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# Pacific white shrimp and tomato production using water effluents and salinity-tolerant grafted plants in an integrated aquaponic production system

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ABSTRACT

A vital goal of cleaner production involves developing agricultural production systems capable of ensuring sufficient yields of highly necessary foods to meet the increasing needs of the global population while minimizing the associated economic and ecological costs. Integrated agri-aquaculture systems (IAAS) offer a number of advantages for sustainable agriculture, including water reutilization, discharge mitigation, and increased profitability by leveraging the symbiotic relationship between organic waste, bacterial mineralization, and plant filtration. The aim of this study was to assess the production of two food items of global socio-economic importance cultivated at different salinities: Pacific white shrimp (*Penaeus vannamei*) and tomatoes (*Solanum lycopersicum* L.) grafted to salinity tolerant wild tomatoes. Pacific white shrimp were cultured at a density of 125 organisms/m$^3$ and tomatoes at a density of 3 plants/m. The shrimp growth test consisted of three salinity levels: 2, 4, and 6 g/L. The corresponding tomato salinity treatments were conducted using shrimp water effluents; Steiner's universal nutrient solution (SNS) was used for the control treatment. The experimental period lasted 175 days. The highest tomato production (77.46 t/ha) was attained with SNS, with no significant difference from the salinity of 2 g/L. Shrimp final mean weight, survival, and production were higher at the 6 g/L salinity; and all the other zootechnical parameters decreased with lower salinity. When compared with the hydroponic system using a cost-benefit analysis (CBA), the production costs associated with the IAAS were lower primarily because of the reduced (or null) costs of fertilizer, and irrigation water. When grafted plants were used, the salinity tolerance of the commercial hybrid increased and shrimp could be cultured at appropriate salinity to facilitate osmoregulation. Chemical fertilization requirements were reduced and acceptable yields were obtained for the tomato crop by grafting to salinity-tolerant rootstocks.


## 1. Introduction

Producing sufficient food with high nutrient value to support an increasing global population under conditions of climate change (e.g., increasing salinity) while also mitigating the impact to the environment and reducing production costs is a pressing global problem requiring immediate attention. One alternative for optimizing the use of natural resources demand in sustainable

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**Abbreviations**

| | |
|---|---|
| CBA | Cost-benefit analysis |
| dat | Days after transplant |
| DO | Dissolved oxygen |
| EC | Electrical conductivity |
| FCR | Feed conversion ratio |
| FW | Final mean weight |
| GR | Mean growth rate |
| HS | Hydroponic system |
| IAAS | Integrated agri-aquaculture systems |
| $NO_2^-$ | Nitrite |
| $NO_3^-$ | Nitrate |
| RAS | Recirculating aquaculture system |
| S | Survival rate |
| SGR | Specific growth rate |
| SNS | Steiner's universal nutrient solution |
| SS | Settleable solids |
| TAN | Total ammonia nitrogen |
| TN | Total nitrogen |
| TSS | Total suspended solid |
| TWG | Total weight gain |

agriculture and aquaculture involves the development of integrated aquaponics systems that recirculate nutrient-rich waste effluents from aquaculture to nourish hydroponic plants, cultivating eco-efficient crops while also reducing effluents pollution and promoting more biorational use of minerals and water.

Shrimp and tomato are nutrient-rich highly necessary foods; however, their production involves the use of costly and limited resources like feed, fertilizer, and water. Pacific white shrimp (*Penaeus vannamei*) aquaculture requirements are: water 1–1.60 $m^3$/shrimp-kg (Boyd and Clay, 2002; Tantu et al., 2020) and dry feed protein (±0.49–0.59 kg/shrimp-kg) at high density; and only 21–24% of N is incorporated into shrimp (Chaikaew et al., 2019) while the remainder is discharged to the adjacent area. *P. vannamei* prefer temperatures >27 °C and an isosmotic salinity point of 25 g/L (Charmantier et al., 1989; Bückle et al., 2006); however, Pacific white shrimp are euryhaline and can adapt to culture in low salinity or fresh water (Fierro-Sañudo et al., 2018). Tomato (*Solanum lycopersicum* L.) water productivity (fruit yield/volume of irrigation) is 0.92 kg/$m^3$ (Harmanto et al., 2005). The optimal temperature for tomatoes grown under shade nets is 25 °C during the day and 15–18 °C at night. Humidity should range between 60 and 80%; higher values affect fertilization and favor diseases in foliage and fruit (Alcorta et al., 2006; Castellanos, 2009). Previous studies of tomato soilless culture estimated a threshold N incorporation of 300 kg N/ha (Steiner, 1966; Muñoz et al., 2008) under normal cultivation practice (563–667 kg N/ha) with the excess being lost to the environment (Muñoz et al., 2008). Salinity is one of the major constraints to the integrated production of shrimp and tomato; the ion load even in low salinity water (Rahman et al., 2018) exceeds the parameters for eco-efficient tomato production. One solution involves the use of euryhaline shrimp and salt-tolerant tomato graft plants capable of developed in limiting conditions. *S. lycopersicum* is a crop sensitive to saline stress; however, other wild Solanum species are tolerant (Salinas-Cornejo et al., 2019). Wild Solanum populations can constitute a source of genetic resistance for plant breeding (Olmedo-López et al., 2019); however, few studies have been conducted to evaluate their use as a source of plant resistance. Wild populations of *Solanum lycopersicum* var. *Cerasiforme* are common in the dry forest of coastal Mexico (Sánchez-Peña et al., 2006). Salinity-tolerant wild tomato from the state of Sinaloa, Mexico, were selected and the resultant seedlings were subjected to salinity levels to evaluate the resistance (survival) prior to selection for use as rootstock (Sanjuan-Lara et al., 2015). The scion was the commercial tomato (*Solanum lycopersicum* L. hybrid Canek F1). Grafted tomato plants can be more resistant to soil diseases like *Fusarium*, *Phytophthora*, and nematodes (Kubota and McClure, 2007), can also tolerate low temperatures and drought, have a better absorption of water and nutrients, and can mitigate the impact of salinity (Xiaohui et al., 2019). The IAAS and hydroponic systems were compared in terms of their economic performance following previous studies where a cost-benefit analysis (CBA) was used to assess profit (Papendiek et al., 2016) and as a social indicator of sustainability (Haputta et al., 2020).

In order to ensure long-term sustainability, the intensive agricultural production systems necessary to meet the global population's increasing nutritional needs must be designed in such a way that diminishes our dependence on finite natural resources and mitigates the deleterious effects to the ecosystem. The aim of this work was to evaluate the production of shrimp and hybrid tomato grafted on salinity tolerant wild tomato rootstocks at different levels of salinity with the novel advantage of using autochthonous plants while maximizing recycling capabilities in the production processes.

## 2. Materials and methods

### 2.1. Experimental design

This study was conducted at the *IPN-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (IPN-CIIDIR) Unidad Sinaloa* (28°32′48″ N and 108°28′53″W) with an elevation of 15 m a.s.l. Tomato was cultivated during 20 weeks in a net house (80% mesh cover) of 3.5-m in height, and shrimp in a greenhouse covered with plastic for temperature control. Two simple randomized designs were used; one for the shrimp growth test with three salinity treatments of 2 (electrical conductivity [EC] = 3.9 ± 0.28), 4 (7.7 ± 0.12), and 6 g/L (11.5 ± 0.22 dS/m) and three replicates each (Fig. 1 and Fig. S1); and the second, the grafted tomato production was irrigated with shrimp water effluents with the corresponding salinity for each treatment (2, 4 and 6 g/L) supplemented only at the beginning with 100 mg/L of $NO_3^-$ (commercial $KNO_3$) and three replicates per treatment (Fig. 1 and S1). The control was one hydroponic non-grafted treatment (Romero et al., 1997; Estan et al., 2005; Etehadnia et al., 2008; Colla et al., 2010) using SNS (EC = 2 dS/m [Steiner, 1961]). The total experiment lasted 25 weeks, including plant production and grafting (Week 0–5), biofilters nitrification (Weeks 5–9), and ending with shrimp growth (Weeks 9–25) and tomato production (Weeks 6–25).

### 2.2. Aquaponic system

A recirculation aquaculture system (RAS) was used and equipped with nine tanks of 1 $m^3$ each (0.8 $m^3$ working volume of water), three solid sedimentation tanks (1.1 $m^3$ working volume each), and three biological filters (0.4 $m^3$ working volume each) (Fig. 1 and S1). The hydroponic system (HS) consisted of hydraulic PVC pipes 3.5 m long and 20.3 cm diameter with 7.5 cm in diameter holes every 29 cm. The separation between each pipe was 1.2 m. Zeolite (2–6 mm grain size) was used as substrate (Zeomex, San Luis Potosí, SLP, Mexico). Drainage was directed through a PVC pipe (1.25 cm diameter) to collecting tanks outside the net house. After passing through the HS, the effluent water was retained in 0.45 $m^3$

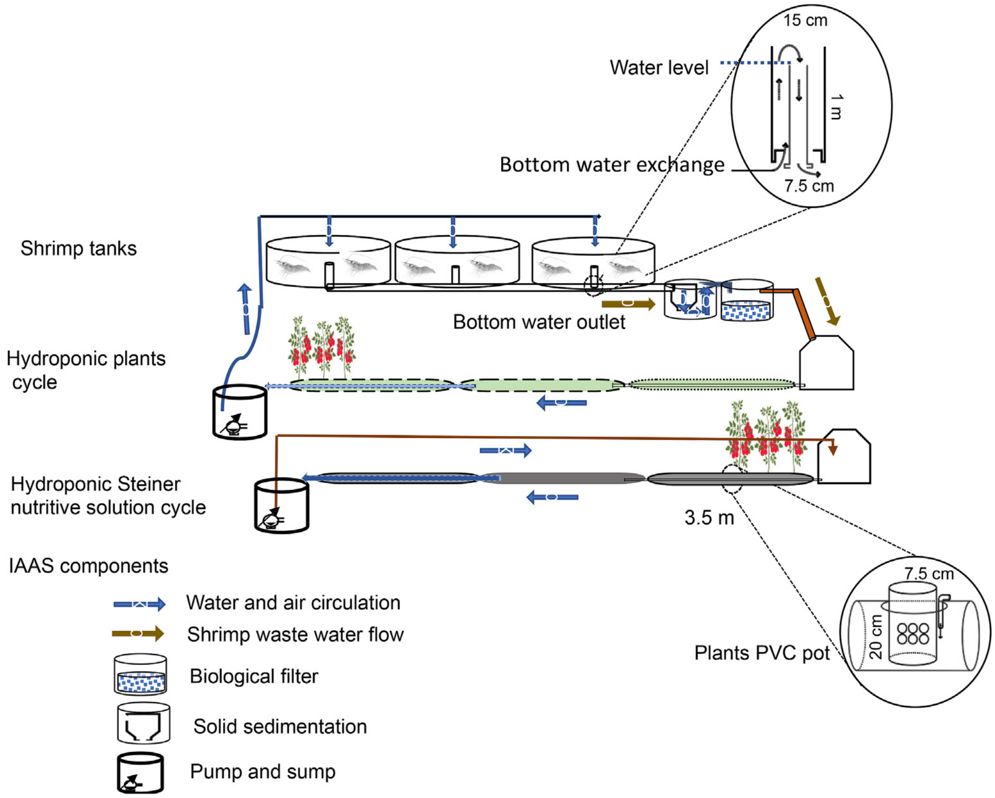


**Fig. 1.** Components of an integrated closed recirculation system and a hydroponics unit for cultivation of Pacific white shrimp (*Penaeus vanamei*) and tomato (*Solanum lycopersicum* hybrid Canek F1). Each salinity experiment was designed with one hydroponic plants-shrimp cycle plus the continuous SNS control treatment. The figure is not to scale and components are illustrative only. Insets show details of bottom water exchange and plant pot. Salinity treatments = 2 g/L, 4 g/L, and 6 g/L; SNS = Steiner's universal nutrient solution. Supplemental Fig. S1 shows the complete integrated closed recirculation system.

containers equipped with submersible pumps (Evans® Aqua60w) and deposited in the culture tanks (Fig. 1 and S1). The water in the shrimp tanks (2.4 $m^3$ total volume) was renewed approximately 2.1 $m^3$/day with a flow rate of 1.5 L/min.

### 2.3. Selection of salinity-tolerant wild tomatoes for grafting

Fruits of wild native tomatoes were collected from Guasave (25.37° N; 108.45° W), Ahome (25.74° N; 108.83° W), and El Fuerte (26.07° N; 108.77° W) regions in northern Sinaloa, Mexico. Salinity tolerance in the seedling stage was evaluated using a $4 \times 4$ factorial design, obtaining 16 treatments with 20 repetitions. The four-levels factors were ecotype (three wild tomatoes plus the hybrid [Canek F1]) and the salinity factor (2, 4, 6, and 9 g/L), following a completely randomized experimental design. Seed germination was at $35 \pm 4$ °C in August in plastic trays with artificial substrate (peat moss, Pro-Moss TBK, Saint-Paul, MN, USA). One week after germination, seedlings were subjected to conductivity tests to facilitate selection for grafting. The severity of damage was assessed each day based on the percentage of dead seedlings.

### 2.4. Tomato plant seedling and grafting

Seeds were planted in germination trays (holes 3.6 cm diameter and 11 cm depth), and peat moss (Pro-Moss TBK) was used as substrate. To optimize germination, trays were covered with black polyethylene and placed at room temperature of $30 \pm 5$ °C for 120 h. After 5 days, germinated plants were transferred to net house conditions and irrigated with SNS (Steiner, 1961). At 25 days post-germination, grafting was performed by the lateral bonding by making a cross section 1 cm below the cotyledons in the rootstock and above those in the scion. A silicone clasp was attached to hold both components together. Subsequently, the grafted seedlings were subjected to an acclimatization process (24 °C, 90% humidity, and total darkness) in a chamber for 8 days. Plants were gradually exposed to ambient light and, finally, taken to the net house for their final transplant. Seedling and grafting lasted approximately 5 weeks.

### 2.5. Agronomic management

Plants were irrigated by gravity with a stock solution for each treatment; irrigation frequency was every 30 min with a 5-min duration. Sulfuric acid ($H_2SO_4$ at 1 meq/L) was added to adjust the pH to 6 for each treatment. After transplanting, the plants were supported using a raffia line with plastic rings; periodic removal of axillary shoots and pruning of older leaves was conducted to achieve a single vertical stem. The first harvest was performed at 98 days after transplanting (dat), and subsequent harvests were made each week.

### 2.6. Shrimp stocking and tank management

Juvenile shrimp were obtained from a commercial shrimp farm and reared at a greenhouse aquaculture facility to acclimatize to the experimental environment. Nitrification biofilters were established in the system in the prior five weeks, with finely ground shrimp feed at 5% of the initial biomass added. The nitrogen cycle was considered established following Somerville et al. (2014): "when the $NO_3^-$ level is steadily increasing" and the nitrite ($NO_2^-$) and total ammonia (TAN) levels were <0.1 mg/L.

Once nitrification was established, juvenile shrimp (weight 0.28 ± 0.08 g) were stocked at 125 organism/$m^3$ in each tank. Shrimp were uniformly sized, with no evidence of disease or reddish discoloration. Three equivalent feed rations were added daily at 0800, 1200, and 1600 h to each tank. During the study, the feeding rates were gradually adjusted (5-2%) based on the body weight of the shrimp and observations of demand monitored with feed trays (Cuadros and Beltrame, 1998). Biometrics were recorded weekly (weight individually) for 30 shrimp from each tank using a digital balance (precision 0.01 g; Ohaus Corporation, Parsippany, NJ, USA), after which the shrimp were returned to their respective tanks.

The water was continuously aerated using a regenerative air blower (4 hp, Sweetwater®, Aquatic Ecosystem Inc., Apopka, FL, USA) to maintain a dissolved oxygen (DO) of 6 mg/L in all tanks. The pH was sustained between 8.0 and 8.5. Thermostatically controlled immersion heaters (Finnex 800 Watt Titanium Heating Tube, Chicago, IL, USA) were used to maintain the water temperature between 27 and 28 °C. Shrimp were reared under a light regime of approximately 14 h light: 10 h darkness. Settleable solids (SS) were removed in order to maintain the system at < 500 mL/L, the water was filtered (within the biological filter) for a 12-h period, and solids waste was removed from the filters. Water was added to recover the levels lost to SS removal.

### 2.7. Water quality parameters

During the experiment, water pH (Hanna 213 pH meter, Hanna Instruments, Woonsocket, RI, USA), temperature (°C), and DO (mg/L) (YSI 55 digital oxygen meter with an integrated thermometer, Yellow Springs, OH, USA) were monitored in each tank twice a day (0800 and 1600 h). Water salinity was monitored daily with an Atago refractometer (Novatech International, Houston, TX, USA). The TAN (mg/L), $NO_2^-$ (mg/L), $NO_3^-$ (mg/L), phosphate (mg/L), alkalinity (mg/L of $CaCO_3$), and total suspended solids (TSS; mg/L) were determined weekly following the methods described by Strikland and Parsons (1972) and APHA (1998). The TSS assessment consisted of filtering (Whatman, 0.45 μm) the water volume and subsequently oven drying to constant weight. An Imhoff cone (Scienceware 1000−0.01 mL, Swedesboro, NJ, USA) was used to monitor settleable solids (SS, mL/L) daily.

### 2.8. Bacterial analyses

From the beginning of the experiment, water samples for bacterial analyses were collected every two weeks following standard practices (APHA, 1998). Water was spread-plated on nutrient-based agar medium to obtain bacterial counts: thiosulphate citrate bile salt agar (TCBS agar; Difco, USA) was used for the presumptive *Vibrio* spp. Count, and mannitol egg yolk polymyxin agar (MYP Agar; Difco, USA) was used for the enumeration of presumptive *Bacillus* spp. The plates were supplemented with 2.5% NaCl and incubated for 24 h at 30 °C before counting (CFU/mL).

### 2.9. Shrimp growth parameters

At the end of the experiment, all shrimp in each tank were individually weighted and counted (n) to estimate growth (final mean weight [FW, g]; Eq. (1), mean growth rate [GR, g/week]; Eq. (2), and specific growth rate [SGR, % weight increased/day]; Eq. (3)), total weight gain (TWG, g/$m^3$; Eq. (4)), feed conversion ratio (FCR; Eq. (5)), survival (S, %; Eq. (6)), and productivity (final biomass). The production parameters were calculated with the successive procedures:

$$FW = (Wt\ /n), \tag{1}$$

$$GR = ([Wf - Wi]/t) \tag{2}$$

$$SGR = 100 \text{ x } (\text{Ln } Wf - \text{Ln } Wi)/t), \tag{3}$$

where Wt = total final weight, Wf = mean individual weight at experiment end, and Wi = mean individual weight at the beginning, and t = time in days of the period (Ricker, 1979).

$$TWG = (W_{Bf} - W_{Bi}), \tag{4}$$

where $W_{Bf}$ = total final weight of the experiment and $W_{Bi}$ = the total initial weight

$$FCR = (\textit{feed provided } [\text{dry weight, g}])/(\textit{live weight gain } [\text{wet weight, g}]), \tag{5}$$

and

$$S = ([\textit{Final shrimp number}]/[\textit{initial shrimp number}] \text{ x } 100). \tag{6}$$

The survival data were arcsine square root transformed before analysis.

### 2.10. Foliar nutrient analysis

Foliar total nitrogen concentration (TN, %) was measured using the micro-Kjeldahl method following Alcantar and Sandoval (2014) which consisted of distillation with NaOH, followed by wet digestion, and finally, titration was carried out with $H_2SO_4$ (0.05 meq/L). Concentration of TN was obtained according to Eq. (7),

$$TN = (\textit{mL of } H_2SO_4 \textit{ used})\,(\textit{normality of the acid})\,(1.4)/(\textit{plant sample in g}). \tag{7}$$

Phosphorus (%) was determined using the vanadate-molybdate method following to Tandon et al. (1968) with a UV light spectrophotometer (Genesys 20) at 470 nm. Potassium (%) was determined with a Buck Scientific® flamometer (PFP-7) (Buck Scientific Inc., Norwalk, CT, USA) at an absorbance of 766.5 nm (Gaines and Mitchell, 1979). Micronutrients concentrations (Ca, Mg, Fe, Cu, Zn, and Mn) were measured using an atomic absorption spectrometer (Varian® SpectrAA 50 B, Varian, Inc., CA, USA) Alcantar and Sandoval (2014).

### 2.11. Tomato growth and yield

Morphological plant variables were recorded at harvest stage (140 dat) and measured from 15 randomly selected plants per treatment. The variables were plant height (cm, from the stem base to the apex) and stem thickness (mm). The number of fruits per bunch was recorded and ripe fruits were sorted into labeled bags for each plant-replicate and treatment; individual fruit were weighed (g) using a digital scale (TORREY®, Monterrey, NL, Mexico) to estimate yields (t/ha).

### 2.12. Techno-economic analysis

Based on collected field data (Papendiek et al., 2016), the IAAS and hydroponic systems were compared using a CBA (Haputta et al., 2020). Both systems were analyzed based on the benefits of the end products (outputs) and the production costs (inputs) using the benefit/cost ratio, where values > 1 (Garcia-Ruboca et al., 2016) indicate the benefit per unit invested. The costs of materials and supplies (Tables S1 and S2) were based on local market prices; for shrimp and tomato (per kg), prices were obtained from the USDA database (USDA, 2019).

### 2.13. Statistical analyses

Normality and homoscedasticity were evaluated using the Anderson-Darling and Levene's tests, respectively. The treatment effects on the water quality parameters, bacterial counts, and shrimp parameters were evaluated by one-way repeated measures ANOVA, with treatment as the main factor and the sampling date as the repeated measures factor (Gomez and Gomez, 1984). The effects of selecting of salinity-tolerant wild tomato for grafting were evaluated using two-way ANOVA, and the effects on foliar nutrients and tomatoes production were evaluated using one-way ANOVA; significant differences were evaluated using post-hoc Tukey tests. A 5% significance level were selected. All analyses were performed using Statistica package v6 (StatSoft, Tulsa, OK, USA).

## 3. Results

### 3.1. Selection of salinity-tolerant wild tomatoes for grafting

Survival (%) of plants materials was measured in the hybrid (Canek F1) used for scion; the percentage of dead plants was 100% in the 9 g/L salinity (EC = 16.1 ± 0.32 dS/m) and 25% in the 2 g/L (3.9 ± 0.28 dS/m) during the first 24 h (Fig. 2). The Guasave wild tomato presented the highest percentage of surviving plants (Fig. 2). This genotype with better tolerance was selected to be used as the standard; mortality was only 8% in 6 and 9 g/L salinity (EC = 11.5 ± 0.22 and 16.1 ± 0.32 dS/m), over 4 weeks (Fig. 2).

### 3.2. Water quality parameters of shrimp culture

Physical, chemical, and biological parameters were analyzed (Table 1) throughout the shrimp cultivation period. All physical parameters including TSS and alkalinity were within the levels recommended for shrimp culture with the exception of the salinity treatment, which was intentionally maintained at values of 2–6 g/L (Table 1). The $NO_2^-$ and $NO_3^-$ ($p < 0.05$) concentrations were significantly different between treatments. Based on the bacteriological analysis, the concentration of *Vibrio* spp. during the experimental period (Fig. 3) reached concentrations of 10–40 × $10^3$ CFU/mL; low salinity presented an unstable environment for *Vibrio* spp. Counts of *Vibrio* and *Bacillus* spp. increased with higher water salinity (Fig. 3).

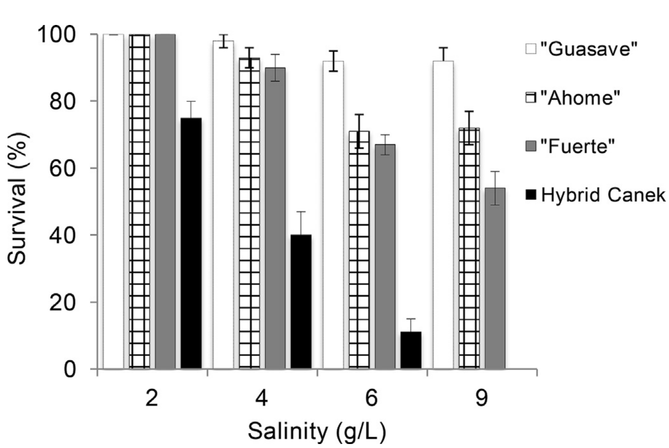


**Fig. 2.** Survival of wild tomatoes (*Solanum lycopersicum* var. *Cerasiforme*) identified by the names of the three sites in Sinaloa from which they were collected (Guasave, Ahome, and El Fuerte) and hybrid tomato (*Solanum lycopersicum* hybrid Canek F1) subjected to salinities of 2, 4, 6, and 9 g/L. Duration was over four weeks in wild ecotypes and 24 h for the hybrid (Canek F1).

**Table 1**
Physical and chemical parameters of the water culture of *Penaeus vannamei* in an integrated closed recirculation system at salinities of 2, 4, and 6 g/L. Mean ± SD of three replicates. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

| Parameter | Salinity | | |
|---|---|---|---|
| | 2 g/L | 4 g/L | 6 g/L |
| Temperature (°C) | 29.20 ± 0.90 | 28.50 ± 1.30 | 28.6 ± 1.4 |
| pH | 8.30 ± 0.07 | 8.3 ± 0.01 | 8.20 ± 0.07 |
| DO (mg/L) | 6.93 ± 0.40 | 6.89 ± 0.6 | 7.03 ± 0.5 |
| TAN (mg/L) | 0.08 ± 0.01 | 0.09 ± 0.02 | 0.08 ± 0.01 |
| $NO_2^-$ (mg/L) | $0.45 \pm 0.7^a$ | $1.38 \pm 2.9^b$ | $1.48 \pm 1.9^b$ |
| $NO_3^-$ (mg/L) | $42.12 \pm 7.1^a$ | $23.62 \pm 11.0^b$ | $17.37 \pm 7.2^c$ |
| Phosphate-P (mg/L) | 0.12 ± 0.03 | 0.07 ± 0.02 | 0.05 ± 0.01 |
| TSS (mg/L) | 129.9 ± 35.4 | 116.7 ± 37.8 | 116.7 ± 37.8 |
| Alkalinity (mg/L) | 288.5 ± 22.3 | 277.9 ± 10.9 | 274.9 ± 16.9 |
| *Vibrio* spp. (CFU/mL) x$10^3$ | $8.0 \pm 7.0^a$ | $13.0 \pm 8.0^b$ | $17.5 \pm 1.6^b$ |
| *Bacillus* spp. (CFU/mL) x$10^3$ | $17.8 \pm 4.2^a$ | $53.3 \pm 7.3^b$ | $80.4 \pm 15.4^c$ |

*DO = dissolved oxygen; TAN = total ammonia; TSS = total suspended solids.

### 3.3. Foliar nutrients

Three different stages (flowering, fruiting, and senescence) were analyzed for nutrients content in tomato plants foliage (Table 2). The TN content showed a significant interaction ($p < 0.05$) between the phenological stage and salinity. The mean TN for the SNS control treatment plants was equal to that of the grafted tomato in 2 g/L salinity and decreased when exposed to 6 g/L (Table 2). Foliar determinations of P and Cu were only significantly different in plants of different age. The foliar concentration of K increased during the fruit development stage without apparent effect from salinity. Interaction between age and salinity were significant ($p < 0.05$, Table 2) for Ca, Zn, Mg, Fe, and Mn; for the first two, concentration increased during senescence, while the latter three reached their highest concentration during flowering (Table 2).

### 3.4. Tomato morphology

The stem diameter of tomato plants was found to be significantly different under different salinities (Fig. 4). Higher salinity (6 g/L) induced a gradual decrease in the stem diameter of plants. Plant height and number of bunches were not different between the control and different salinity. The number of fruits per bunch declined at higher salinity, but was not significantly different from that under the SNS control treatment (Fig. 4).

### 3.5. Production, fruit weight, and blossom-end rot

The production from six harvests was obtained; the highest production (77.46 t/ha) corresponded to the treatment irrigated with the SNS control treatment, without statistical differences at 2 g/L salinity. For the 4 and 6 g/L salinities, production was reduced by 40 and 54%, respectively, without significant differences (Fig. 5). Fruit weight (g) was lower as salinity increased, the reduction at 6 g/L salinity was 45% of the mean weight compared to plants under the SNS control treatment (Fig. 5). Finally, apical fruit rot increased by up to 40% at 6 g/L salinity (Fig. 5).

### 3.6. Shrimp growth, survival, and production parameters

Water salinity had a direct effect in shrimp yield parameters (Table 3). Production, FW, and S, were higher at 6 g/L salinity; as the

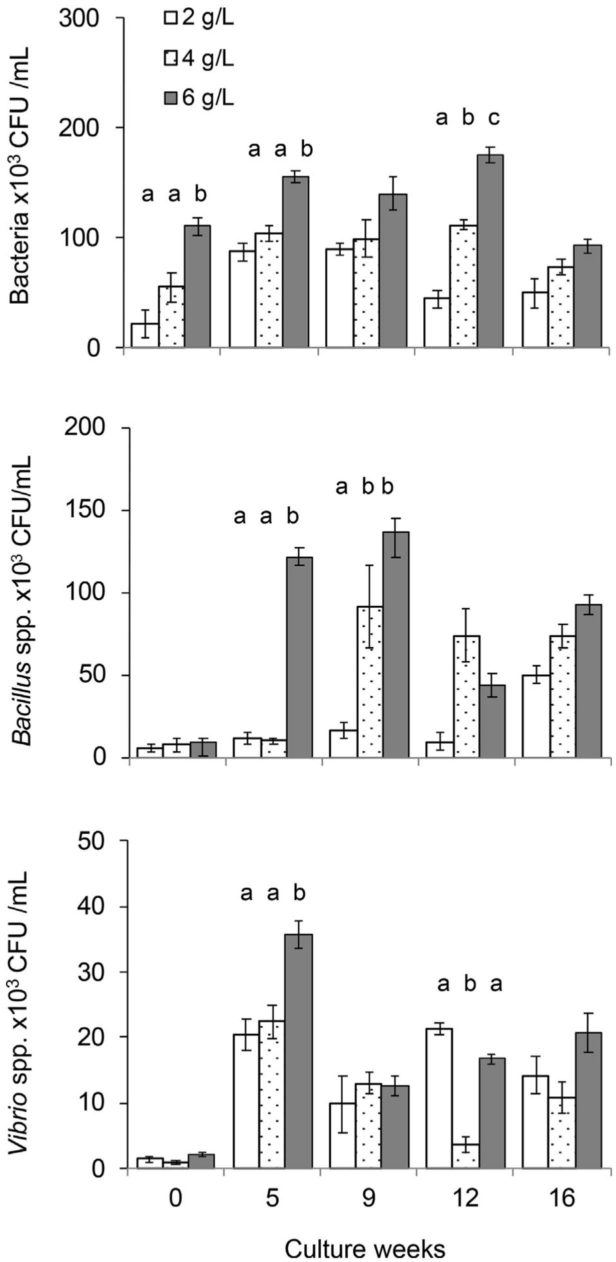


**Fig. 3.** Total bacterial, *Bacillus* spp., and *Vibrio* spp. (x $10^3$ CFU/mL) growth in shrimp culture water with salinities of 2, 4, and 6 g/L over 16 weeks. Different letters above the bar indicate significant differences between salinities ($p < 0.05$).

salinity decreased so did all zootechnical parameters (Table 3 and Fig. 6). Significant differences were found between the 2 and 6 g/L salinity treatments; however, no significant difference existed between either of these two and the 4 g/L treatment (Table 3).

### 3.7. Techno-economic analysis

The CBA for hydroponic tomato was 1.72 (Table S1); for IAAS at 2 and 4 g/L salinity, the CBA ratio was 1.42 and 1.05, respectively (Table S1), and coincided with reduced tomato and shrimp yields due to the effects of salinity. The reduction in production costs for the IAAS (Table S1) was primarily due to differences in the cost of fertilizer, irrigation water, and disease control. Apical blossom-end rot reduced the volume (kg) of the output and was therefore discounted from the final exportation weight (Table S1) in order to perform the analysis.

## 4. Discussion

Performance and production are the main parameters that describe the efficacy of an integrated aquaponics culture system in terms of optimizing water and fertilizer while simultaneously producing animals and plants at reduced socio-economic and ecological cost. The plants take nutrients from mineralized surplus food and excreta and filter the water; meanwhile, shrimp are grown in recycled water of a quality adequate for cultivation (Gichana et al., 2018). Current challenges for aquaponic development include selection of tolerant plants, stress-resistant aquatic animals, and nitrifying bacteria, and the use of appropriate systems in order to ensure optimal conditions (Goddek et al., 2015). Shrimp optimal development requires salt concentrations of 25 g/L in water (Ponce-Palafox et al., 2019). It exceeds the tolerance of non-halophyte plants, such as Solanaceae, with negative effects on the vegetative development and fruit production.

Grafted tomato plants can reduce abiotic saline stress (Al-Harbi et al., 2017), dependence on chemicals and fertilizers, and increase fruit quality (Colla et al., 2010; Xiaohui et al., 2019). The productive performance of a particular scion-rootstock combination (Grieneisen, 2018) is influenced by scion compatibility as well as biotic and abiotic pressures (Xiaohui et al., 2019). The grafted "Guasave" tomato attenuated salinity up to 4 g/L and there was a 13% difference in production between the SNS control treatment (744 mg/L $NO_3^-$) and 2 g/L salinity. Only the stem diameters were significantly different at salinity >4 g/L, signaling tolerance and less adverse effect to production at the lowest salinity. The aquaponic system had limited macro- (N, P, K, Ca) and micro-nutrient (Fe, Mn, Zn, Cu, B, Mo) concentration to fertilize the plants (Somerville et al., 2014). A genome study with tomato rootstock demonstrated that 3–8 loci controlled the transportation and absorption of Ca, Cu, K, Mg, Mn, Na, P, S, Zn, and Mo (Fazio et al., 2013), regulating salt tolerance, and were related to "increased yield in the scion" (Asins et al., 2015). The N content in the grafted tomato had a tendency to decrease in plants subjected to 6 g/L salinity. Salinity increases the osmotic effect and disrupts the absorption of nutrients. However, under limited supply of nutrients, the productive potential decreased even in grafted plants and the resistance to the biotic stress expected from grafting was affected at higher salinity.

### 4.1. Water quality parameters of shrimp culture

Shrimp cultivated in low salinity waters must maintain an osmotic concentration close to medium (Péqueux, 1995), which requires additional metabolic energy (Camacho-Jiménez et al., 2018); thus, less energy is available for growth and the elimination of toxic metabolites (Kuhn et al., 2012; Akbarzadeh et al., 2019). $NO_2^-$ ions are toxic and a concentration of 0.5–5 mg/L constitutes a stressor in catfish and tilapia. Ammonium ($NH_4^+$) must be maintained below 0.1 mg/L to avoid negative effects in most aquaculture animal species (Fregoso-López et al., 2018), with the combined effects of nitrogen compounds, high density (120 shrimp/$m^2$), and low salinity (1.9 g/L) inducing low water quality, survival (11.9%), and performance (0.10 kg/$m^2$).

In aquaponic crops nitrification, pH, alkalinity, and DO are important so that the compounds generated do not damage the health status of aquatic animals or hinder the absorption of minerals by plants (Rakocy et al., 2006; Zaghdoud et al., 2016). Tomato plants demand high $NO_3^-$ (744 mg/L based on the SNS control treatment) specifically during the fruiting stage; meeting the plants' requirements within the range of tolerance of shrimp to

**Table 2**
Foliar nutrient concentration in plants at different phenological stage of grafted tomato under hydroponic conditions in an integrated culture system. The SNS control treatment corresponds to universal nutrient solution. Salinity treatments of 2, 4, 6 g/L were attained for an initial concentration of 100 mg/L of $NO_3^-$ in water.

| Plant age | Salinity g/L | Total N (%) | P | K | Ca | Mg | Fe (mg/kg) | Cu | Zn | Mn |
|---|---|---|---|---|---|---|---|---|---|---|
| Flowering | SNS | $3.8^{ab}$ | $1.6^{a**}$ | $1.7^{a**}$ | $1.2^{ef}$ | $0.53^{abc}$ | $99.2^{abc}$ | $50.7^{a**}$ | $70.3^{bc}$ | $63.1^{a}$ |
| | 2 | $3.9^{a}$ | $1.8^{a}$ | $1.5^{a}$ | $1.1^{f}$ | $0.53^{ab}$ | $98.1^{a}$ | $50.7^{a}$ | $70.1^{e}$ | $63.7^{ab}$ |
| | 4 | $3.7^{abc}$ | $1.6^{a}$ | $1.8^{a}$ | $1.2^{ef}$ | $0.51^{abc}$ | $95.3^{ab}$ | $51.1^{a}$ | $84.3^{cd}$ | $65.7^{a}$ |
| | 6 | $2.5^{c}$ | $1.7^{a}$ | $1.6^{a}$ | $1.2^{ef}$ | $0.48^{bcd}$ | $88.3^{abc}$ | $53.7^{a}$ | $78.3^{d}$ | $64.1^{ab}$ |
| Fruiting | SNS | $2.8^{bc}$ | $1.2^{b**}$ | $2.1^{b**}$ | $1.5^{cd}$ | $0.39^{def}$ | $92.2^{bc}$ | $62.1^{b**}$ | $95.8^{abc}$ | $49.5^{b}$ |
| | 2 | $2.9^{bc}$ | $1.4^{b}$ | $2.1^{b}$ | $1.3^{de}$ | $0.42^{cdef}$ | $93.3^{abc}$ | $51.7^{b}$ | $96.6^{ab}$ | $68.3^{a}$ |
| | 4 | $2.4^{c}$ | $1.3^{b}$ | $2.3^{b}$ | $1.2^{ef}$ | $0.44^{bcde}$ | $98.1^{a}$ | $50.7^{b}$ | $98.1^{a}$ | $68.3^{a}$ |
| | 6 | $2.4^{c}$ | $1.2^{b}$ | $1.7^{b}$ | $1.4^{cd}$ | $0.36^{ef}$ | $87.3^{bc}$ | $59.7^{b}$ | $96.3^{ab}$ | $63.1^{ab}$ |
| Senescence | SNS | $3.1^{abc}$ | $1.1^{b**}$ | $1.2^{c**}$ | $1.6^{a}$ | $0.34^{a}$ | $89.1^{abc}$ | $66.7^{b**}$ | $89.1^{abc}$ | $53.5^{ab}$ |
| | 2 | $3.3^{abc}$ | $1.4^{b}$ | $1.1^{c}$ | $1.5^{bc}$ | $0.32^{f}$ | $84.7^{c}$ | $67.3^{b}$ | $93.3^{ab}$ | $60.1^{ab}$ |
| | 4 | $2.9^{abc}$ | $1.3^{b}$ | $1.3^{c}$ | $1.5^{bc}$ | $0.33^{ef}$ | $90.3^{abc}$ | $70.3^{b}$ | $93.6^{ab}$ | $53.7^{ab}$ |
| | 6 | $2.5^{c}$ | $1.4^{b}$ | $1.1^{c}$ | $1.5^{bc}$ | $0.36^{ef}$ | $90.1^{abc}$ | $69.7^{b}$ | $95.1^{ab}$ | $56.4^{ab}$ |
| *PSD | | 0.37 | 0.14 | 0.20 | 0.06 | 0.04 | 3.47 | 4.93 | 2.76 | 9.01 |
| Interaction ($p$-value) | | 0.028 | 0.338 | 0.146 | 0.003 | 0.001 | 0.003 | 0.106 | 0.001 | 0.050 |

* PSD = pooled standard deviation. ** Age significance ($p < 0.05$). Means with the same letter for each column are statistically identical, Tukey ($p < 0.05$).

$NO_3^-$ is a serious restriction. The productivity reach of shrimp and tomato fruit were supported by nourishment generated in the symbiosis of the plants as biofilters and the input of nutrients from the aquaculture component.

## 4.2. Foliar nutrients

Nitrogen deficiency hinders protein synthesis and affects the growth and reproduction of plants. A typical symptom includes pallid yellowish-green leaves caused by a decrease in chlorophyll and extending quickly to young leaves as nitrogen is a high mobility nutrient (Bénard et al., 2009). In modern agriculture, 50–70% of the nitrogen applied to the soil is lost (Montemurro and Diacono, 2016) and in most cases, it reaches aquifers used for human consumption (Granados et al., 2013; Soto et al., 2018). Fruit plants demand more nutrients, and complex aquaponic systems (Goddek and Vermeulen, 2018) and deficiencies impact vegetable development (Bénard et al., 2009).

The concentrations in the N, P, and K were significantly different during flowering and fruiting relative to the rest of the vegetative cycle. The same elements were affected by the increase in the salinity of the irrigation water treatments. Osmotic pressure affects the transport flow of $Ca^{2+}$ and $Mg^{2+}$ in particular (Fan et al., 2011); salinity and age were found to have the strongest interaction effects on nutrient concentrations. In horticultural hydroponic crops, non-optimum concentrations of the nutrient solution give rise to slow growth, weak stems, premature death of old leaves, falling of flower buds, small fruits, and significantly reduced yield (Maucieri et al.,

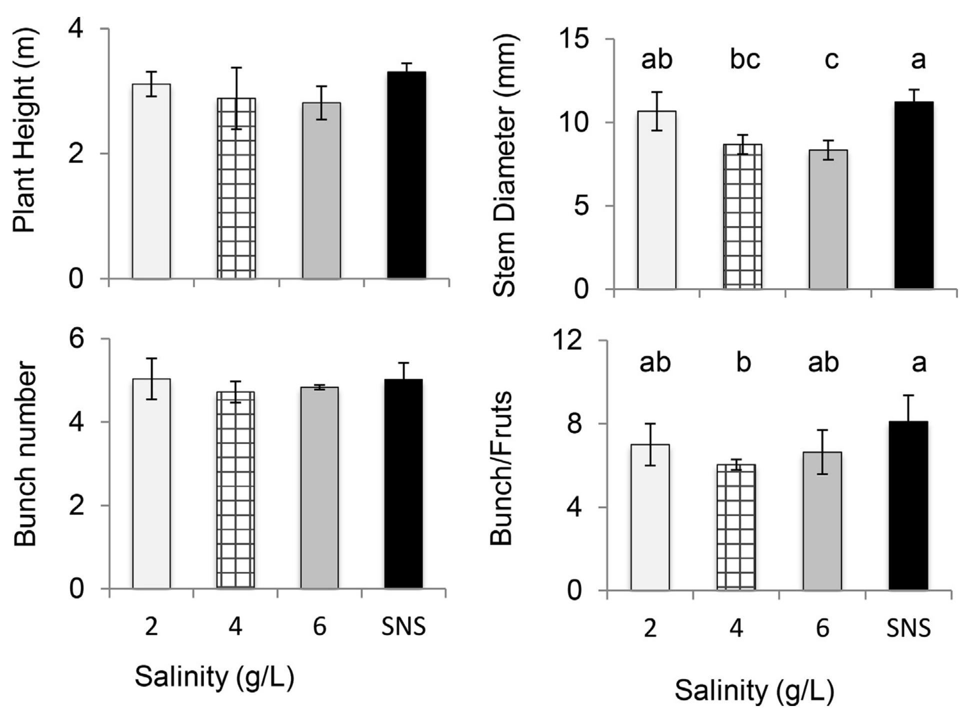


**Fig. 4.** Plant height (m), stem diameter (mm), bunch number, and fruits/bunch rate of grafted tomato subjected to salinities of 2, 4, 6 g/L, and the Steiner's universal nutrient solution (SNS) control treatment. Different letters above the bar indicate significant differences between salinities ($p < 0.05$).

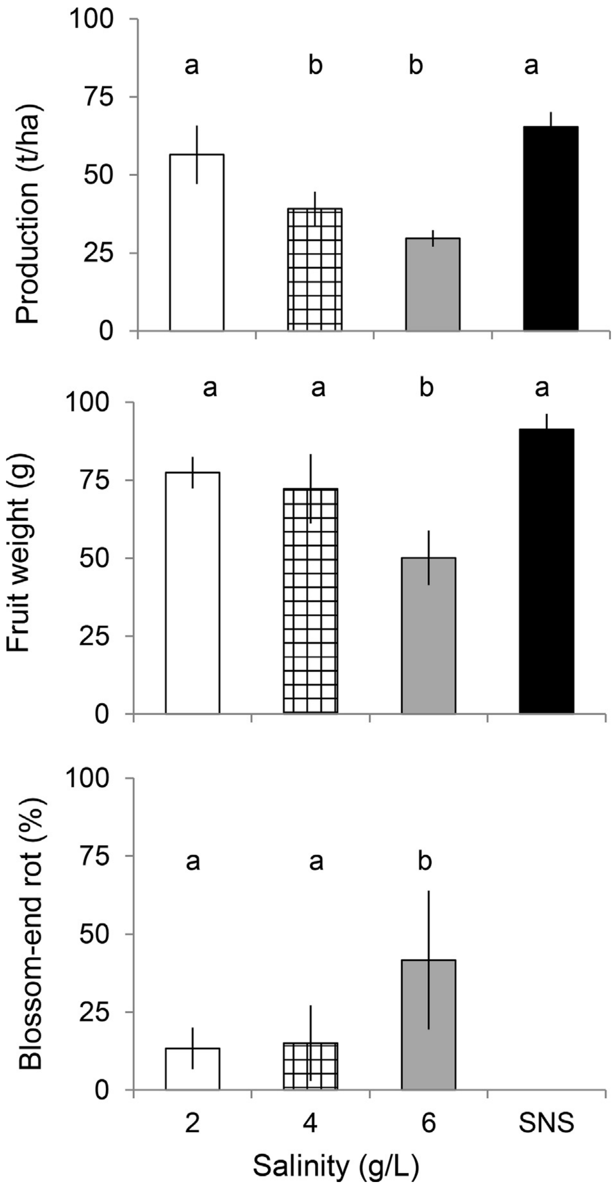


**Fig. 5.** Production (t/ha), fruit weight (g), and blossom-end rot (%) of grafted tomato subjected to salinities of 2, 4, and 6 g/L, and the Steiner's universal nutrient solution (SNS) control treatment. Different letters above the bar indicate significant differences between salinities ($p < 0.05$).

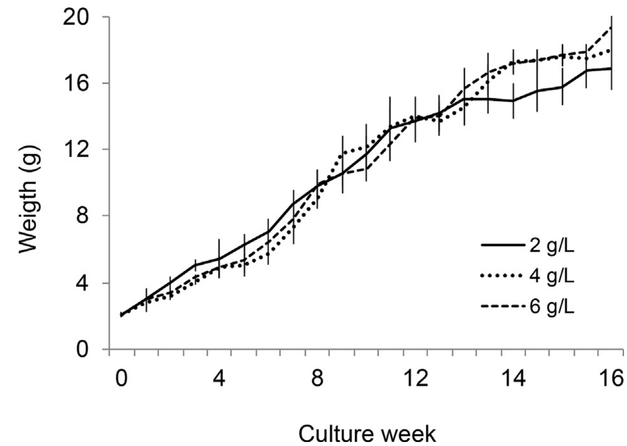


**Fig. 6.** Mean weights of Pacific white shrimp (*Penaeus vannamei*) stocked at 125 shrimp/$m^3$ in salinities of 2 (solid), 4 (point), and 6 g/L (dash) during the experimental period (16 weeks).

2019). The latter was observed mainly at salinity >2 g/L even with application of initial synthetic fertilizer.

### 4.3. Tomato morphology and production

Grafted tomato stem diameter (26%) was negatively affected by increased salinity. Costan et al. (2020) reported that the tomato stem diameter was reduced by 19% and plant height by 13% at EC = 7.5 dS/m. Changes in tomato stem diameter are related to the water status (Meng et al., 2017) and saline stress (El-Mogy et al., 2018) and correlated with growth rate, plant size, and fruit diameter and number (Noor et al., 2019). The rootstocks stem diameter can affect the delivery of nutrients to the shoots, and altering the transpiration rate (Albornoz et al., 2020). The height and bunch number were not significantly different between salinities treatments. Albornoz et al. (2020) have reported that the total number of fruits per bunch (22.25/4) remained unaffected with EC. The morphological characteristics of graft plants expressed tolerance to salinity and possibility the fruit number was established for the hybrid plant.

Tomatoes need nutrient balance and excessive salt or ions ($SO_4^{2-}$, $Ca^{2+}$, and $Mg^{2+}$) increase EC and interrupt nutrition (Savvas et al., 2009). Hydroponic tomatoes require good quality water and the nutrient solution must have a pH of 5.5–6.5, EC of 1.5–3.5 dS/m, and the minerals must dissociate in ionic form to avoid precipitates and antagonisms (Adams, 2004). Saline water also limits nutrient uptake (Zhang and Sonnewald, 2017), affecting shoot growth, root weight and yield. Marketable size is one important parameter of

**Table 3**
Growth indicators of Pacific white shrimp *Penaeus vannamei* reared at salinities of 2, 4, and 6 g/L, and stocking at a density of 125 shrimp/$m^3$ during the experimental period (16 weeks).

| Parameter | Salinity | | |
|---|---|---|---|
| | 2 g/L | 4 g/L | 6 g/L |
| Initial mean weight (g) | 2.03 ± 0.17 | 2.11 ± 0.14 | 2.03 ± 0.29 |
| Initial stocking density (g/$m^3$) | 244 ± 7.9 | 247 ± 4.9 | 244 ± 10.1 |
| Final mean weight (g) | 16.8 ± 1.3 | 17.9 ± 1.3 | 18.3 ± 1.4 |
| Survival rate (%) | 61.4 ± 8.4 | 66.7 ± 10.2 | 72.2 ± 14.5 |
| Final biomass (g/$m^3$) | 1019 ± 118.7$^a$ | 1156 ± 224.6 $^{ab}$ | 1360 ± 140.2$^b$ |
| Specific growth rate (%/day) | 1.47 ± 0.01$^a$ | 1.49 ± 0.07 $^{ab}$ | 1.53 ± 0.02$^b$ |
| Weight gain (g) | 14.7 ± 0.2$^a$ | 15.3 ± 1.8 $^{ab}$ | 16.2 ± 0.4$^b$ |
| Total weight gain (g/$m^3$) | 775.3 ± 126.6$^a$ | 902.1 ± 119.7 $^{ab}$ | 1116.7 ± 175.9$^b$ |
| Food conversion ratio | 1.78 ± 0.19 | 1.88 ± 0.15 | 1.71 0.23 |

*Each value represents mean ± S.D. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

fresh saladette tomato. In our study, fruit size was small to medium (61 g) at salinities of 2–6 g/L, and large (91 g) for the SNS control treatment. Only one recent study has been published on integrated shrimp and tomato production (Mariscal-Lagarda et al., 2012); with water EC = 1.3 dS/m, all tomato was large and extra-large (mean 94 and 110 g, respectively). According to Delaide et al. (2019), fruit weight was not significantly different in decoupled aquaponic (6.0 dS/m) versus hydroponic (5.1 dS/m) systems. In our study, the highest fruit weight was obtained with the SNS control treatment; the effluents water ≤4 g/L salinity was adequate to irrigate grafted tomato plants without significant reduction in fruit weight and quality.

This work is the first to develop an IAAS where grafted tomato was used to mitigate the effect of salinity stress on yields. Suhl et al. (2016) and Delaide et al. (2019) added nutritive solutions to irrigation water in a decoupled recirculating system, finding no significant difference in tomato yield using fish waste in aquaponic versus hydroponics systems: 293 and 316 t/ha without an EC effect and 483 and 480 t/ha with EC = 4.3–6.9 dS/m, respectively. Rahman et al. (2018) report that the strong salinity effect at 4 dS/m made no plant suitable for cultivation and the decrease in fruit size and number was accentuated during the harvest period. In our study, grafting helped mitigate the negative effect on the majority of the anatomical, plant growth, and productive traits evaluated in the presence of salt with nutrient limitation; the one exception was apical damage due to growing in higher salinity. Future research should evaluate performance in terms of biofiltration effects on water quality and clean fruit production.

### 4.4. Shrimp growth, survival, and production parameters

Pacific white shrimp *P. vannamei* can survive and grow at low salinities with adequate composition or ratio of dissolved ions (Esparza-Leal et al., 2010; Ray and Lotz, 2017). Tomato production requires a high $NO_3^-$ concentration, which is undesirable for shrimp culture in an integrate system. Kuhn et al. (2012) cultivated *P. vannamei* at a salinity of 2.0 g/L and 400 mg of $NO_3^-$/L, obtained a 100% of mortality and low GR of 0.73 g/week; at 11.0 g/L salinity and 220 mg of $NO_3^-$/L, mortality was 35.8% and GR 0.84 g/week. Without considering the effect of $NO_3^-$, the shrimp GR was depressed (0.73 and 0.81 g/week) at 2 and 6 g/L salinity and survival was between 60 and 70%. In commercial aquaculture, competitive GR is approximately 1 g/week. Shrimp performance ([GR, g/week], [TWG, kg/m$^3$], [FCR] and [S, %]) can be compared with other studies involving IAAS (Table 4). For example, at 36 g/L salinity, nitrogen recovery by *Sarcocornia ambigua* was 39.3% (Pinheiro et al., 2017); however, lower salinity (20 g/L) affected production (Poli et al., 2019). Moreover, *Ocimum basilicum* production at 1.7 g/L salinity (Fierro-Sañudo et al., 2018) and tomato production at 0.65 g/L salinity (Mariscal-Lagarda et al., 2012) were found to be feasible for IAAS with low-salinity waters. All previous authors concluded that shrimp performance parameters (except TWG) were not significantly limited by water characteristics. In contrast, our experiment revealed the disadvantages of poor water quality control and the salinity effects that occur in intensive crop systems.

During water quality verification, bacteria of the genus *Vibrio*, opportunistic parasites of shrimp, were observed; the non-pathogenic genus *Bacillus* was the most abundant group. Aquaculture effluents include feces, food residues, and bacterial biomass, all compounds rich in nutrients (Henares et al., 2020). In our recirculating system, effluent water solids were removed with two sedimentation devices and then flowed to the hydroponic growing areas for nutrients water treatment. During the removal of solids, it is estimated that an average of 21% of the dry weight originated by the feed during a production cycle is eliminated (Rakocy et al., 2006). The increased surface area to volume ratio (approximately 5000 L) of the IAAS was an efficient substrate for bacterial colonization, obtaining as result a positive exclusion between both groups of microorganisms as indicated by the low CFU of the genus *Vibrio*.

The most promising advantages of IAAS include the use of dissolved nutrients in aquaculture water for plants to avoid discharge into water bodies, minimize water exchange, and reduce the operating costs of the RAS, as design modifications can eliminate the need for costly biofilters and solids separators (Pantanella, 2018). In addition, the use of protected crops permits longer harvest periods when it is not possible to cultivate outdoors (Pantanella, 2018). The goal of modern sustainable agricultural is to reduce the impact on the environment (Somerville et al., 2014; Cole et al., 2018) and minimize dependence on non-renewable resources.

### 4.5. Techno-economic analysis

Other studies also have demonstrated that shrimp and tomato crops cultivated in controlled-climate environments are more rentable. With one annual crop grown in an aquaponic system, Suhl et al. (2016) reported that production was comparable to tomato yields from conventional hydroponics. Under similar climate conditions as those in the present study, the profitability of greenhouse tomato systems have a rentability assessment of CBA = 1.89 (García-Ruboca et al., 2016). Typically, two or more shrimp crops/year are necessary to reach CBA >1; in the techno-economic analysis presented here, shrimp profitability was relatively low as only one crop/year was produced for our study. The second reason for the relatively low profitability was that the required greenhouse (see Table S1) increased the initial construction costs (Mariscal-Lagarde et al., 2012). In rotational farming systems involving three plants (Ni et al., 2020) and evaluated using an economic benefit analysis, the net profits were 1.63–2.26 times higher than those obtained from shrimp monoculture.

In this study, resource optimization and IAAS were examined as a means of achieving this goal. The success of the productive relationship between shrimp and grafted tomato was exemplified by the fact that the stress tolerance of Solanaceae in 2 g/L (>4 dS/m) salinity doubled when wild rootstock was used. Water exchange to

**Table 4**
Performance indicators of Pacific white shrimp (*Penaeus vannamei*) reared in integrated agri-aquaculture (IAAS) with varying characteristics.

| Salinity (g/L) | GR | TWG | FCR | S | Reference |
|---|---|---|---|---|---|
| | (g/week) | (kg/m$^3$) | | (%) | |
| 36.00 | 1.00 ± 0.03 | 2.10 ± 0.10 | 1.7 ± 0.10 | 73.5 ± 1.9 | Pinheiro et al. (2017) |
| 20.00 | 1.50 ± 0.05 | 3.20 ± 0.75 | 1.7 ± 0.05 | 88.0 ± 2.0 | Poli et al. (2019) |
| 1.70 | 1.15 ± 0.06 | 0.65 ± 0.02 | – | 86.0 ± 2.0 | Fierro-Sañudo et al. (2018) |
| 0.65 | 0.73 ± 0.04 | 0.39 ± 0.02 | 1.60 ± 0.03 | 56.3 ± 1.1 | Mariscal-Lagarda et al. (2012) |

*GR = growth rate; TWG = total weight gain; FCR = feed conversion ratio; S = survival rate.

eliminate toxic by-products was avoided with continuous recycling during more than 150 days of shrimp-tomato culture. The maximum $NO_3^-$ of 40 mg/L was not toxic in water by nutrients incorporating into plants and without increase by permanent feed addiction. Shrimp and grafted tomato culture were productive at a threshold value of 4 g/L salinity for irrigation with effluent water. Further research should focus on whether aquaponic production may be developed as an agrotechnology that could enhance specific limited resources in this region and in areas with similar socio-economic and environmental characteristics.

## 5. Conclusions

A closed aquaculture system integrated with hydroponic and mineralization components was operated with the novel advantage of employing local plants as genetic resources, water and waste recycling capabilities, and productive symbiosis. Wild tomato from saline soil local to the microclimate of northwest Mexico was tested in varying salinity concentrations; the best "Guasave" generated 100% of resilient plants to 4 g/L salinity and was used as a salinity-tolerant rootstock. The advantage was a continuous cycle without exchange to purify the shrimp rearing water, populations of bacteria in the biofilter did not "crash off", and there were no significant or sudden changes in the pH, levels of TAN, $NO_2^-$, or $NO_3^-$, avoiding massive shrimp mortality.

To evaluate productivity, at 2 and 6 g/L salinity the shrimp yield (TWG = 0.77 and 1.12 kg/m$^3$) and tomato production (5.64 and 2.96 kg/m$^2$) were extrapolated to t/ha; results based on extrapolated productivity must be interpreted with caution. The productive performance of shrimp is boosted in salinity of 6 g/L and the tomato improves in 2 g/L; extremes are predominant since each developed better approaching its isosmotic value. In our study, 4 g/L salinity was found adequate for future studies focused on enhancing productivity using wild species and local vegetal resources. One problem associated with the tomato production was apical fruit rot due to high salinity; however, the associated benefit was the reduction of chemical fertilization to only initially support. This research solves the difficulty of integrating marine shrimp with tomato susceptible to salinity, closes the cycle with recycled water, and recovers the nutrients added to system. A cost-benefit analysis can be used in future research to assess the initial investment costs of building a scalable productive prototype and solving the technical deficiencies mentioned here. Future studies also should focus on improving plant and animal selection and increasing knowledge of nutrient and micronutrient dynamics in order to further develop IAAS as an efficient production alternative.

## CRediT authorship contribution statement

**Adolfo Dagoberto Armenta-Bojórquez:** Conceptualization, Methodology, Resources, Funding acquisition, Writing - original draft, Project administration. **Alba Rosario Valenzuela-Castañeda:** Conceptualization, Methodology, Investigation, Writing - original draft. **Kevin Fitzsimmons:** Conceptualization, Writing - review & editing. **Ely Sara López-Alvarez:** Methodology, Validation, Writing - original draft. **Gerardo Rodríguez-Quiroz:** Methodology, Validation. **Wenceslao Valenzuela-Quiñónez:** Conceptualization, Methodology, Resources, Funding acquisition, Writing - original draft, Writing - review & editing, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.124064.

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