the influence of nitrogen-rich sap in plants' vessels. Aquaponic water was poorer in nitrogen than hydroponics at the end of the trial despite the higher stock available at transplant (114 g). This difference was mainly due to the higher nitrogen loss (20.4%) of aquaponics against a 5.4% loss observed in hydroponics, which was only due to natural denitrification. Nitrogen uptake efficiency, which included also roots, was similar in both aquaponics and hydroponics (Tab. 3). Harvest index was similar in both aquaponics and hydroponics and resulted in a 44-49% yield from the total plant. Aquaponic nitrogen uptake by fruit was however lower than hydroponics ($p<0.05$) (Tab.3), as already confirmed by the kjeldahl analysis on fruits (Tab. 2).

Discussion

Cucumber production did not show significant differences between aquaponics and hydroponics. Aquaponics was able to compete against hydroponics despite higher nutrient concentrations should have favoured higher yields (Jasso-Chaverria et al., 2005; Schon and Peggy-Compton, 1997). The nitrogen concentrations measured suggested that optimal growth could be obtained with nitrate levels 8-10 lower than those used by Savidov (2005), who observed higher yields in mature aquaponics than hydroponics. Higher yields observed by Savidov (2005) could have found support in the optimal nitrogen to potassium ratio (1:2), which enhanced fruiting. In the present trial addition of potassium up to a N:K ratio close to 1:1 at transplant could have supported aquaponic yields. Graber and Junge (2009) in aquaponic trials with cucumber under nitrate levels at 12.1–95 mg L^{-1}, observed a 36% yield reduction due to low potassium levels, which were 45 times lower the concentrations available in hydroponics.

Similar values in nitrogen uptake efficiency (Tab. 3) suggested that aquaponic plants were not limited by reduced nutrient concentrations. NUE values, 16% higher than those measured by Grewal et al. (2001), suggested that higher nitrogen concentrations in water did not favour plants growth nor nitrogen recovery, which was always similar in both aquaponics and hydroponics. Nevertheless fruits in hydroponics were richer in nitrogen ($p<0.05$) presumably due to direct translocation of nutrients in sap. Likewise, fruit quality was not affected by any nitrogen pattern. Brix and pH values in cucumber resulted in fact similar and were presumably not influenced by any differences in nitrogen uptake, which could have modified fruit pH and tritatable acidity (Gomez-Lopez et al., 2006).

The nitrogen budget (Fig. 4) accounted for 103.7 g of nutrients entering the system as feed and 29.4 g taken by fish. Aquaponic systems revealed lower nitrogen levels into remaining water than hydroponics. This was justified by the percentage of nitrogen lost, which was 20.4% and corresponded to 17.3 g of the total stock available for plants. The higher loss in aquaponics not only accounted for natural denitrification, which might have resulted in 5.4% as in hydroponics, but also for waste disposal (feed and solid), fish metabolism and ecosystem maintenance of the complex aquaponic habitat (plankton, bacteria/floc, fungi etc). Plant uptake was similar ($p>0.05$) between aquaponics and hydroponics and corresponded to 5.68 ± 1.72 grams of nitrogen in aquaponics and 5.98 ± 0.80 grams in hydroponics for the whole crop cycle. The balance between nitrogen levels from initial and final water concentrations (Fig. 1) gave for aquaponics a surplus of 21 g per system, which corresponded to further 3.7 plants to meet the nitrogen break even. Given a standing fish biomass of 3.3 kg the optimal number of plants to meet the nitrogen balance was 10. This resulted that each plant was the sink for 3g feed (44% protein) supplied to fish on a daily basis.

Water productivity resulted similar in both aquaponics and hydroponics. Apparently fish management did not have a sensitive effect in water budget due to the intense plant evapotranpiration. However, a comparison against hydroponic productions in greenhouses accounted for 20.4 L kg fruit^{-1} (Grewal et al., 2011), which was sensitively lower than the volume used in the trial.

Conclusions

Aquaponics showed comparable yields to hydroponics despite ideal nitrogen concentrations above 200 mg L^{-1} are suggested in hydroponic literature. Fruit quality in aquaponics was similar to hydroponics and confirmed the good nutritional status of plants. A comparison with other aquaponic trials suggested a 1:1 nitrogen to potassium ratio as an ideal condition for optimal productivity. The optimal fish/plant ratio resulted in 330g fish per plant. Similar nutrient uptake

efficiency and harvest index suggested aquaponics as a valid production system for sustainable horticulture.

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Tables

Table 1 Plant biomass production. Mean values ± standard deviation

	Plant mean weight g plant-1	Mean stem weight g plant-1	Mean leaf weight g plant-1	Stem dry matter (%)	Leaf dry matter (%)	mean root dry weight g plant-1	Leaf stem ratio
Aquaponics	843.7 ± 219.1	374.2 ± 85.9	458.7 ± 134.9	7.1 ± 0.6	12.7 ± 1.1	7.0 ± 4.4	1.21 ± 0.11
Hydroponics	750.4 ± 106.2	325.9 ± 47.7	412.1 ± 56.8	7.2 ± 0.2	14.4 ± 1.7	6.5 ± 0.9	1.27 ± 0.01
Significance	ns	ns	ns	ns	ns	ns	ns

Table 2 Plant nitrogen concentrations. Mean values ± standard deviation

	Leaf g kg^{-1} DW	Stem g kg^{-1} DW	Root g kg^{-1} DW	Fruit g kg^{-1} DW
Aquaponics	40.06 ± 2.17	24.23 ± 0.47	42.37 ± 6.00	31.13 ± 0.51
Hydroponics	40.78 ± 2.01	30.82 ± 0.91	45.52 ± 6.82	36.62 ± 3.15
Significance	ns	***	ns	*

Table 3 Nitrogen Uptake efficiency (NUE), apparent N recovery, N harvest index, harvest index and standardized N removal of aquaponic and hydroponic systems. Mean values ± standard deviation

	NUE	Apparent N recovery	N harvest index	harvest index	standardized N removal
Aquaponics	47.0 ± 14.3	19.8 ± 4.5	44.9 ± 2.7	47.9 ± 2.6	3.1 ± 0.1
Hydroponics	52.5 ± 4.3	24.0 ± 1.0	48.1 ± 2.4	49.0 ± 2.4	3.7 ± 0.3
Significance	ns	ns	ns	ns	*

Figures

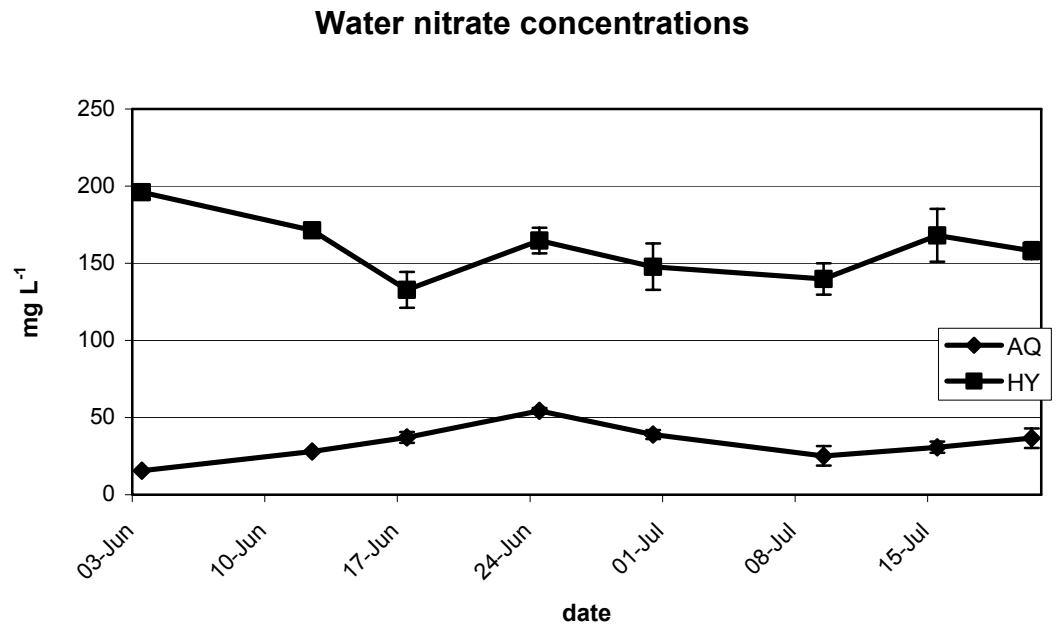

Figure 1 Water NO_3-N concentrations. Mean values and standard error

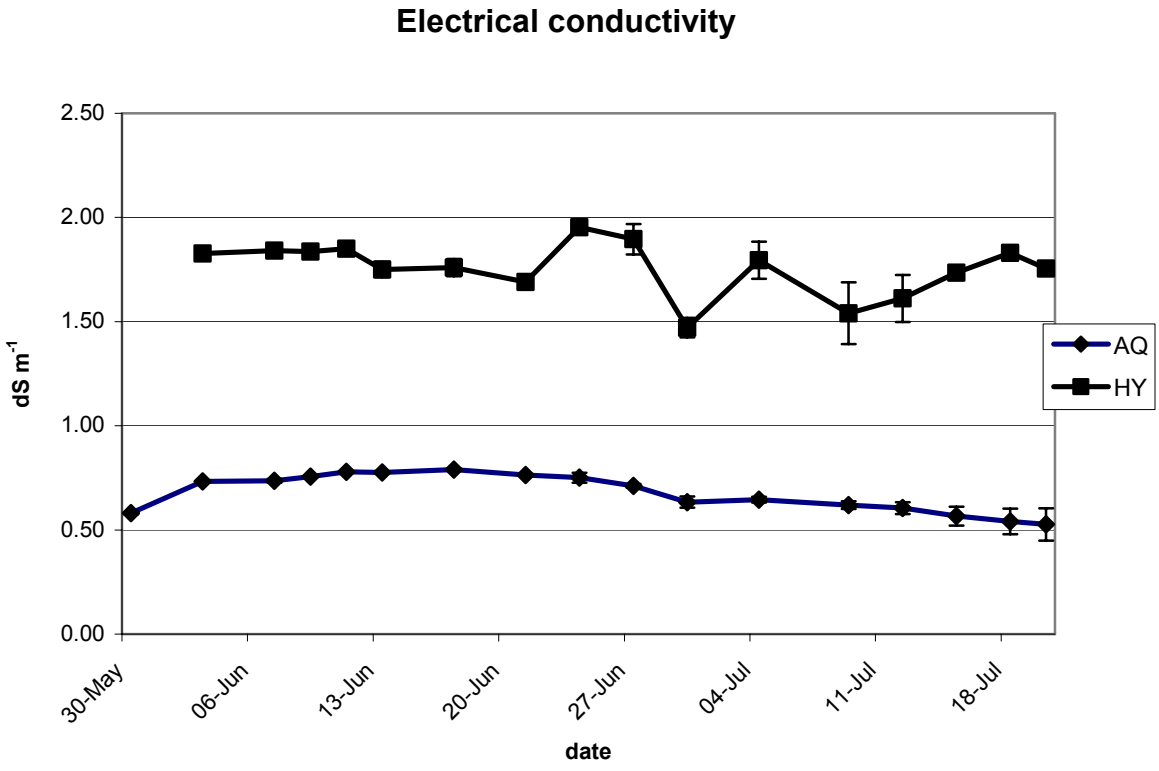

Figure 2 Water EC concentrations. Mean values and standard error

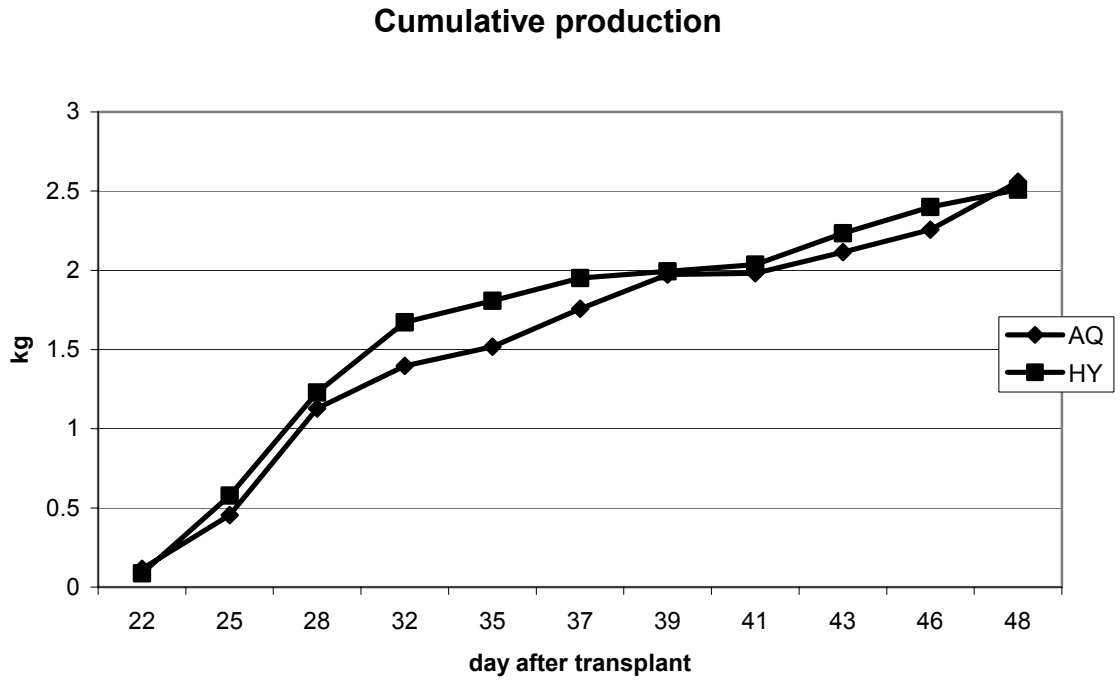

Figure 3 Plant cumulative fruit production. Mean of all plants

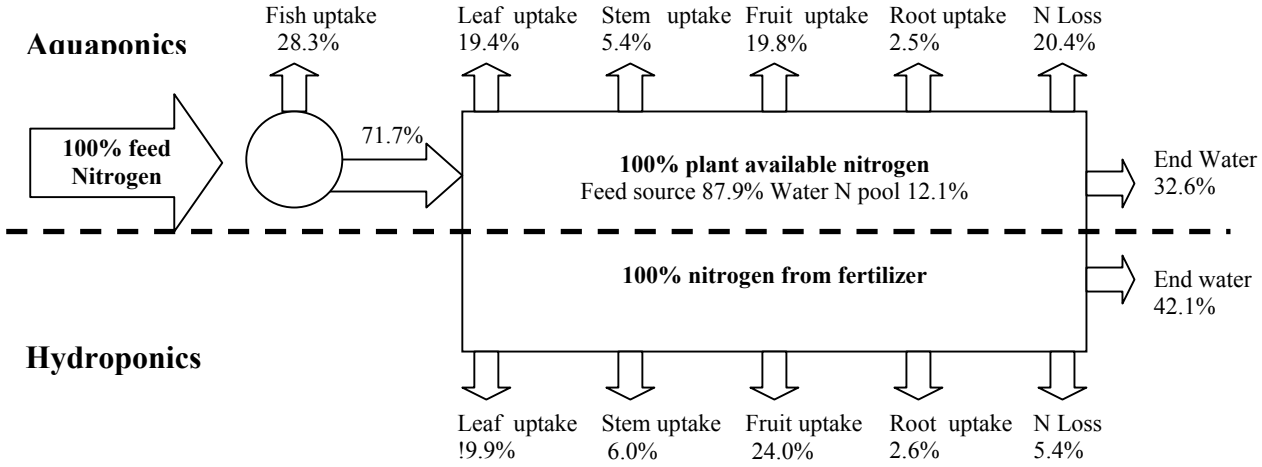

Figure 4. Nitrogen budget in aquaponics and hydroponics

Chapter 6

Saline Horticulture and Water Reclamation: Production and Quality Challenges in *Salsola soda* from Marine Aquaponics

Abstract

Saline agriculture unveils new opportunities to expand farming systems in marginal areas, where traditional agriculture cannot develop. Halophytes, which are well adapted to drought conditions, nutrient scarcity and salt, are ideal crops for saline water and can be used to recover nutrients from crop-animal farming. Beyond many halophytes the genus Salsola is well known around the world as a specialty vegetable. Its high profitability as well as rapid growth makes this plant the perfect candidate for reclaiming water from marine aquaculture operations. Three consecutive trials in winter-spring 2011 assessed *Salsola soda* growth and quality under raising salinity. Plants were tested in aquaponic systems under 5 g L^{-1}, 10 g L^{-1} 20 g L^{-1} and 30 g L^{-1} salt and were compared against hydroponic controls set with 5 g L^{-1} or 10 g L^{-1} of salt. Optimal salinity was assessed at 10g L^{-1}, but Salsola perfomed fairly well up to 20 g L^{-1}. Aquaponics was more productive than hydroponics under same salinity whenever nitrogen concentrations were above 30 mg L^{-1}. Maximum biomass production in aquaponics was 3.2 – 5.1 kg m^{-2} in a 30 day crop. Proximate analysis of shoot and roots did not outline major differences in salsola between aquaponics and hydroponics. Nevertheless increased salinity improved sodium and chloride uptake by plants.

Keywords: Phytoremediation, integrated aquaculture, halophytes, mariponics, saline agriculture

Introduction

Saline agriculture and wastewater can greatly contribute to raise agricultural output and support future demand in food and energy. Bruinsma (2009) estimates a 50% raise in food demand to meet the needs of a growing world population, which is expected to be 8.3 billions in 20 years (UNDESA, 2009) Therefore competition for freshwater, energy, land as well as the need to improve agricultural productivity will be sensitive issues in future development planning. According to UN-Water (2012) 70% of the available freshwater is already withdrawn by agriculture, which severely limits the choice for further uses. Resources scarcity may hamper world sustainability unless major

changes occur in the direction of cross-integration and recycling. In the case of land a possible solution can be found in the conversion of marginal areas, where traditional agriculture cannot be developed, to the production of drought or salt-resistant plants. Use of low-quality water, such as saline or nutrient-rich wastewater, not only would contribute to irrigation, but also reduce oil-derived fertilization needs. Although advances in technology help supporting traditional crop productivity, saline agriculture can overtake problems of soil/water salinity, land competition or deforestation. Many halophytes are easy sources of food, drugs, essential oils, fibers and biofuels (NRC, 1990; Glenn et al., 1994; Jaradat, 2003; Masters et al. 2007; Khan and Ansari, 2008; Abideen et al. 2011). Seeds from halophytes are rich in proteins (Wu and Sessa, 2004) or essential amino acids, which are in higher concentrations than common staples (Ruales and Nair, 1992).

Optimal growths in salt-tolerant plants are obtained within specific salinity ranges. According to Masters et al. (2007) electrical conductivity (EC) values below 15 dS m^{-1} favour dry matter productions in halophytes up to 5-10 ton $year^{-1}$. On the contrary EC values above 25 dS m^{-1} reduce yields to 0.5-5 ton $year^{-1}$. Beyond biomass production salinity determines changes in plant mineral composition, particularly for sodium, potassium, calcium and magnesium, and affect plant assimilation (Masters et al., 2007).

Among many halophytes the genus Salsola is widespread in the world and grows spontaneously in arid, semiarid and temperate climates. Salsola has been studied as fodder for animals (Towhidi et al., 2011; Osman et al., 2006), fuel production (Foster, 1979; Foster and Karpiscak, 1983; Abideen et al. 2011), as a bioremediation plant for land reclamation in sandy areas (Shekhawat et al., 2006; Toderich et al., 2009) or as a companion plant to reduce the salinity effects in horticultural crops (Colla et al., 2006; Graifenberg et al., 2003). Salsola is also a specialty salad in Europe and in Japan and is well known for its high profitability (Hammer et al., 1990).

Optimal growth and succulence was assessed in *Salsola kali* for salinities within 50-100 mM (NaCl), but biomass was seen decreasing at 200 mM (Reimann and Breckle, 1995). In the case of *Salsola orientalis* yields in desert areas were 2.0-2.8 ton ha^{-1} (f.w.) and 1.0-2.2 ton ha^{-1} (d.w.) (Toderich et al., 2009). *Salsola* spp, showed lower ions content than other halophytes due to excretion at epidermal level (Toderich et al., 2009). According to Duke (1983) ash proximate analysis of *Salsola kali* was 40% of carbonate, 20% of potassium, 18% calcium, 6% of phosphate, 3% magnesium and 2% of chloride. Nevertheless a variability in plant behaviour was observed among species or subspecies. In the case of *Salsola kali* raising salinity increased salt content in plants, but lowered K/Na and Na/Cl ratios (Reimann and Breckle, 1995). Redman and Fedec (1987) classified *Salsola pestifer* as alkali halophyte, whose tissues were rich in calcium, magnesium and potassium but low in chloride and sodium.

Water reclamation and use of saline water are seen a reliable resource for alternative farming systems or landscape management. Use of low-quality water with moderate salinity can sustain landscape restoration and ecosystem maintenance in arid areas (Gerhart et al., 2006). On the other hand half-strength seawater (17 g L^{-1} salt) could support plant growth, providing that sufficient concentrations of nitrogen and phosphorus are supplied (Sleimi and Abdelly, 2002). Therefore use of wastewater in agriculture is a reliable strategy to dispose nutrients without polluting aquifers. In the case of aquaculture consistent amounts of nutrients in wastewater (Piedrahita, 2003) have significant environmental impact (Handy and Poxton, 1993; Wu, 1995). Up to now aquaculture operations have diverted consistent investments in water treatment (Martins et al., 2010), which significantly reduced aquaculture profitability. Halophytes can efficiently remove up to 94-99% of nitrogen and phosphorus from saline aquaculture wastewater (Brown et al., 1999) and convert the cost for water treatments into revenues. Behind traditional agricultural systems aquaponics, the soilless plant cultivation on aquaculture water (Rakocy and Hargreaves, 1993), can enhance quality of productions through controlled growing conditions, plant nutrient optimization and increased levels of food safety. In the vision of sustainability reuse of wastewaters for fertilization could halve greenhouse gases emissions in agriculture (Franks and Hadingham, 2012) and create opportunities for value-added environmental services in marginal lands.

The objective of the present research was to assess the production, quality and nutrient uptake of Salsola soda under raising salinity. A nutrient uptake assessment was used to monitor aquaponic performance.

Materials and methods

Three consecutive trials were performed in a heated greenhouse at the experimental farm of the University of Tuscia, central Italy, between January 2011 and May 2011. Six independent 160 L aquaponic systems were used for the trials. Each system consisted of a 100L fish tank, a 5 L biofilter made with expanded clay and a 0.85 m^2 plant trough housing a 0.75 m^2 polystirene raft. Every trough was 7 cm deep and contained a water volume of 60 L. Water circulated in each system by means of an aquarium pump under a continuous flow rate of 180L h^{-1}.

Aquaponic systems were stocked on 11 January with mullet (*Mugil cephalus* L.) at 8.07 ± 0.11 kg m^{-3} to raise water nutrient levels prior trials commencement. For the second and the third trial fish stocking density was respectively 7.37 ± 0.11 kg m^{-3} and 7.40 ± 0.15 kg m^{-3}.

Two different salinities were set: a low salinity (LS) treatment with 5 g L^{-1} of marine salt at 10dS m^{-1} electrical conductivity (EC) and a high salinity (HS) treatment with 10 g L^{-1} salt and EC at 19dS m^{-1}. For the second trial salinity was raised up to 10 g L^{-1} (LS) and 20 g L^{-1} (HS), which

corresponded to 19 dS m^{-1} and 36 dS m^{-1} respectively. In the third trial HS salinity was raised up to 30 g L^{-1} (equivalent to 52 dS m^{-1}). Fish were fed a 54% protein diet (Nutra Pro 0, Skretting S.p.A., Mozzecane, Verona, Italy). Water temperature in all tanks was kept on average at 18°C ± 1 in the first and 22°C ± 1 in the second and third trial.

At startup each aquaponic system was added with small quantities of potassium (18.2 mg L^{-1}), phosphorus (12.4 mg L^{-1}), sulphur (20.3 mg L^{-1}), magnesium (14.2 mg L^{-1}) and iron (0.7 mg L-1) to raise the electrical conductivity (EC) by 0.15 dS m^{-1} and meet the nutrient concentrations of mature aquaponic systems. Systems pH was maintained at 6.5 ± 1.0 by addition of $CaCO_3$, KOH and $Ca(OH)_2$ in both aquaponic and hydroponic tanks.

For all trials aquaponics was compared against a hydroponic control containing: NO_3-N 196 mg L^{-1}, NH_4-N 21 mg L^{-1}, phosphorus 37.2 mg L^{-1}, potassium 195 mg L^{-1}, calcium 180 mg L^{-1}, sulphur 60 mg L^{-1}, magnesium 42 mg L^{-1}, manganese 0.2 mg L^{-1}, iron 0.7 mg L^{-1}, boron 0.5 mg L^{-1}, zinc 50 µg L^{-1}, copper 20 µg L^{-1} and molybdenum 3.2 µg L^{-1}. Electrical conductivity from fertilization alone was equivalent to 1.9 dS m^{-1}. Each hydroponic tank was added with 5 g L^{-1} of marine salt in the first trial and 10 g L^{-1} in the successive two trials. EC values in hydroponics were 12 dS m^{-1} in the first trial and 22 dS m^{-1} in the second and third trials. Hydroponic pH was maintained at 6.0 ± 1.0. Every treatment was replicated three times.

For each trial 3-week old *Salsola soda* seedlings were transplanted on floating rafts at 144 plants m^{-2}. Crop length was 35 days (1st trial) 32 days (2nd trial) and 30 days (3rd trial). Plant were harvested at flowering. The water-plant ratio set for each aquaponic and hydroponic system in the first two trials was 1.5 L $plant^{-1}$. The water-plant ratio in the third trial raised to 2.5 L $plant^{-1}$ in HS and 3.5 L $plant^{-1}$ in LS due to the reduced number of plants.

Plants were assessed for fresh weight. Roots were collected from each treatment and washed in distilled water to remove salt residues. Roots and shoots were dried in a forced-air oven at 80 °C for 72 h for dry biomass determination.

Electrical conductivity and pH were measured twice a week with Hanna HI98130 meter. Spectrophotometric measurement of water ammonia and nitrate occurred once a week following the ammonia nitrogen method by Anderson and Ingram (1989) and the salicil-solphoric acid method for nitrate nitrogen by Cataldo et al. (1975). Nitrogen levels in tissues were determined following kjeldhal method. Plants, fish, feed and system water were analyzed for their mineral profile with ICP-MS.

System performance was determined by measuring plants' nutrient uptake efficiency (NUE) (Van Eerd, 2007). NUE was calculated by dividing the nutrients stocked in plant shoot by net supplied nutrients, according to the equation:

NUE= [Nutr plants] / [Nutr supplied]

Net supplied nutrients accounted for the amount of mineral already stocked into the water and the quantity supplied by feed after fish uptake.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 16 for Windows). Percentages values were analyzed with square root or arc sine transformation. Duncan's multiple range test was performed at $p = 0.05$ on each of the significant variables measured.

Results

In the first trial salsola production (Tab. 1) was similar among treatments. Yields in every treatment were above 2.2 kg m^{-2}. Differences in biomass were noticed in the second trial, when LS aquaponic (10 g L^{-1} salt) outperformed by 45% ($p<0.05$) HS (20 g L^{-1} salt) and hydroponics (10 g L^{-1} salt). Similar results were obtained in the third trial with LS (10 g L^{-1} salt) obtaining 2.5 times more biomass than hydroponics under 10 g L^{-1} salt ($p<0.001$). HS on the contrary obtained half of the hydroponic production due to higher water salinity (30 g L^{-1} salt). No differences were noticed in root dry weight in the first two crops, but aquaponics LS had almost double root biomass ($p<0.01$) than the other two treatments in the third crop. In the first trial dry matter content was higher in hydroponics than HS and LS aquaponics ($p<0.01$) despite higher salt concentration in HS water. Plant dry matter content was not different among treatments in the second crop, but was 27% higher in HS ($p<0.001$) than hydroponics in the third crop due to very high salinity conditions.

Water macronutrients in aquaponics were constantly below hydroponic concentrations. Nitrate nitrogen in aquaponics (Fig. 1) at the beginning of the first trial was almost 10 times less the initial nitrate concentration of hydroponics ($p<0.001$). During the trials aquaponic nitrate raised constantly from 14.0 ± 1.8 mg L^{-1} (HS) and 19.1 ± 1.3 (LS) of the first trial up to 24.7 ± 6.1 (HS) and 23.8 ± 2.6 (LS) at the beginning of the second trial. Nevertheless nitrate values almost settled from the end of the second trial (39.0 ± 7.1 HS, 32.0 ± 8.1 LS) to the end of the third trial (42.4 ± 8.1 HS, 39.9 ± 11.7 LS).

Ammonia nitrogen levels in hydroponics were consistently higher than aquaponics ($p<0.05$) in all trials (Fig.1). A constant decline of hydroponics ammonia was observed due to nitrification. Values at the end of each trial were in fact 14% (1st trial), 22% (2nd trial) and 33% (3rd trial) lower than the initial concentration set at 21 mg L^{-1}. Ammonia levels in aquaponics were below 1 mg L^{-1} in the first and third trial, but higher in the second trial, with an average range of 5 mg L^{-1}.

Phosphorus (Fig.1) concentrations in aquaponics were 2-3 times lower than in hydroponics ($p<0.001$) and followed a downward trend that halved initial concentrations. Likewise aquaponics showed 5-10 times less potassium (Fig.1) than hydroponics ($p<0.01$). In fish-plant systems

potassium followed a negative trend with 70-85% decrease from the initial values in just 2 crops. Potassium concentrations were restored in the third crop to initial values due to pH adjustments with KOH. On the contrary calcium had a positive trend in aquaponics due to the continue use of $CaCO_3$ and $Ca(OH)_2$ to counterbalance the acidifying effect of nitrifying bacteria. Calcium (Fig.1) values in aquaponics raised 2-3 times the initial stock and reached similar levels of hydroponics ($p>0.05$) from the end of the first trial. Magnesium concentrations (Fig.1) in aquaponics raised to values similar to hydroponics ($p>0.05$) by the end of the first trial, However differences were noticed in LS ($p<0.01$) at the end of the third crop due to higher plant uptake as a consequence of increased yields.

Iron (Fig.2) concentrations in aquaponics were 5-10 times below hydroponics levels ($p<0.001$). Similarly boron (Fig.2) values were 4-7 times lower than hydroponics ($p<0.001$), but they appeared constant during the trials. Copper increased during the first trial due to fish treatment with $CuSO_4$ against saproleigna (0.16 mg L^{-1} of copper), however successive readings were seen in constant decline and sensitively lower than hydroponics ($p<0.001$). Similarly zinc raised in the first trial but decreased at the end of the third trial to levels that were significantly lower than hydroponics ($p<0.001$).

Elemental analysis in shoots (Tab. 2) did not show significant nitrogen differences. Values appeared constant in all three trials, irrespectively to the level of nitrogen or salt in water. Phosphorus and potassium concentrations were not different among treatments (Tab.2), although a 25-30% difference was observed in aquaponics in the third trial ($p<0.05$) due to higher accumulation in hydroponic shoots (Fig.1). Calcium levels were similar in the first two trials (Tab.2), but higher in LS aquaponics in the third trial ($p<0.001$). The sensitive increase in yields observed in LS (Tab.1) could have favoured a higher calcium accumulation in shoots due to evapotranspiration. No sodium differences were observed in shoots above water salt concentrations of 10 g L^{-1} (Tab.2). On the contrary chloride strictly followed water concentrations, with maximum shoot values observed in HS treatment ($p<0.05$). Micronutrients did not show significant differences among treatments for iron, copper, manganese and boron ($p>0.05$) (Tab.2). Nevertheless zinc was higher in aquaponic shoots ($p<0.01$) in the last two trials despite higher water concentrations were observed in hydroponics (Fig.2).

Roots (Tab.3) showed similar nitrogen concentrations of shoots (Tab.2), but differences were seen in higher-yielding treatments in the second and third trial ($p<0.05$). Although phosphorus and potassium levels (Tab.3) were similar to shoots (Tab.2), roots markedly followed water concentrations (Fig.1). Aquaponic phosphorus (Tab.3) was constantly 18% lower than hydroponics

($p<0.05$). Likewise a 45%-65% reduction in potassium (Tab.3) occurred when aquaponic water concentrations were below 17 mg L^{-1} (Fig.2).

No differences were noticed among treatments for calcium ($p>0.05$) (Tab.3), but higher levels of magnesium were found in aquaponic roots ($p<0.05$), which were also 30-40% higher than shoots (Tab.2). Sodium in aquaponics and hydroponics roots (Tab.3) was similar among treatments ($p>0.05$) for water salinity of 10-20 g L^{-1}, but changed sensitively with lower or higher water concentrations. Chloride followed a similar pattern to sodium, with no differences observed in the 10-20 g L^{-1} range

For both iron, copper, manganese and zinc (Tab.3) the level of metals present in roots was sensitively higher than shoots (Tab.2). In particular iron in roots was fairly constant among treatments but concentrations were 6-11 times higher than shoots, regardless water concentrations. A similar trend was noticed for copper, whose roots concentrations were 37 times (1^{st} crop) and 11 times (3^{rd} crop) higher than shoots. Copper in aquaponic roots was higher than hydroponics ($p<0.05$) and followed water concentrations (Fig.2). Zinc in root was 2-5 times higher than shoots and higher concentrations were always observed in aquaponics ($p<0.001$) regardless water concentrations (Fig.2).

Nutrient uptake efficiency (NUE) (Tab.4) assessed system/plant performance in utilizing a specific element. It was calculated as the ratio between the amount of nutrients immobilized by shoots from a given nutrient stock. For the first and second trials the system water volume available per plant was 1.5 L $plant^{-1}$ for both aquaponics and hydroponics. In the last trial the ratio in aquaponic systems changed into 2.5 L $plant^{-1}$ (HS) and 3.5 L $plant^{-1}$ (LS) due to the limited number of available seedlings. Nitrogen NUE were consistently higher in aquaponics ($p<0.01$) due to lower water nutrient concentrations (Fig.1). This advantage for aquaponics was maintained despite the increase in water nitrates and the raise in water/plant ratio occurred in LS and HS in the third trial. Phosphorus showed higher uptake values in aquaponics ($p<0.05$) in the second trial, but no differences were noticed in the third crop, following a fall in shoot concentrations (Tab.2) and the increase in water/plant ratio. Calcium, magnesium, sodium and chloride uptake efficiency (Tab.4) was proportional to yields (Tab.1) or to the higher aquaponic shoot concentrations. Iron and boron uptake was higher in aquaponics ($p<0.05$) due to lower water supplement (Fig.2).

Crop sink (Tab.5) refers to the whole amount of nutrients used to produce 1 kg of harvestable shoot (f.w.). The calculated inclusion accounted for both shoot and root uptake, which are shown in the table as a percentage of the total stripped nutrient. Aquaponics stripped less nitrogen than hydroponics in the first ($p<0.01$) and third trial ($p<0.001$). On the other side phosphorus use was similar in the first two trials but lower in LS in the last crop. Sodium and chloride sink was

proportional to salinity, but no differences were noticed among treatments for water sodium concentrations of 10-20 g L^{-1}. For all macronutrients the percentage of minerals retained by shoots was around 90% of the total. Metal uptake was not significantly different among treatments with the exception of zinc, whose uptake was higher in aquaponics. The percentage of micronutrients retained in roots was higher than macronutrients and accounted for 30-40% of the total uptake.

Discussion

Optimal Salsola soda yields (Tab. 1) were obtained with salt concentrations between 5 g L^{-1} and 10 g L^{-1}, equivalent to EC of 10 dS m^{-1} and 20 dS m^{-1}. The harvested biomass corresponded to a monthly production of 20-51 ton ha^{-1}, equivalent to 1.6-3.6 tom ha^{-1} in dry matter. The raise in yields observed in salsola from the first to the third trial was also due to increasing daylight, which was on average 10h 35m in the first crop, 12h 13min in the second crop, 13h 45min in the third crop. Salsola yields at 20 g L^{-1} salt (36 dS m^{-1}) were still comparable to hydroponics grown at lower salinity (10 g L^{-1}) and corresponded to 21.7 ton $ha^{-1}crop^{-1}$ of production (1.73 ton ha^{-1} d.w.). Increase in salinity up to 30 g L^{-1} (52 dS m^{-1}) in HS in the third trial depressed yields, which were 50% of hydroponics and 20% of LS aquaponics. Biomass data followed Masters et al. (2007) conclusions on optimal halophytes growth for salinity below 15 dS m^{-1}, although in the current trials EC values up to 20 dS m^{-1} appeared still competitive. Nevertheless *Salsola soda* showed similar growth responses to *Salsola kali*, for which moderate salinity (50-100 mM NaCl) enhanced production but higher salinity (200 mM NaCl) depressed biomass growth (Reinman and Breckle, 1995). Floating systems seemed a favouring factor for plants growing with high salinity: evidences from *Salsola kali* outlined infact that 400 mM of NaCl (24 g L^{-1}) was not vital for plants growing on substrate (Rilke, cited by Reinman and Breckle, 1995). In the present trials *Salsola soda* not only grew under very high salt conditions but showed optimal salinity ranges higher than 0.5% NaCl suggested by Onal (1969) for water culture of *Salsola kali*.

Na/Cl ratios assessed in shoots (Table 2) settled at costant values of 0.5-0.7 in both three trials. K/Na ratio fell with increased salinity, but fraction settled at 0.5-0.6 in the 10-20 g L-1 range to reach 0.35 under maximum salinity. Results followed Reinman and Breckle (1995) conclusions for some *Salsola kali* subspecies with falling K/Na trends and stable Na/Cl ratios below 1 under raising water salinity. Increased water salinity decreased potassium and magnesium and increased sodium levels, which normally occurs also in other salt tolerant species such as *Beta vulgaris* (Marschner et al., 1981), *Atriplex hortensis* (Jeschke & Stelter, 1983) or in most Chenopodiacee under saline stress (Jeschke, 1984; Flowers & Yeo, 1988).

Salsola soda showed good productivity with low nitrogen concentrations. The standard hydroponic fertilization rates did not give significant advantages to yields (Tab.1), but negatively affected NUE performances (Tab. 4). The nitrogen uptake efficiency, which was calculated under a water/plant ratio of 1.5 L plant^{-1} in both aquaponics and hydroponics, was from 50% to 300% higher in aquaponics than hydroponics. However, in the third trial NUE in aquaponic systems showed lower percentages due to higher water/plant ratios (2.5 L plant^{-1} in HS, 3.5 L plant^{-1} in LS against 1.5 L plant^{-1} in hydroponics), which may have suggested for a +60% NUE increase for nitrogen in aquaponics under the same water/plants conditions of hydroponics. Phosphorus uptake efficiency (Tab.4) in aquaponics was similar (1st trial) or higher (2nd crop) than hydroponics. Likewise phosphorus uptake efficiency in the third aquaponic trial would have been higher under similar water/plant ratios to hydroponics. Aquaponics proved a better metal uptake efficiency despite lower water concentrations (Tab.4). Contrarily to traditional vegetables salsola did not suffer from iron chlorosis, which suggests for a higher adaptability to oligotrophic conditions.

Crop sink assessment (Tab. 5) suggested that roots play an important role in metal removal, irrespectively to the salt content and metal concentrations. In the perspective of water reclamation strategies the contextual harvest of roots is fundamental to take metals off recirculating aquaculture systems.

Conclusions

Saline agriculture is as a valid strategy to produce food and energy in marginal areas where no alternative land uses are possible. The use wastewaters with moderate or high salinity can open opportunities to crop halophytes with relative nil impact on environment. Trials with *Salsola soda* grown on aquaponics showed that quality crops are possible with salinity levels compatible with marine fish. The high biomass production, the high NUE as well as the low levels of nutrient required in the nutritive solution makes salsola a candidate crop for food, energy or fodder productions. Nevertheless the high revenues obtainable from this vegetable, open up opportunities to develop integrated farming systems that can make better use of by-products from saline aquaculture operations.

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Tables

Table 1 Plant production in high salinity (HS) and low salinity (LS) aquaponics and hydroponics. Means ± SD.

treatment	Yields (kg m^{-2})	Plant fresh weight (g $plant^{-1}$)	Plant dry matter (%)	Root dry weight (g $plant^{-1}$)	shoot root ratio
			1st crop cycle		
aquaponics HS	2.35 ± 0.46	16.31 ± 3.19	6.25 ± 0.28 a	0.097 ± 0.009	10.43 ± 0.85 b
aquaponics LS	2.22 ± 0.24	15.44 ± 1.68	5.92 ± 0.07 a	0.109 ± 0.014	8.42 ± 0.16 ab
hydroponics	2.38 ± 1.65	16.54 ± 11.47	7.41 ± 0.44 b	0.164 ± 0.078	6.90 ± 1.62 a
significance	ns	ns	**	ns	*
			2nd crop cycle		
aquaponics HS	2.17 ± 0.41 a	15.10 ± 2.85 a	7.98 ± 0.07	0.117 ± 0.014	10.22 ± 0.91
aquaponics LS	3.16 ± 0.21 b	21.94 ± 1.44 b	6.36 ± 0.24	0.134 ± 0.008	10.38 ± 0.68
hydroponics	2.15 ± 0.43 a	14.90 ± 2.96 a	8.00 ± 1.52	0.120 ± 0.023	9.89 ± 1.70
significance	*	*	ns	ns	ns
			3rd crop cycle		
aquaponics HS	1.02 ± 0.25 a	7.09 ± 1.72 a	10.10 ± 0.07 c	0.08 ± 0.02 a	9.08 ± 0.42
aquaponics LS	5.14 ± 0.60 c	35.71 ± 4.15 c	6.36 ± 0.07 a	0.20 ± 0.03 b	11.32 ± 0.51
hydroponics	2.02 ± 0.30 b	14.04 ± 2.11 b	7.96 ± 0.77 b	010 ± 0.02 a	11.30 ± 3.09
significance	***	***	***	**	ns

Table 2 Plant macro and micronutrients in high salinity (HS) and low salinity (LS) aquaponics and hydroponics. Means ± SD.

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Cu	Mn	B	Zn
	g kg-1 d.w.							mg kg-1 d.w.				
						1st crop						
aquaponic HS	42.6 ± 0.7	5.9 ± 1.7	32.1 ± 2.3 ab	3.5 ± 0.6	1.7 ± 0.1	30.3± 3.7 b	57.0 ± 0.9 b	29.4 ± 9.8	2.5 ± 0.3	94.0 ± 28.3	40.6 ± 9.7	112.0 ± 28.6
aquaponic LS	41.4 ± 1.5	4.4 ± 1.6	21.1 ± 9.5 a	4.6 ± 2.0	2.2 ± 1.0	24.2 ± 1.2 a	45.0 ± 4.6 a	18.8 ± 8.5	3.0 ± 1.7	60.5 ± 23.9	27.5 ± 13.7	79.6 ± 42.3
hydroponics	41.4 ± 3.9	5.5 ± 0.6	38.0 ± 5.7 b	4.5 ± 0.9	1.7 ± 0.5	20.9 ± 1.4 a	40.6 ± 2.3 a	21.9 ± 3.2	3.7 ± 0.7	40.7 ± 5.5.	28.9 ± 3.9	51.1 ± 5.1
significance	ns	ns	*	ns	ns	*	*	ns	ns	ns	ns	ns
						2nd crop						
aquaponic HS	36.8 ± 0.9	5.6 ± 0.1	20.8 ± 3.5	2.5 ± 2.2	1.3 ± 0.0 b	39.5 ± 5.1	70.3 ± 4.9 b	20.8 ± 7.1	3.9 ± 0.1	88.1 ± 19.7	28.4 ± 4.3	97.5 ± 8.4 b
aquaponic LS	40.1 ± 1.3	5.9 ± 1.2	20.1 ± 3.6	5.4 ± 0.5	1.7 ± 0.1 b	35.3 ± 5.8	47.2 ± 9.8 a	21.5 ± 2.5	3.4 ± 1.0	72.1 ± 10.1	33.4 ± 0.4	104.9 ± 20.6 b
hydroponics	40.4 ± 2.3	5.1 ± 2.5	23.1 ± 14.6	3.3 ± 2.1	0.7 ± 0.5 a	31.0 ± 6.7	54.6 ± 4.8 a	17.9 ± 10.5	2.5 ± 1.7	44.6 ± 31.3	24.0 ± 12.5	29.3 ± 17.8 a
significance	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	**
						3rd crop						
aquaponic HS	34.5 ± 0.8	5.2 ± 0.9 a	15.5 ± 4.5	3.9 ± 0.6 a	1.3 ± 0.0 b	43.8 ± 6.0	76.2 ± 8.9 b	15.5 ± 3.3	3.9 ± 2.4	30.8 ± 17.2	22.0 ± 2.4	66.6 ± 8.4 b
aquaponic LS	38.9 ± 4.1	5.0 ± 0.6 a	23.9 ± 7.5	8.0 ± 0.4 b	1.9 ± 0.2 c	40.9 ± 8.7	54.1 ± 6.7 a	13.7 ± 1.7	5.6 ± 0.7	26.7 ± .16	27.7 ± 6.9	71.8 ± 4.2 b
hydroponics	38.2 ± 3.8	7.0 ± 0.8 b	28.7 ± 3.2	4.1 ± 0.4 a	0.9 ± 0.2 a	37.4 ± 5.4	69.2 ± 1.9 b	18.8 ± 2.6	3.8 ± 1.0	42.8 ± 8.9	32.4 ± 2.6	33.6 ± 4.4 a
significance	ns	*	ns	***	***	ns	*	ns	ns	ns	ns	***

Table 3. Root macro and micronutrients in high salinity (HS) and low salinity (LS) aquaponics and hydroponics. Means ± SD.

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Cu	Mn	B	Zn
	g kg-1 d.w.							mg kg-1 d.w.				
						1st crop						
aquaponic HS	39.6 ± 2.3	4.8 ± 0.7 a	34.5 ± 8.1	6.3 ± 2.8	2.5 ± 0.5 ab	28.0 ± 6.0 b	49.0 ± 5.4 b	182.9 ± 77.5	93.7 ± 3.5 b	228.2 ± 45.2	11.9 ± 1.4 a	494.4 ± 67.7 b
aquaponic LS	44.0 ± 2.0	5.1 ± 0.8 a	24.8 ± 5.6	5.2 ± 0.8	3.2 ± 0.5 b	15.5 ± 3.5 a	30.2 ± 3.6 a	157.5 ± 22.7	78.8 ± 17.3 b	277.3 ± 45.7	14.6 ± 1.5 b	414.1 ± 50.3 b
hydroponics	42.3 ± 2.6	6.5 ± 0.5 b	29.7 ± 4.5	5.2 ± 0.5	1.8 ± 0.3 a	15.9 ± 1.8 a	34.9 ± 3.9 a	184.5 ± 31.2	37.3 ± 8.2 a	166.6 ± 45.7	16.8 ± 0.7 b	53.0 ± 5.1 a
significance	ns	*	ns	ns	*	*	**	ns	**	ns	**	***
						2nd crop						
aquaponic HS	38.4 ± 2.9 a	5.9 ± 0.3 a	17.4 ± 4.9 a	8.0 ± 2.2	2.0 ± 0.2 b	27.9 ± 5.6	57.9 ± 7.4	123.1 ± 3.2	72.1 ± 5.0 b	141.5 ± 10.6	11.5 ± 0.3	524.4 ± 45.4 b
aquaponic LS	43.2 ± 0.6 b	5.9 ± 0.5 a	14.0 ± 4.8 a	8.0 ± 0.2	2.3 ± 0.4 b	21.8 ± 5.4	43.6 ± 5.3	186.1 ± 38.6	81.9 ± 28.2 b	118.6 ± 28.9	12.3 ± 1.5	577.7 ± 95.8 b
hydroponics	37.2 ± 2.2 a	7.3 ± 0.7 b	31.4 ± 5.0 b	7.0 ± 0.6	1.5 ± 0.1 a	18.1 ± 4.5	51.6 ± 4.7	135.8 ± 27.0	29.7 ± 10.6 a	103.2 ± 17.1	13.8 ± 0.2	63.5 ± 9.4 a
significance	*	*	*	ns	*	ns	ns	ns	*	ns	ns	***
						3rd crop						
aquaponic HS	35.6 ± 1.7 a	7.5 ± 0.4 a	31.6 ± 10.7	8.9 ± 5.0	2.4 ± 0.6 b	34.4 ± 1.2 b	91.0 ± 12.9 b	174.5 ± 52.4	49.2 ± 9,3	64.1 ± 30.0 a	10.9 ± 0.2 a	228.8 ± 71.7 b
aquaponic LS	41.9 ± 2.4 b	7.7 ± 0.5 a	25.4 ± 4.0	6.8 ± 1.3	3.1 ± 0.1 c	23.5 ± 1.2 a	52.2 ± 3.8 a	116.0 ± 45.4	64.9 ± 4.7	35.0 ± 8.0 a	11.7 ± 0.4 a	169.6 ± 52.8 b
hydroponics	40.7 ± 1.6 b	10.4 ± 0.4 b	33.8 ± 7.2	5.3 ± 0.8	1.6 ± 0.1 a	24.4 ± 1.4 a	53.6 ± 7.4 a	113.2 ± 15.7	55.7 ± 23.3	141.3 ± 41.2 b	14.8 ± 1.0 b	57.0 ± 7.5 a
significance	*	***	ns	ns	**	***	**	ns	ns	*	***	*

Table 4 Nutrient Uptake efficiency (NUE) in high salinity (HS) and low salinity (LS) aquaponics and hydroponics. Means ± SD

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Cu	B	Zn
	1st crop							1st crop			
aquaponics HS	41.4 ± 11.3 b	21.0 ± 7.2	58.3 ± 17.9 b	0.5 ± 0.1	4.6 ± 1.2	0.8 ± 0.3 a	0.7 ± 0.1	12.0 ± 3.3 b	0.9 ± 0.1 a	35.2 ± 12.5 b	41.2 ± 8.9
aquaponics LS	32.8 ± 10.3 b	13.9 ± 7.1	42.8 ± 7.9 b	0.6 ± 0.3	5.6 ± 3.4	1.6 ± 0.4 b	1.0 ± 0.2	6.7 ± 4.1 a	0.9 ± 0.6 a	19.1 ± 11.2 ab	28.3 ± 20.2
hydroponics	10.8 ± 4.4 a	8.3 ± 3.1	11.2 ± 5.0 a	0.6 ± 0.1	2.0 ± 0.2	0.6 ± 0.2 a	0.8 ± 0.3	3.1 ± 1.4 a	4.6 ± 1.6 b	4.2 ± 1.4 a	23.6 ± 9.2
significance	**	ns	**	ns	ns	*	ns	*	**	*	ns
	2nd crop							2nd crop			
aquaponics HS	27.5 ± 2.7 b	32.8 ± 12.4 b	na	0.9 ± 0.1 a	4.6 ± 0.8 b	0.7 ± 0.0 a	0.6 ± 0.1	10.5 ± 3.5 b	4.9 ± 1.5	45.4 ± 8.6 b	27.2 ± 0.9
aquaponics LS	36.5 ± 1.3 c	37.6 ± 8.9 b	na	3.1 ± 0.5 b	8.7 ± 1.6 c	1.9 ± 0.3 b	1.1 ± 0.3	13.3 ± 2.5 b	5.1 ± 1.3	63.8 ± 10.8 c	38.6 ± 8.4
hydroponics	17.6 ± 4.6 a	12.3 ± 5.5 a	10.4 ± 4.2	1.2 ± 0.7 a	1.5 ± 0.8 a	0.9 ± 0.4 a	0.9 ± 0.2	4.0 ± 2.4 a	5.1 ± 3.5	5.9 ± 3.3 a	21.2 ± 11.6
significance	**	*	na	*	***	**	ns	*	ns	***	ns
	3rd crop							3rd crop			
aquaponics HS	10.9 ± 1.5 a	12.6 ± 4.6	14.2 ± 6.1 a	0.3 ± 0.0 a	0.9 ± 0.3 a	0.1 ± 0.0 a	0.1 ± 0.1 a	3.9 ± 1.3	2.1 ± 1.5 a	10.0 ± 4.4 a	10.1 ± 2.4 a
aquaponics LS	28.9 ± 9.9 b	21.2 ± 4.6	40.6 ± 18.3 b	1.6 ± 0.2 b	3.8 ± 0.2 c	0.9 ± 0.2 b	0.4 ± 0.4 b	8.2 ± 4.1	7.6 ± 2.5 b	32.0 ± 5.6 b	25.0 ± 7.9 b
hydroponics	13.8 ± 2.1 a	14.5 ± 3.5	11.2 ± 2.1 a	1.5 ± 0.2 b	1.6 ± 0.1 b	0.9 ± 0.3 b	1.0 ± 0.2 b	3.6 ± 0.9	6.3 ± 0.7 b	6.7 ± 1.5 a	20.9 ± 4.1 b
significance	*	ns	*	***	***	***	***	ns	*	***	*

Table 5 Crop uptake per kg of shoot produced (f.w.) and shoot-root partition in high salinity (HS) and low salinity (LS) aquaponics and hydroponics. Means ± SD

Treatments	N	P	K	Ca	Mg	Na	Cl	Fe	Cu	B	Zn
	g Kg-1 (fw)							mg Kg-1 (fw)			
	1st crop							1st crop			
aquaponics HS	2.90 ± 0.10 a (92% - 8%)	0.40 ± 0.13 (93% - 7%)	2.21 ± 0.12 a (91% - 9%)	0.26 ± 0.07 (86% - 14%)	0.12 ± 0.01 (88% - 12%)	2.05 ± 0.18 b (92% - 8%)	3.85 ± 0.16 c (92% - 8%)	2.99 ± 1.17 (63% - 37%)	0.72 ± 0.09 (22% - 78%)	2.62 ± 0.69 (97% - 7%)	10.05 ± 2.73 (70% - 30%)
aquaponics LS	2.76 ± 0.09 a (89% - 11%)	0.29 ± 0.09 (86% - 14%)	1.74 ± 0.03 a (90% - 10%)	0.31 ± 0.12 (86% - 14%)	0.15 ± 0.07 (83% - 17%)	1.54 ± 0.06 a (93% - 7%)	2.88 ± 0.29 a (93% - 7%)	2.22 ± 0.45 (48% - 52%)	0.73 ± 0.22 (23% - 77%)	1.73 ± 0.82 (92% - 8%)	7.65 ± 2.80 (58% - 42%)
hydroponics	3.54 ± 0.30 b (86% - 14%)	0.48 ± 0.07 (85% - 15%)	3.16 ± 0.39 b (89% - 11%)	0.39 ± 0.09 (85% - 15%)	0.14 ± 0.04 (86% - 14%)	1.75 ± 0.17 a (90% - 10%)	3.41 ± 0.16 b (88% - 12%)	3.74 ± 0.66 (45% - 55%)	0.71 ± 0.21 (41% - 59%)	2.34 ± 0.42 (92% - 8%)	4.37 ± 0.13 (86% - 14%)
significance	**	ns	***	ns	ns	*	**	ns	ns	ns	ns
	2nd crop							2nd crop			
aquaponics HS	3.23 ± 0.06 (91% - 9%)	0.49 ± 0.01 (91% - 9%)	1.79 ± 0.30 (92% - 8%)	0.34 ± 0.07 (82% - 18%)	0.12 ± 0.00 b (87% - 13%)	3.37 ± 0.39 (93% - 7%)	6.06 ± 0.48 c (93% - 7%)	2.62 ± 0.51 (62% - 38%)	0.88 ± 0.07 (36% - 74%)	2.35 ± 0.32 (96% - 4%)	11.87 ± 0.67 b (65% - 35%)
aquaponics LS	2.81 ± 0.09 (91% - 9%)	0.42 ± 0.09 (91% - 9%)	1.36 ± 0.21 (94% - 6%)	0.39 ± 0.05 (87% - 13%)	0.12 ± 0.01 b (88% - 12%)	2.37 ± 0.33 (94% - 7%)	3.28 ± 0.73 a (92% - 8%)	2.54 ± 0.57 (55% - 45%)	0.71 ± 0.19 (30% - 70%)	2.20 ± 0.06 (97% - 3%)	10.21 ± 1.38 b (65% - 35%)
hydroponics	3.53 ± 0.66 (91% - 9%)	0.46 ± 0.18 (85% - 15%)	2.01 ± 0.88 (85% - 15%)	0.31 ± 0.16 (78% - 22%)	0.07 ± 0.03 a (79% - 21%)	2.81 ± 0.93 (95% - 5%)	4.74 ± 0.59 b (91% - 9%)	2.52 ± 1.21 (52% - 48%)	0.44 ± 0.24 (40% - 60%)	2.00 ± 1.04 (93% - 7%)	2.78 ± 1.38 a (78% - 22%)
significance	ns	ns	ns	ns	*	ns	**	ns	ns	ns	***
	3rd crop							3rd crop			
aquaponics HS	3.89 ± 0.06 b (90% - 10%)	0.61 ± 0.09 b (86% - 14%)	1.92 ± 0.57 (82% - 18%)	0.49 ± 0.11 ab (80% - 20%)	0.16 ± 0.01 b (83% - 17%)	4.80 ± 0.57 b (92% - 8%)	8.63 ± 1.00 c (89% -11%)	3.51 ± 0.68 b (45% - 55%)	0.94 ± 0.24 (39% - 61%)	2.35 ± 0.26 (95% - 5%)	9.26 ± 1.59 c (73% - 27%)
aquaponics LS	2.71 ± 0.24 a (91% - 9%)	0.36 ± 0.03 a (88% - 12%)	1.66 ± 0.51 (91% - 9%)	0.54 ± 0.03 b (93% - 7%)	0.14 ± 0.01 b (87% - 13%)	2.73 ± 0.54 a (95% - 5%)	3.72 ± 0.39 a (92% - 8%)	1.52 ± 0.32 a (58% - 42%)	0.72 ± 0.08 (49% - 51%)	1.83 ± 0.45 (96% - 4%)	5.53 ± 0.55 b (83% - 17%)
hydroponics	3.32 ± 0.05 c (91% - 9%)	0.63 ± 0.06 b (88% - 12%)	2.52 ± 0.12 (90% - 10%)	0.37 ± 0.01 a (90% - 10%)	0.08 ± 0.01 a (85% - 15%)	3.18 ± 0.67 a (94% - 6%)	5.91 ± 0.64 b (93% - 7%)	2.31 ± 0.08 a (64% - 36%)	0.68 ± 0.06 (44% - 56%)	2.68 ± 0.27 (96% - 4%)	3.07 ± 0.17 a (86% - 14%)
significance	***	**	ns	*	***	*	**	**	ns	ns	***

Figures

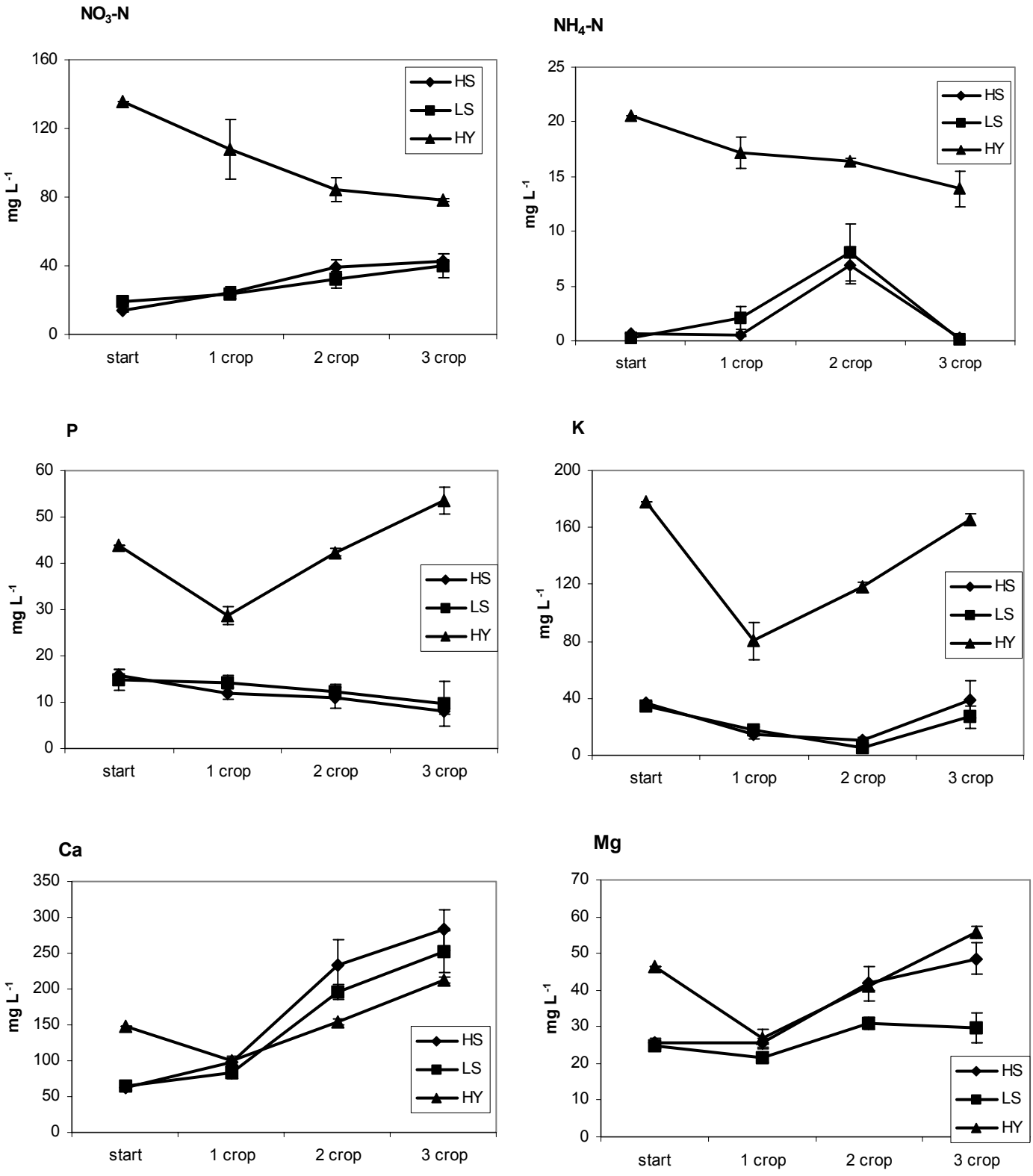

Figure 1 Nitrate (NO_3-N), ammonia (NH_4-N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) concentrations (mg L^{-1}) in high salinity aquaponics (HS), low salinity aquaponics (LS) and hydroponics (HY). Means and standard error

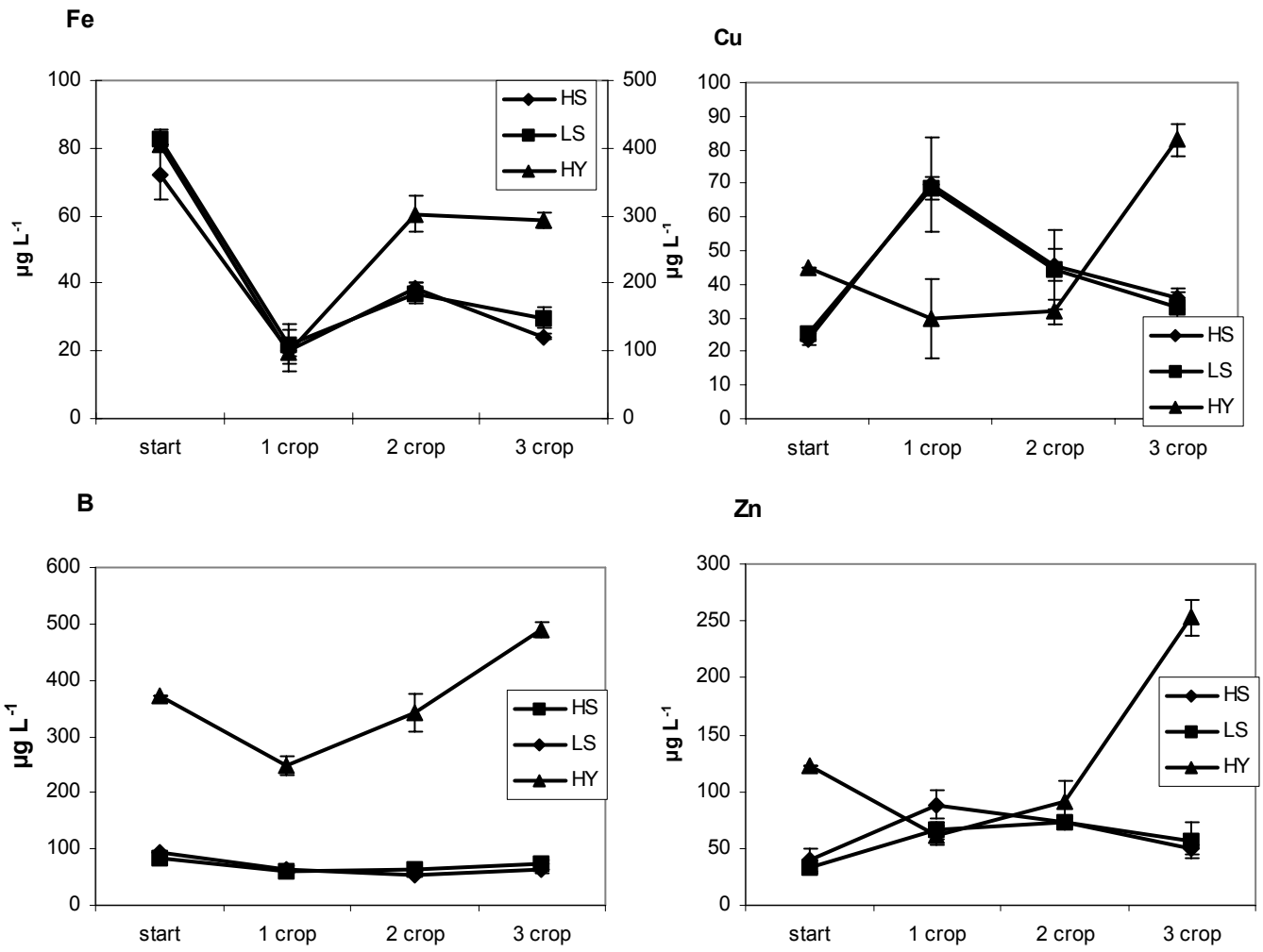

Figure 2. Iron (Fe), copper (Cu), boron (B) and zinc (Zn) concentrations (µg L^{-1}) in high salinity aquaponics (HS), low salinity aquaponics (LS) and hydroponics (HY). Means and standard error

General conclusions

Aquaponics is as productive as hydroponics. The experimental observations from most trials outlined that nitrogen levels 3-7 times lower than those used in hydroponics were sufficient to support very high productivity. In the case of lettuce results showed that above certain concentrations plant uptake was constant and supported growth fairly well.

In the case of fruity plants production was similar to hydroponics despite levels of nutrients 3-6 times lower than optimal concentrations suggested in literature. Nevertheless nutrition balance played a key role in plant production. In the case of cucumber similar yields observed in both aquaponics and hydroponics was the result of good N:K balance. Literature data from other aquaponic trials confirm a correlation between low N:K ratios and fruit productivity. Being aquaponics a potassium limiting environment, adeguate nutrient management is thus a fundamental aspect that must be taken into account.

Integrated system do have the big potential to improve agroecosystems' sustainability. Nevertheless performances are always a factor influenced by sub-system design and management. Beyond optimal pools of nutrients the delivery system plays a key role in productivity. A clear example was provided by comparing plants' performances in floating system, NFT and soilless substrate system. Productivity was not only the result on how much concentrated the nutrients were or how deeply roots were in contact with the nutritive solution, but also in how fast the nutrients flew through the roots. In the goal of enhancing system performance these three conditions should be carefully accounted for planning efficient aquaponic sub-system designs.

On the qualitative point of view aquaponics did not differ from hydroponics. No significant differences were noticed neither in chlorophyll, leaf area and nitrates of leafy vegetables, nor in the acidity, sweetness and dry matter of fruity vegetables. As far as food safety is concerned sterilization can indeed guarantee for bacteria-free productions, even with high contamination levels by fish excreta. Nevertheless sub-system choice would favourably improve either quality of productions and food safety issues. In the case of NFT the small amount of water used by the system would be a cost-saving choice for sterilization, but similar results might be also achievable by flowing clean water before harvest to get rid of bacteria at root level and reduce leaf nitrate content through plant starvation.

Increased productivity and sustainability of agroecosystems should further consider input and plant management an important factor to improve overall system efficiency. In the case of sweet basil high leaf/steam ratios resulting from optimal planting density would avoid excessive waste of unusuable/unmarketable parts of the plants. At the same time waste management/recycle of plant by-products, as in the case of micro-nutrients rich roots, would allow the recovery of a consistent

percentage of the total stripped nutrients back into the systems. Nevertheless, nutrient efficiency is the key to raise performances and sustainability. On this point an important aspect outlined by aquaponics was the higher water volume needed for fish/plant integrated management. If lower water volumes would be a risk factor for fish due to reduced dilution of toxic metabolites, it would undoubtely enhance nutrients concentrations to the benefit of plant uptake and bioremediation effect of plants. Floating system would thus appear a less performing system for its higher volume of water par rapport to less-consuming systems such as NFT .

In the envision of sustainability system integration is the key factor for low input productions. However, agroecosystems' complexity derived by fish and plant integration should take into account of the increased management and environmental needs of plants, animals and their surrounding habitat. Aquaponics is as efficient as hydroponics in producing high quality food. However the full expansion of every integrated system would be only possible when products will show lower production costs than traditional agriculture, or when aquaponics will prove higher and faster retuns on investments than hydroponics. The key for agroecological sustainability would eventually be the perfect trade-off among environment, social and economic sustainability.