

Advances in the use of photoheterotrophic, mixotrophic and multi-trophic systems in marine shrimp farming

Laenne Barbara Silva de Moraes*, Débora Louise Barros Silva, Jéssika Lima de Abreu, Elizabeth Pereira dos Santos, Renata da Sliva Farias, Luis Otávio Brito and Alfredo Olivera Gálvez

Aquaculture Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil.

*Corresponding author: laennemoraes@gmail.com

Abstract

The growth of aquaculture can cause different environmental impacts, from the use of large amounts of water to the excessive release of effluent. Shrimp farming can be done in different systems: extensive, semi-intensive, intensive and super-intensive. Extensive systems with low productivity (<500 kg ha⁻¹ year⁻¹), exhibit low technological levels and less control of environmental conditions. The semi-intensive system requires food supplementation, reasonable natural productivity, biomass yield of 3,000 - 5,000 kg ha⁻¹ year⁻¹ and water change (5 to 10 % day⁻¹). To increase production, different culture systems are used, such as photoheterotrophic, heterotrophic and mixotrophic, with minimal water exchange (0.5 to 3 % day⁻¹), providing greater biomass yield (intensive 10 to 20 ton ha⁻¹ cycle⁻¹ and super-intensive 20 up to 40 ton ha⁻¹ cycle⁻¹). These can be developed in nursery or grow-out tanks, but with high operating costs, high technological levels, disease control and better control of environmental conditions. However, intensive and super-intensive systems accumulate nitrogen, phosphorus and solid waste that can be transformed into biomass in multi-trophic aquaculture systems. In this review, we will discuss the effects of these systems on water quality and productivity of marine shrimp. The photoheterotrophic, mixotrophic and multi-trophic systems are interesting alternatives to the photoautotrophic systems in shrimp production, demonstrated by the higher zootechnical performance, as well as the environmental quality, since these models promote the minimum exchange of water and the transformation of nutrient residues in biomass.

Keywords: Aquaculture, Intensive system, Biofloc, Algae

Introduction

With declining fishing resources due to unsustainable fishing, fisheries production has been relatively stagnant since the 80's, making aquaculture an alternative to fish production. In 2016, according to data from FAO (2018), global aquaculture production reached 110.2 million tons, of which 7.9 million tons came from crustacean production.

Shrimp farming is one activity that is showing a significant development in several countries. In Brazil, it developed in the mid-70's when cultures started at experimental levels, but only in the 90's, the activity began to be economically viable (Rocha, 2011). The success of Brazilian shrimp farming was achieved with the introduction of the exotic species, *Litopenaeus vannamei*, native to the Pacific coast. This species has a high tolerance to environmental conditions such as temperature, pH and salinity among others. It has good zootechnical performance, and a well-developed technological package allowed a great boost to this activity (Vinatea-Arana, 2004).

In shrimp farming, culture can be grown in different systems: extensive, semi-intensive, intensive, and super-intensive. Extensive systems are considered an important source of aquaculture production, especially in developing countries. In this system, maintaining water quality is essential for the development of the natural ecosystem, of primary (photoautotrophic) and secondary producers, that

are a source of crustacean live food (Tacon et al., 2004; Wasielesky et al., 2006). They are developed with low biomass (<500 kg ha⁻¹ year⁻¹), being able to support animals with little aeration, presenting low operating costs, low technological levels, without controlling diseases and environmental conditions (Southgate and Lucas, 2019).

The semi-intensive system is between extensive and intensive cultivation, requires food supplementation, but still depends on natural productivity, with biomass production of 3,000 to 5,000 kg ha⁻¹ year⁻¹ and water renewal of 5 -10 % per day⁻¹, presenting low operating costs, intermediate technological levels, intermediate control of environmental conditions and greater spread of diseases (Southgate and Lucas, 2019). In this system, most of the nutrients originated from formulated diets, thus feeding strategies must be optimized considering the chemical fluxes, water quality and shrimp production (Casillas-Hernández et al., 2007).

In intensive and super-intensive systems all the nutrients for the farmed stock come from introduced feeds, requiring highly balanced diet and providing higher yields per unit area or volume (intensive 10 to 20 ton ha⁻¹ cycle⁻¹ and super-intensive 20 to 40 ton ha⁻¹ cycle⁻¹), with minimal exchange of water (0.5 to 3 % day⁻¹) (Avnimelech, 2009). The stocking density depends upon being able to maintain the water quality conditions required by the organism, in the shrimp culture the biomass could be 1 to 2 kg m⁻³ (Avnimelech, 2009; Southgate and Lucas, 2019). These

systems can be developed in nursery or grow-out tanks, presenting high operating costs, high technological levels, high energy inputs (power, aeration, filtration, pumping), without energy recycling, low energy losses with feeding and disease control and environmental conditions (Southgate and Lucas, 2019).

However, in recent years, with the aim of reducing the environmental and economic impacts generated by the activity, studies have been initiated using other types of culture methods with minimal water exchange. These systems can be classified as heterotrophic, photoheterotrophic and mixotrophic, according to the energy sources used by the producing organisms. The heterotrophic system is characterized by the use of organic compounds as an energy source in the absence of light, being classified as photoheterotrophic when it occurs in the presence of light, while the mixotrophic system is characterized by the use of light, inorganic and organic compounds as energy sources (Pérez-García and Bashan, 2015). Combined or not with these systems is the use of the multi-trophic system, where species of different trophic levels take advantage, as a source of energy, compounds that would be rendered useless by cultivating only a single species (Troell et al., 2009).

Photoautotrophic (algal based systems)

Algal based systems, also known as traditional or conventional culture systems, usually built on land, consist of culture systems that have as primary base the development of systems based on phytoplankton. These are photosynthetic microorganisms that absorb carbon dioxide and supply oxygen from nutrient assimilation. They play an important role in the water quality of the system where the dynamics of oxygen production and nutrient cycle occurs but maintains a balance between photosynthetic activity and cellular respiration, varying seasonally due to temperature, light, and concentration of these nutrients in the substrate (Hargreaves, 2006). In such systems, the phosphorus and nitrogen are the most important limiting nutrients. The development of phytoplankton may require concentrations of 0.01 to 0.1 mg L⁻¹ soluble inorganic phosphorus and 0.1 to 0.75 mg L⁻¹ inorganic nitrogen (Boyd, 2015).

Phytoplanktons, represented by microalgae, have a rich species diversity and are adapted to different environments, including freshwater, saltwater or even the sediment (Nigam and Singh, 2011). They also have the abilities of rapid multiplication and increasing the biomass (Martínez-Córdova et al., 2015). In addition to the important role played in the water quality of photoautotrophic systems, the phytoplankton is also a nutritional source for cultured animals, since it is rich in lipids, carbohydrates, vitamins, pigments, and minerals, whose content varies according to species (Abdelnour et al., 2019; Sahni et al., 2019).

Phytoplankton production is mainly stimulated through system fertilization, such as the use of agricultural residues or inorganic and mineral fertilizers, which also contribute to the development of zooplankton and benthic species that can be directly consumed by farmed animals (Hargreaves, 2006). Swine, cattle, and poultry waste are the commonly used organic manures (Martínez-Córdova et al., 2015), constituting sources of Carbon (C), Nitrogen (N), and Phosphorus (P), but this organic material can be nutritionally inadequate and unpalatable to the animals.

When only chemical (inorganic) fertilizers are used, carbon is derived from photosynthetic processes (Boyd and Tucker, 1998), which is highly recommended. Inorganic fertilizers commonly used in agricultural crops include urea, sodium nitrate, ammonium nitrate, triple superphosphate, and ammonium phosphate (Lin et al., 1997). As for the ratio of nitrogen and phosphorus (N:P), this can be established between 5:1 and 10:1, varying with site specificities (Boyd, 1997). According to Biró (1995) the optimal amount of fertilizer addition depends on water and soil characteristics, and for organic fertilizer amounts are around 100 - 200 kg ha⁻¹. As for the inorganic fertilizer, Lin et al. (1997) considers the application rate of 28 - 56 kg ha⁻¹ of N and 7 - 14 kg ha⁻¹ of P, often every two weeks. In practice, the ponds are usually treated with liming materials to reduce acidity, increase total alkalinity and improve the system's fertilization response (Diana et al., 1997).

Phytoplankton biomass can also be generated by assimilating unconsumed nutrients from inert feed and animal excretion (Martínez-Córdova et al., 2015). Fertilizer use should be established at appropriate levels as dense phytoplankton limit light penetration in water (Schroeder, 1978), and the seasonal variation of these organisms due to environmental conditions and ecological successions is emphasized. Its presence highly depends on aquaculture type, intensification level, cultivated species, and food management practices (Moriarty, 1997).

Phytoplankton is distributed among the Cyanophyta, Chlorophyta, Heterokontophyta, Pyrrophyta and Euglenophyta divisions. The "blooms" give the water its characteristic color, and the highest growth rates usually occur during spring and summer and are slower in colder periods (Boyd, 2015). The presence of diatoms (Heterokontophyta) and green algae (Chlorophyta) is considered desirable, especially for the feeding of most aquatic invertebrates, and fish and shrimp larvae. When growing in outdoor farms with enough light and natural photoperiod, it may be the dominant community (Yusoff et al., 2002; Godoy et al., 2012). The presence of cyanobacteria (Cyanophyta) and dinoflagellates (Pyrrophyta) reflects poor water quality and eutrophication, where a reduction in diatom populations can be observed under these conditions (Yusoff et al., 2002). The presence of these two groups is considered undesirable due to toxin production and taste alteration, besides their potential to generate mortality in the culture (Boyd, 2015; Souza et al., 2012).

Conventional culture can be developed in different production systems. When established in still water it presents low yield, with less than 10 pl m⁻², equivalent to the production of less than 700 kg ha⁻¹ year⁻¹, using the refined feed, whose residues can be assimilated by natural recycling (Joffre et al., 2018). As the stocking density of shrimp and the shrimp biomass increase, more feed is used, generating an increase in the production of metabolites such as carbon, nitrogen and phosphorus, because 75 % of N and P are not used and these remain as residues in water. This requires high water exchange rates (5-20 %) because phytoplankton have a limited rate of carbon assimilation in such ponds, on the order of 2 - 10 g of carbon / m² per day (Avnimelech, 2009). Thus, to control waste, this effluent is released into adjacent water bodies. Some studies have shown improved shrimp zootechnical performance with exchange of water (Green et al., 1999; Mohanty et al., 2015). However, these nutrient loads (N, P and organic carbon) were equivalent to

6.79 - 71.9 kg of inorganic N, 0.63 - 14.3 kg of P and 13.04 kg of total OC per ton of shrimp (Sahu et al., 2013; Boyd and Queiroz, 2001; Jackson et al. 2003).

In traditional semi-intensive culture system, with densities of 10 - 29 shrimp m⁻² and production of 3.5 tons ha⁻¹ ciclo⁻¹ (Table 1), nutrition and good zootechnical performance results become dependent on diet supplementation with inert food (Martínez-Cordova et al., 2015; Joffre et al., 2018). In the case of penaeid, Nunes et al. (1997) estimated that 29.7 % of the carbon present in shrimp muscle comes from inert food and the others from natural productivity, highlighting the importance of its establishment in aquaculture. In intensive culture, especially shrimp larviculture, there is also a predominance of photoautotrophic conditions, because microalgae rich in polyunsaturated fatty acids are daily addition (Lorenzo et al., 2015).

Table 1. Zootechnical performance of marine shrimp reared in photoautotrophic systems (algal based systems).

Shrimp specie	Phase	Stocking density (shrimp)	Yield	Final weight (g)	Survival (%)	Time (days)	Reference
<i>L. vannamei</i>	N	108 m ⁻²	0.095 kg m ⁻²	1.20	73.3	32	Ogle (1992)
<i>P. monodon</i>	N	288 m ⁻³	0.218 kg m ⁻³	0.78	97	30	Rodriguez et al. (1993)
<i>L. vannamei</i>	N	1,521 m ⁻³	2.699 kg m ⁻³	1.9	93.4	42	Otoshi et al. (2001)
<i>P. semisulcatus</i>	N	180 m ⁻³	0.111 kg m ⁻³	0.67	77.4	35	Nour et al. (2004)
<i>L. vannamei</i>	N	6,250 m ⁻³	2.408 kg m ⁻³	0.428	90	42	Silva et al. (2009)
<i>L. vannamei</i>	N	250 m ⁻³	0.475 kg m ⁻³	0.87	68.0	63	Becerra-Dorame et al. (2012)
<i>L. schimitti</i>	N	50 m ⁻²	0.244 kg m ⁻³	5.38	90.8	105	Márquez et al. (2012)
<i>L. vannamei</i>	N	1,500 m ⁻³	0.378 kg m ⁻³	-	60	30	Supono et al. (2014)
<i>L. vannamei</i>	N	1,100 m ⁻³	0.719 kg m ⁻³	0.82	79.7	20	Lara et al. (2016)
<i>L. vannamei</i>	N	67,000 m ⁻³	0.07 kg m ⁻³	0.0014	94.4	13	Schweitzer et al. (2017)
<i>L. vannamei</i>	G	10 m ⁻²	0.130 kg m ⁻³	18.1	72	77	Wyban et al. (1987)
<i>L. vannamei</i>	G	20 m ⁻²	0.126 kg m ⁻³	9.69	65	84	Freeman et al. (1992)
<i>L. vannamei</i>	G	30 m ⁻²	0.334 kg m ⁻³	15.97	69.8	133	Martinez-Cordova et al. (1998)
<i>L. vannamei</i>	G	16 m ⁻²	0.179 kg m ⁻³	12.93	88.8	112	Martinez-Cordova et al. (2002)
<i>P. monodon</i>	G	25 m ⁻²	0.424 kg m ⁻³	16.8	84	56	Smith et al. (2002)
<i>L. vannamei</i>	G	15 m ⁻²	0.181 kg m ⁻³	12.52	96.6	102	Peixoto Jr et al. (2003)
<i>F. paulensis</i>	G	15 m ⁻²	0.148 kg m ⁻³	11.17	88.3	102	Peixoto Jr et al. (2003)
<i>L. vannamei</i>	G	35 m ⁻²	0.207 kg m ⁻³	8.5	69.7	131	Brito et al. (2006)
<i>L. vannamei</i>	G	37.5 m ⁻²	0.445 kg m ⁻³	12.29	96.7	88	Silva et al. (2008)
<i>F. subtilis</i>	G	16 m ⁻²	0.124 kg m ⁻³	8.49	91.7	87	Souza et al. (2009)

N: Nursery phase; G: Grow-out phase.

Despite the emphasis on the importance of phytoplankton domain in this system, there are certain disadvantages present in the photoautotrophic condition, such as diurnal variations in pH, ammonia and oxygen levels, mainly due to bloom (Burford et al., 2003; Ebeling et al., 2006). There is a net increase in dissolved oxygen and a net reduction in carbon dioxide during the day and vice versa at night, becoming a stressor, causing mortality as a result of the formation of oxygen-free layers and accumulation of toxic compounds such as ammonia, nitrite and hydrogen sulfide (Yusoff et al., 2002). The greater the abundance of phytoplankton, the greater the magnitude of the daily

variation of these factors (Boyd, 2015). Therefore, adequate management of the system is recommended, especially in water quality and control of the phytoplankton community (Souza et al., 2012). Measurements for phytoplankton abundance evaluation can be done through microscopic analysis or indirect methods such as determination of particulate organic matter, chlorophyll *a* and Secchi disc analysis (Boyd, 2015). This control can lead to improved growth, optimizing productivity and financial return for farms (Moriarty, 1997).

Photoheterotrophic System

Photoheterotrophic systems are characterized by the use of organic compounds and light as energy sources (Pérez-García and Bashan, 2015). Among the variations of this type of culture is the biofloc system (BFT). Studies on BFT were initiated in the 70's by Ifremer - COP (French Research Institute for Sea Exploration, Pacific Ocean Center), where different species of marine shrimp such as *Litopenaeus stylirostris* and *L. vannamei* were examined (Hopkins, 1994; Emerenciano et al., 2013b). Already in the 80's, Ifremer started a French scientific program called "Ecotron" to better understand the system (Serfling, 2006). Several studies have contributed to a comprehensive approach to the biofloc system and clarified the interrelationships within the system, such as water and bacteria, as well as nutrition and physiology (Avnimelech, 2007). Already in the late 80's and early 90's, USA (Waddell Mariculture Center) and Israel conducted research with the biofloc system using *L. vannamei* and Tilapia, respectively, and the main motivations for starting studies were limitation costs, land acquisition costs, and concerns about environmental impact (Serfling, 2006; Avnimelech, 2009).

The biofloc system is a super-intensive system with high stocking densities (300 to 1500 shrimp m⁻²) where the strong aeration and zero or minimal water exchange make possible the formation of microbial floc. The development of the system occurs through the manipulation of the carbon:nitrogen ratio (C:N) in the cultivation environment, thus stimulating the growth of a microbial community, where the floc is formed by a heterogeneous mixture of bacteria, phytoplankton, zooplankton, remains of food and feces, exoskeletons and other invertebrates, predominantly the heterotrophic and aerobic biota (De Schryver et al., 2008; Avnimelech, 2009; Samocha et al., 2017). The floc formed in the system has a high protein content (Khatoon et al., 2016), which can be an alternative food for the organisms, improving the zootechnical performance and decreasing the feed conversion rate (Avnimelech, 2009).

This culture system can be implemented in small areas, favoring the good use of the area and greater productive efficiency, since one of the obstacles to the development of aquaculture is the availability and cost of the land. In addition to the low use of water, it is feasible to construct cultivation units both near and far from the coastline, enabling the interiorization of farms using low salinity waters (Samocha et al., 2012). Another advantage presented by the system is the possibility of reusing water for several production cycles without a negative impact on crop yield (Krummenauer et al., 2014).

The biofloc system was developed mainly to control the nitrogenous compounds (ammonia) that accumulate and when in high concentrations, they are toxic to aquaculture organisms (Avnimelech et al., 2007). The main nitrogen

cycling processes in closed culture systems are made by microalgae that absorb nitrogen and bacteria responsible for the nitrification process (Hargreaves, 1998). The heterotrophic bacteria present in the biofloc system can also act on nitrogen removal in water, both organic (free and combined amino acids) and inorganic (ammonia and nitrate) forms, in addition to being involved in the decomposition process (Wheeler and Kirchman, 1986).

However, in order for bacteria to be able to synthesize organic carbon from proteins and ammonia, it is necessary that C:N ratio is suitable in the water for use, as they do not have good efficiency in decomposing organic material when there are high levels of carbon or nitrogen. With a C:N ratio of approximately 10-20:1 (weight-based ratio) digestion is relatively easy (Chamberlain et al., 2001; Hargreaves et al., 2006; De Schryver et al., 2008; Avnimelech, 2009).

The stabilization of bacteria in the biofloc system can be divided into two phases: In the first, there is a predominance of heterotrophic bacteria in the system. This is because they are fast-growing, and in the second phase there is a predominance of autotrophic bacteria, as they have slower growth (Avnimelech, 2009). The autotrophic bacteria begin to stabilize between 2nd and 3rd weeks after the carbon source introduction (Wasielesky et al., 2013). These bacteria work in the system, converting nitrogen compounds to nitrate (Krummenauer et al., 2014).

According to Wasielesky et al. (2006), the presence of bacteria at the beginning of culture is of great importance in preventing the development and competition for the substrate with the bacteria responsible for ammonia oxidation. Bacteria responsible for the oxidation of ammonia to nitrite, a highly toxic compound, may cause harm to the producer by reducing animal growth and increasing mortality. The complexity and dynamics of this system require intensive management and should always be aware of the physicochemical and biological parameters of water (Wasielesky et al., 2006).

Several studies related to this type of system have been carried out in the last years, aiming to improve the zootechnical performance of the culture animals, as well as to better understand the functioning of the system. Brito et al. (2015) got satisfactory results when post-larvae of *L. vannamei* were cultured in a biofloc system in terms of the zootechnical performance of the animals with the average final weight of 0.68 g; the survival was 71.3 % and yield was 1.21 kg m⁻³ for 35 days of culture. Marinho et al. (2014) cultured the post-larvae of the same species and obtained a final weight of 0.24 g, 41.5 % of survival and weight gain of 0.22 g, at 20 days of culture. Abreu et al. (2019) also had good zootechnical results for post-larvae of *L. vannamei* cultured in the biofloc system, which reached a final average weight of 0.69 g, survival 93 % and yield of 1.95 kg m⁻³, in 42 days of culture (Table 2).

Table 2. Zootechnical performance of marine shrimp reared in photoheterotrophic systems.

Shrimp specie	Phase	Stocking density (shrimp)	Yield	Final weight (g)	Survival (%)	Time (days)	Reference
<i>F. brasiliensis</i>	N	500 m ⁻²	0.15 kg m ⁻²	0.3	94	30	Fóes et al. (2011)
<i>F. brasiliensis</i>	N	1000 m ⁻³	0.02 kg m ⁻³	0.24	67	30	Emerenciano et al. (2012)
<i>L. vannamei</i>	N	1500 m ⁻²	0.65 kg m ⁻²	0.45	96.3	30	Wasiolesky et al. (2013)
<i>L. vannamei</i>	N	2500 m ⁻³	0.25 kg m ⁻³	0.24	41.5	20	Marinho et al. (2014)
<i>L. vannamei</i>	N	250 m ⁻³	3.22 kg m ⁻³	14.47	89.2	49	Jatobá et al. (2014)
<i>F. brasiliensis</i>	N	750 m ⁻³	0.622 kg m ⁻³	1.03	80.5	30	Souza et al. (2014)
<i>L. vannamei</i>	N	2500 m ⁻³	1.21 kg m ⁻³	0.68	71.3	35	Brito et al. (2015)
<i>L. vannamei</i>	N	1200 m ⁻²	1.44 kg m ⁻²	1.22	98.6	35	Serra et al. (2015)
<i>L. vannamei</i>	N	2500 m ⁻³	0.46 kg m ⁻³	0.205	91.3	20	Marinho et al. (2017)
<i>L. vannamei</i>	N	3000 m ⁻³	1.95 kg m ⁻³	0.69	93.6	42	Abreu et al. (2019)
<i>F. duorarum</i>	G	38 m ⁻²	0.32 kg m ⁻²	13.3	63	210	Emerenciano et al. (2013a)
<i>L. vannamei</i>	G	238 m ⁻³	1.0 kg m ⁻³	6.2	70.6	34	Schweitzer et al. (2013)
<i>L. vannamei</i>	G	300 m ⁻²	0.586 kg m ⁻²	2.17	90	42	Silva et al. (2013)
<i>P. monodon</i>	G	21 m ⁻³	0.132 kg m ⁻³	6.6	95	60	Anand et al. (2014)
<i>L. vannamei</i>	G	425 m ⁻³	1.30 kg m ⁻³	5.42	56	28	Brito et al. (2014a)
<i>P. monodon</i>	G	100 m ⁻³	0.622 kg m ⁻³	7.5	83	75	Kumar et al. (2015)
<i>L. vannamei</i>	G	130 m ⁻³	0.674 kg m ⁻²	5.97	86.9	60	Rajkumar et al. (2016)
<i>L. vannamei</i>	G	300 m ⁻³	2.83 kg m ⁻³	9.99	95.5	42	Xu et al. (2016)
<i>L. vannamei</i>	G	250 m ⁻³	1.7 kg m ⁻³	11.1	69	55	Ray et al. (2017)
<i>L. vannamei</i>	G	300 m ⁻³	2.48 kg m ⁻³	8.75	96.7	35	Xu et al. (2018)

N: Nursery phase; G: Grow-out phase.

Table 3. Zootechnical performance of marine shrimp reared in mixotrophic systems.

Shrimp specie	Phase	Stocking density (shrimp)	Yield	Final weight (g)	Survival (%)	Time (days)	Reference
<i>F. paulensis</i>	N	300 m ⁻²	0.206 kg m ⁻²	0.72	95.4	30	Ballester et al. (2007)
<i>P. monodon</i>	N	40,000 m ⁻³	-	-	56.3	19	Khatoon et al. (2009)
<i>F. paulensis</i>	N	250 m ⁻²	0.151 kg m ⁻²	0.68	89	45	Ballester et al. (2010)
<i>F. paulensis</i>	N	10,000 m ⁻³	0.325 kg m ⁻³	0.068	47.8	15	Emerenciano et. al 2011
<i>L. vannamei</i>	N	1000 m ⁻³	1.077 kg m ⁻³	1.11	97	43	Godoy et al., 2012
<i>L. vannamei</i>	N	2500 m ⁻³	0.84 kg m ⁻³	0.348	96	20	Marinho et al., 2014
<i>L. vannamei</i>	N	390 m ⁻²	0.943 kg m ⁻²	2.43	99.5	30	Martins et al., 2014
<i>L. vannamei</i>	N	2500 m ⁻³	2.46 kg m ⁻³	1.08	91.7	35	Brito et al., 2015
<i>L. vannamei</i>	N	2500 m ⁻³	0.67 kg m ⁻³	0.27	98.3	20	Marinho et al., 2017
<i>L. vannamei</i>	N	3000 m ⁻³	2.42 kg m ⁻³	0.86	93.6	42	Abreu et al., 2019
<i>L. stylirostris</i>	G	20 m ⁻²	1,886 kg ha ⁻¹	15.16	66.5	140	Cordova et al., 2002
<i>L. vannamei</i>	G	130 m ⁻²	21,001 kg ha ⁻¹	18.4	88	90	Taw & Chandaeng, 2005
<i>L. vannamei</i>	G	550 m ⁻²	51,893 kg ha ⁻¹	13.8	66	57	Taw & Chandaeng, 2005
<i>P. monodon</i>	G	5000 m ⁻³	1.11 kg m ⁻³	0.9	60.6	49	Arnold et al., 2009
<i>L. vannamei</i>	G	115 m ⁻²	16,300 kg ha ⁻¹	16.7	85	113	Avnimelech et al., 2009
<i>L. vannamei</i>	G	403 m ⁻³	9.59 kg m ⁻³	25.22	94	38	Samocha et al., 2010
<i>L. vannamei</i>	G	130 m ⁻²	22,514 kg ha ⁻¹	18.78	89.2	90	Taw et al., 2011
<i>F. paulensis</i>	G	60 m ⁻²	0.287 kg m ⁻²	5.98	93.3	75	Wasiolesky Jr et al., 2012
<i>L. vannamei</i>	G	224 m ⁻³	2.219 kg m ⁻³	10.70	92.6	30	Xu & Pan, 2014
<i>L. vannamei</i>	G	300 m ⁻³	2.81 kg m ⁻³	9.84	97.3	42	Xu et al., 2016

N: Nursery phase; G: Grow-out phase.

Mixotrophic system

The mixotrophic system is a variant of the heterotrophic and photoautotrophic while using light and organic compounds as energy sources, and both respiratory and photosynthetic metabolism operating concurrently (Perez-García and Bashan, 2015). This type of system has been widely used in aquaculture, whether for the cultivation of microalgae, crustaceans or fish, with good yield results (Perez-García and Bashan, 2015; Brito et al., 2015).

Shrimp culture using biofloc technology in mixotrophic system had been reported to have achieved better growth rates, yield, and FCR, benefitting from microalgae dominance (Marinho et al., 2014; Marinho et al., 2017), association of microalgae and rotifers (Brito et al., 2015) or bacteria (Xu et al., 2016). The addition of microalgae contributes to the good performance of *Litopenaeus vannamei* when cultured in the biofloc system, as shown by Marinho et al. (2014, 2017). When added to microalgae *Navicula* sp., a final weight of 0.348 g, yield of 0.84 kg m⁻³ and FCR of 0.9 after 20 days of culture were achieved, while the addition of three diatom species (*Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricorutum*) resulted in a final weight of 0.27 g, yield of 0.67 kg m⁻³ and FCR of 0.61. Values higher than the treatment without the addition of microalgae amounting to a final weight of 0.27 and 0.18 g, yield of 0.63 and 0.59 kg m⁻³ and FCR of 0.64 and 1.2 were obtained for both the experiments, respectively (Table 3). Because of the good results with *Navicula*, research was carried out with the different concentrations of this microalga: 25,000, 50,000 and 100,000 cells mL⁻¹, where it was verified that concentrations of 50,000 and 100,000 cells mL⁻¹ achieved the best results for the final weight (0.80 and 0.86 g, respectively) and the SGR (15.92 and 16.08 % day⁻¹, respectively), but all the treatments had good results with *Navicula*, with a yield of 2.19 to 2.42 kg m⁻³ and FCR of 0.77 to 0.82 (Abreu et al., 2019). In addition, it is also observed that the diatoms, *C. calcitrans* and *P. tricorutum*, were outside the biofloc while the *Navicula* sp. was part of biofloc (Marinho et al., 2017). Abreu et al. (2019) found that biofloc plus *Navicula* had concentrations of 50,000 and 100,000 cells mL⁻¹ achieved high concentrations of PUFA, mainly DHA and the shrimp presented a high amount of fatty acids when used *Navicula* since it had the high concentration of PUFA, mainly EPA and DHA (Khatoon et al., 2009).

The biochemical composition of microalgae varies according to the species and cultivation conditions used, such as temperature, pH, salinity, luminosity, culture medium and culture system (George et al., 2014). Cultivation can be done in an autotrophic system, when using inorganic carbon and light as energy sources; heterotrophic by inserting organic carbon and source of organic energy in the absence of light; and mixotrophic, in the presence of organic and inorganic carbon, light and organic compounds as energy sources (Perez-García and Bashan, 2015). Some species are able to grow under all these conditions, and there may even be an increase in biomass and the production of

organic molecules when grown in heterotrophic and mixotrophic systems (Perez-García and Bashan, 2015). Among the compounds produced by these microorganisms, lipids have a large participation, being polyunsaturated fatty acids that are important for shrimp larvae nutrition of (Martins et al., 2014). Thus, diatoms are preferred due to their high content of PUFAs (Ju et al., 2009).

Another way to offer microalgae and provide better utilization of their nutritional content is through bioencapsulation in a zooplankton organism, such as rotifers. Rotifers are important food for larvae of fish and crustacean in aquaculture. Their biochemical composition is influenced by the microalgae used in their production and can be manipulated according to the larvae nutritional requirements (Hoff and Snell, 2001). Brito et al. (2015) reported good results by adding *Navicula* sp. (50,000 cells mL⁻¹) and the *Brachionus plicatilis rotifer* (30 ind mL⁻¹) in a biofloc for *L. vannamei* where a final weight of 1.08 g, yield of 2.46 kg m⁻³ and FCR of 0.92 were observed after 35 days of cultivation, while control treatment (without addition of organisms) resulted in a final weight of 0.68 g, yield of 1.2 kg m⁻³, and FCR of 1.9 (Table 3).

These results confirmed that the combination of *Navicula* plus rotifer represents an excellent natural diet for the shrimp post-larvae, as they provide important nutritional compounds such as essential amino acids and highly unsaturated fatty acids that are important for the proper development of shrimp (Martins et al., 2014). The addition of these organisms also contributes to improving the biofloc composition and digestive enzyme activity, given the high protein content of shrimp and the highest protein efficiency ratio (2.73) (Brito et al., 2015). The biochemical composition of diatoms and rotifers may change under different cultural conditions.

According to Lavens and Sorgellos (1996), mixotrophic systems have a characteristic of being self-sustaining due to the formation of algae and also because they are less subject to deficiencies, such as food shortages. But water quality variables may be less subject to controls due to system dynamics. In these systems, added diatoms act by controlling the proliferation of cyanobacteria, and absorbing nitrogen and phosphate compounds from the environment, which can be detrimental to cultivation (Khatoon et al., 2009; Marinho et al., 2014, 2017). Also, there is the action of bacteria in the autotrophic nitrification process, where ammonia is transformed into nitrite and nitrite into nitrate (Ebeling et al., 2006), besides the absorption of ammonia by heterotrophic bacteria provided by the increased C/N ratio, with the addition of carbohydrates (Crab et al., 2007). However, the rapid increase in total suspended solids (TSS) and volatile suspended solids (VSS) caused by high growth of microbial biomass may impair nutrient uptake by microalgae as a result of reduced light conditions, affecting water quality and shrimp production, particularly when the nitrification process is not well established (Ray et al., 2010; Gaona et al., 2017). In addition, high energy inputs and critical power

failures (maximum one hour at any time) are intrinsic to this system (Taw and Chandaeng, 2005).

Thus, the main advantages of mixotrophic systems are: minimal or zero water exchange, high biosecurity (from water), production 5-10 % better than photoautotrophic (algae based systems), shrimp size bigger by about 2.0 g than photoautotrophic systems, FCR low (1.0 - 1.3), production cost lower by around 15-20 %. In grown phase of *L. vannamei* farming, when using densities of 130 and 550 shrimp m⁻², the production can reach 22,514 and 51,893 kg ha⁻¹, with shrimp weight of 18.78 and 13.8 g, respectively (Taw and Chandaeng, 2005; Taw et al., 2011).

Multi-trophic system

Integrated multi-trophic aquaculture (IMTA) is based on the use of two or more species belonging to different trophic levels where metabolic wastes by one species are used by other organisms as a source of energy (Troell et al., 2009). Success of the IMTA system depends on the choice of species that will be integrated into the culture and other factors. It is necessary to assess the function that each organism plays in the system and to take into account the economic and commercial potential of each stocked species (Troell et al., 2009; Barrington et al., 2010). The use of IMTA enables a reduction of solid waste and better utilization of dissolved nutrients (Granada et al., 2016), resulting in an improvement in the water quality in the culture system. Although initially the concept of IMTA was developed to minimize environmental damage in offshore cultivation (Troell et al., 2009), it is possible to use its guidelines to improve water quality in systems that promote degradation of water quality throughout cultivation, as occurs in systems with minimal water exchange.

Characteristic water management of the BFT system such as minimal water exchange and the reuse of different proportions of liquid effluent from previous culture provides the accumulation of dissolved nutrients in the water (Burford et al. 2003; Krummenauer et al., 2014). These compounds are derived from the dissociation of uneaten food and by-products of animal metabolic reactions (Viadero et al., 2005). Silva et al. (2013) documented that over 60 % of nitrogen and phosphate compounds used for feeding are not assimilated by the shrimp, and these are in the form of ammonia, nitrite, nitrate, and phosphate. Maximum values for concentrations of phosphate compounds are not known in marine shrimp under super-intensive systems, unlike nitrogen compounds, which negatively influence the growth and survival of *L. vannamei* due to their toxicity to these animals (Ebeling et al., 2006; Samocha et al., 2017). Moreover, the use of a high C:N ratio favors the development of heterotrophic bacteria. This community has higher nutrient conversion rates in bacterial biomass than nitrifying bacteria, thus contributing to the formation of particles called bioflocs (Ebeling et al., 2006), where rich concentrations of these suspended solids present in water can affect the development of marine shrimp (Schweitzer et al., 2013).

Given this problem, some studies have been conducted to evaluate the bioremediation potential, through integrating several organisms that are able to assimilate compounds dissolved in water such as mollusks (Petersen et al., 2017), fish (Shpigel et al., 2016), microalgae (Magnotti et al., 2016) and marine macroalgae (Brito et al., 2013, 2014a, 2014b; Samocha et al., 2015), integrating them into the culture of marine shrimp, offering good productive results (Table 4).

Among these organisms, macroalgae have a high potential for use in integrated systems in aquaculture due to their bioremediation action. In addition, according to Attasat et al. (2013), an increase in shrimp biomass of 15 % is possible by using an integrated system between these and macroalgae. Besides enabling this increase, it has high efficiency in removing nitrate and phosphate compounds from the environment in which they are inserted. Decreases in ammonia concentrations from 35 to 100 % (Castelar et al., 2015; Rahardjo et al., 2018), nitrite 26 to 84 % (Rahardjo et al., 2018; Brito et al., 2018a), nitrate 17 to 99 % (Castelar et al., 2015; Rahardjo et al., 2018), in addition to the removal of 25 to 63.1 % of phosphate compounds have been reported (Macchiavello and Bulboa, 2014; Brito et al., 2018a) (Table 5). In addition, the improvement in water quality resulting from the use of the macroalgae can be seen through the influence on the microbiological and phytoplanktonic community of the culture systems, due to the growth of beneficial microalgae for the shrimp culture in the system where the macroalgae are used (Elle et al., 2017).

Macroalgae, besides contributing to their bioremediation potential, may also favor the zootechnical performance of the animals, as they can be used as a food source in the culture of aquatic animals (Fleurence et al., 2012), and may contribute in the form of substrate for biofilm formation when stored in the culture units or acting as a supplementary food source. This is because of their rich amounts of protein, lipids, and essential amino acids among other nutrients. (Tabarsa et al., 2012). The macroalgae of the genus *Gracilaria* sp. may have 6.4 to 37.6 % of protein and 0.2 to 12.9 % of lipid (Haslun et al., 2012; Øverland et al., 2019), serving not only as a source of high nutritional value but also as a functional ingredient due to their probiotic or antioxidant properties (Niu et al., 2019).

In assessing the effect of the presence of several organisms, the benefits are related not only to water quality parameters but also their contribution to the marine shrimp zootechnical performance indexes (Table 6). Furthermore, it is even possible to reduce the amount of feed protein in the nursery phase by virtue of the presence of these organisms in the system. Thus, integrating two or more organisms with super-intensive culture is a promising strategy for reducing nutrient concentrations in the farming effluents, and contributing through food supplementation directly (used as a food source) or indirectly (used as adhesion substrate) for the growth and condition of the stocked species of the shrimp.

Table 4. Zootechnical performance of marine shrimp reared in multi-trophic systems.

IMTA System	Shrimp specie	Phase	Stocking density (shrimp)	Yield	Final weight (g)	Survival (%)	Time (days)	Reference
<i>Sargassum plagyophyllum</i>	<i>P. monodon</i>	N	50 m ⁻³	563 kg ha ⁻¹	1.33	85	30	Izzati (2011)
<i>Gracillaria verrucosa</i>	<i>P. monodon</i>	N	50 m ⁻³	740 kg ha ⁻¹	1.66	89	30	
<i>Caulerpa sertularioides</i>	<i>F. californiensis</i>	N	28 m ⁻²	0.18 kg m ⁻²	-	98	60	Portillo-Clark et al. (2012)
<i>G. birdiae</i>	<i>L. vannamei</i>	N	500 m ⁻³	1.96 kg m ⁻³	4.12	95	42	Brito et al. (2014b)
<i>G. birdiae</i>	<i>L. vannamei</i>	N	500 m ⁻³	1.71 kg m ⁻³	3.87	93	42	Brito et al. (2018b)
<i>Ulva lactuca</i>	<i>L. vannamei</i>	N	10 m ⁻³	0.02 kg m ⁻³	2.08	96	28	Elizondo-Gonzalés et al. (2018)
<i>G. dichotoma</i>	<i>L. vannamei</i>	N	300 m ⁻³	1.12 kg m ⁻³	3.9	96	30	Anaya-Rosas et al. (2019)
<i>G. vermiculophylla</i>	<i>L. vannamei</i>	N	300 m ⁻³	1.13 kg m ⁻³	4.0	94	30	
<i>G. tenuistipitata</i>	<i>L. vannamei</i>	N	3000 m ⁻³	2719 ind m ⁻³	0.31	91	30	Anh et al. (2019)
<i>Crassostrea gigas</i>	<i>L. vannamei</i>	N	200 m ⁻²	0.51 kg m ⁻²	2.73	94	30	Omont et al. (2020)
<i>Mugil liza</i>	<i>L. vannamei</i>	N	2500 m ⁻³	3.34 kg m ⁻¹	1.37	98	41	Borges et al. (2020)
<i>U. lactuca</i>	<i>L. vannamei</i>	G	132 m ⁻²	3.72 kg m ⁻²	7.04	93	28	Brito et al. (2013)
<i>G. birdiae</i>	<i>L. vannamei</i>	G	425 m ⁻³	1.4 kg m ⁻³	6.57	50	28	Brito et al. (2014a)
<i>G. domingensis</i>	<i>L. vannamei</i>	G	425 m ⁻³	1.37 kg m ⁻³	5.75	56	28	
<i>G. tikvahiae</i>	<i>L. vannamei</i>	G	92 m ⁻²	3.2 kg m ⁻²	26.7	100	67	Samocha et al. (2015)
<i>G. corticata</i>	<i>L. vannamei</i>	G	50 m ⁻²	0.47 kg m ⁻²	13.4	71	45	Fourooghifard et al. (2017)
<i>U. clathrata</i>	<i>F. californiensis</i>	G	30 m ⁻²	0.28 kg m ⁻²	12.0	79	126	Peña-Rodríguez et al. (2017)
<i>U. prolifera</i>	<i>L. vannamei</i>	G	500 m ⁻³	4.78 kg m ⁻³	10.2	93.8	35	Ge et al. (2018)
<i>Mugil cephalus</i>	<i>L. vannamei</i>	G	60 m ⁻³	0.40 kg m ⁻³	10.7	63	75	Hoang et al. (2018)
<i>C.cuttackensis + Entreromorpha</i>	<i>P. monodon</i>	G	30000 ha ⁻¹	781 kg ha ⁻¹	35.9	-	150	Biswas et al. (2019)
<i>Oreochromis niloticus</i>	<i>L. vannamei</i>	G	10 m ⁻²	0.12 kg m ⁻²	15.1	78	106	Juárez-Rosales et al. (2019)
<i>O. niloticus + S. ambigua</i>	<i>L. vannamei</i>	G	312 m ⁻³	3.9 kg m ⁻³	14.6	88	57	Poli et al. (2019)

N: Nursery phase; G: Grow-out phase.

Table 5. Nutrient uptake by macroalgae in IMTA systems.

Species	Ammonia (%)	Nitrite (%)	Nitrate (%)	Phosphate (%)	Reference
<i>Ulva lactuca</i>	94	-	-	40	Alencar et al. (2010)
<i>Ulva fasciata</i>	50	31	70	-	Ramos et al. (2010)
<i>Gracilaria manilaensis</i>	83	33	68	-	Shukri and Surif (2011)
<i>Gracilaria vermiculophylla</i>	90	-	-	82	Skriptsova and Miroshnikova (2011)
<i>Undaria pinnatifida</i>	72	-	-	74	
<i>Gracilaria caudata</i>	23	57	70	-	Marinho-Soriano et al. (2011)
<i>Gracilaria vermiculophylla</i>	100	-	58	-	Abreu et al. (2011)
<i>Gracilaria verrucosa</i>	54	50	76	49	Huo et al. (2011)
<i>Gracilaria verrucosa</i>	61	48	47	58	Huo et al. (2012)
<i>Ulva lactuca</i>	83	-	-	41	Al-Hafedh et al. (2012)
<i>Gracilaria arcuata</i>	80	-	-	41	
<i>Hydropuntia cornea</i>	88	23	-	-	Robledo et al. (2012)
<i>Ulva lactuca</i>	88.2	-	-	-	Ben-Ari et al. (2014)
<i>Gracilariopsis longissima</i>	97	87	87	77	He et al. (2014)
<i>Gracilaria edulis</i>	70	-	-	-	Lavania-Baloo et al. (2014)
<i>Ulva lactuca</i>	45	-	-	-	
<i>Ulva lactuca</i>	100	-	83	65	Macchiavello and Bulboa (2014)
<i>Gracilaria chilensis</i>	100	-	88	38	
<i>G. birdiae + Ulva spp.</i>	98	87	98	62	Castelar et al. (2015)
<i>Macrocystis pyrifera</i>	75	-	-	-	Hadley et al. (2015)
<i>Gracilaria sp.</i>	36	11 - 27	18	-	Rahardjo et al. (2018)
<i>Caulerpa sp.</i>	25	4 - 21	12	-	
<i>Euclima sp.</i>	12	1 - 25	9	-	

Table 6. Zootechnical performance of marine shrimp reared in IMTA systems and monoculture.

Shrimp specie	Phase	Monoculture / IMTA system	Final weight (g)	SGR (% d ⁻¹)	Yield	Survival (%)	Reference
<i>P. monodon</i>	N	Monoculture	1.25	-	445 kg ha ⁻¹	71	Izzati (2011)
		<i>S. plagyophyllum</i>	1.33	-	563 kg ha ⁻¹	85	
		<i>G. verrucosa</i>	1.66	-	740 kg ha ⁻¹	89	
<i>F. californiensis</i>	N	Monoculture	-	-	0.09 kg m ⁻²	84	Portillo-Clark et al. (2012)
		<i>C. sertularioides</i>	-	-	0.18 kg m ⁻²	98	
<i>L. vannamei</i>	N	Monoculture	3.12	5.42	1.41 kg m ⁻³	90	Brito et al. (2014b)
		<i>G. birdiae</i>	4.12	5.86	1.96 kg m ⁻³	95	
<i>L. vannamei</i>	N	Monoculture	3.21	-	1.39 kg m ⁻³	83	Brito et al. (2018b)
		<i>G. birdiae</i>	3.87	-	1.71 kg m ⁻³	93	
<i>L. vannamei</i>	N	Monoculture	1.82	6.42	0.02 kg m ⁻³	83	Elizondo-Gonzalés et al. (2018)
		<i>U. lactuca</i>	2.08	6.91	0.07 kg m ⁻³	96	
<i>L. vannamei</i>	N	Monoculture	3.9	-	1.17 kg m ⁻³	100	Anaya-Rosas et al. (2019)
		<i>G. vermiculophylla</i>	4.0	-	1.13 kg m ⁻³	94	
		<i>D. dichotoma</i>	3.9	-	1.12 kg m ⁻³	96	
<i>L. vannamei</i>	N	Monoculture	0.31	3.86	2178 ind m ⁻³	72.6	Anh et al. (2019)
		<i>G. tenuistipitata</i>	0.31	3.89	2719 ind m ⁻³	90.6	
<i>L. vannamei</i>	N	Monoculture	1.5	5.17	3.75 kg m ⁻¹	98.7	Borges et al. (2020)
		<i>Mugil liza</i>	1.37	4.96	3.34 kg m ⁻¹	97.2	
<i>L. vannamei</i>	N	Monoculture	2.47	6.2	0.46 kg m ⁻²	94	Omont et al. (2020)
		<i>C. gigas</i>	2.73	6.48	0.51 kg m ⁻²	94	
<i>L. vannamei</i>	G	Monoculture	6.55	0.98	3.56 kg m ⁻³	96	Brito et al. (2013)
		<i>U. lactuca</i>	7.04	1.38	3.72 kg m ⁻³	93	
<i>L. vannamei</i>	G	Monoculture	5.42	1.56	1.3 kg m ⁻³	56	Brito et al. (2014a)
		<i>G. birdiae</i>	6.57	2.12	1.4 kg m ⁻³	50	
		<i>G. domingensis</i>	5.75	1.81	1.37 kg m ⁻³	56	
<i>L. vannamei</i>	G	Monoculture	12.5	1.7	0.32 kg m ⁻²	51	Fouroughifard et al. (2017)
		<i>G. corticata</i>	13.4	1.85	0.47 kg m ⁻²	71	
<i>L. vannamei</i>	G	Monoculture	10.1	-	0.38 kg m ⁻³	62	Hoang et al. (2018)
		<i>Mugil cephalus</i>	10.7	-	0.40 kg m ⁻³	63	
<i>L. vannamei</i>	G	Monoculture	5.1	3.0	0.71 kg m ⁻²	81	Laramore et al. (2018)
		<i>U. lactuca</i>	5.2	3.2	0.69 kg m ⁻²	83	
<i>P. monodon</i>	G	Monoculture	32.4	-	662 kg ha ⁻¹	-	Biswas et al. (2019)
		<i>C. cuttackensis</i> + <i>Entreromorpha</i> spp.	35.9	-	781 kg ha ⁻¹	-	
<i>L. vannamei</i>	G	Monoculture	14.8	3.0	0.10 kg m ⁻²	69	Juárez-Rosales et al. (2019)
		<i>O. niloticus</i>	15.1	3.0	0.12 kg m ⁻²	78	
<i>L. vannamei</i>	G	Monoculture	14.1	-	3.9 kg m ⁻³	89.3	Poli et al. (2019)
		<i>O. niloticus</i> + <i>S. ambigua</i>	14.6	-	3.9 kg m ⁻³	88	

N: Nursery phase; G: Grow-out phase; SGR: Specific Growth Rate.

Conclusions

Photoheterotrophic, mixotrophic and multi-trophic culture systems are interesting alternatives to conventional (photoautotrophic) systems for marine shrimp production. The good zootechnical performance and environmental benefits provide models that promote the minimum exchange of water and the best utilization of the nutrients available in the environment, thereby reducing the effluent generation. It should be mentioned that the algal biomass obtained can be used in food preparation technologies for aquafeed. On the other hand, it will be possible to explore the presence of biomolecules produced by the dominant algae that could be of application in nutraceutical and pharmaceutical industries. Of course, this will require investment in research and development for purposeful outcomes.

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