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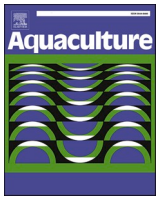

Effects of water salinity in an aquaponic system with rainbow trout (*Oncorhynchus mykiss*), black bullhead catfish (*Ameiurus melas*), Swiss chard (*Beta vulgaris*), and cherry tomato (*Solanum lycopersicum*)

Francesco Bordignon [a,*], Marco Birolo [a], Cecilia Fanizza [a,b], Angela Trocino [a,c], Giulia Zardinoni [a], Piergiorgio Stevanato [a], Carlo Nicoletto [a], Gerolamo Xiccato [a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy
[b] Institute of Animal Science and Technology, Group of Aquaculture and Biodiversity, Universitat Politècnica de València, Camino de Vera 14, 46071 València, Spain
[c] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy

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ABSTRACT

This study evaluated the role of increasing salinity in brackish-water aquaponics, also defined as haloponics, during an 8-month cycle characterized by two fish monoculture phases in autumn and spring and a polyculture phase in winter. The effects of three water salinity levels (low: 0.5‰; medium: 3.0‰; high: 6.0‰) were assessed on the health, growth performance and carcass traits of black bullhead catfish (*Ameiurus melas*) and rainbow trout (*Oncorhynchus mykiss*), and the concurrent production of Swiss chard (*Beta vulgaris*, ssp. vulgaris) and cherry tomato (*Solanum lycopersicum*). During 268 days, from September to June, a total of 261 catfish (initial weight 147 ± 22 g) were distributed in the nine units of an experimental aquaponic system (three units per treatment; initial stocking density 8.50 kg m^{-3}). In December, 150 trout (initial weight 153 ± 22 g) were added to the system (initial stocking density 5.06 kg m^{-3}) and reared for 103 days with catfish. During the trial, two growing cycles of Swiss chard and one of cherry tomato were carried out. Water microbiota communities were dominated by the phyla of Proteobacteria (60%) and Bacteroidota (19%), followed by Actinobacteriota, Cyanobacteria, Fusobacteriota, Patescibacteria, and Firmicutes. The Shannon entropy index for alpha-diversity decreased when the water salinity increased, where low and high salinities groups significantly differed. As for the 20 most abundant genera, differences according to water salinities were found on their relative abundance. Water salinity did not affect the final weight (348 g, on average), eviscerated carcass yield (87.5%) and fillet (52.0%) yields of trout, as well as the final weight (193 g), eviscerated carcass yield (84.1%) and fillet yield (48.3%) of catfish. Water salinity did not affect the fillet chemical composition in both species. At low salinity, the fillet fatty acid profile of catfish showed lower (−6.5%; $P < 0.05$) MUFA and higher PUFA (+6.8%; $P < 0.05$) and n-6 (+5.9%; $P < 0.05$) proportions compared with high salinity. At high and medium salinity, Swiss chard showed higher total yield (+61%; $P < 0.01$) in the first production cycle and higher total (+28%; $P < 0.01$) and marketable (+32%; $P < 0.001$) yield in the second production cycle compared with low salinity, whereas cherry tomato truss weight was lower (−32%: $P < 0.001$) at high compared with medium and low salinity.

The use of brackish water until 6‰ proved to be a viable alternative to reduce freshwater consumption, without impairing fish and leafy vegetable growth or the overall balance of the aquaponic system.

1. Introduction

The long-term success of aquaculture production is closely related to sustainable farming practices (Stevens et al., 2018), based on a reduced use of non-renewable resources (e.g. water, land, and fossil fuels) and on the recycling of nutrients (Lennard and Goddek, 2019). Aquaponics, combining recirculating aquaculture systems (RAS) with hydroponic cultivation, perfectly integrates with the concept of sustainable development of agriculture (Asciuto et al., 2019). Then, since freshwater availability for food production (agriculture and aquaculture) is diminishing and soil salinity is progressively increasing in many parts of the world (Gunning et al., 2016; Turcios and Papenbrock, 2014), the

* Corresponding author.
E-mail address: francesco.bordignon@unipd.it (F. Bordignon).

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interest in the use of brackish waters increases (Verma et al., 2023), together with the interest in farming of euryhaline fish (Gunning et al., 2016; Rossi et al., 2021). Until now, freshwater aquaponic systems have given good results in terms of fish and vegetable production and quality (Birolo et al., 2020; Bordignon et al., 2022; Fischer et al., 2021), whereas haloponics, using brackish water, would be the next step for innovation and sustainability. Nevertheless, to date, only few studies have been carried out in haloponics setups, using seabream (*Sparus aurata*) and halophyte vegetables (rock samphire; *Crithmum maritimum*) (Vlahos et al., 2019), Nile tilapia (*Oreochromis niloticus*) and lettuce (Lenz et al., 2017) or spinach (Thomas et al., 2019, 2021), or tilapia in combination with various vegetables, herbs, and plants (Kotzen and Appelbaum, 2010). In fact, a few edible horticultural species can be cultivated at medium salinity (5–10‰), such as Swiss chard (*Beta vulgaris* var. *cicla*), sea beet (*Beta vulgaris* var. *maritima*), common tomato (*Lycopersycon esculentum*), cherry tomato (*Lycopersycon esculentum* var. *cerasiformee*), and basil (*Ocimum basilicum*) (Kotzen and Appelbaum, 2010). Differently, several reared fish species are able to adapt to changes in water salinity, such as rainbow trout (*Oncorhynchus mykiss*) (Tian et al., 2022), European seabass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), mullets (*Mugil/Chelon* spp.), and croaker (*Umbrina cirrosa*) (Rossi et al., 2021; Verma et al., 2023). All these species, however, are demanding for high water quality. On the other hand, the black bullhead catfish (*Ameiurus melas*) is highly adaptable to water turbidity, low oxygen concentration, and high temperature (Roncarati et al., 2014). Despite no specific information is available about its salinity tolerance, wild specimens have been found in brackish environments with a salinity of up 13.8‰ (Bringolf et al., 2005; García-de-Lomas et al., 2009). Thus, the black bullhead catfish could be and interesting candidate for haloponics, particularly in systems with low technological input and environmental control. However, few data are available on its growth performance in closed systems (Roncarati et al., 2014) and no information is available about its flesh traits. On the other hand, the farming of rainbow trout could improve the competitiveness and profitability of haloponic productions.

The setting up and operation control in aquaponic systems can be challenging due to the asynchronous growth cycles of fish and plants. Additionally, especially in low-input systems characterized by low or null water quality control, fish and plant species selection mainly depends on temperature. Additionally, in haloponic systems, another issue is related to water salinity, which influences fish movement, feed intake (Mattioli et al., 2017), and digestive enzyme activity (Kemp, 2009), besides nitrogen excretion and oxygen consumption (Altinok and Grizzle, 2003, 2004).

Thus, the present study evaluated the effect of increasing water salinity content on water characteristics and microbiota, fish adaptability, production performance, and fillet characteristics of black catfish and rainbow trout, and on the Swiss chard and cherry tomato yields in a haloponic system, where the rearing cycles of the two fish partially overlapped, resulting in a polyculture phase during winter.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethical Committee for Animal Experimentation (Organismo per la Protezione del Benessere Animale, OPBA) of the University of Padova (project no. 17/2021; prot. no. 15481 of February 1st, 2021). Fish were handled by animal specialists (PhD or MSc in Animal Sciences) and veterinary practitioners.

2.2. Equipment

The trial lasted from September to June (268 days). The haloponic system was placed in a greenhouse tunnel at the Experimental Farm "L. Toniolo" of the University of Padua.

The system used nine independent experimental units, each made up of different functional components: a tank for rearing fish (PVC, volume 500 L, height 0.80 m, diameter 0.90 m); a 100-L settler; two tanks for growing plants in hydroponics (volume 275 L each, height 0.35 m, diameter 1.00 m, total cultivation area 1.6 m^2) filled with 225 L of expanded clay (LECA Laterlite, Solignano, Italy; specific surface 250 m^2 m^{-3}, packing density 300 kg m^{-3}, total porosity 0.55 m^3 m^{-3}); a water collection tank (volume 50 L; height 0.45 m); a 33-W immersion pump (NEWA Jet 1700, NEWA Tecno Industria Srl, Loreggia, Italy); two 106-W aerators (Scubla, Remanzacco, Udine, Italy). The tanks were filled with municipal water without any pre-treatment. Inside each experimental unit (Fig. 1), water continuously flowed between the components, which were positioned at different heights. In details, the water flowed from the tank for rearing fish to the settler for decanting the solid particles (removed at the end of each crop cycle). From the settler, the water overflowed into the hydroponic tanks filled with the expanded clay. The clay worked as a growing substrate for plants and as a biofilter. Finally, the water flowed inside a water collection tank, in which there was an immersion pump which returned the water to the tank with fish. The decrease in the water level, mainly due to evapotranspiration, was daily measured at the level of the water collection tank and the water level was restored accordingly using municipal water. The salinity of the water added to the experimental units to restore water level was checked once a week.

2.3. Production cycle

The production cycle was divided into three phases (Fig. 2), i.e. autumn, winter, and spring, designed considering the expected yearly temperature fluctuations in the experimental site (Legnaro, PD, Italy) (45°20′ N, 11°57′ E, 6 m above s.l.) (ARPAV, 2022).

In the first phase, (Autumn: October–December; 94 days) the black bullhead catfish were reared in monoculture and a first crop cycle with Swiss chard ran. Then, during the second phase (Winter: December–March; 103 days), rainbow trout were added in the tanks resulting in a polyculture phase with black bullhead catfish and a second cycle of Swiss chard. During winter, the expected temperature range was below the feeding temperature range of most of the catfish species, whereas optimal for rainbow trout growth (10–15 °C), therefore guaranteeing a continuity in the production of organic and nitrogenous substances, useful for the functioning of the biofilter and for plant nutrition. At the end of the second (polyculture) phase, trout reached the commercial size and were removed from the tank. Finally, during the third phase (spring: March–June; 74 days), black bullhead catfish were kept in monoculture and a concurrent cherry tomato (*Solanum lycopersicum*) crop cycle started in March which continued after catfish rearing ended (until September).

2.4. Animals, plants, and experimental design

The fish used in the experimental trial were purchased from two Italian commercial farms (black bullhead catfish: Vicenzi Persici, Finale Emilia, Modena; rainbow trout: Troticoltura Santa Cristina, Quinto di Treviso, Treviso). Both species were transported to the experimental farm by authorized transport means with constantly monitoring of the water temperature and oxygen levels. Once arrived at the experimental facilities, the fish were mildly anesthetized with a 10% clove essential oil solution (0.4 ml L^{-1} of water), weighed, and distributed in the fish rearing tanks, by balancing the initial biomass.

A randomized block experimental design was used with three replicates per treatment, i.e., low, medium, and high water salinity.

The catfish (261 fish; initial weight 147 ± 52 g) were transported to the experimental facilities in September, randomly allocated in the tanks, and assigned to the three experimental treatments (29 fish per tank, 87 per experimental treatment). After an adaptation period of 21 days (0.5 ‰ of water salinity in all the experimental tanks), the water

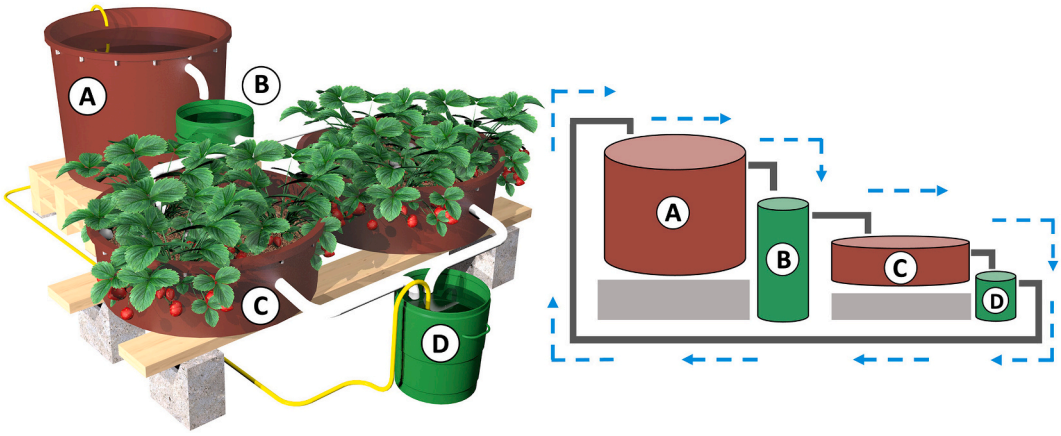

Fig. 1. Working diagram of the experimental aquaponic unit; A) fish tank; B) settling vessel; C) tanks for vegetables/biofilters; D) storage tank in which the water was collected before returning into the fish tank. Blue arrows represent the water flow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

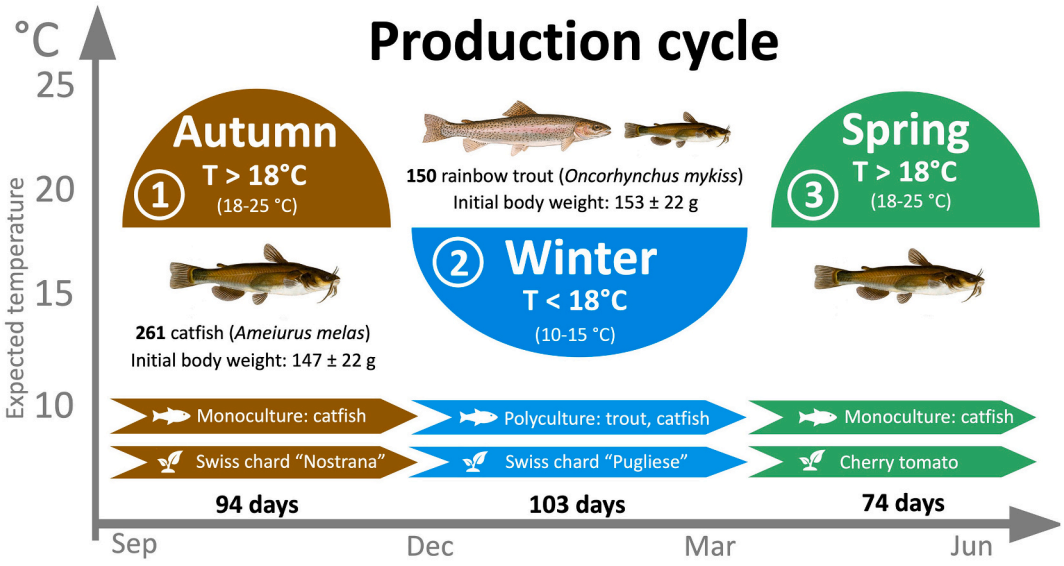

Fig. 2. Scheme of the three phases of the production cycle.

salinity was gradually increased (0.25 salinity points per day) until 3‰ in the medium- and 6‰ in the high-salinity systems. At the end of the first phase, 150 rainbow trout (initial average weight 153 ± 22 g) were added to the tanks with fish (16–17 trout per tank, 50 trout for experimental treatment).

The fish were weighed at the beginning of the trial and, then, once per month. Before each weighing, the fish were placed in a tray with the same water salinity used for farming and slightly anesthetized. Once weighed, the fish were put back into the corresponding rearing tank. Health status of the fish were checked daily, together with removing and recording any deaths.

In the initial and final periods, when the black bullhead catfish were alone, fish were fed with an extruded commercial diet for catfish (Veronesi, San Martino Buon Albergo, Verona, Italy) characterized by a crude protein content of 38%, crude fat 14%, ash 5.2%, crude fibre 4% (as-fed basis). During the second phase (polyculture) rainbow trout and catfish were fed with an extruded commercial diet for rainbow trout (Aller Aqua, Christiansfeld, Denmark) characterized by a crude protein content of 43%, crude fat 22%, ash 7%, crude fibre 2% (as-fed basis).

The feed was distributed twice a day (9:00 h and 14:00 h), six days a week, until apparent visual satiation. Feed conversion ratio (per tank) was calculated as total feed intake (g) / [final biomass (g) – initial biomass (g)].

Two tanks for the horticultural plants were associated with each tank for fish rearing, in which the two cycles of Swiss chard (*Beta vulgaris* L. subsp. *vulgaris* Cicla group) were carried out. Twelve plants per tank were transplanted (24 plants per experimental unit).

After harvesting, Swiss chard plants were weighed, and the parts of the plant separated to measure the total and marketable yields. Cherry tomato plants were transplanted at the stage of four expanded leaves (5 plants/m^2; 8 plants per experimental unit). After 76 days, a collection of fruits was performed starting from the base of the plant, collecting one truss per plant. The truss was weighed and the number of mature fruits counted.

2.5. Water quality

During the trial, the water temperature, dissolved oxygen content, and oxygen saturation were daily monitored using a portable multimeter (Handy Polaris, Oxyguard International, Farum, Denmark). Twice per week, the water pH was measured with a laboratory pH meter (Sension Ph1, Hach Lange S.r.l., Lainate, Milano, Italy) and the water

turbidity was assessed with a portable turbidimeter (HACH 2100P, Hach Lange S.r.l.). Once a week, total ammonia nitrogen (TAN) was measured with a specific kit (Ammonia Assay Kit, Megazyme, Bray, Co. Wicklow, Ireland). To determine salinity, electrical conductivity was measured once a week using a portable multi-parameter apparatus (HQ40d Portable Multi-Parameter Meter, Hach Lange GmbH, Germany). Then, electrical conductivity was converted into salinity using a calibration curve prepared in our lab following the method described in Comina et al. (2013).

2.6. Microbiota analysis of water

At the end of the second phase, i.e., at the end of the polyculture phase, 30 ml of water was sampled from each tank in a sterile Falcon tube and stored at −80 °C. Bacteria DNA extraction was performed with the following protocol: Falcon tubes were centrifuged at 5500 rpm for 10 min. The first supernatant was removed and 300 μL of RLT buffer was added. After this, the samples were transferred to a 2 mL Eppendorf tube and subjected to enzymatic treatment with Proteinase K (Thermo Fisher Scientific, Waltham, MA, USA) at 50 °C for 1 h. In the end, samples were again centrifuged for 5 min at 6000 ×*g*, after which the supernatant was collected. The DNA purification of samples involved the use of Biosprint 96 (Qiagen, Hilden, Germany) as follows: samples lysate was transferred in an S-block with 200 μL of isopropanol and 20 μL of MagAttract magnetic beads suspension (Qiagen) and loaded into the instrument. The protocol provides the use of other 5 plates: one S-block with 500 μL of RPW buffer (guanidine hydrochloride, 1.31 M) (Qiagen), two plates with 500 μL of ethanol (96%), another S-block with 500 μL of tween solution at 0.02% and a last flat 96-well plate with 100 μL of nuclease-free water where the DNA was eluted. The final concentration of DNA was measured with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

Library preparation started with the amplification of hypervariable regions (V2, V4, V8 and V3, V6–7, V9). To perform this first step, we utilized the *16S* Ion Metagenomics Kit (Thermo Fisher Scientific), and the following amplification program: 95 °C for 10 min, 25 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 20 s, and a hold stage at 72 °C for 7 min. After PCR, a cleaning step was carried out and the libraries were normalized to 30 ng μ^{-1}. For barcode ligation, the Ion Xpress Plus 9 Fragment Library and Ion Express Barcode Kits (Thermo Fisher Scientific) were used, and each sample was ligated with a distinctive barcode. Another amplification step was performed with the following program: 95 °C for 5 min, 7 cycles of 95 °C for 15 s, 58 °C for 15 s, and 70 °C for 1 min. Amplificated libraries were quantified using a Qubit 3.0 Fluorometer with Qubit™ DNA HS Assay Kit to pool libraries at a final concentration of 100 pM. Then, the pool was processed with the Ion 520™ & Ion 530 ™ Kit – OT2 400 bp (Thermo Fisher Scientific) following the manufacturer's instructions. In the end, the samples were loaded on the Ion 520 chip, and the sequencing runs were performed with Ion™ GeneStudio S5 System (Thermo Fisher Scientific).

2.7. Slaughter yield and morphometric and somatic indices of fish

The rainbow trout were slaughtered after 103 days of rearing, at the end of the second phase (in polyculture), whereas the black bullhead catfish were slaughtered at the end of the third phase, i.e., after 268 days of farming.

At slaughtering, fish were weighed and then sacrificed by percussion (i.e., sharp blow delivered to the head of fish by a trained personnel). Subsequently, the fish were submitted to morphometric measurements (total length, head length, and maximum height).

Then, all fish were eviscerated and 144 fish (72 fish per species; 24 per experimental treatment; 8 fish per tank) were filleted. The weights of visceral packs (heart, gas bladder, and gonads), livers, eviscerated (gutted) carcasses, and fillets with skin were collected. Data were used to calculate the following indices:

$$\text{Cranial index} = \text{head length (cm)}/\text{total length (cm)} \quad (1)$$

$$\text{Relative profile} = \text{maximum height (cm)}/\text{total length (cm)} \quad (2)$$

$$\text{Condition factor} = \left[\text{final weight (g)}/(\text{total length (cm)})^3\right] \times 100 \quad (3)$$

$$\text{Hepatosomatic index} = [\text{liver weight (g)}/\text{final weight (g)}] \times 100 \quad (4)$$

$$\text{Viscerosomatic index} = [\text{visceral pack weight (g)}/\text{final weight (g)}] \times 100 \quad (5)$$

$$\text{Eviscerated carcass yield} = [\text{eviscerated carcass weight (g)}/\text{final weight (g)}] \times 100 \quad (6)$$

$$\text{Fillet yield} = [\text{fillet weight (g)}/\text{final weight (g)}] \times 100 \quad (7)$$

2.8. Quality of fish fillets: Rheological traits, chemical composition, and fatty acid profile

Soon after dissection, the skin was separated from the fillets and the L* a* b* colour indices (Commission International de l'Eclairage, 1976) were measured at three points on the dorsal side of the right fillets using a Minolta CM–508C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). Then, both the right and left fillets were minced. The right fillets were freeze-dried, placed under vacuum in plastic bags, and stored at 4 °C until used for analysis of proximate composition. The left fresh fillets were stored under vacuum at −18 °C until used for analysis of fatty acid profiles.

As for proximate composition, freeze-dried fillets were analysed according to AOAC (2000) methods to determine the contents of dry matter (934.01), ash (967.05), and crude protein (2001.11). Ether extract contents were measured after acid hydrolysis treatment (EC, 1998).

As for fatty acid profile, samples (4 g) were defrosted and then fat extracted with accelerated solvent extraction (ASE®, Dionex 350, Sunnyvale, CA, USA; Application Note 334). A hexane/isopropanol (3:2) solution was used as solvent and the procedure consisted of two production cycles at 100 °C (5-min heating phase and 1-min extraction phase).

Then, the extracted solution was evaporated with Genevac EZ-2 evaporator (Genevac Ltd., Ipswich, UK) and the lipid content weighed and esterified at 50 °C. An acid esterification (H_2SO_4) was performed to evaluate fatty acids esters, following Christie (1982). After that, the sample was injected into a gas chromatograph (Agilent Technologies 7820 A, Santa Clara, CA, USA) with a split/splitless injector and two capillary columns: Omegawax (Supelco, Merck KGaA, Darmstadt, Germania) (30 m × 0.25 mm internal diameter, 0.25 μm film thickness); and JeW 19091S 431 HPS ms second (3.8 m × 0.25 mm internal diameter, 0.25 μm film thickness), with a 1.4 mL min^{-1} flow. Hydrogen was used as the carrier gas and the oven temperature was initially set at 50 °C and held for 2 min, then increased to 220 °C at 4 °C $minute^{-1}$, and kept for 17 min. The injector and detector temperatures were both 250 °C. Fatty acids were identified by comparing their retention times with a standard mixture of 37 fatty acids methyl esters (FAMEs; standard 37-Component FAME Mix, 47,885-U Supelco; PUFA-3, Menhaden Oil, 47,085-U). The concentration of individual FAMEs was expressed as a percentage of the total area of eluted FAMEs (known plus unknown). The Chemstation software (Agilent Technologies) was used for data analysis.

2.9. Statistical analysis

Data relating to water quality and production performance (live weight, weight gain, feed intake and feed conversion ratio), slaughter yields and fillet quality, and vegetable yields were subjected to ANOVA,

using the GLM procedure of SAS (SAS, 2013) with water salinity as the main effect. Fish survival was analysed with the PROC CATMOD procedure of SAS with water salinity as the main effect. Bonferroni's test was used to compare means. Differences among means with $P \leq 0.05$ were assumed to be statistically significant.

Regarding the water microbiota analysis, raw reads were processed and analysed using the Microbial Genomics workflow of the CLC Genomics workbench (version 22.0.2) (Qiagen, Hilden, Germany). Primers were removed by trimming 20 base pairs on both ends. Samples were filtered based on the number of reads to have comparable coverage. The minimum number of reads was set at 100 and the minimum percent from the median at 50%. High-quality reads were clustered at a 97% level of similarity into Operational Taxonomical Units (OTUs). SILVA SSU v138.1 database was used as a reference for the taxonomic assignation of OTUs (Quast et al., 2012). Low abundance OTUs were eliminated (<10 reads for combined abundance). Rarefaction analyses of diversity measures of the number of total OTUs were calculated, to check the sequencing depth. Alpha diversity was estimated using the Shannon entropy index, and the Kruskal-Wallis statistical test was performed to identify statistical differences ($P \leq 0.05$). Beta diversity among samples was analysed using the Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distances, to which the Permutational MANOVA (PERMANOVA) was subsequently applied to investigate the statistical significance of sample division. Differential abundance analysis was used to highlight significant differences (Wald test, $P \leq 0.05$) within bacteria taxa.

3. Results

3.1. Water quality

The water temperature (Fig. 3a) ranged from a minimum of 7.4 °C in February to a maximum of 30.2 °C in June, averaging 16.9 ± 3.83 °C. During the polyculture phase (December–March), the average temperature was 13.8 ± 2.08 °C. The content of dissolved oxygen averaged at 9.09 ± 1.16 mg L^{-1}. The highest value (12.6 mg L^{-1}) was reached in

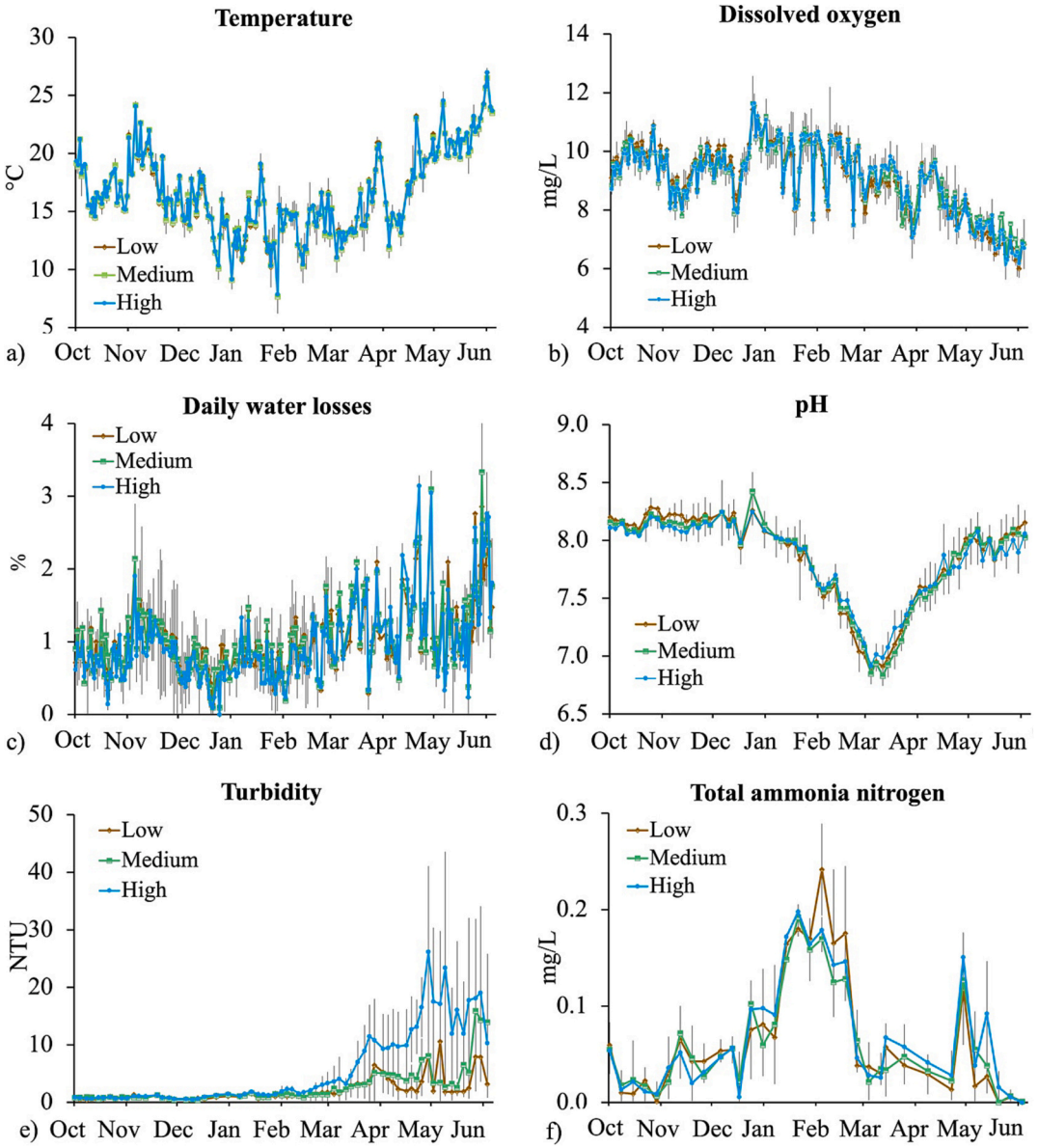

Fig. 3. Water values (mean ± SE) of temperature (a), dissolved oxygen (b), water losses due to evapotranspiration (c), pH (d), turbidity (e) and total ammonia nitrogen (f) during the rearing cycle of rainbow trout and black bullhead catfish at different water salinities. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity.

January and the lowest (5.64 mg L^{-1}) in June (Fig. 3b). Daily water losses for evapotranspiration, 6.84 ± 3.96 L d^{-1} (Fig. 3c) were equal to 0.9% of the total volume of the aquaponic unit.

Water pH (Fig. 3d) was on average 7.8 ± 0.40. During the first two months of the trial (October–December) pH remained stable around 8.16 ± 0.10, whereas along with the introduction of rainbow trout, it gradually decreased to a minimum value of 6.83 and then raised again until 8.0–8.1 during the second period with the catfish alone. The water turbidity ranged from a minimum of 0.22 NTU to a maximum of 44.2 NTU (Fig. 3e), whereas the highest content of total ammonia nitrogen (TAN, Fig. 3f) was found in the systems during the polyculture phase and the lowest water temperatures (0.09 ± 0.06 mg L^{-1}).

No difference in water quality was recorded according to salinity, apart from a numerical (not significant) higher turbidity in the 6‰ salinity units during the final period on trial with catfish alone.

3.2. Water microbiota

The 16S multi-amplicon sequencing produced 2,836,855 raw reads, with an average number of sequences per sample of 306,206 ± 42.7. The

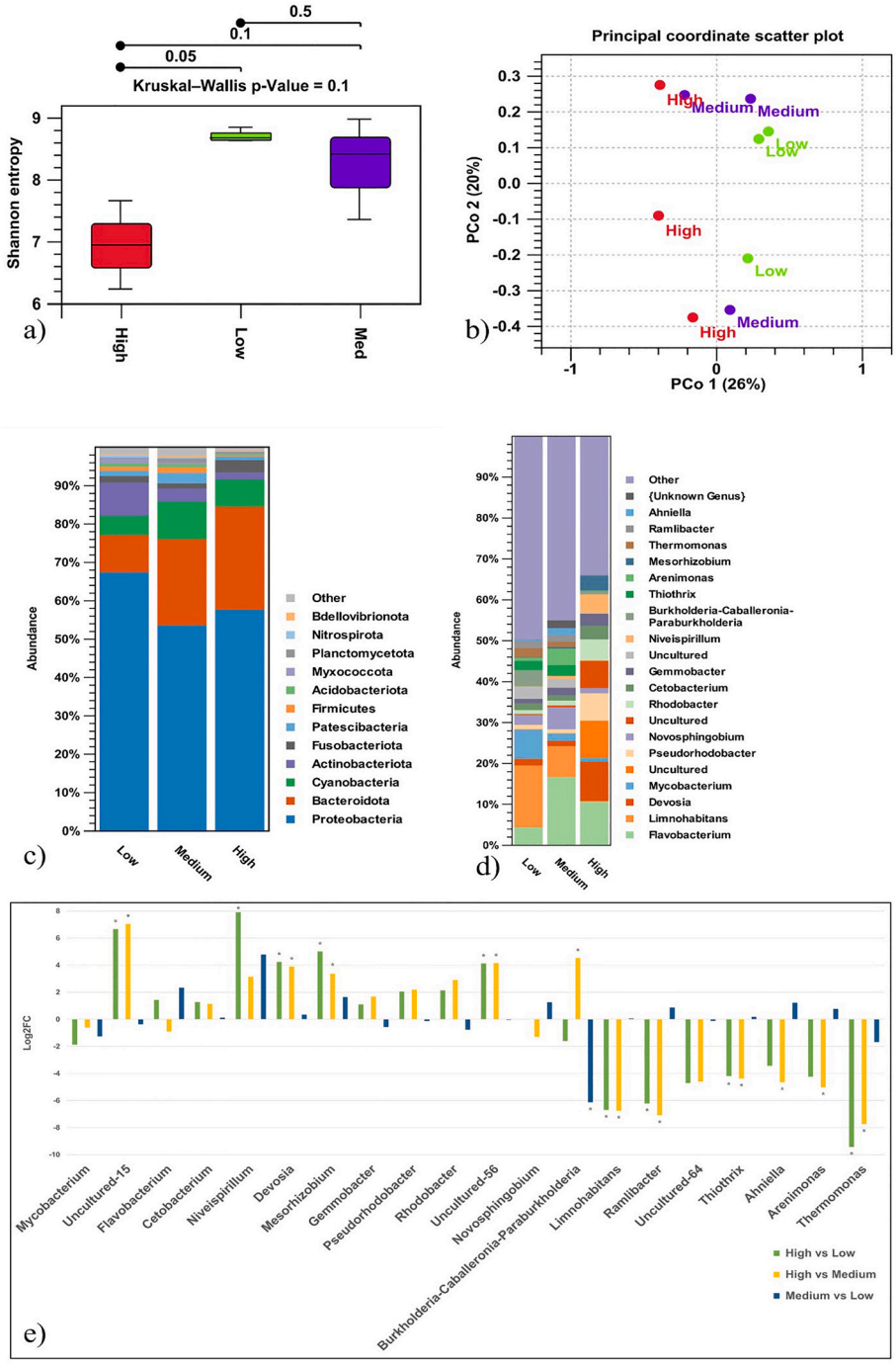

Fig. 4. Alpha diversity (Shannon entropy index) (a), beta-diversity (PCoA) (b), classification of reads to phylum level indicated as percentage of population, only the most 10 abundant phyla for each group are reported(c), classification of reads to genus level indicated as percentage of population, only the most 10 abundant genera for each group are reported (d), Clustered bar chart with the Log_2 fold change values of the 20 most abundant genera between the salinity groups pairs, * symbol above bars represent significant differences (Wald test, $P \leq 0.05$) (e).

Shannon entropy index for alpha-diversity (defined as the richness and the evenness of the ecological community) decreased when the water salinity increased, where low and high salinities groups significantly differed (Kruskall-Wallis test, $P \leq 0.05$) (Fig. 4a). On the other hand, beta diversity was not affected by the salinity (Fig. 4b).

Water communities were dominated by the phyla of Proteobacteria (60%) and Bacteroidota (19%), followed by Actinobacteriota, Cyanobacteria, Fusobacteriota, Patescibacteria, and Firmicutes (Fig. 4c). The phylum of Proteobacteria was mainly represented by the families of *Rhodobacteraceae* and *Comamonadaceae*. *Flavobacteriaceae* were the dominant family of the Bacteroidota phylum. At the genus level, the microbial community was composed mainly of *Flavobacterium* and *Limnohabitans* (Fig. 4d). Minor genera from other families, including *Devosia*, *Mycobacterium*, *Pseoudorhodobacter*, *Novosphingobium*, *Rhodobacter*, *Cetobacterium*, *Thiothrix*, *Thermomonas*, and *Aeromonas* were also detected.

As for the 20 most abundant genera, the differential abundance comparisons between the water pairs of salinities (high vs. low, high vs. medium, and medium vs. low water salinity) revealed the presence of statistically significant genera (Wald test, $P \leq 0.05$) (Fig. 4e). When the water salinity increased from low to medium, only the abundance of one genus significantly decreased. From medium to high salinity, 5 genera increased their abundance while 6 genera decreased. Comparing the highest salinity to the lowest caused the downshifting of the abundance of 4 and the increase of 5 genera.

3.3. Fish growth performance and survival

Neither growth nor survival of rainbow trout and black bullhead catfish during the experiment were affected by water salinity (Fig. 5). As for growth, rainbow trout showed an average weight gain of 195 g, reaching a final weight of 347 ± 77 g (Fig. 5a). Catfish grew slowly during the autumn period and did not grow at all during the winter period from mid-November to mid-February. Then, during spring, catfish showed an increasing growth, for an overall weight gain of 41 g and a final live weight of 192 ± 50 g (Fig. 5b).

As for the winter polyculture phase, at the end of the 103 days, the average biomass growth was 3612 g and tank feed conversion ratio was 1.32 without differences among treatments (Table 1). However, during the last 2 weeks (88–103 d) growth ($P = 0.06$) and feed conversion ($P = 0.13$) were numerically worse with the high than with the low salinity. Final survival rate averaged 89.7% for rainbow trout (Fig. 5c) and 75.8% for black bullhead catfish, the latter showing a higher mortality

Table 1
Tank performance during polyculture farming of rainbow trout and black bullhead catfish at different water salinities for 103 days (second phase; December–March).

	Salinity			*P*-value	RMSE
	Low	Medium	High		
Tanks, n	3	3	3		
Feed intake, g					
0–32 d	913	913	914	0.99	22
33–60 d	1275	1301	1270	0.73	50
61–88 d	1805	1917	1740	0.45	162
89–103 d	717	661	600	0.33	87
Total (0–103 d)	4710	4792	4523	0.47	259
Daily feed intake g/d					
0–32 d	28.5	28.5	28.6	0.99	0.7
33–60 d	45.5	46.5	45.3	0.73	1.8
61–88 d	64.5	68.5	62.1	0.45	5.8
89–103 d	47.8	44.1	40.0	0.33	5.8
Total (0–103 d)	43.9	45.7	46.5	0.47	2.5
Fish biomass growth, g					
0–32 d	434	372	362	0.21	47
33–60 d	1049	1209	975	0.50	233
61–88 d	1738	1915	1516	0.30	283
89–103 d	540	433	294	0.06	95
Total (0–103 d)	3761	3929	3147	0.25	537
Feed conversion ratio					
0–32 d	2.12	2.46	2.58	0.24	0.31
33–60 d	1.26	1.08	1.38	0.48	0.28
61–88 d	1.05	1.01	1.18	0.45	0.16
89–103 d	1.35	1.61	2.08	0.13	0.37
Total (0–103 d)	1.26	1.22	1.47	0.23	0.17

RMSE: Root mean square error. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity.

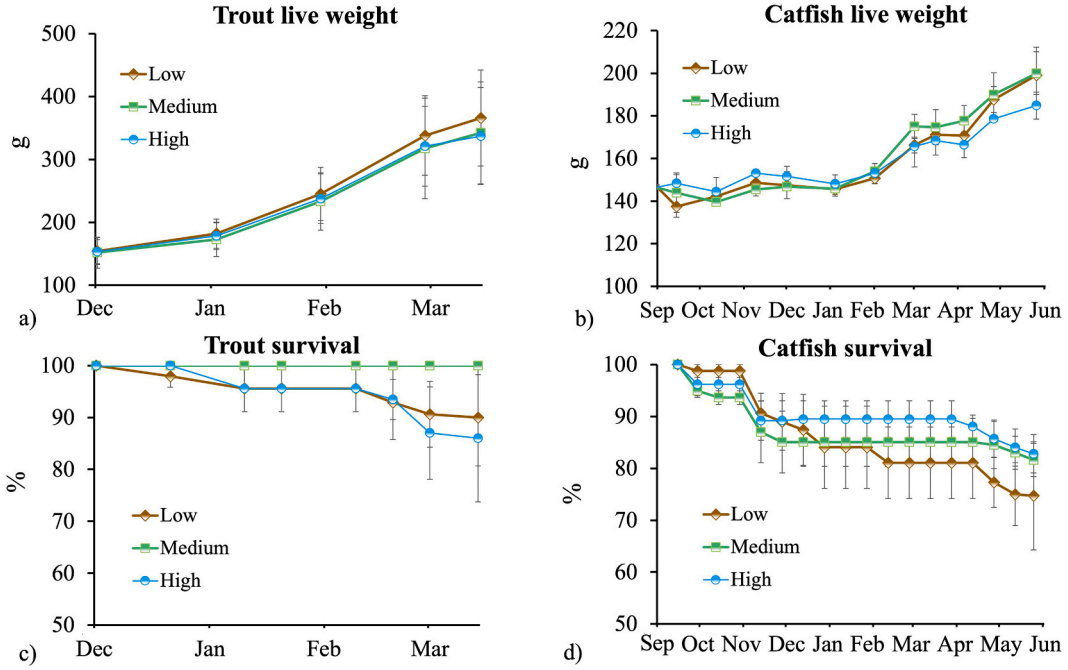

Fig. 5. Live weight of rainbow trout (a) and black bullhead catfish (b), and survival rate of rainbow trout (c) and black bullhead catfish (d) in an aquaponic system with different water salinities (Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity).
Values are expressed as mean ± SE.

in the spring period, especially for the low salinity treatment (Fig. 5d).

3.4. Morphometric and somatic indices and slaughter results

Neither the morphometric indices nor the slaughter results of rainbow trout were affected by water salinity (Table 2). On average, relative profile was 0.249, condition index 1.41, and cranial index 0.190. Gutted yield (87.5%), viscerosomatic index (12.8%), hepatosomatic index (1.6%), and trout fillet yields (52%) were in accordance with the slaughter weight. Fillets showed an average lightness of 47.4, a red index (a*) of −0.35, a yellow index (b*) of 11.5.

As for the black bullhead catfish, the highest condition index was observed in fish farmed at medium salinity (1.61), while the lowest in those kept with high salinity (1.52). Fish farmed at high salinity also showed a lower relative profile (−5%; $P < 0.01$) and a higher cranial index (+4%; $P < 0.001$) than those reared at medium and low salinities. Gutted yield averaged 84.1% (Table 2).

3.5. Fillet chemical composition and fatty acid profile

Water salinity did not affect the chemical composition of rainbow trout fillets (on average, moisture 72.2%, ash 1.50%, crude protein 20.6%, and crude fat 4.00%) (Table 3) or that of black bullhead catfish fillets (moisture 76.9%, ash 1.32%, crude protein 17.4%, and crude fat 1.87%) (Table 4). Similarly, fillet fatty acid profile of rainbow trout fillets (Table 3) did not change, whereas in catfish MUFA were lower (−6.5%; $P < 0.05$) at low salinity compared with medium salinity, with intermediate values at high salinity, whereas PUFA and n-6 showed an opposite trend (+6.8% and + 5.9%, respectively; $P < 0.05$) (Table 4).

Table 2
Morphometric indices, carcass yield and fillet quality traits of rainbow trout (after 103 days of farming) and black bullhead catfish (after 268 days of farming): effect of water salinities in an aquaponic system.

	Salinity			P-value	RMSE
	Low	Medium	High		
Rainbow trout					
Fish, n (initial n)	45 (50)	50 (50)	43 (50)		
Morphometric indices					
Slaughter weight, g	366	342	337	0.19	78
Relative profile	0.249	0.251	0.243	0.09	0.019
Condition index	1.42	1.44	1.37	0.10	0.14
Cranial index	0.188	0.191	0.190	0.11	0.012
Carcass yield and somatic indices					
Eviscerated carcass yield, %	88.4	85.9	88.2	0.10	6.4
Viscerosomatic index, %	12.9	13.1	12.4	0.31	2.1
Hepatosomatic index, %	1.59	1.70	1.61	0.22	0.33
Fillet traits					
Fillet, n	24	24	24		
Fillet yield, % carcass	53.1	51.4	51.6	0.44	0.1
Colour					
L*	47.3	47.6	47.1	0.82	2.9
a*	−0.27	−0.56	−0.21	0.42	0.97
b*	11.3	11.4	11.8	0.73	2.1
Black bullhead catfish					
Fish, n (initial)	65 (87)	71 (87)	72 (87)		
Morphometric indices					
Slaughter weight, g	199	200	185	0.26	51
Relative profile	0.218[b]	0.222[b]	0.211[a]	<0.01	0.016
Condition index	1.54[ab]	1.61[b]	1.52[a]	<0.01	0.15
Cranial index	0.230[a]	0.227[a]	0.236[b]	<0.001	0.011
Carcass yield and somatic indices					
Eviscerated carcass yield, %	84.4	82.2	85.7	0.06	7.7
Viscerosomatic index, %	5.82	5.63	6.14	0.06	1.14
Hepatosomatic index, %	2.29	2.33	2.24	0.44	0.43
Fillet traits					
Fillet, n	24	24	24		
Fillet yield, % carcass	48.5	49.5	47.0	0.07	3.5
Colour					
L*	42.1	42.7	43.6	0.08	2.2
a*	1.30	0.60	0.63	0.06	1.10
b*	10.8	10.3	11.0	0.22	1.4

RMSE: Root mean square error. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity.

Table 3
Fillet proximate composition and fatty acid profile of rainbow trout reared in an aquaponic system: effect of water salinities.

	Salinity			P-value	RMSE
	Low	Medium	High		
Fillets, n	36	36	36		
Proximate composition					
Moisture, %	72.0	72.2	72.5	0.93	1.7
Ash, %	1.4	1.7	1.4	0.37	0.6
Crude protein, %	20.6	20.4	20.7	0.42	8.4
Crude fat, %	3.9	4.6	3.5	0.32	1.7
Fatty acids (% of total FAME)					
C14:0	0.98	0.98	0.96	0.82	0.07
C16:0	11.2	11.0	11.1	0.83	0.6
C18:0	2.74	2.72	2.73	0.87	0.12
C16:1 n-7	1.97	1.88	1.80	0.50	0.34
C18:1 n-9	42.1	42.6	42.2	0.56	1.3
C18:1 n-7	2.85	2.86	2.84	0.80	0.07
C20:1 n-7	1.52	1.52	1.51	0.54	0.13
C18:2 n-6	17.3	17.5	17.6	0.67	0.7
C18:3 n-3	4.44	4.47	4.45	0.97	0.28
C18:4 n-3	0.99	0.90	0.98	0.21	0.13
C20:4 n-6	1.02	0.93	1.01	0.19	0.12
C20:5 n-3	1.22	1.18	1.20	0.87	0.19
C22:6 n-3	6.00	5.73	6.04	0.67	0.93
SFA[1]	15.7	15.5	15.5	0.79	0.7
MUFA[1]	49.7	50.2	49.6	0.57	1.6
PUFA[1]	34.6	34.3	34.9	0.69	1.7
∑n-6	21.0	21.0	21.2	0.67	0.8
∑n-3	13.6	13.3	13.6	0.72	1.3
∑n-6/n-3	1.55	1.61	1.56	0.16	0.69

RMSE: Root mean square error. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity. FAME: Fatty acid methyl esters; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.
[1] Including minor FAs.

3.6. Swiss chard and cherry tomato yields

At the end of the first cycle, Swiss chard total yield was higher (+61%; $P < 0.01$) when water salinity increased from low to medium and high, whereas no significant effects were found on the marketable yield (Fig. 6a, b). At the end of the second cycle, both total yield (+28%; $P < 0.01$) and marketable yield (+32%; $P < 0.001$) increased with water salinity (Fig. 6c, d).

As for cherry tomato, truss weight was lower (−32%; $P < 0.001$) when water salinity increased from low and medium to high, whereas no differences were found in the number of mature fruits per truss (15 fruits, on average) (Fig. 6e, f).

4. Discussion

This study explored the effectiveness of an haloponic system working in continuous over a long cycle using two fish species, which rearing cycles partially overlapped during the winter season resulting in a polyculture phase.

Aquaponic farming offers significant advantages in terms of water preservation compared to conventional rainbow trout raceways systems, with water losses ranging from 20% to 50% (Love et al., 2015).

Table 4
Fillet proximate composition and fatty acid profile of black bullhead catfish reared in an aquaponic system: effect of water salinities.

	Salinity			P-value	RMSE
	Low	Medium	High		
Fillets, n	36	36	36		
Proximate composition					
Moisture, %	77.5	76.7	76.6	0.71	1.4
Ash, %	1.3	1.3	1.3	0.46	0.1
Crude protein, %	17.3	17.4	17.6	0.50	0.7
Crude fat, %	1.7	2.1	1.9	0.26	0.6
Fatty acids (% of total FAME)					
C14:0	1.21	1.24	1.24	0.86	0.12
C16:0	14.7	14.3	14.9	0.39	0.9
C18:0	4.16	3.84	4.16	0.37	0.64
C16:1 n-7	2.52	2.76	2.66	0.35	0.41
C18:1 n-9	34.0[a]	36.7[b]	34.4[ab]	<0.05	2.4
C18:1 n-7	3.01	3.09	3.00	0.38	0.17
C20:1 n-7	2.30	2.33	2.28	0.80	0.19
C18:2 n-6	21.7	20.6	21.5	0.07	1.2
C18:3 n-3	3.58	3.57	3.45	0.58	0.35
C18:4 n-3	0.21	0.20	0.21	0.85	0.02
C20:4 n-6	0.78	0.69	0.77	0.38	0.18
C20:5 n-3	1.34	1.25	1.32	0.15	0.12
C22:6 n-3	4.43	3.71	4.29	0.14	0.91
SFA[1]	20.8	20.1	20.9	0.33	1.43
MUFA[1]	43.0[a]	46.0[b]	43.5[ab]	<0.05	2.7
PUFA[1]	36.2[b]	33.9[a]	35.6[ab]	<0.05	1.8
∑n-6	25.0[b]	23.6[a]	24.7[ab]	<0.05	1.3
∑n-3	11.1	10.2	10.8	0.07	0.9
∑n-6/n-3	2.27	2.32	2.29	0.81	0.19

RMSE: Root mean square error. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity. FAME: Fatty acid methyl esters; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.
[1] Including minor FAs.

Achieving and maintaining long-term equilibrium and stability in aquaponic systems pose significant challenges that involve various factors, including bacteria populations, which exert considerable influence on the overall ecosystem balance. Nevertheless, the higher TAN we measured during the polyculture phase, without reaching threatening levels for fish (Molony, 2001), boosted the activity of the biofilter, resulting in a gradual decrease of water pH (Villaverde et al., 1997).

On the other hand, there is few information about water microbiota in aquaponics systems (Kasozi et al., 2020). Under our conditions, a mild effect of water salinity was found on the microbial communities in the water of fish tanks. In detail, the diversity of the bacteria ecosystem measured by the Shannon index decreased with 6‰ salinity, which aligns with previous studies that have also shown a reduction in microbial species richness and diversity, as well as changes in normal bacterial colonization resulting from increased water salinity (Ya et al., 2023; Hieu et al., 2022). However, the PCoA analysis did not reveal any differences in the community structure between the three salinity levels, suggesting a certain stability in the microbial communities. Noteworthy, changes in environmental microbial communities were previously observed even with small changes in the salinity of other environments such as sludge (Ya et al., 2023), coastal lakes (Lew et al., 2022), besides at the gut level in catfish (Hieu et al., 2022). As for the taxonomic classification, our sequencing data detected Proteobacteria and Bacteroidota as the two major groups, as previously found in other aquaponic systems, specifically in the root zone, biofilter, and periphyton samples (Schmautz et al., 2017; Eck et al., 2021; Kasozi et al., 2020). We found *Rhodobacteraceae*, *Comamonadaceae*, and *Flavobacteriaceae* families, known to benefit plant growth and health (Fischer et al., 2021). In particular, the *Flavobacterium* genus is often found in association with plant roots and plant leaves (Eck et al., 2021). Regarding the nitrification processes, *Nitrospira* and *Nitrosomonas* were present in all the fish tanks, but with a low relative abundance (<1%), as these genera are mainly found in the biofilter compartment (Spradlin and Saha, 2022). Interestingly, another nitrogen-fixing bacteria, *Novosphingobium*, was detected among the 10 most abundant genera in all experimental groups. The genus *Cetobacterium,* commonly part of the gut of fish, was detected in all samples, which can be associated with the contamination of fish faeces in the low-tech aquaponic system used in our study (Eck et al., 2021). Lastly, pathogenic bacteria for fish and human health, such as *Escherichia coli* and *Salmonella*, were not detected except for the *Mycobacterium* genus, an opportunistic pathogen inducing infection-necrotizing granulomas like tuberculosis, morbidity, and mortality in fish (Hashish et al., 2018). The different salinity levels also led to a specific change in the abundance of several genera within the 20 most abundant taxa. In particular, *Devosia* and *Mesorhizobium*, potential nitrogen-fixing bacteria establishing mutualistic symbiosis with plants (Schmautz et al., 2022), presented a higher abundance at 6‰ salinity with respect to the other two water conditions. Concerning other important bacteria involved in the nitrogen cycle, *Thermomonas*, *Arenimonas*, and *Thiothrix*, three denitrifying bacteria, resulted in higher abundance in the low- and medium-salinity tanks with respect to the high- one.

As for fish, during the first monoculture rearing period (October–December), black bullhead catfish showed poor growth, regardless of water salinity. This result could be likely due either to the acclimation to the new farming system or to the rather low water temperature (17 ± 2.3 °C), which was below the recommended minimum feeding temperature for most catfish species (>17 °C) (Holloway, 1993). Then, during the polyculture phase in winter (December–March), water temperature (14 ± 2.1 °C) kept falling, furtherly decreasing catfish metabolic activity and feed intake (Jaćimović et al., 2019). Therefore, all the feed distributed during the polyculture phase was likely consumed by the trout, as suggested by the overall feed conversion ratio recorded during this period (1.32), which was somewhat higher than the data recorded on rainbow trout reared in freshwater RAS (1.0–1.2) (d'Orbcastel et al., 2009; Aubin et al., 2009), but lower than the values (2.26) measured in rainbow trout reared in seawater (32‰ salinity) (Serrano et al., 2021). Feed conversion measured in the present study was also lower than those recorded in previous studies with the same aquaponic system, i.e. 1.51–1.65 (Birolo et al., 2020) and 1.50–1.55 (Bordignon et al., 2022).

As for fish health, under our conditions, a lower survival was recorded for black bullhead catfish. Indeed, at 0.5‰ salinity, few cases of fungal infections caused by Saprolegnia where observed, which might have played a role in the higher mortality, although not statistically significant. To mitigate these risks, saline water baths with salinity between 4‰ and 6‰ have been shown to be effective in controlling skin parasitosis and fungal infections in freshwater fish (Ali, 2005; Dinçtürk et al., 2019). Therefore, the use of brackish water (2‰ to 6‰ salinity) for farming freshwater fish could also provide an advantage for preventing skin infections. This would be useful in aquaponic systems where the use of chemical sanitizers negatively impacts the performance of the biofilter and may challenge vegetable quality (Ovissipour et al., 2019).

Slaughter results and morphometric indexes of rainbow trout were consistent with previous reports from the same aquaponics system (Birolo et al., 2020; Bordignon et al., 2022) and not affected by water salinity. Differently, as for catfish, at the highest water salinity (6‰) fish condition index, relative profile, and cranial index significantly worsened, and final weight ($P > 0.10$) and fillet yield ($P = 0.07$) decreased. Based on these results, the black bullhead catfish likely thrive better at low-salinity waters (0.5‰ to 3‰), as other stenohaline catfish species like channel catfish (*Ictalurus punctatus*) (Altinokand and Grizzle, 2005) and African catfish (*Clarias gariepius*) (Borode et al., 2008), which also showed reduced growth at water salinities higher than 5–6‰. Nevertheless, this study added information about a new candidate species for aquaponics, for which information regarding the morphometric indices,

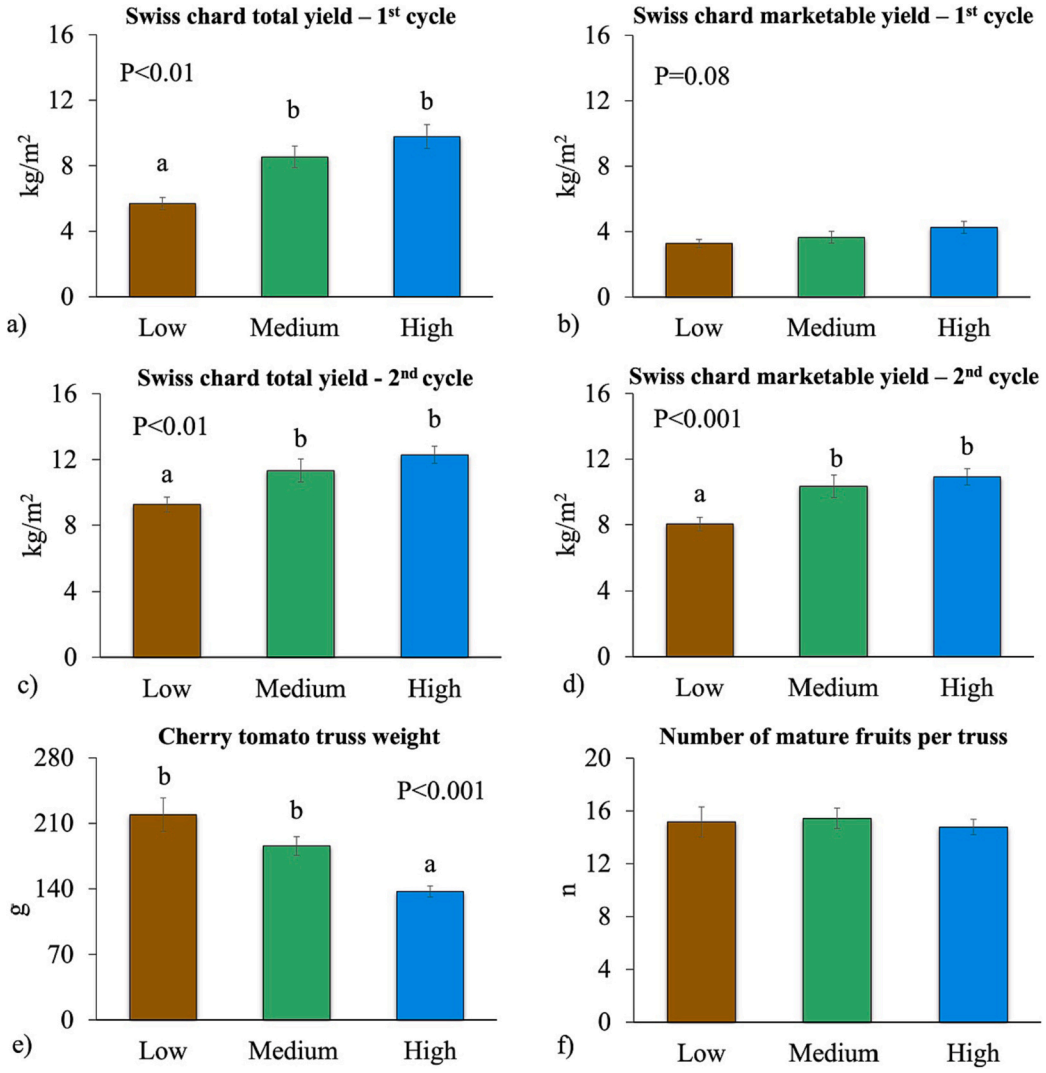

Fig. 6. Total yield (a) and marketable yield (b) of Swiss chard var. Nostrana (first production cycle), and total yield (c) and marketable yield (d) of Swiss chard var. Pugliese (second production cycle), and cherry tomato truss weight (e) and average number of mature fruits per truss (f) in an aquaponics system. Different letters above bars represent significant differences between means. Low: 0.5‰ salinity; medium: 3.0‰ salinity; high: 6.0‰ salinity.

the slaughter results, and the fillet quality traits is completely missing.

In freshwater species like Nile tilapia (*Oreochromis niloticus*) (Cheng et al., 2022; Gan et al., 2016), grass carp (*Ctenopharyngodon idellus*) (Zhang et al., 2021), and largemouth bass (*Micropterus salmoides*) (Du et al., 2022), an increase in water salinity improved fillet quality by enhancing the proportion of polyunsaturated fatty acids. In black bullhead catfish we found a different trend, with a slight increase of monounsaturated fatty acids at the highest salinity (6‰), as found also in grass carp and black carp (*Mylopharyngodon piceus*) with salinity increasing from 0‰ to 7.5‰ (Qu et al., 2022). On the other hand, euryhaline salmonids, such as rainbow trout (Haliloğlu et al., 2004) and Atlantic salmon (*Salmo salar*) (Tocher et al., 1995), tend to be more conservative in their flesh fatty acids when subjected to changes in water salinity, which aligns with our results in rainbow trout fillets.

Finally, looking also at the essential, vegetal component of an aquaponic system, Swiss chard seemed to adapt well to haloponics systems (Kaburagi et al., 2020), obtaining results similar or even better, as in our study, to plants grown in freshwater (Kotzen and Appelbaum, 2010). However, further investigation is necessary to validate these findings, as previous reports showed better yields (Tahjib-UI-Arif et al. (2019), or, conversely, reduced growth (Deveci et al., 2019), when salinity increased up to 5‰ salinity. Nevertheless, different results might be attributed to the varieties of chard used and to the cultivation method employed. Cherry tomato showed the lower truss weight at the highest salinity, consistently with previous studies in soil-less culture (Roșca et al., 2023; Zhang et al., 2022) which could be associated with a reduction of fruit size (Zhang et al., 2022).

5. Conclusions

The use of brackish water until 6‰ did not affect fish growth performance and the overall balance of the haloponic system. Rainbow trout demonstrated excellent adaptability to both haloponics and polyculture farming together with black bullhead catfish. However, the latter fish did not acclimate as effectively to our systems, showing on average poor growth with slightly better performance in freshwater, suggesting potential stenohaline characteristics of this species. Swiss chard thrived at medium-high salinities, confirming its high adaptability to moderate brackish environments.

Incorporating polyculture phases into aquaponic production could represent a viable and sustainable strategy. By aligning seasonal temperature variations with fish temperature requirements, this approach would eliminate the need for costly and impactful temperature-controlling devices and would maintain system functionality and

nutrient supply year-round.

This study could serve as a starting point for further investigations on different fish and vegetable combinations in haloponics, with the ultimate goal of reducing freshwater consumption and, at the same time, preserving the competitiveness and profitability of aquaponic systems.

Authors' contributions

GX, FB, MB, AT and CN conceived and designed the experiment. GX and CN acquired the financial and logistic support for the project. GX supervised the experiment. FB, MB, CN performed the in-vivo trial, registered the experimental data, collected and prepared samples for chemical analyses. GZ and PS analysed water microbiota. FB and GZ performed the statistical analyses. FB, CF, GZ, AT analysed the literature, and interpreted the data and wrote the first draft of the manuscript. All authors critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

CRediT authorship contribution statement

Francesco Bordignon: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Marco Birolo:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Cecilia Fanizza:** Formal analysis, Writing – original draft, Writing – review & editing. **Angela Trocino:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Giulia Zardinoni:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Piergiorgio Stevanato:** Formal analysis, Writing – review & editing. **Carlo Nicoletto:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Gerolamo Xiccato:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data will be made available on request.

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