

# Practical and essential information on water reuse systems in experimental aquaculture production: a descriptive review

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Research

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## ABSTRACT

The present study reviewed the literature published in recent years on the topic “Recirculating Aquaculture Systems (RAS)”, a technology that makes it possible to grow aquatic organisms more sustainably and environmentally friendly, reducing water consumption, environmental pollution and land use. Commercial and academic application of recirculating aquaculture systems has increased considerably in recent years. The RAS is considered a master key for the increase of sustainable aquaculture, with the possibility of flexibility in its productive structure, ability to control environmental variables and the possibility of production throughout the year. This review provides an overview of aquaculture, RAS, sustainability and research perspectives.

Keywords: Environment; aquaculture; intensive production; blue revolution; fish; aquatic organisms.

## 1. INTRODUCTION

### State-of-the-art

The aquaculture sector is booming. In 2016 alone, world aquaculture production surpassed 110 million tons, of which 80 million tons came from farmed fish and 30 million tons from algae cultivation, representing a commercial movement of approximately US \$ 243 billion. Fish farmed in aquaculture systems accounted for approximately 42% of the total 171 million tonnes obtained in the sum of catch and aquaculture in 2016 (FAO 2018). In order to increase productivity, many producers choose to intensify production, using high densities, many times above the carrying capacity of their systems. The increase in aquaculture productivity directly implies the need for greater stocking of animals per cubic meter of water in production systems. This leads to intensification of food supply, which in turn when poorly managed can deteriorate water quality, causing loss of sanity in farms (Wu et al. 2013; Kayansamruaj et al. 2014; Subasinghe 2005). Conventional aquaculture systems will become unsustainable in the long run, whether due to environmental problems or biosecurity (Tal et al. 2009; Timmons and Ebeling 2010). Environmental problems related to aquaculture are generally measured on a global scale, ie, how aquaculture activity affects the planet using indicators such as greenhouse gas emissions (CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>2</sub>), energy consumption and surface area needed to activity development. In addition to global indicators, aquaculture environmental problems can be scaled at the regional level, close to the sites where the activity is developed, with indicators of eutrophication and acidification being the amount of nitrogenous and phosphorus compounds released into ecosystems (Martins et al. 2010).

Another factor to be considered is biosafety, since exotic species are used in aquaculture and the possibility of these species escaping into the environment can impact wild populations either by intraspecific crosses or by resource competition (Tal et al. 2009). Health problems highlighted the fragility of the aquaculture sector in the 1990's, where outbreaks of viral pathogens shook the shrimp

industry, causing major economic losses ever since (Otoshi et al. 2003). According to FAO (2014) the number of people who depend on fishing and aquaculture as a source of income and food is increasing, estimating that around 10% to 12% of the world population depend directly on these activities. However, the mismanagement of environmental resources threatens the sustainability of the activity. Per capita consumption of fish products is expected to increase over the coming decades, making the sector increasingly dependent on aquaculture (Rocha et al. 2013). According to Godfray et al. (2010), to increase food production in the same area size requires sustainable intensification in order to reduce environmental impacts. Following these production strategies, recirculating aquaculture systems, known by the acronym "RAS", emerge, which enable high production rates to be achieved (Badiola et al. 2017a; Legarda et al. 2019; Long et al. 2019), minimizing impacts on the environment (Gómez et al. 2019; Yogev and Gross 2019). Sustainable aquaculture, which advocates food production in harmony with nature, can be achieved when production systems are designed with minimal impact on the environment. Recirculating aquaculture systems provide reductions in water use and physical area, improving waste management, making intensive fish production compatible with environmental sustainability (Martins et al. 2010; Suantika et al. 2018; Calone et al. 2019).

RAS is seen as sustainable, environmentally friendly, with the possibility of flexibility in its production structure, ability to control environmental variables and the possibility of production throughout the year. The evolution process of these systems has been happening over the last three decades and many of these technologies have been developed through research in universities or other sectors, which are dedicated to the investigation of methodologies, techniques and equipment that contribute to the refinement of processes for purification and reuse of water in aquaculture (Timmons and Ebeling 2010). Currently, the recirculation systems have been presented as a master key to the development of a more sustainable aquaculture, because

physicochemical variables of water such as temperature, pH, dissolved oxygen, alkalinity, among others may remain more stable throughout the production period in these systems (Suantika et al. 2018; Calone et al. 2019; Gómez et al. 2019). Given all these facts, we can say that they are more biosecure, because they allow greater control of water quality, avoiding possible environmental contamination through the almost total elimination of water changes. (Suantika et al. 2018). In addition, RAS can contribute to preventing leakage of exotic species to nature, which also minimizes the risk of biological contamination and prevents the spread of pathogens. Research shows that the implementation of recirculation systems is becoming an increasingly common practice, as sustainable and environmentally friendly activities are needed today, as they provide an extreme reduction in the volume of water used, optimizing breeding spaces and mainly allowing a better control over the environmental variables of the systems. We can consider these systems to be an innovation for aquaculture, since we relinquish outdoor breeding in exposed tanks and make use of tanks allocated in smaller, protected areas, increasing fish stocking densities in a more controlled environment (Helfrich and Libey 1991; Heldbo 2015).

Closed systems are presented as a great commercial opportunity as they are based on high stocking densities using up to 99% less water than traditional aquaculture production systems. Costs involved in the design and execution phase of recirculating aquaculture system are typically higher than those practiced in conventional systems and any misconceptions in sizing or choosing equipment can result in catastrophic system-wide failures. Although, when properly sized, recirculation systems offer great advantages, allowing greater autonomy and production control (Nazar et al. 2013). Recirculation systems can be modeled in many ways using a wide range of structural components to remove unwanted waste in water (Bijo et al. 2007; Pfeiffer and Wills 2011; Masłoń and Tomaszek 2015; Pungrasmi et al. 2016). The various forms of nitrogen compounds can be removed from production systems through

mechanical, physicochemical and biological processes (Crab et al. 2007; Wik et al. 2009; Whang et al. 2012; Pungrasmi et al. 2016; Calone et al. 2019). However, biological processes are more economical and efficient, since they follow the same decomposition routes existing in nature, but under controlled conditions.

Ammonia removal from these systems is achieved through nitrification and denitrification processes where nitrifying bacteria oxidize ammonia to nitrate under aerobic conditions and nitrate can be simultaneously reduced to gaseous nitrogen ( $N_2$ ) under anaerobic conditions. Nitrifying bacteria such as the genera *Nitrobacter*, *Nitrosomonas*, *Nitrospira* among others, are mostly chemoautotrophic and from the oxidation of ammonia or nitrite gets their energy. Oxidized nitrogen compounds can be used by denitrifying bacteria as electron receptors, alternatively. Bacteria that perform denitrification are mostly heterotrophic, such as *Pseudomonas*, *Rhizobium* and *Paracoccus* (Mateju et al. 1992) and need the availability of dissolved organic carbon (DOC) as an energy source (Sliemers et al. 2002).

When we focus on the aquaculture field, biological processes are the most important for wastewater treatment, with nitrification being the most relevant one. The nitrification that occurs in the biofilter can be affected by a number of variables, such as the type of media used for bacterial biological support, dissolved oxygen concentrations, amount of organic matter, temperature, pH, alkalinity, salinity. Nitrifying bacteria are sensitive and susceptible to a variety of water quality factors such as high ammonia and nitrous acid concentrations, low dissolved oxygen levels, and pH out of comfort range (Malone and Pfeiffer 2006; Crab et al. 2007; Brown et al. 2013; Attramadal et al. 2014; Chun et al. 2018; Owatari et al. 2018).

In recirculating aquaculture systems, challenges are associated with ammonia and nitrite accumulation. The nitrification process is substantially important for aquaculture, especially in RAS where effluent discharge into the environment is close to zero and water quality is maintained through biofiltration

(Brown et al. 2013; Summerfelt et al. 2015; Von Ahnen et al. 2019). It occurs in two steps, in which ammonia is oxidized to nitrite by ammonia oxidizing bacteria, called nitrification, and then nitrite is oxidized to nitrate by nitrite oxidizing bacteria, known as nitrification. The genera *Nitrosomonas*, *Nitrosospira* are examples of bacteria that oxidize ammonia to nitrite and the genera *Nitrosococcus*, *Nitrobacter*, *Nitrospira*, *Nitrococcus* and *Nitrospina* are some of the nitrite oxidizing bacteria (Timmons and Ebeling 2010; Brown et al. 2013; Attramadal et al. 2014; Brailo et al. 2019).

Production systems are usually classified into three categories according to the degree of availability of organic carbon and nitrogen: they may be oligotrophic (high nutrient limitation); mesotrophic (medium nutrient limitation); or eutrophic (excess nutrients). However, from the point of view of aquaculture engineering, one first observes the natural habitat of the species to be cultivated and then defines the water quality standard to be adopted, forming an important link between production expectations, and the characteristics that the biofilter must have to perform well. Biological support materials, also called filter media, are commonly evaluated and compared across the total available surface area per cubic meter of media (Lekang and Kleppe 2000; Yoon et al. 2003; Pfeiffer and Wills 2011; Von Ahnen et al. 2019). However, attention should be paid to the theoretical surface area of any material used as a biological support, since biofilm when covering the media alters the characteristics of the structures and creates a “new” surface area. This may reduce the area available for nitrifying bacteria (Guerdat et al. 2010).

The efficiency of biological filters and the nitrification processes involved in this type of system are directly related to the types of media used as biological support for bacteria adhesion. It is important that the media used offer greater surface area, allowing for greater bacterial growth per unit volume of the filter media and therefore increase ammonia removal. In addition, the construction of biological filters using large surface area media can

be cost-effective, since we will need less physical space to install them (Lekang and Kleppe 2000).

The useful life of alternative media for use in biological filters should be observed, considering the composition of their structures, for example. Media such as wood can be used, however it has a reduced useful life when compared to plastic media (Saliling et al. 2007) and may decompose causing secondary system problems such as changes in physicochemical parameters of water quality. The RAS system is dependent on efficient biofilters that are capable of oxidizing toxic ammonia in nitrates, considered less toxic to aquatic organisms (Liu et al. 2013).

Currently in aquaculture there is a constant search for new forms of production aimed at reducing the consumption of water, land and environmental damage. Therefore, a good project must foresee and contemplate the use of technologies to achieve these goals (Losordo et al. 1999; Saliling et al. 2007). One of the factors limiting this practice is the high cost of materials, especially when we choose to use traditional plastic media for biological support, where the cost of this material can reach close to \$ 1000 / m<sup>3</sup> (Saliling et al. 2007). In RAS, infinite natural and artificial biological supports can be used in biofilters as long as they favor the growth of nitrifying bacteria and do not harm farmed aquatic organisms (Ridha and Cruz 2001; Liu et al. 2013; Owatari et al. 2018; Von Ahnen et al. 2019). The choice of the RAS components, as well as the biological support to be used, can directly influence the implantation costs and its efficiency (Summerfelt, 2006). For example, submerged filters compared to percolator filters, have the advantage of being able to reduce up to ten times the volume of filter media required to operate (Blancheton 2007). Generally, in aquaculture production systems, biological filters should have as main function the removal of excess nitrogen compounds in the system and avoid their accumulation. Biofilters should reduce total ammonia (TAN) concentration through nitrification, and thus RAS projects should maximize and improve TAN removal rate in order to reuse water while minimizing the impacts of nitrogen compounds on farmed animals (Guerdat et al. 2010). However,

understanding the control parameters of the different arrangements of recirculation systems, filters and their biological supports is a very important step in defining operational processes, configuring the layout and sizing of filters. It is essential to develop protocols for evaluation of cultivation systems, biofilters and filter media, in order to contribute information for the industry to make use of these technologies in the production chain.

Thus, based on the content addressed so far, this review aimed to gather information in an enlightening way about the recirculation systems used in experimental aquaculture, as well as the materials constituting the physical structure of the arrangements; the challenges that limit the use of RAS in the aquaculture industry; the possible environmental impacts generated by the aquaculture industry and the strategies to minimize them.

## 2. METHODOLOGY

This study was developed using a comprehensive review of the literature on recirculating aquaculture systems published in leading international journals and books. The publications were selected on the Science Direct platform using the keywords: Aquaculture, RAS, Sustainability, Recirculating Aquaculture Systems, Fish Farm and Environment. The publications were selected in four distinct searches. The first search used the keywords: RAS; Aquaculture; Sustainability; Environmental, 547 results were found in publications from 10 journals on the search platform. In a second keyword combination: Aquaculture; sustainability, 21,490 results were found in 10 journals on the platform. A third combination using the keywords: Recirculating aquaculture systems and Sustainability, 1652 results were presented by the platform. In the fourth keywords combination: Aquaculture; sustainability and Fish Farm, 7,290 results were presented. Based on the search results, publications with a significant theme for this review were selected, ie they contained material related to current aquaculture challenges and recirculating aquaculture systems. The publications were categorized according to the theme of published research such as removal of unwanted residues and

maintenance of water quality, media for physical and biological filtration, layout components and species of farmed animals.

## 3. RESULTS

The literature review showed that only in the last three years (2017, 2018 and 2019) 222 publications with the theme RAS, Aquaculture, Environment and Sustainability. 6,254 with the theme aquaculture and sustainability. With the theme recirculation aquaculture systems and sustainability were 485 and 2,340 with the theme Aquaculture; sustainability and Fish Farm, characterizing the importance and relevance of the subject.

## 4. DISCUSSION

Recirculating Aquaculture Systems (RAS) began to develop in the 1970's with technology derived from sewage treatment units (Asche 2008). RAS are technified systems, where a set of structural components treat water in a closed and continuous recirculation circuit, keeping the physicochemical variables within the desired parameters (Blancheton 2007). The use of this system is a relatively modern concept, very unique and extremely necessary, where we can create an infinite range of species of aquatic organisms at high densities, with autonomy over the environmental variables of a controlled environment (Attramadal et al. 2014; Orellana et al. 2014; Ngoc et al. 2016; Badiola et al. 2017a; Chun et al. 2018; Owatari et al. 2018; Long et al. 2019; Pedrosa et al. 2019). Recirculation systems advocate the reuse of water by filtering and removing unwanted waste in the tanks, thus maintaining the ideal conditions for the comfort and growth of target species (Martins et al. 2010). Water, continuously treated and recycled, is constantly monitored and strict control over pollutant levels is measured, thus offering greater environmental sustainability, enabling integration with other agricultural activities and different species (Summerfelt et al. 2006; Badiola et al. 2017b; Poli et al. 2019; Legarda et al. 2019). Another important advantage is that RAS technology is adaptable to species, which allows operators to switch species to follow the market trend for aquatic products (Bijo 2007; Martins et al. 2010; Timmons and Ebeling

2010). However, we realize that in countries like Brazil, the migration of production to this system model is still a paradigm, which comes up against the high costs of electricity and inputs for building the systems. This makes the implementation of RAS systems slow and unviable in many situations.

Aquaculture has been and is still widely criticized for its potentially negative impacts on the environment and the welfare issues of farmed organisms. One solution to these problems is a promising development of sustainable production methods as the consumer market has some clear expectations for sustainable aquaculture. Therefore, the aquaculture sector has a duty to disseminate sustainable aquaculture practices in a reliable and understandable way for consumers (Feucht and Zander 2015; Milewski and Smith 2019). One of the challenges of the scientific society is to make the results of research not only come to the knowledge of society, but also through a language that is easily understood by the productive sector. Countries where aquaculture is based on small production models, should receive special attention, since the profits obtained from these properties may not cover investments in new technologies.

The background and main principles of sustainable intensification of aquaculture pose particular challenges to its use. Aquaculture can be considered as part of food systems that go beyond production issues to cover storage, distribution, trade, processing and consumption. Sustainable intensification is a useful insight to interpret the challenges and opportunities of the trade in seafood grown between regions (Little et al. 2018). In most of the manuscripts analyzed in this review, the sustainability provided by the recirculation systems is constantly highlighted with a perspective of improvement in reducing environmental impacts. The technology offers opportunities to reduce water use and improve waste management and nutrient recycling, making intensive fish production compatible with environmental sustainability.

However, it is necessary that the operation of these systems is managed honestly and correctly. There is no point in constantly highlighting the water savings provided by the RAS, if mismanagement in production generates forced water changes in the systems, as well as not knowing how to dispose of the solid waste generated during the productive cycles.

Recent research aims to improve the efficiency of water treatment to reduce water renewal rates (Suantika et al. 2018). However, the nature of intensive breeding provides intensive use of energy, making sustainability and profitability difficult (Badiola et al. 2017b). Results presented by Kucuk et al. (2010) show that the energy efficiencies of the system components are highly affected by the variation of the incoming energy flows as a function of the ambient temperature and the operating period of the water cooling equipment. The most efficient projects are the least energy dependent, ie optimized unit processes, integrating systems, selecting appropriate equipment and renewable energy, are of potential use in RAS (Kucuk et al. 2010; Badiola et al. 2017b; Badiola et al. 2018; Poli et al. 2019).

Studies that verify the use of RAS technology for aquaculture of freshwater species (Al-Hafedh et al. 2003; Guerdat et al. 2010; Owatari et al. 2018; Pedrosa et al. 2019; Poli et al. 2019 ) and marine species (Orellana et al. 2014; Sterzelecki et al. 2017; Legarda et al. 2019; Mota et al. 2019) are being developed in many parts of the world. Other farming methods such as Bioflocs (Poli et al. 2019), IMTA Integrated Multitrophic Aquaculture Systems (Legarda et al. 2019) and Aquaponics (Calone et al. 2019) use this technology. The combination of all the knowledge involved in these emerging forms of integrated cultures will give us a deeper understanding of the interaction between aquatic organisms and the environment in which they are being cultivated and the recirculation systems will help us meet these challenges.



**Figure 1:** Components that make up a basic recirculation system described by Owatari et al. (2018). Synthetic fibers used as biological support (A); Expanded clay used as material for physical filtration (B); Hairdresser rolls act as adhesion material for nitrifying bacteria (C); A low-cost system for purifying and recirculating water for aquaculture. Designed with discarded drums from the natural juice industry (D).

### Components and processes of recirculating aquaculture systems

The components that make up a basic low cost recirculation system have already been described by Owatari et al. (2018) (Figure 1) and Lepine et al. (2018) and may be made from alternative materials. The basic structure for efficient removal of undesirable waste should include the installation of compartments for decanting suspended solids, physical filtration, biological filtration, ultraviolet radiation, air diffusers, foam fractionators and motor pumps (Hutchinson and Jeffrey 2004; Bijo 2007 Timmons and Ebeling 2010). However, the structure composition of the systems and the filter material may vary according to the needs of each researcher or producer (Colt et al. 2006; Guerdat et al. 2010; Pfeiffer and Wills 2011; Van Rijn 2013; Lepine et al. 2018; Poli et al. 2019). It is important that procedure development be standardized and classified. Designs for biological filters require that filter performance be evaluated and reported in a standardized manner, ie a standard in terms of definition, variable names, and units should be used. Basic principles of experimental design, statistical analysis and randomization should be followed (Colt et al. 2006).

Regardless of the structural configuration of a recirculation system, we must always consider the microbiological characteristics of bacterial biomass

that acts on the decomposition processes of nitrogen compounds. The kinetics related to the activities of the microorganisms involved in these processes can change and reflect directly on parameters that are closely linked to the biological reactions involved in the variations of dissolved oxygen, pH, temperature or alkalinity (Lekang and Kleppe 2000; Van Rijn 2013; Owatari et al. 2018).

Plastic structures were used as biological supports and studied by Al-Hafedh et al. (2003). They verified the performance of different biofilters for Nile tilapia farmig. In this research, the physicochemical variables of water quality remained within the allowed limits without affecting fish health. Singh et al. (1999) tested percolating filters and aerated submerged filters respectively and obtained means ranging between  $0.67 \pm 0.17$  and  $0.90 \pm 0.36$  mg L<sup>-1</sup> for TAN;  $0.26 \pm 0.19$  and  $0.89 \pm 0.93$  mg L<sup>-1</sup> for N-NO<sub>2</sub>;  $5.75 \pm 0.77$  and  $6.92 \pm 0.60$  mg L<sup>-1</sup> for OD, highlighting the good performance of nitrification processes in experimental systems through water quality results. It is important to know that a biofilter can be considered stable when it is able to keep TAN and N-NO<sub>2</sub> levels below 0.7 mg L<sup>-1</sup> by receiving a daily ammonia load of 8.64 g for 7 consecutive days or when the TAN load removed is equal to the load entering the system (Zhu and Chen 2001; Sandu et al. 2002).

Al-Hafedh et al. (2003) emphasize the importance of alkalinity on the biochemical process of nitrification in recirculation systems. Nitrification is an acidifying process in the system and consumes alkalinity. Maintaining pH in a range between 7.0 and 8.0 is considered optimal for nitrifying bacteria metabolism. Alkalinity and carbon dioxide levels should be closely monitored, as they directly interfere with pH maintenance (Timmons and Ebeling 2010).

The efficiency of the nitrification process in biological filters is described by Lekang and Kleppe (2000), where they tested variations of surfaces for biological support in RAS, expended clay in three sizes; plastic rings; plastic tubes; plastic synthetic grass. In the research, ammonium chloride ( $\text{NH}_4\text{Cl}$ ) was used to obtain the appropriate concentrations of ammonia in the system without the use of animals. All tested media showed decrease in the amount of TAN between days 50 and 60 after peaks caused by ammonium chloride. The phenomenon was related to a rapid increase in the number of nitrifying bacteria. Owatari et al. (2018) evaluated the use of synthetic fiber as a low cost alternative biological support for adhesion of nitrifying bacteria in a recirculating aquaculture system, providing stability in water quality, allowing good fish growth in the system and maintaining up to  $11.19 \text{ kg m}^{-3}$  of fish biomass. Martins et al. (2010) report that a healthy microbial community contributes to maintaining water quality within recirculation systems and the ability to maintain this constant quality is critical and contributes to improved animal health. Thus, the media used as biological supports for bacteria adhesion directly interfere with the nitrification processes involved in this type of system (Lekang and Kleppe 2000). A wide variety of biological supports can be used in biofilters (Lekang and Kleppe 2000; Ridha and Cruz 2001; Yoon et al. 2003; Summerfelt et al. 2015; Owatari et al. 2018).

Designing a recirculation system and its components is a complicated task, in which the designer needs to make several choices to find the balance between deployment and operation costs, always keeping in mind the risks involved. Some criteria such as system volume, density applied to organisms, target species,

feed rates may influence the design of the biofilters. From the total amount of feed supplied daily to the fish, 3% will be converted to TAN, ie for every 1000 g of feed 30 g of TAN will be produced in the system. For each 0.3 g of TAN generated in the system would require  $1.0 \text{ m}^2$  of surface area as biological support for the oxidation of ammonia to nitrate to occur effectively (Timmons and Ebeling 2010).

Strategies that can optimize the maturation and colonization of biological filters in aquaculture production systems are desirable since the natural establishment of nitrifying bacterial communities is slow (Kuhn et al. 2010). Owatari et al. (2018) using the NITE-OUT II<sup>®</sup> commercial product containing *Nitrosomonas*, *Nitrobacter* and *Nitrospira* found nitrification activity after 3 days of inoculation. Thus, using a wide range of commercial components and products can be a good alternative to maximize biofilter efficiency and lower costs in setting up Recirculating aquaculture systems. Maintaining a viable bacterial culture for biofilter inoculation seems to be a viable and most welcome strategy to adopt.

RAS systems involve a diverse microbial community that inhabits nitrifying and denitrifying biofilters. They are responsible for the oxidation processes of the remaining nitrogenous compounds in the system (Van Rijn 2013; Yep and Zheng 2019). Nitrifying bacteria mainly convert TAN to nitrate ( $\text{NO}_3$ ). The transformation of nitrogenous compounds occurs in distinct and diversified microbial communities of bacteria capable of ammonia and nitrite oxidation, and may be more abundant in nitrification biofilters. The proportion of known bacterial communities to perform heterotrophic and autotrophic denitrification and participate in sulfur cycling can be found in the denitrification bioreactor (Crab et al. 2007; Brailo et al. 2019).

Prior to bacterial action total ammonia must be available in water (Owatari et al. 2018). Total ammonia sources are also composed of fish excretion in the form of urine and feces or released through gills such as ammonia (Timmons and Ebeling 2010).



Once in water, ammonia can be used as a source of energy by ammonia oxidizing bacteria. Ammonia is reduced to nitrite form which is in turn used as an energy source by nitrite oxidizing bacteria and converted to nitrate. Nitrifying bacteria can adhere to and grow throughout the system, but are typically concentrated in biofilters. Nitrification is ideal when the temperature is between 25 and 30 ° C, the pH is between 7 ~ 9, optimizing at pH 7.8 (Yep and Zheng 2019).

The three main commonly known nitrifying bacteria are *Nitrobacter*, *Nitrosomonas* and *Nitrospira*. However, the concept that *Nitrosomonas* is the main oxidizing bacteria of nitrite and *Nitrobacter* is the main oxidizing bacteria of ammonia is changing. Some research has shown that the genus *Nitrospira* can appear to a greater extent in the biofilter (Schramm et al. 1998; Brown et al. 2013) and the genera *Nitrobacter* and *Nitrosomonas* to a lesser extent (Crab et al. 2007; Masłóń and Tomaszek 2015; Chun et al. 2018; Brailo et al. 2019; Yep and Zheng 2019). Even with all the available information about the various bacterial communities that act on ammonia cycling in biofiltration systems, we have to keep in mind that the microorganisms involved in this process establish mutualistic relationships with each other. This relationship is perennial, causing dependence, ie it is indispensable for the survival of different groups of bacteria that make chemosynthesis. The product resulting from the metabolism of bacteria in the nitrification phase is the substrate consumed by bacteria in the nitrification phase. This interrelation must be kept in balance to achieve the maximum efficiency of the biofilter.

### Target species and research performance

The present research has found that the manuscripts address a wide range of species used in recirculation systems. Badiola et al. (2012) reported that in Europe, at the beginning of the decade, the number of tilapia producing companies was the most common, representing 37.5% of diversity. Undoubtedly the genus *Oreochromis* is one of the most investigated in aquaculture. Sterzelecki et al. (2017) found that Brazilian sardines *Sardinella brasiliensis* appears to

tolerate a wide range of dietary carbohydrate and lipid inclusion like other omnivorous marine species. In Brazil, Brazilian sardines are emerging as a promising species in experimental aquaculture for the generation of live bait for tuna fishing. Ngoc et al. (2016) seeking an answer to achieve environmental sustainability of *Pangasius* farming in Vietnam, investigated the viability of cultivating this species in recirculation systems, since *Pangasius* is one of Vietnam's main export products. The research group considered that adopting RAS systems could be an important step towards achieving compliance with sustainability and disease control certifications on *Pangasius* farms. Improvement in water quality using microalgae *Ettlia* sp. in freshwater recirculation systems for *Danio rerio* has been demonstrated by Chun et al. (2018). At the time a highly sedimentable microalgae was applied to a freshwater RAS to improve the treatment of nitrogenous compounds, producing beneficial effects on water quality.

K-selection of microbial communities to improve survival in *Gadus morhua* cod larviculture has been investigated. Opportunistic microorganisms are rapidly growing and may be negative for the performance of marine fish larvae. K-selection can be established through resource competition by maintaining a low nutrient supply for a given group of microorganisms in an immature biofilter. The results showed a high potential for increasing fish survival using K-selection of bacteria and was considered an inexpensive and easy method that can be used in all types of aquaculture systems (Attramadal et al. 2014).

Owatari et al. (2018) used Nile tilapia *Oreochromis niloticus* with initial weight of  $32.11 \pm 7.6$  g and initial total biomass of 3.8 kg and Jundiá *Rhamdia quelen* initial weight  $11.34 \pm 2.4$  g initial total biomass 5.4 kg for 60 days in recirculation systems (Figure 2). For *O. niloticus* and *R. quelen* a final biomass of 12.6 kg and 21.5 kg was verified, with specific growth of 1.98 and 2.29 g day<sup>-1</sup>, respectively. Already Ridha and Cruz (2001) found that Nile tilapia with initial average weight of 62 g raised in simple recirculation systems can reach the

weight of 264 g in 172 days. Von Ahnen et al. (2018) treated effluents on *Oncorhynchus mykiss* rainbow trout farms, where three denitrifying bioreactors containing full scale wood chips (350, 650 and 1250 m<sup>3</sup>) were evaluated. Results for N-NO<sub>3</sub> effluent removal in freshwater recirculation systems were satisfactory. Woodchip bioreactors appear to achieve

relatively stable N-NO<sub>3</sub> removal rates throughout the year within the temperature range of 4.5 to 15.6 °C examined in the study. A multitude of low cost alternative materials deserve attention as the costs of deploying these systems are high. Research evaluating new materials such as biofilter biological supports is most welcome.



**Figure 2:** Experimental recirculation system for aquaculture designed with residential water tanks (Volume 100L). It can be successfully used in the experimental farming of several species of fish, respecting the limit of the load capacity of the system.

Biological filtration or biofiltration is the key-technology in RAS. Ammonia removal with sequential microsphere biofilters was efficient in the cultivation of *Perca Barcoo Scortum Barcoo*. When the water temperature was  $29 \pm 1.2$  °C and the fish biomass  $7 \text{ kg m}^{-3}$  the establishment of the experimental filter nitrification function was about 8 days. Culture density was gradually increased to  $38 \text{ kg fish m}^{-3}$  and at the 52-day stable stage TAN and N-NO<sub>2</sub> concentrations were maintained at levels  $1.6 \text{ mg L}^{-1}$  and  $0.9 \text{ mg L}^{-1}$  (Liu et al. 2013). The development of research to introduce new species for commercial production of RAS was approached by Orellana et al. (2014) when verifying that yellowtail *Seriola lalandi* is a promising candidate for Chilean aquaculture diversification. Growth performance, feed conversion, feed rate, condition factor and mortality

were determined for fish with an average initial weight of  $0.7 \pm 0.2 \text{ g}$  to a final average weight of  $2006 \pm 339.0 \text{ g}$ . The RAS configuration with drum filter, ozone protein skimmer, nitrification and biological denitrification, carbon dioxide removal and oxygenation kept the water quality stable and with low renewal.

The Amazon giant Pirarucu *Arapaima gigas* is commonly grown in excavated nurseries. Nevertheless, Pedrosa et al. (2019) investigated the effects of feeding strategies on growth, biochemical parameters and excretion of juvenile pirarucus reared in RAS. All feeding strategies evaluated by the research group were valid to be used without compromising pirarucu growth in these cultivation systems. Juvenile Chinese sturgeon

*Acipenser sinensis* grown in recirculation systems may have its growth negatively affected by high population density as well stress changes and immune responses. The appropriate stocking density recommended for growing juvenile Chinese sturgeon in a RAS is between 4.80 kg m<sup>-2</sup> and 8.99 kg m<sup>-2</sup> (Long et al. 2019).

Dalsgaard et al. (2013) evaluated the published literature along with unpublished practices for breeding different species in the Nordic countries. The manuscript identified species with practical experiments performed in recirculation systems. Atlantic salmon *Salmo salar*, Rainbow trout *Oncorhynchus mykiss*, Eel *Anguilla anguilla*, Perch *Stizostedion lucioperca*, Arctic Char *Salvelinus alpinus*, Sturgeon (order Acipenseriformes), Nile tilapia *O. niloticus* and Lagil *Homarus gammarus*, had the performance described. Improving water management in European catfish farms *Silurus glanis* can be optimized by integrating catfish and lettuce, contextualizing the definition of sustainable aquaculture, where activity plays an important role in food security, employment and in economic development. The sustainability of the RAS can be enhanced by using aquaponics, a circular production system in which RAS wastewater is recovered for growing crops and returned to fish ponds (Calone et al. 2019).

Similarly Legarda et al. (2019) investigated a recirculating water system integrating the breeding of Pacific white shrimp *Litopenaeus vannamei* and *Mugil curema* mullet into an experimental scale biofloc system. After 53 days, the final shrimp Weight  $12.56 \pm 0.22$  g and survival rate 91.8%  $\pm$  2.9% were similar between treatments. The fish had a survival rate of  $91.1\% \pm 10.2\%$  and adequate growth for *Mugil* species  $0.71 \pm 0.05$  g week<sup>-1</sup> in the integrated system. In shrimp + fish treatment productivity was increased by 11.9% by combining shrimp + mullet biomass. Shrimp + mullet treatment also showed a 16.8% increase in phosphorus retention compared to shrimp-only treatment.

Integrated multitrophic aquaculture applied to shrimp

farming in a closed recirculating biofloc system was investigated by Poli et al. (2019). The researchers evaluated the performance of an integrated multitrophic aquaculture system (IMTA) applied to rearing *L. vannamei* shrimp in a rearing tank (800 L), a tilapia *O. niloticus* breeding tank (90 L) and a hydroponic bench with 0.33 m<sup>2</sup> of planted area for the *Sarcocornia ambigua* crop. The study demonstrated an increase in yield up to 21.5% by multitrophic integration of the three species into a biofloc system.

High concentrations of carbon dioxide (CO<sub>2</sub>) negatively affect fish. Growth, welfare and health of post-smolted Atlantic salmon (*Salmo salar*) in RAS showed maximum performance at CO<sub>2</sub> concentrations below 12 mg L<sup>-1</sup> (Mota et al., 2019). The farming of Atlantic cod *Gadus morhua* in recirculation systems was verified in the Basque region of northern Spain by Badiola et al. (2017a). At the opportunity 2500 cod individuals were reared in two different thermal regimes in two pilot RAS systems for 430 days. The study was the first attempt at technical viability of cod in land base aquaculture in northern Spain and demonstrated the technical feasibility for producing cod in land base crops.

The development of a land base marine RAS was considered to have virtually no environmental impact due to the result of efficient biological waste treatment and water recycling. Over 99% of water volume can be recycled daily, integrating aerobic nitrification to eliminate toxic ammonia. The viability of the system was validated by *Sparus aurata* farming from 61 g to 412 g. A total of 1.7 tonnes was achieved in a 131 days record with a 99% survival rate. The system is location-independent, biosecure and not restricted to a single species (Tal et al. 2009).

The capital costs involved in structuring aquaculture recirculation systems are high and are one of the biggest challenges for their sustainability (Kucuk et al. 2010; Dalsgaard et al. 2013; Badiola et al. 2018). Feasibility requires intensive large-scale production to reduce investment and operating costs. However,

the necessary management including waste maintenance and efficient nitrogen removal and much of the applied RAS technology is well known and available for applicability in the farming of a variety of species. Successful production of new species in the RAS is mostly limited to the lack of information on the minimum biological requirements of the target species, and the RAS project should accommodate and support these requirements. Continuous supply of juvenile forms is a prerequisite for successful RAS production of most species (Dalsgaard et al. 2013). Successful operations of less intensive RAS such as aquaponic systems (Calone et al. 2019) or integrated systems (Ilegarda et al. 2019; Poli et al. 2019) appear to be viable, especially when involving species of differentiated value and intended for selected clients (Dalsgaard et al., 2013).

### RAS Prospects

The promising future for aquaculture, once mentioned many times within the production chain in past decades, has arrived. The generation of food through aquaculture and the rapid development of the activity is being termed as the *blue revolution*. However, the so-called *blue revolution* brings with it endless environmental concerns. To increase the production of aquatic organisms, aquaculture must grow sustainably and its environmental impacts must be significantly reduced (Ahmed and Thompson 2018). Canada, for example, is a signatory to the United Nations (UN) conventions on sustainable development and has set sustainability goals through legislation and public policies related to the natural resources sector, including aquaculture. This requires measures to assess and create sustainability indicators. However, decision makers, ie the government, must translate the aspirations of sustainable aquaculture policies into measurable sustainability indicators that assess whether the results of those policies have the expected effects on practice. The absence of policy measures of global, national and local sustainability endanger the stated objectives of aquaculture sustainability and the risk of being reduced to mere political slogans is a consequence (Milewski and Smith 2019).

The development of new sustainability systems and strategies is a constant within aquaculture research lines. The RAS system can have a significant reduction in energy consumption by up to 75% when a microaerophilic assimilation activated sludge bioreactor has been coupled to the system for single stage waste treatment. In addition, solid waste discharge was eliminated as it was fully recovered as microbial biomass that can be used as an ingredient in protein-rich foods, replacing fishmeal (Yogev and Gross 2019).

Perspectives that consider the whole, not only as a junction of its parts, and that seek to understand the phenomena of the fullness of sustainability emerge as necessary to describe and interpret the structure of activities undertaken by companies at the global level, rather than limited and oriented visions for the production. Animal protein production and consumption must remain within sustainable limits, requiring a rapid move toward incentives to support sustainable intensification. Future challenges will spur the emergence of increasingly efficient systems that use technologies and precision management (Little et al. 2018). Sustainable aquaculture produces food while preserving natural resources and is only achieved when production systems with minimal ecological impact are used. Recirculating aquaculture systems offer opportunities to reduce water use and improve waste management and nutrient recycling. Fish feed, waste and energy are the main components that explain the ecological impact of RAS. Technical improvements within the recirculation cycle and the adoption of integrated aquaculture are trends that contribute to improvements in RAS environmental sustainability (Martins et al. 2010).

The exergetic aspects of an RAS show that the exergetic efficiencies of the system components are highly affected by the variation of the input exergetic fluxes as a function of ambient temperature. It is recommended that the operating conditions of the components, particularly the pumps, be optimized and improved based on the system's fish production capacity (Kucuk et al. 2010). With the introduction of a hydroponic component into the system, water

discharged into a RAS can be recovered and used as a nutrient solution for vegetable cultivation, since the RAS has a daily water consumption, where 86% may be related to direct discharge of water. While system evaporation and moisture shrinkage losses account for 14% (Calone et al. 2019).

Alternatives aimed at reducing and treating sludge generated in recirculation systems are global priorities for the sustainable development of aquaculture. The use of polychaete *Abarenicola pusilla* for the removal of organic residues present in aquaculture sludge can achieve significant efficiency in the consumption of organic components, producing a production of about 9 g m<sup>-2</sup> per day and almost 400 g m<sup>-2</sup> in 45 days, suggesting the possibility of success in the integrated culture with this species (Gómez et al. 2019).

The performance of multitrophic systems can be optimized with the integration between *L. vannamei*, *S. ambiguous* and *O. niloticus* in biofloc systems without influence of *S. ambiguous* on nitrogen and phosphorus recovery in the system (Poli et al. 2019). The integration of *M. curema* juvenile with Shrimp *L. vannamei* in biofloc systems increased productivity by 11.9% and phosphorus retention by 16.8%, thus increasing overall system efficiency. Mulletts can be kept in biofloc systems with restricted diet, without apparent impairment in their physiology (Legarda et al. 2019).

As we have seen, there are a wide variety of emerging possibilities in aquaculture that seek sustainability and productivity optimization. This is desirable and demonstrates that activity enthusiasts are investigating alternatives for a healthy and prosperous future of the planet through more sustainable aquaculture practices. In this context, Recirculating aquaculture systems are indispensable tools to make the difference between past and future aquaculture.

## 5. CONCLUSION

In Brazil, it is known that the most recurring strategy used in land base fish production is continuous flow in excavated tanks that use large amounts of available water and high energy demand for water pumping. These conditions become threatening when we realize that under these conditions farms are subject to weather conditions such as excessive rainfall, as well as long periods of drought (as recently observed in Brazil) and the constant threat of biological agents. On the other hand, the migration of the productive sector to the use of RAS technology is a viable alternative, but it foresees several challenges such as implementation costs, specialized labor for equipment operation, constant suppliers of young forms of organisms for growth, besides the availability of water. Perspectives related to environmentally friendly practices in aquaculture necessarily contemplate the versatility and use of recirculation systems, which provide a variety of configurations adaptable to the use of different species. Environmental sustainability in the use of RAS will define how we will use natural resources and implement solutions for future aquaculture needs.

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