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# Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology

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ABSTRACT

The aim of this study was to evaluate the integrated culture of *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc. The experiment was performed for 73 days and two treatments were evaluated: plants and control (without plants), with four replicates. Each experimental unit consisted of an 800 L tank, a 40 L conical bottom settling chamber, and a hydroponic bench with 0.4 $m^2$ of planting area and a capacity for 40 plants. The water from the shrimp tank was pumped continuously to the settling chamber and the overflow was distributed to the channels to irrigate the plants, then returned to the tank by gravity. Tanks were stocked with 250 shrimp $m^{-3}$ (1.4 ± 0.0 g). Shrimp were fed a commercial diet containing 35% crude protein, four times per day. At the end of the experiment, total nitrogen was determined in the shrimp, the plants and the ration, as well as antioxidant activity and total phenolic compounds in *Sarcocornia*. The water quality remained within the acceptable limits for the culture of marine shrimp. No significant differences were observed in the performance of *L. vannamei*. The final biomass of shrimp was 2.1 ± 0.1 kg $m^{-3}$, with a survival rate of 73.5 ± 1.9%, a final average weight of 11.7 ± 0.4 g, and a feed conversion ratio of 1.7 ± 0.1. The production of plants was 8.2 ± 0.3 kg $m^{-2}$. The antioxidant activity in *S. ambigua* was 38.3 ± 1.3 μmol TEAC 100 $g^{-1}$ FM, which characterizes this species as a functional food. The recovery of nitrogen supplied to the system through the ration was higher in the plants treatment (39.3%) than in the control (31.4%). In the proposed aquaponic system it was possible to produce 2 kg of plants for each kilogram of shrimp, integrating the production of *L. vannamei* and *S. ambigua*, and thus improve the use of nutrients in the culture.



## 1. Introduction

The increment of aquaculture production depends on the development and application of new technologies aimed at maximizing production with less environmental impact (Hu et al., 2015). In this context, biofloc technology (BFT) has proven to be a rational alternative to the intensification of production (Crab et al., 2012). The BFT system has characteristics, such as high stocking densities, limited water exchange, intensive aeration and oxygenation, and the accumulation of microbial flakes (Ebeling et al., 2006; Ray et al., 2010). In this system, resources are used more efficiently, compared with conventional production (Avnimelech, 2006; Burford et al., 2004).

In most conventional aquaculture systems, both extensive and semi-intensive, cultured animals retain only part of the nitrogen and phosphorus added to the system through the feed, and the rest is released to the environment (Buhmann et al., 2015). In biosecure intensive systems, such as the BFT, less effluent is formed, but with higher concentrations of nitrogen compounds (Quintã et al., 2015). This excess of nutrients can be reduced with the use of plants in an integrated culture system (Buhmann et al., 2015).

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Aquaponics is an innovative and sustainable solution because it combines the culture of aquatic animals with the hydroponic production of plants (Tyson et al., 2011). The aquaponic system can improve nutrient removal efficiency, reduce the use of water and effluent disposal to the environment, and also improve profitability through the simultaneous production of two crops (Diver, 2006). Rather than being discarded into the environment, residual nitrogen and phosphate compounds may be absorbed by plants, reducing nutrient waste (Hu et al., 2015). However, for the integration of hydroponics with marine aquaculture, salt-tolerant plants, or halophytes, should be used (Buhmann and Papenbrock, 2013a).

In this context, halophyte plants are known for been cultivated in areas where the salt concentration would be lethal to most other species (Flowers and Colmer, 2008). These species grow over mangroves and salt marshes, and in Brazil, there is an occurrence of species such as *Sarcocornia ambigua* (synonymous *Salicornia gaudichaudiana*) (Alonso and Crespo, 2008; Costa et al., 2006). Plants of the genus *Sarcocornia* (family Amaranthaceae) are characterized by simple morphology, since they produce only succulent shoots without leaves and have a perennial life cycle (Ventura and Sagi, 2013; Ventura et al., 2011).

*Salicornia* was introduced to the European market for human consumption as a vegetable similar to green asparagus, being consumed like salad and also as a spice (Bertin et al., 2014). The young and succulent shoots have gained prominence not only for their taste but also for their high nutritional value in terms of mineral composition and bioactive compounds, such as phenolics (Bertin et al., 2014; Ventura and Sagi, 2013).

Some authors have obtained positive results for the production of vegetables irrigated with effluents from shrimp farms (Dufault and Korkmaz, 2000; Dufault et al., 2001; Mariscal-Lagarda et al., 2012; Miranda et al., 2008) and for the treatment of marine effluent using halophyte plants of the genera *Sarcocornia* and *Salicornia* (Buhmann et al., 2015; Glenn et al., 2013; Rozema et al., 2013; Shpigel et al., 2013; Webb et al., 2012). Thus, the aim of this study was to evaluate the culture of the halophyte *Sarcocornia ambigua* and shrimp *Litopenaeus vannamei* in an aquaponic system with biofloc.

## 2. Material and methods

### 2.1. Biological material

#### 2.1.1. Shrimp

The experiment was conducted at the Marine Shrimp Laboratory (LCM) of the Federal University at Santa Catarina, southern Brazil. Specific pathogen- free post-larvae of *Litopenaeus vannamei* where purchased from a commercial hatchery (Aquatec Ltda., Canguaretama, RN, Brazil) and grown under an intensive biofloc system in a matrix circular fiberglass tank of 50 $m^3$, until they reached the weight required to begin the experiment.

#### 2.1.2. Plants

Seedlings were grown through vegetative propagation by cuttings, in an aquaponic system with floating rafts. Matrix plants of *Sarcocornia ambigua* from LCM were cut into 10 cm cuttings without leaves and beveled at the bottom. These cuttings were planted in polystyrene sheets, leaving approximately 2 cm submerged for rooting. The sheets remained floating in a circular fiberglass tank with aeration and 20 $m^3$ of water from the shrimp culture in biofloc, where they remained for 60 days. After this period, 160 seedlings were individually weighed (average weight of 4.4 ± 3.0 g) and transferred to the aquaponic system.

### 2.2. Experimental design, experimental units and system management

Each aquaponic experimental unit consisted of an 800 L circular polyethylene tank with a heater, aeration and artificial substrates, a settling chamber and a hydroponic bench for the plants (Fig. 1).

One day before the experiment began, the tanks were filled with water from the 50 $m^3$ matrix tank. This water had a concentration of total suspended solids of 425.0 mg $L^{-1}$, total ammonia nitrogen of 0.0 mg $L^{-1}$, nitrite of 0.8 mg $L^{-1}$, and nitrate of 65. mg $L^{-1}$. The biofloc in the matrix tank was already in a chemotrophic stage, with the nitrification process established. During the trial, the addition of organic carbon was therefore not necessary for the maintenance of water quality parameters (Ebeling et al., 2006). Each tank was stocked with 200 shrimp with an average weight of 1.4 g, representing a stocking density of 250 shrimp $m^{-3}$.

The structure for growing the plants was constructed 0.5 m above the water level of each shrimp tank. This structure was based on the nutrient film technique (NFT) system, where the roots of the plants are partially submerged in a film of water passing through the irrigation channel (Lennard and Leonard, 2006). These channels were composed of five PVC pipes of 75 mm diameter and 1.10 m length, arranged side by side. The pipes were painted with aluminum enamel paint to reflect the light and avoid heating of the water film (Rodrigues, 2002) and placed on wooden supports with a 4% slope. Each channel contained eight plants, spaced 12 cm apart (Izeppi, 2011). Each bench had 0.4 $m^2$ of planting area with capacity for 40 seedlings of *S. ambigua*, which is equivalent to a density of 100 plants $m^{-2}$. Plants were placed in supports made of 50 mm diameter PVC pipe and nylon screen, with perlite added as a substrate (Ventura et al., 2011).

To protect the roots from the excess solids produced by shrimp culture (Hu et al., 2015), before irrigation a 40 L conical bottom settling chamber was used (Baloi et al., 2013). The water was pumped continuously at a flow rate of 3.0 L $min^{-1}$ using a submerged pump (Sarlo Better model SB650, São Caetano do Sul, SP, Brazil). A PVC hose of 1 “connected the upper output of the settling chamber to a 50 mm diameter PVC pipe arranged perpendicularly on the bench, and PVC hoses of 3/8” distributed the water in each channel by gravity. After irrigating the plants, the water was collected in the channel at the end of the bench and returned to the tank by gravity.

To maintain the proper concentration of suspended solids in the culture water (Schveitzer et al., 2013), every hour the accumulated sludge in the settling chamber was pumped for 40 s back to the culture tank, with a flow of 15 L $min^{-1}$ through an electric bomb (Emicol, Itu, SP, Brazil) connected to the bottom outlet of the settling chamber. Thus, until the water level was restored in the settling chamber the channels remained without irrigation.

In the aquaponic experimental units, two treatments were evaluated, plants and control. The control treatment consisted of the same system and operating mode, but without plants in the bench. Each treatment had four replicates, totaling eight experimental units that were randomized in a 243 $m^2$ greenhouse.

Shrimp were fed four times per day (08:00, 11:00, 14:00 and 17:00) with a commercial feed containing 35% crude protein (Guabi Potimar, Campinas, SP, Brazil). The amount of feed provided was calculated weekly based on weight gain, survival and the expected feed conversion ratio (Ray et al., 2010). Calcium hydroxide was added when the alkalinity was under 120 mg $L^{-1}$, at a rate of 20% of the daily feed intake (Schveitzer et al., 2013). Throughout the experimental period there was no renewal of water and only the volume lost by evaporation was replaced.

The light intensity inside the greenhouse (at 14:00), which ranged from 67.0 to 1254.7 μmol photons $m^{-2}$ $s^{-1}$, and the temperature, which ranged from 27 to 43 °C, were determined weekly.

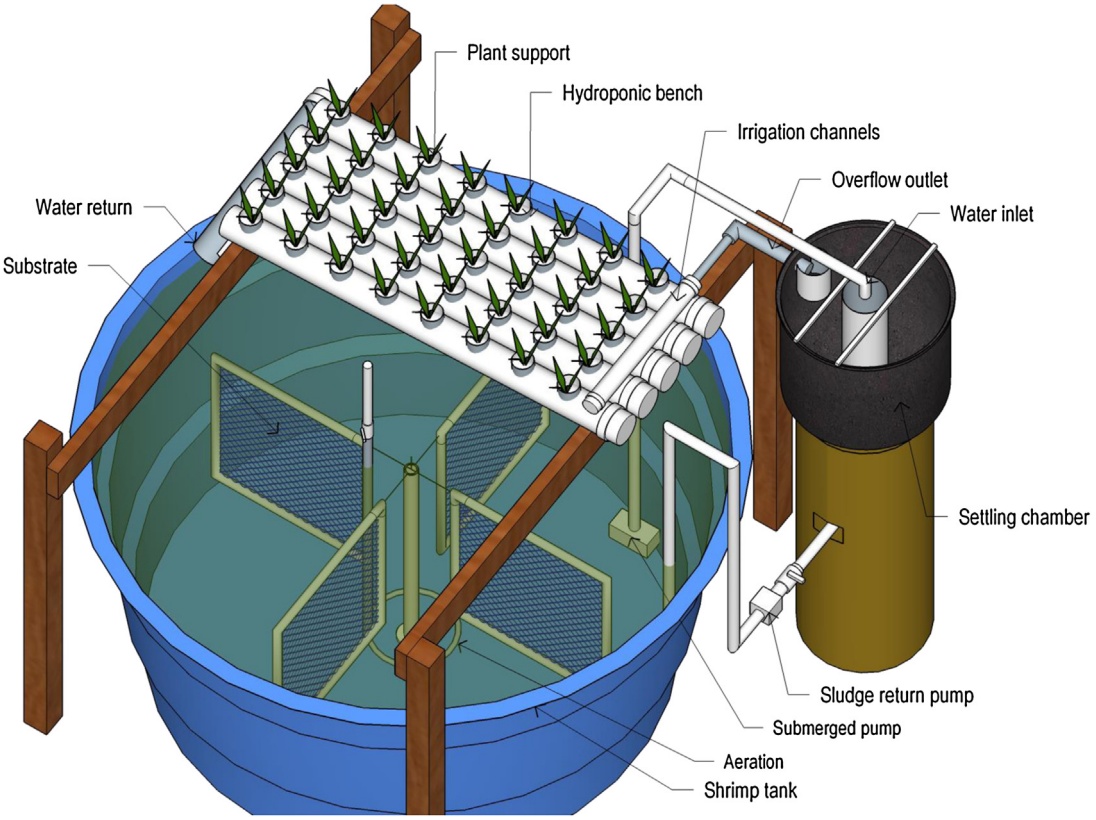


**Fig. 1.** Aquaponic experimental unit used in the experiment.

The photoperiod during the experiment was 14 h light and 10 h dark.

### 2.3. Water quality variables

During the experiment, the dissolved oxygen and temperature were measured twice per day, at 08:00 and 17:00 (Dissolved Oxygen Meter YSI, model Pro20). The pH (pH meter YSI, model pH100), total suspended solids, nitrate, alkalinity (APHA, 2005), total ammonia nitrogen (TAN), nitrite, orthophosphate (Strickland and Parsons, 1972), and salinity (EcoSense YSI, model EC300A) were measured twice per week.

### 2.4. Performance indexes of shrimp

To evaluate the weekly growth rate, 20 shrimp were sampled from each experimental unit. The experiment lasted ten weeks. After the conclusion of the experimental period, the following performance indexes were evaluated:

average final weight (g) = biomass (g)/final number of animals

weekly weight gain (grams per week) = {(average final weight (g) − average initial weight (g))/days of culture * 7}

production ($g.m^{-3}$) = final biomass (g)/tank volume ($m^3$)

survival (%) = (final number of animals/initial number of shrimp) * 100

feed conversion ratio = feed intake (g)/gain of biomass (g)

### 2.5. Plant production indexes

At the end of the experimental time, all plants were weighed individually. The average final weight (g), final biomass (kg), gain in biomass (kg), and production ($kg\,m^{-2}$) were then calculated.

### 2.6. Analysis of nitrogen in plants, shrimp and rations

To determine the content of total Kjeldahl nitrogen (TKN), at the end of the culture 15 shrimp and 1 kg of *Sarcocornia ambigua* were collected from each experimental unit, as well as a sample of the ration used in the experiment. The analyses were performed according to the total nitrogen content determined by the Kjeldahl method (code 920.87) described by AOAC (2005).

### 2.7. Analysis of antioxidant activity and total phenolic compounds of Sarcocornia ambigua

Plants extracts from each experimental unit were prepared using 10 g of fresh sample (aerial part) with 25 mL of methanol in an ultrasonic bath (Unique, 1400a, São Paulo, SP, Brazil) at room temperature for 1 h. The extracts were then centrifuged at 14000 RPM for 5 min (Eppendorf MiniSpin Plus). The supernatant was recovered for analysis.

Antioxidant capacity was estimated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Willians et al. (1995) and was expressed in fresh weight as micromoles of Trolox equivalent antioxidant capacity per 100 g of fresh matter (μmol TEAC $100\,g^{-1}$ FM) (Bertin et al., 2014).

Total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Absorbance was read at 765 nm and results were compared to a standard curve of gallic acid solution and expressed in fresh weight as milligrams of gallic acid equivalents per 100 g of fresh matter (mg GAE $100\,g^{-1}$ FM).

**Table 1**
Water quality variables in tanks of *Litopenaeus vannamei* cultivated in an aquaponic system for 73 days at a stocking density of 250 shrimp $m^{-3}$.

| Parameter | Treatment | | ANOVA | | |
|---|---|---|---|---|---|
| | Plants | Control | T | W | TxW |
| Temperature (°C) | 29.5 ± 0.6 (27.8–32.9) | 29.8 ± 0.6 (28.5–33.4) | – | – | – |
| Dissolved Oxygen ($mg\,L^{-1}$) | 5.67 ± 0.17 (4.82–7.16) | 5.57 ± 0.19 (4.7–7.11) | – | – | – |
| Salinity ($g\,L^{-1}$) | 36.2 ± 1.6 (31–38) | 36.4 ± 1.5 (34–39) | ns | * | ns |
| pH | 8.0 ± 0.1 (7.8–8.2) | 8.0 ± 0.1 (7.8–8.1) | ns | * | ns |
| Alkalinity ($mg\,L^{-1}$) | 163.5 ± 18.5 (136–208) | 150.8 ± 13.8 (118–182) | * | * | * |
| Total Suspended Solids ($mg\,L^{-1}$) | 333.8 ± 53.9 (257–459.5) | 371.3 ± 53.2 (266–445) | * | * | * |
| Total ammonia – N ($mg\,L^{-1}$) | 0.3 ± 0.1 (0.0–0.5) | 0.2 ± 0.1 (0.0–0.6) | * | * | * |
| Nitrite ($mg\,NO_2^-$-N $L^{-1}$) | 0.6 ± 0.3 (0.1–1.1) | 0.4 ± 0.3 (0.0–1.0) | * | * | * |
| Nitrate ($mg\,NO_3^-$-N $L^{-1}$) | 21.4 ± 17.0 (6.0–79.2) | 22.9 ± 16.7 (10.2–80.6) | ns | * | * |
| Orthophosphate ($mg\,PO_4^{3-}$-P $L^{-1}$) | 5.3 ± 1.1 (2.5–7.5) | 5.5 ± 1.0 (3.1–7.4) | ns | * | ns |

Values are means ± standard deviation (range). Repeated measures ANOVA: T (treatment), W (weeks). TxW (TxW interaction), *Significant difference ($p < 0.05$); ns: not significant.

### 2.8. Statistical analysis

Water quality variables were analyzed by one-way repeated measures ANOVA. Performance indexes and nitrogen recovery were compared by one-way ANOVA. Homoscedasticity and normality were tested by Bartlett and Shapiro-Wilk tests, respectively. All statistical tests were evaluated at a 5% significance level.

## 3. Results

### 3.1. Water quality variables

Temperature and dissolved oxygen were similar in the two treatments. pH remained constant over culture and was not significantly different between treatments ($p > 0.05$). No statistically significant difference was detected in salinity, which showed similar means between the two treatments. Alkalinity was higher in the plants treatment than in the control (Table 1).

The concentration of total suspended solids was lower in the plants treatment than in the control. There was no significant difference in orthophosphate between treatments ($p > 0.05$). Total ammonia nitrogen (TAN) increased over culture time in both treatments, but was higher during weeks seven and eight in the plants treatment (Fig. 2-a). Nitrite was higher in the plants treatment between weeks five and nine (Fig. 2-b). No significant differences were observed in nitrate concentration ($p > 0.05$) between the two treatments (Fig. 2-c).

### 3.2. *Performance of* Litopenaeus vannamei

No significant differences were observed ($p > 0.05$) between treatments for any of the shrimp performance parameters analyzed (Table 2).

### 3.3. Sarcocornia ambigua *production*

At the end of the experiment, 3.3 ± 0.1 kg of *S. ambigua* were harvested in each experimental unit, which is equivalent to a production of 8.2 ± 0.3 $kg\,m^{-2}$. The average final weight of the plants was 85.08 ± 59.07 g and the plant biomass gain in each aquaponic unit was 3.1 ± 0.2 kg.

**Table 2**
Production indexes of *L. vannamei* cultivated in an aquaponic system with biofloc at a stocking density of 250 shrimp $m^{-3}$ for 73 days.

| Parameter | Treatment | |
|---|---|---|
| | Plants | Control |
| Final average weight (g) | 11.6 ± 0.3 | 11.7 ± 0.5 |
| Average weight gain ($g\,week^{-1}$) | 1.0 ± 0.03 | 1.0 ± 0.05 |
| Production ($g\,m^{-3}$) | 2159.4 ± 0.0 | 2121.9 ± 0.1 |
| Survival (%) | 74.5 ± 2.1 | 72.5 ± 1.2 |
| Feed conversion ratio | 1.7 ± 0.1 | 1.7 ± 0.1 |
| Produced biomass (g) | 1450.5 ± 29.8 | 1423.5 ± 46.7 |

Values are means ± standard deviation.

### 3.4. Nitrogen utilization

The nitrogen contents of the ration, shrimp and plants were 5.2%, 2.7 ± 0.1%, and 0.4 ± 0.0%, respectively. During the culture, 2418 ± 37.1 g of feed were added per experimental unit, which correspond to 124.7 ± 1.9 g of nitrogen per tank. The nitrogen recovery as product was higher in the plants treatment ($p < 0.05$), where 37.8 ± 1.7 g and 11.5 ± 0.7 g were recovered in shrimp and *S. ambigua* biomass, respectively, representing 39.3% of the nitrogen added (Fig. 3). In the control treatment, 38.9 ± 3.2 g were recovered in shrimp biomass, corresponding to 31.4% of the nitrogen intake from the feed.

### 3.5. *Total phenolic compounds and antioxidant activity of* Sarcocornia ambigua

The phenolic content of *S. ambigua* extract determined from the standard curve of gallic acid was 41.34 ± 1.67 mg GAE 100 $g^{-1}$ FM. The antioxidant activity measured by DPPH was 38.30 ± 1.28 μmol TEAC 100 $g^{-1}$ FM.

## 4. Discussion

### 4.1. Water quality variables

Temperature, dissolved oxygen, pH, and salinity remained within the proper limits for the culture of *L. vannamei* in biofloc system (Baloi et al., 2013; Ray et al., 2010; Vinatea et al., 2010).

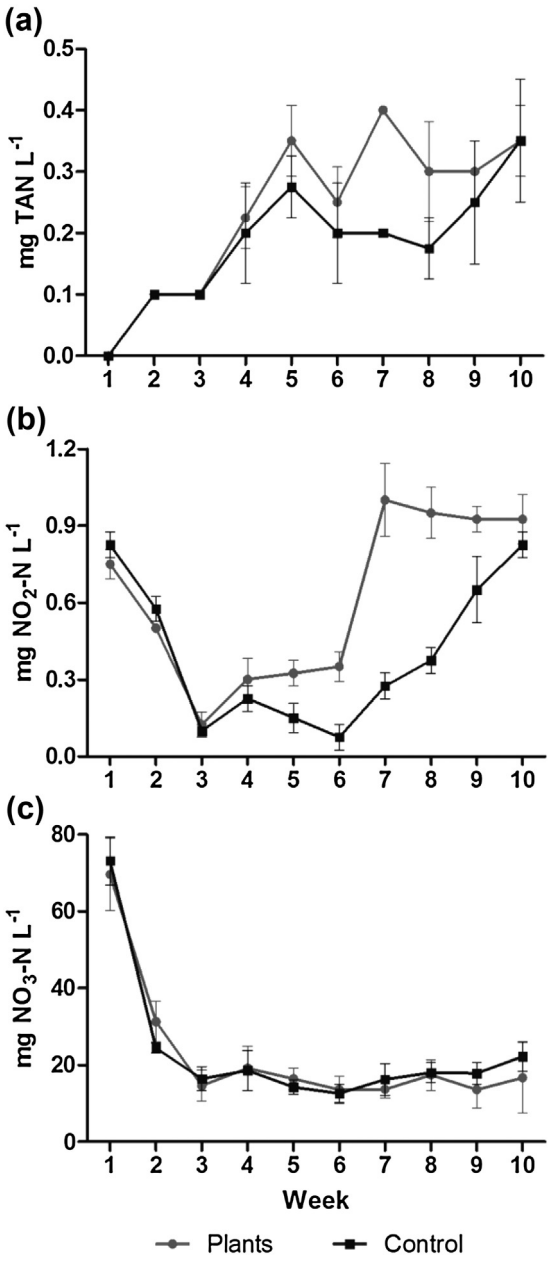


**Fig. 2.** (a) Total ammonia nitrogen (TAN), (b) nitrite and (c) nitrate in tanks of *Litopenaeus vannamei* cultivated in aquaponic system with biofloc at a stocking density of 250 shrimp $m^{-3}$ for 73 days.

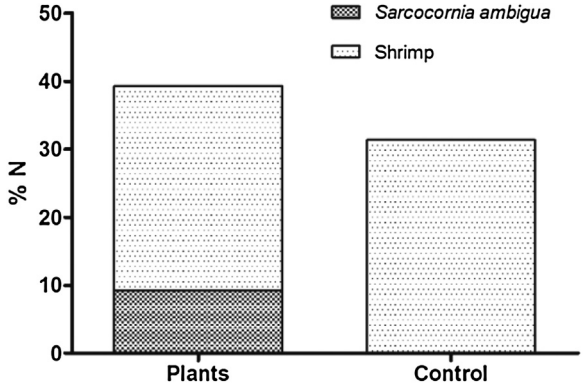


**Fig. 3.** Nitrogen recovery (%) in shrimp and plant biomass cultivated in an aquaponic system for 73 days.

In BFT culture, the use of settling chambers reduces the solid concentration and improves the water quality and performance of shrimp (Ray et al., 2010; Schveitzer et al., 2013). During the experimental period, no removal of solids from the system was carried out because the solids were returned to the tank from the bottom of the settling chamber. Nevertheless, retention and degradation of solids may have occurred inside the settling chamber and in the channels of the bench, since there was a reduction in the concentration of TSS in the culture water (Rakocy, 2012; Ray et al., 2010). This reduction was observed in both treatments, but was more pronounced in the plants treatment, possibly due to the retention of TSS in the root zone (Jewell et al., 1983). However, the accumulation of organic matter in the irrigation channels may be beneficial, since the decomposition of solids can release inorganic nutrients essential to plant growth, in a process known as mineralization (Rakocy, 2012).

The decrease in the concentration of TSS was probably also responsible for the reduction in nitrifying bacterial biomass in the system (Hu et al., 2015) leading to the gradual accumulation of TAN in the two treatments during the weeks. With the growth of ammonia-oxidizing bacteria, the accumulated TAN was gradually oxidized to $NO_2$ (Hu et al., 2015). Oxidation of nitrite to nitrate is a process that occurs more slowly, resulting in the accumulation of $NO_2$ and the maintenance of low concentrations of $NO_3$ in the system (Ebeling et al., 2006; Ray et al., 2011). The concentration of nitrate, in turn, decreased during the first weeks of the experiment. This reduction may have been due to the simultaneous occurrence of denitrification within the settling chamber and the decreased rate of nitrification caused by the reduction in solid concentration (Ray et al., 2011). After that, contrary to what we expected, there was no reduction in $NO_3$, suggesting preferential $NH_4$ absorption by *S. ambigua*. Species of the *Salicornia* and *Sarcocornia* genera can use both $NO_3$ and $NH_4$ as nitrogen sources (Quintã et al., 2015), however, under conditions of high salinity, as were present in this experiment, the absorption of $NO_3$ can be reduced and the use of $NH_4$ becomes more favorable for the growth of halophytes (Kudo and Fujiyama, 2010; Quintã et al., 2015).

### 4.2. *Performance of* Litopenaeus vannamei *and production of* Sarcocornia ambigua

The performance of *L. vannamei* was similar to that observed in other cultures with biofloc systems. Final weight, production and survival were close to those obtained by Ray et al. (2010) ($11.6 \pm 1.1$ g, $2.21 \pm 0.12$ kg $m^{-3}$ and 71.8% respectively). Weekly weight gain was similar to that obtained by Jatobá et al. (2014) in the same experimental conditions and the feed conversion ratio was close to that obtained by McIntosh et al. (2001). In this study, no differences were found in the production indexes between the treatments, indicating that the presence of plants in the proposed aquaponic system does not affect the performance of marine shrimp (Lennard and Leonard, 2006; Mariscal-Lagarda et al., 2012).

The productive potential of a halophyte may vary according to the species and salinity to which it is subjected during culture (Ventura and Sagi, 2013; Ventura et al., 2011). Species of the genus *Sarcocornia* are characterized by slow growth and low productivity when irrigated with seawater (Ventura et al., 2011). Nevertheless, after 73 days of experimentation, the average production of fresh biomass of *S. ambigua* was 8 kg $m^{-2}$. This value is higher than that obtained in high salinity cultures of species from the genus *Salicornia*. Ventura et al. (2011) reported a production of 6 kg $m^{-2}$ in hydroponic culture with seawater enriched with fertilizers. In an experimental culture of *S. ambigua* irrigated with shrimp farm effluent held in Brazil, up to 2 kg $m^{-2}$ of fresh biomass were obtained after 150 days of culture (Izeppi, 2011).

### 4.3. Nitrogen recovery in the system

One of the features of an intensive culture system is the provision of a high amount of food rich in nutrients. Protein is the most expensive component of the ration, and feed represents more than half of the production costs (Casillas-Hernández et al., 2007). However, only about 30% of the nitrogen added to the system through the feed is recovered in the biomass of fish and shrimp harvested (Endut et al., 2013). Thus, the efficiency of an aquaculture system may be evaluated based on the conversion of N in harvested biomass (Endut et al., 2013). In this study, the nitrogen content of shrimp and plants were 2.7% and 0.4%, respectively, in close agreement with those reported in the literature (Bertin et al., 2014; Jackson et al., 2003; Lu et al., 2010). The N recovered as *L. vannamei* biomass (16.2 g per kg of added feed) is close to the values reported by other authors in semi-intensive culture of marine shrimp (Casillas-Hernández et al., 2006; Páez-Osuna et al., 1997). Nevertheless, the use of the nitrogen added to the system through the feed was 25% more efficient in the integrated culture of shrimp and plants, even with the N low content found in *S. ambigua*.

### 4.4. Total phenolic compounds and antioxidant activity of Sarcocornia ambigua

The phenolic compounds are the main secondary metabolites of plants and are directly related to antioxidant activity in halophytes (Essaidi et al., 2013; Gargouri et al., 2013). In the samples of *S. ambigua* grown in an aquaponic system, the content of phenolic compounds was moderate ($41.34 \pm 1.67$ mg GAE $100\,g^{-1}$ FM) and was close to that reported by Gargouri et al. (2013) in *Sarcocornia perennis* extract, but lower than the values found in extract of *Salicornia* species that, depending on growing conditions, have values close to 200 mg GAE $100\,g^{-1}$ FM (Essaidi et al., 2013; Ventura et al., 2011). However, the values found in *S. ambigua* are close to those found in other leafy vegetables classified as rich in phenolic compounds (>50 mg GAE $100\,g^{-1}$ FM) (Isabelle et al., 2010a), because the production of these compounds may vary according to the species and the environmental conditions to which plants are subjected, including salinity, water and nutrient availability, and light intensity (Buhmann and Papenbrock, 2013a; Ventura and Sagi, 2013).

The *Sarcocornia ambigua* extract analyzed showed moderate antioxidant activity ($38.30 \pm 1.28$ μmol TEAC $100\,g^{-1}$ FM), which is related to the phenolic compound content found in the samples (Gargouri et al., 2013). The results obtained in this study are close to those presented by Bertin et al. (2014) for the same species irrigated with the effluent of *L. vannamei* culture, though the same authors reported higher values ($135.83 \pm 5.00$ μmol TEAC $100\,g^{-1}$ FM) for *S. ambigua* samples collected in their natural habitat.

The synthesis of antioxidant compounds is one of the plant's metabolic responses to oxidative damage (Bertin et al., 2014; Ksouri et al., 2008). Salt stress in halophytes can lead to the production of reactive oxygen species, affecting cell membrane integrity and enzyme activity (Buhmann and Papenbrock, 2013b; Ventura et al., 2011). Growth under suboptimal conditions can induce stress and consequently, result in an increase in secondary metabolites, such as antioxidants (Boestfleisch et al., 2014). According to the results of this study, it is possible that plants grown in an aquaponic system were not subjected to stress or had already adapted to the conditions imposed by the culture (Boestfleisch et al., 2014).

Notwithstanding, the concentrations of phenolic compounds and antioxidant activity of *S. ambigua* grown in this study are comparable to those found in some vegetables, such as asparagus, green pepper, onion, and tomato (Isabelle et al., 2010a; Kähkönen et al., 1999), and fruits such as nectarine, papaya, and pineapple (Isabelle et al., 2010b), and indicate that the culture of *S. ambigua* in seawater can be a viable option for secondary metabolite production (Buhmann and Papenbrock, 2013b).

## 5. Conclusions

Using the proposed aquaponic system, it is possible to produce 2 kg of plants for each kilogram of shrimp and to demonstrate that the production of *S. ambigua* in an integrated system with intensive shrimp culture does not affect the growth performance of animals. The use of nitrogen is 25% more efficient in the integrated culture of shrimp and plants. In this production system, quantities of phenolic compounds and antioxidant activity found in *Sarcocornia ambigua* characterize it as a promising source of natural antioxidants for human consumption.

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## References

AOAC, 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington.

Alonso, M.Á., Crespo, M.B., 2008. Taxonomic and nomenclatural notes on south american taxa of *Sarcocornia* (Chenopodiaceae). Ann. Bot. Fenn. 45, 241–254, http://dx.doi.org/10.5735/085.045.0401.

Avnimelech, Y., 2006. Bio-filters: the need for an new comprehensive approach. Aquac. Eng. 34, 172–178, http://dx.doi.org/10.1016/j.aquaeng.2005.04.001.

Baloi, M., Arantes, R., Schveitzer, R., Magnotti, C., Vinatea, L., 2013. Performance of Pacific white shrimp *Litopenaeus vannamei* raised in biofloc systems with varying levels of light exposure. Aquac. Eng. 52, 39–44, http://dx.doi.org/10.1016/j.aquaeng.2012.07.003.

Bertin, R.L., Gonzaga, L.V., Borges, G. da S.C., Azevedo, M.S., Maltez, H.F., Heller, M., Micke, G.A., Tavares, L.B.B., Fett, R., 2014. Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC–ESI–MS/MS. Food Res. Int. 55, 404–411, http://dx.doi.org/10.1016/j.foodres.2013.11.036.

Boestfleisch, C., Wagenseil, N.B., Buhmann, A.K., Seal, C.E., Wade, E.M., Muscolo, A., Papenbrock, J., 2014. Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. AoB Plants 6, http://dx.doi.org/10.1371/.

Brand-Willians, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. Leb. Technol. – LWT 28, 25–30.

Buhmann, A., Papenbrock, J., 2013a. Biofiltering of aquaculture effluents by halophytic plants: basic principles, current uses and future perspectives. Environ. Exp. Bot. 92, 122–133, http://dx.doi.org/10.1016/j.envexpbot.2012.07.005.

Buhmann, A., Papenbrock, J., 2013b. An economic point of view of secondary compounds in halophytes. Funct. Plant Biol. 40, 952–967, http://dx.doi.org/10.1071/FP12342.

Buhmann, A.K., Waller, U., Wecker, B., Papenbrock, J., 2015. Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. Agric. Water Manag. 149, 102–114, http://dx.doi.org/10.1016/j.agwat.2014.11.001.

Burford, M. a., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. Aquaculture 232, 525–537, http://dx.doi.org/10.1016/s0044-8486(03)00541-6.

Casillas-Hernández, R., Magallón-Barajas, F., Portillo-Clarck, G., Páez-Osuna, F., 2006. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: trays and mechanical dispersal. Aquaculture 258, 289–298, http://dx.doi.org/10.1016/j.aquaculture.2006.03.027.

Casillas-Hernández, R., Nolasco-Soria, H., García-Galano, T., Carrillo-Farnes, O., Páez-Osuna, F., 2007. Water quality, chemical fluxes and production in semi-intensive Pacific white shrimp (Litopenaeus vannamei) culture ponds utilizing two different feeding strategies. Aquac. Eng. 36, 105–114, http://dx.doi.org/10.1016/j.aquaeng.2006.09.001.

Costa, C.S., Armstrong, R., Detres, Y., Koch, E.W., Bertiller, M., Beeskow, A., Neves, L.S., Tourn, G.M., Bianciotto, O.A., Pinedo, L.B., Blessio, A.Y., San Roman, N., 2006. Effect of ultraviolet-B radiation on salt marsh vegetation: trends of the genus *Salicornia* along the Americas. Photochem. Photobiol. 82, 878–886, http://dx.doi.org/10.1562/2005-10-30-RA-729.

Crab, R., Defoirdt, T., Bossier, P., Verstraete, W., 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 356 (-357), 351–356, http://dx.doi.org/10.1016/j.aquaculture.2012.04.046.

Diver, S., 2006. Aquaponics—Integration of hydroponics with aquaculture. ATTRA Natl. Sustain. Agric. Inf. Serv. Natl. Cent. Appropr. Technol. 28.

Dufault, R., Korkmaz, A., 2000. Potential of biosolids from shrimp aquaculture as a fertilizer in bell pepper production. Compost Sci. Util. 8, 310–319.

Dufault, R., Korkmaz, A., Ward, B., 2001. Potential of biosolids from shrimp aquaculture as a fertilizer for broccoli production. Compost Sci. Util. 9, 107–114.

Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture 257, 346–358, http://dx.doi.org/10.1016/j.aquaculture.2006.03.019.

Endut, A., Jusoh, A., Ali, N., 2013. Nitrogen budget and effluent nitrogen components in aquaponics recirculation system. Desalin. Water Treat. 52, 744–752, http://dx.doi.org/10.1080/19443994.2013.826336.

Essaidi, I., Brahmi, Z., Snoussi, A., Ben Haj Koubaier, H., Casabianca, H., Abe, N., El Omri, A., Chaabouni, M.M., Bouzouita, N., 2013. Phytochemical investigation of Tunisian Salicornia herbacea L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. Food Control 32, 125–133, http://dx.doi.org/10.1016/j.foodcont.2012.11.006.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963, http://dx.doi.org/10.1111/j.1469-8137.2008.02531.x.

Gargouri, M., Magne, C., Dauvergne, X., Ksouri, R., El Feki, A., Metges, M.A., Talarmin, H., 2013. Cytoprotective and antioxidant effects of the edible halophyte *Sarcocornia perennis* L. (swampfire) against lead-induced toxicity in renal cells. Ecotoxicol. Environ. Saf. 95, 44–51, http://dx.doi.org/10.1016/j.ecoenv.2013.05.011.

Glenn, E.P., Anday, T., Chaturvedi, R., Martinez-Garcia, R., Pearlstein, S., Soliz, D., Nelson, S.G., Felger, R.S., 2013. Three halophytes for saline-water agriculture: an oilseed, a forage and a grain crop. Environ. Exp. Bot. 92, 110–121, http://dx.doi.org/10.1016/j.envexpbot.2012.05.002.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C., Khanal, S.K., 2015. Effect of plant species on nitrogen recovery in aquaponics. Bioresour. Technol., http://dx.doi.org/10.1016/j.biortech.2015.01.013.

Isabelle, M., Lee, B.L., Lim, M.T., Koh, W.P., Huang, D., Ong, C.N., 2010a. Antioxidant activity and profiles of common vegetables in Singapore. Food Chem. 120, 993–1003, http://dx.doi.org/10.1016/j.foodchem.2009.11.038.

Isabelle, M., Lee, B.L., Lim, M.T., Koh, W.P., Huang, D., Ong, C.N., 2010b. Antioxidant activity and profiles of common fruits in Singapore. Food Chem. 123, 77–84, http://dx.doi.org/10.1016/j.foodchem.2010.04.002.

Izeppi, E.M., 2011. Efeitos da densidade de plantio na sobrevivência, desenvolvimento e produção de biomassa da halófita Sarcocornia ambigua (Michx.) Alonso & Crespo. Master Sci. Aquac. (Universidade Federal do Rio Grande).

Jackson, C., Preston, N., Thompson, P.J., Burford, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture 218, 397–411, http://dx.doi.org/10.1016/s0044-8486(03)00014-0.

Jatobá, A., da Silva, B.C., da Silva, J.S., Vieira, F. do N., Mouriño, J.L.P., Seiffert, W.Q., Toledo, T.M., 2014. Protein levels for Litopenaeus vannamei in semi-intensive and biofloc systems. Aquaculture 432, 365–371, http://dx.doi.org/10.1016/j.aquaculture.2014.05.005.

Jewell, W.J., Madras, J.J., Clarkson, W.W., DeLancey-Pompe, H., Kabrick, R.M., 1983. Wastewater Treatment with Plants in Nutrient Films (No EPA-600/S2-83-067). US Environmental Protection Agency.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47, 3954–3962, http://dx.doi.org/10.1021/jf990146l.

Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., Smaoui, A., Abdelly, C., 2008. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C. R. Biol. 331, 865–873, http://dx.doi.org/10.1016/j.crvi.2008.07.024.

Kudo, N., Fujiyama, H., 2010. Responses of halophyte *Salicornia bigelovii* to different forms of nitrogen source. Pedosphere 20, 311–317.

Lennard, W.A., Leonard, B.V., 2006. A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. Aquac. Int. 14, 539–550, http://dx.doi.org/10.1007/s10499-006-9053-2.

Lu, D., Zhang, M., Wang, S., Cai, J., Zhou, X., Zhu, C., 2010. Nutritional characterization and changes in quality of *Salicornia* bigelovii Torr during storage. LWT – Food Sci. Technol. 43, 519–524, http://dx.doi.org/10.1016/j.lwt.2009.09.021.

Mariscal-Lagarda, M.M., Páez-Osuna, F., Esquer-Méndez, J.L., Guerrero-Monroy, I., del Vivar, A.R., Félix-Gastelum, R., 2012. Integrated culture of white shrimp (*Litopenaeus vannamei*) and tomato (*Lycopersicon esculentum* Mill) with low salinity groundwater: management and production. Aquaculture 366 (-367), 76–84, http://dx.doi.org/10.1016/j.aquaculture.2012.09.003.

McIntosh, D., Samocha, T.M., Jones, E.R., Lawrence, A.L., Horowitz, S., Horowitz, A., 2001. Effects of two commercially available low-protein diets (21% and 31%) on water and sediment quality, and on the production of *Litopenaeus vannamei* in an outdoor tank system with limited water discharge. Aquac. Eng. 25, 69–82.

Miranda, F.R., Lima, R.N., Crisóstomo, L. a., Santana, M.G.S., 2008. Reuse of inland low-salinity shrimp farm effluent for melon irrigation. Aquac. Eng. 39, 1–5, http://dx.doi.org/10.1016/j.aquaeng.2008.04.001.

Páez-Osuna, F., Guerrero-Galván, S.R., Ruiz-Fernández, a. C., Espinoza-Angulo, R., 1997. Fluxes and mass balances of nutrients in a semi-intensive shrimp farm in north-western Mexico. Mar. Pollut. Bull. 34, 290–297, http://dx.doi.org/10.1016/S0025-326X(96)00133-6.

Quintã, R., Santos, R., Thomas, D.N.N., Le Vay, L., Quinta, R., Santos, R., Thomas, D.N.N., Le Vay, L., 2015. Growth and nitrogen uptake by Salicornia europaea and Aster tripolium in nutrient conditions typical of aquaculture wastewater. Chemosphere 120, 414–421, http://dx.doi.org/10.1016/j.chemosphere.2014.08.017.

Rakocy, J.E., 2012. Aquaponics — integrating fish and plant culture. In: Tidwell, J.H. (Ed.), Aquaculture Production Systems. Wiley-Blackwell, Oxford, pp. 343–386.

Ray, A.J., Lewis, B.L., Browdy, C.L., Leffler, J.W., 2010. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture 299, 89–98, http://dx.doi.org/10.1016/j.aquaculture.2009.11.021.

Ray, A.J., Dillon, K.S., Lotz, J.M., 2011. Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management. Aquac. Eng. 45, 127–136, http://dx.doi.org/10.1016/j.aquaeng.2011.09.001.

Rodrigues, L.R.F., 2002. Técnicas De Cultivo Hidropônico E De Controle Ambiental No Manejo De Pragas, Doenças E Nutrição Vegetal Em Ambiente Protegido, 1st ed. Funep, Jaboticabal.

Rozema, J., Muscolo, A., Flowers, T., 2013. Sustainable cultivation and exploitation of halophyte crops in a salinising world. Environ. Exp. Bot. 92, 1–3, http://dx.doi.org/10.1016/j.envexpbot.2013.02.001.

Schveitzer, R., Arantes, R., Costódio, P.F.S., do Espírito Santo, C.M., Arana, L.V., Seiffert, W.Q., Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. Aquac. Eng. 56, 59–70, http://dx.doi.org/10.1016/j.aquaeng.2013.04.006.

Shpigel, M., Ben-Ezra, D., Shauli, L., Sagi, M., Ventura, Y., Samocha, T., Lee, J.J., 2013. Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 412 (-413), 52–63, http://dx.doi.org/10.1016/j.aquaculture.2013.06.038.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Strickland, J.D., Parsons, T.R., 1972. Practical Handbook of Seawater Analysis, 1st ed. Fish Research Board of Canada, Ottawa.

Tyson, R.V., Treadwel, D.D., Simonne, E.H., 2011. Opportunities and challenges to sustainability in aquaponic systems. Horttechnology 21, 1–13.

Ventura, Y., Sagi, M., 2013. Halophyte crop cultivation: the case for *Salicornia* and *Sarcocornia*. Environ. Exp. Bot. 92, 144–153, http://dx.doi.org/10.1016/j.envexpbot.2012.07.010.

Ventura, Y., Wuddineh, W.A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T.M., Sagi, M., 2011. Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci. Hortic. (Amsterdam). 128, 189–196, http://dx.doi.org/10.1016/j.scienta.2011.02.001.

Vinatea, L., Gálvez, A.O., Browdy, C.L., Stokes, A., Venero, J., Haveman, J., Lewis, B.L., Lawson, A., Shuler, A., Leffler, J.W., 2010. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. Aquac. Eng. 42, 17–24, http://dx.doi.org/10.1016/j.aquaeng.2009.09.001.

Webb, J.M., Quinta, R., Papadimitriou, S., Norman, L., Rigby, M., Thomas, D.N., Le Vay, L., 2012. Halophyte filter beds for treatment of saline wastewater from aquaculture. Water Res. 46, 5102–5114, http://dx.doi.org/10.1016/j.watres.2012.06.034.