



A REVIEW ON RECIRCULATING AQUACULTURE SYSTEM: INFLUENCE OF STOCKING DENSITY ON FISH AND CRUSTACEAN BEHAVIOR, GROWTH PERFORMANCE, AND IMMUNITY

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Abstract

The human population is expected to reach 9.7 billion by 2050. This in turn will put more pressure on the limited available resources such as land and freshwater. Combined with the high food demand, highly virulent pathogens, and worsening effects of climate change, cases of chronic hunger and malnutrition are expected to escalate in the future. Therefore, the implementation of sustainable food production systems is crucial in safeguarding food security. Recirculating aquaculture systems (RAS) have gained much attention today for the intensive production of certain aquatic species in controlled conditions. In these systems, wastewater is purified via several water purification steps and recycled back into the system. As such, water quality parameters such as water temperature, dissolved oxygen, dissolved carbon dioxide, pH, total ammonia-nitrogen, nitrites, nitrates, and total soluble solutes are maintained within the desirable range required for proper growth and survival of the reared species. However, maintenance of good water quality largely depends on certain factors, most noticeably, the stocking density. Stocking densities below and above the recommended optimal levels negatively impact the behavior, growth performance, and immunity of reared animals. As a consequence, huge production losses are incurred. This review, therefore, aims to discuss the effect of stocking density on behavior, growth performance, feed utilization, and immunity of reared species in RAS. Moreover, optimum stocking densities of several aquatic species reared in RAS under certain culturing conditions are highlighted for sustainable production of food.

Key words: recirculating aquaculture system, aquatic species, stocking density, sustainable food production

The greatest challenges threatening the survival of humans are: population increase, limited natural resources, and climate change. An increase in the human population puts pressure on the limited natural resources such as land and freshwater, hence threatening global food security (Moore et al., 2020; Srivastava and Sahai, 1987; Xiao et al., 2019). Furthermore, high food demand, highly virulent plant pathogens, and climate change have added another suppressor to the general wellbeing and survival of humans either through having a direct or an indirect negative influence on food availability. As such, it is imperative to search for viable and sustainable food production systems that will answer the challenges of population increase amidst limited natural resources and climate change.

For the past 20 years, aquaculture has undergone significant developments and changes in infrastructural design with a long-term goal of increasing aquaculture production and economic returns in an environmentally friendly manner, with limited land and freshwater usage amidst several challenges brought by the current global population explosion. It is worth noting that aquaculture is the fastest-growing food production system, contributing about 82 million tons of fish worldwide worth

USD 250 billion. Moreover, it is estimated that aquaculture contributes 156 million tons of fish for human consumption equivalent to an annual supply of 20.5 kg per capita. The People's Republic of China is the leading fish producer across the globe, and by 2018, China was contributing approximately 35 percent of the total fish production. Excluding China, Asia has the highest fish production (34 percent) followed by the Americas (14 percent), Europe (10 percent) with Africa and Oceania contributing the least production (7 and 1 percent respectively) (FAO, 2020).

The grain input in aquaculture for the exchange of animal protein is very minimal, thus international experts have all agreed that aquaculture is the most efficient technology for acquiring animal protein (Ramli et al., 2020). However, there are three challenges mainly associated with traditional aquaculture production systems. These include:

- Limited access to capital and poor infrastructure,
- Poor water quality to sustain the growth of reared species,
- Environmental pollution.

Therefore, newer technologies are urgently needed to sustain aquaculture production with limited or zero environmental pollution and degradation.

Recirculating aquaculture systems (RAS) are aquaculture food production systems where both water and dissolved nutrients are recycled. In contrast to the traditional systems (cages, raceways, and ponds), the water exchange rate in RAS is 0.1–3 m³/kg feed (Moore et al., 2020; Ramli et al., 2020; Xiao et al., 2019). Moreover, this system can accommodate intensive rearing of several aquatic species to maximize production with negligible impacts on the organisms' wellbeing provided that water quality parameters such as water temperature, dissolved oxygen (DO), dissolved carbon dioxide (CO₂), pH, total ammonia-nitrogen (TAN), nitrites (NO₂⁻), nitrates (NO₃⁻), total soluble solids (TSS) as well as other conditions such as feeding regimes and feed type, water exchange rates and flow velocities, water salinity, photoperiod, light intensity and spectrum, tank color, and stocking density are within the desirable range of the aquatic species under intensive production. Growth models of several fish species such as turbot (*Scophthalmus maximus* L. and *Psetta maxima*) (Baer et al., 2011; Lugert et al., 2017), goldfish (*Carassius auratus*) (Tanveer et al., 2020), and gilthead seabream (*Sparus aurata* L.) (Seginer, 2016), have been designed to predict optimum water quality parameters, growth and growth performance of fish in RAS.

RAS provides the opportunity to conduct intensive aquacultural practices with high stocking densities to achieve maximum net production and big profits compared to open aquaculture systems such as ponds. Helfrich and Libey (1990) reported that in RAS, 4.5 × 10⁴ kg of fish can be produced in a 4.64 × 10² m² building compared to an 8 ha outdoor pond to produce the same quantities of the fish harvest. However, high stocking densities can negatively affect immunity, behavior, and growth performance of different stages of fish due to the accumulation of TAN, NO₂⁻ and NO₃⁻ above the recommended range (Gullian-Klanian and Arámburu-Adame, 2013 a).

Optimum stocking density differs from one species to another due to differences in their physiology and biology. In addition, the influence of stocking density on fish performance is not consistent throughout the growth process. For example, van de Nieuwegiessen et al. (2009) indicated that better welfare of African catfish (*Clarias gariepinus*) was achieved by increasing the stocking density in the first stage of development (102.1 ± 3.49 g; initial densities of 6.8–54.4 kg/m³) yet no changes were noted in the effect of stocking density during the second phase (1044.6 ± 31.6 g; initial densities from 70 to 347.9 kg/m³). In the same context, different stages of the fish life cycle exhibit differences in optimum stocking densities due to differences in body sizes and feed requirements. Larval stages are more sensitive to stocking densities compared to later stages of the fish life cycle. Previous studies have shown increased cannibalism, reduced feed intake, and poor growth performance among larvae of several fish species reared under high stocking densities (de Barros et al., 2019; Santos et al., 2020; Slembrouck et al., 2009; Sukumaran et al., 2011).

Furthermore, manipulation of rearing conditions such as feeding regimes, feed composition, water salinity, and light spectrum among others could influence changes in the recommended optimal stocking densities required for the species' survival and performance. There is no explicit evidence indicating an irregularity in the association of stocking density to growth performance. Recently, however, Li et al. (2021) have shown a direct correlation between stocking density and fish body mass and its impact on the growth performance (specific growth rate, feed conversion ratio, and conditioning factor) of several aquatic species. However, to the best of our knowledge, no review study has assessed the influence of stocking density on fish and crustacean behavior, growth performance, and immunity under RAS conditions.

This review, therefore, discusses the effect of stocking density on the growth performance of fish and crustacean species reared in RAS. The first section of the paper gives a detailed overview of the concept of RAS, including major aquatic species reared in RAS and their economic value. The section that follows discusses the effect of stocking density on behavior, growth performance, feed utilization, and immunity of reared species in RAS, highlighting their recommended optimum stocking densities under certain culturing conditions for sustainable aquaculture production. The last section concludes the findings and suggests future perspectives.

Concept of recirculating aquaculture system (RAS)

In this system, wastewater discharge into the environment is controlled hence harmonizing the ecological balance. Likewise, a smaller volume of water per kilogram fish production is utilized and higher biosecurity standards are implemented to sustain the general wellbeing of aquatic species under intensive cultivation. Moreover, a self-cleaning conditioning system and temperature control must be achieved in RAS to provide specific optimum conditions required by specific aquatic organisms for their maximum growth and survival (Gullian-Klanian and Arámburu-Adame, 2013 a). Figure 1 shows a schematic diagram of RAS and the different steps taken in water quality management as described below.

Briefly, physical filtration installed into the system aids in the removal of both organic and inorganic load contributed by uneaten feed, fish excreta, and other particulate matter which would not only damage the gill structure of the reared organisms but also cause blockage of the biological filters.

The biological filtration system, composed of several microorganisms such as *Nitrosomonas* and *Nitrobacter* bacterial species facilitate the reduction of ammonia-nitrogen and nitrites to a less toxic form, nitrates. For survival and normal growth, ammonia-nitrogen should not exceed 1 mg/L whereas nitrate levels should not be above 500 mg/L for marine species and 1,000 mg/L for freshwater species (Gullian-Klanian and Arámburu-Adame, 2013 a).

The next step in water purification is sterilization. This step usually aids in the removal of various aquatic pathogens that would otherwise pose a serious health risk to the reared organisms. Among the water sterilization equipment installed in RAS are the ozone equipment (Schroeder et al., 2011), ultraviolet (UV) equipment (Gullian et al., 2012; Xiao et al., 2019), and a combination of both (Sharrer et al., 2005; Xiao et al., 2019).

Certain RAS system designs have different aeration and oxygenation chambers aimed at improving the levels of dissolved oxygen. Likewise, certain RAS system designs have carbon dioxide (CO₂) removing equipment to control CO₂ levels in the system. Lastly, temperature control equipment is of great importance in all RAS designs to achieve maximum aquaculture production. Different fish species require different optimum temperature conditions for their optimum growth and survival, failure of which will lead to serious economic losses. Different energy sources such as geothermal energy, solar energy, waste heat from industry, hydropower, wind, and wave power have been used in different RAS designs to meet the increasing consumer demands of certain fish species in different countries. Detailed information on these energy sources, their merits, and their demerits in different RAS designs under different geographical locations has been presented in a previous review by Badiola et al. (2018).

Common aquatic species reared in RAS and their economic value

Cyprinids

Cyprinids are the most farmed aquaculture species worldwide thus contributing 38% of the total aquaculture production (FAO, 2020). The most popular representative of cyprinids in aquaculture is the common carp (*Cyprinus carpio*), which is the oldest domesticated aquaculture species around the globe. By 2018, the net production of common carp stood at 4,189.5 tons (7.7%) slightly after silver carp (*Hypophthalmichthys molitrix*) and grass carp

(*Ctenopharyngodon idellus*) whose production stood at 4,788.5 tons (8.8%) and 5,704 tons (10.5%) respectively (FAO, 2020). In Europe, common carp contributed 0.17 million tons (Mt) of the total inland fisheries production between 2015 and 2016. Other major producing common carp countries are: the Russian Federation (0.06 Mt), Czech Republic (0.02 Mt), Poland (0.02 Mt), Ukraine (0.01 Mt), and Hungary (0.01 Mt) (Roy et al., 2020). In landlocked European countries, carp production is mainly concentrated in ponds and the average production of carp in Central European countries ranges between 0.3 and 1 ton/ha. Traditionally, common carp is captured for sport in many European countries and is also used in the preparation of several folk dishes (Vilizzi, 2012). Carp production is not only concentrated in Europe but also in the Mediterranean region. Major producers include Egypt and France (Vilizzi, 2012). In Egypt for example, carp are reared in ponds, cages, fish culture in rice fields, aquaculture in irrigated areas, village fish farms as well as on large-scale commercial farms (Vilizzi, 2012).

Despite successful carp production in the aforementioned countries, the availability of carp fingerlings on market throughout the year is still complex. This is mainly due to inadequate feeding and ecological conditions mostly in mud-pond aquaculture systems. Therefore, research in carp larvae and juvenile production under controlled conditions has been conducted to understand the optimum rearing conditions for carp. For example, Jelkic et al. (2012) found that *Cyprinus carpio* larvae reared at a stocking density of 200 larvae/L in RAS exhibited better survival and growth performance compared to those reared in a mudfish pond (800 000 larvae/ha). However, Mojer et al. (2021) have recently shown that earthen ponds resulted in better growth performance of *Cyprinus carpio* compared to those reared in RAS. The discrepancy in results could be attributed to differences in initial weight with the latter having 0.002 g which resulted in reduced feed intake.

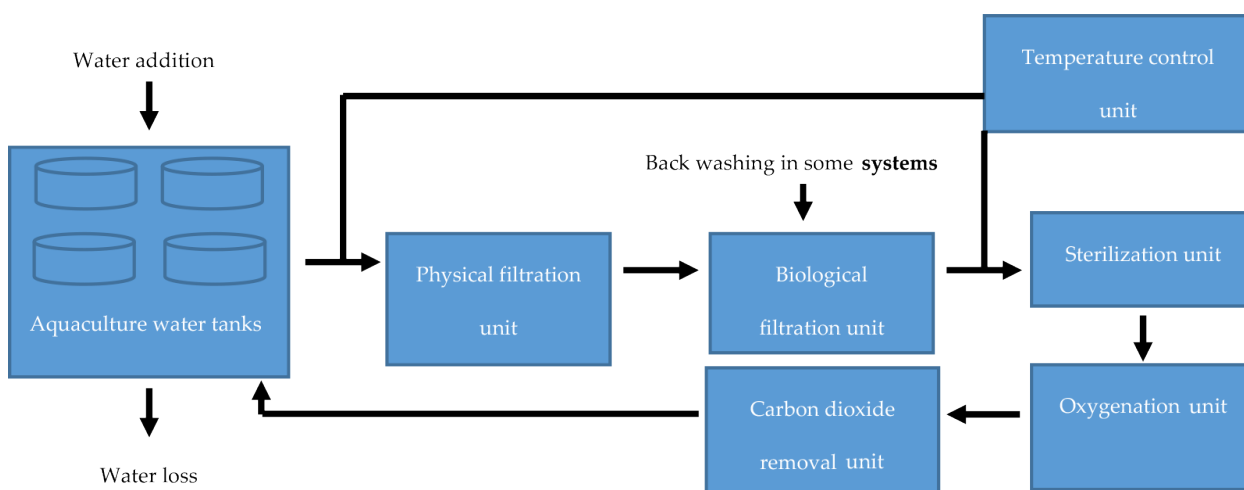


Figure 1. Schematic diagram of a recirculating aquaculture system

In another study, Kristan et al. (2018) observed that rearing grass carp (*Ctenopharyngodon idella*) juveniles in RAS during the first winter cycle resulted in better survival and growth performance of fish compared to those reared in ponds. Moreover, the economic analysis revealed that RAS provided more profit returns (total production 14,669 fish, price per fish € 0.185) compared to pond culture (total production 1642 fish, price per fish, € 0.185).

Cichlids

Tilapia (*Oreochromis* sp.) belongs to the family of Cichlids and is endemic to the freshwater habitats of Africa and the Middle East whose culture dates back to ancient Egypt, 900 B.C (Arechavala-Lopez et al., 2018). Currently, there are more than 140 countries that produce tilapia and it is the second most widely farmed fish around the globe after carp. China is the leading producer, consumer, and exporter of tilapia. Other highly producing countries of tilapia include: Indonesia, the Philippines, Thailand, Bangladesh, and Egypt among others (Ansah et al., 2014; Chen et al., 2018; Jansen et al., 2019). By 2018, the net production of Nile tilapia (*Oreochromis niloticus*) was approximately 4,525 tons thus contributing 8.3 percent of the total aquaculture production in the world (FAO, 2020). Its sales have since increased from USD 7.66 billion in 2012 to USD 12 billion in 2018 and they are projected to reach USD 25 billion in 2028 (FAO, 2020). In developing countries, tilapia is an important source of animal protein mainly consumed in rural areas. Moreover, this fish species is a source of income to many impoverished families and is sold either in markets or at farm gates (Ansah et al., 2014). Due to tilapia's historical, social, and economic benefits, governments of developing countries have prioritized drafting policies and funding research to increase the production of tilapia through intensive aquaculture practices. Land-based RAS for tilapia production has gained momentum due to improved system designs which promote water conservation and maintenance of water quality parameters within the optimum range. Moreover, RAS negates the negative ecological impacts of tilapia rearing on the environment and global biodiversity.

For example, Shnel et al. (2002) assessed the design performance of a zero-discharge tilapia RAS and observed that incorporation of a sedimentation basin in RAS reduces the concentrations of nitrates (i.e. 30.6 mg/L) and phosphorus (i.e. 73 kg recovered) in RAS water which could be reused for fish production without causing adverse effects on fish performance. Likewise, Zachritz et al. (2008) also reported that incorporation of a clarifier and a submerged surface flow (SSF) constructed wetland comprising of soil, gravel, or rock into RAS was effective in purifying tilapia wastewater discharged into the environment. The authors observed that SSF wetlands were able to remove 8.21, 0.58, 0.63, and 0.93 g/(m² day) of TSS, TAN, nitrite-nitrogen (NO₂-N), and nitrate-nitrogen (NO₃-N) respectively.

Salmonids

Atlantic salmon (*Salmo salar*) is among the most famous and widely domesticated fish species around the globe. Due to its fascinating life cycle, this fish species is a highly prized sporting fish that provides a nutritious food product for human consumption. Salmon aquaculture is worth ~9.7 billion euros annually (FAO, 2020; Houston and Macqueen, 2019) thus significantly contributing to food security, economic growth, and employment opportunities. Major producing countries are Norway, Chile, Canada, and the United Kingdom (Houston and Macqueen, 2019). However, since 2014, salmon production growth has stagnated in major producing countries due to high ectoparasite infestations (*Lepeophtheirus salmonis*) of fish reared in sea cages and this has led to difficulty in obtaining permits for sea cage farming (Davidson et al., 2017; Mota et al., 2019). As such, the cultivation of this fish species in land-based RAS is gaining momentum to maximize production and profits. In Norway, these facilities are projected to produce post-smolts up to 1 kg (Davidson et al., 2017; Good et al., 2017). Other economically important salmon species include: chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*).

Rainbow trout (*Oncorhynchus mykiss*) is also another economically important salmonid across the globe whose global production stands at 8.14 thousand tons. Major producing countries are Chile, Denmark, France, Germany, Italy, Turkey Iraq, Norway, Spain, the United Kingdom, and the United States of America (Singh et al., 2020). In Denmark, in-land fish farms are adopting the intensive culture of trout in RAS due to environmental restrictions on effluent discharge. By 2015, there were 33 RAS farms in Denmark thus making her one of the major trout-producing countries in Europe (Lasner et al., 2017).

Clariids

Clariids are air-breathing catfish usually cultured in warm water climates. Domestication of African catfish (*Clarias gariepinus*) in West and Central Africa has existed for decades since the 1950s and was introduced for commercial farming around the world in the early 1980s. In Africa, catfish is an important source of animal protein and a source of income for many impoverished families. It is much more profitable than tilapia due to its ability to breathe in atmospheric oxygen hence can be sold live in markets. Other advantages of this fish species include:

- A fast growth rate and ability to feed on agricultural by-products,
- Its hardiness to environmental stress is higher than that of tilapia,
- It can reproduce effectively even during captivity,
- It is relatively cheap to culture.

Other economically important catfish species domesticated across the globe include: channel catfish (*Ictalurus punctatus*), striped catfish (*Pangasianodon hypophthalmus*, Sauvage), Indian butter catfish (*Ompok bimaculatus*, B.), Chinese longsnout catfish (*Leiocassis*

longirostris, Gunther), and silver catfish (*Rhamdia quelen*). Although catfish have traditionally been reared in ponds, intensive production of catfish in RAS has proved to be more economically viable compared to pond culture. A comparative study on striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) reared in RAS and ponds showed that although the quantity of sludge collected from ponds was higher than that from RAS, solid waste nutrient content was higher in RAS than that of ponds. Moreover, the biogas yield of RAS sludge was higher (58% methane) compared to that of ponds (38% methane) (Nhut et al., 2019). Adamek et al. (2015) investigated the flesh quality of European catfish (*Silurus glanis* Linnaeus, 1758) reared in two different culturing systems: RAS and outdoor farming units. It was found that skinned trunk and fillet yields, as well as deposited fat weight, were significantly higher in RAS compared to those reared in outdoor farming units thus indicating RAS a more preferable food production system for catfish.

Percids

Pikeperch (*Sander lucioperca*) belongs to the family Percidae, fishes that inhabit both freshwater and brackish water regions of western Eurasia. This species is considered one of the most valuable aquatic animal proteins in Europe due to its small number of bones thus making the best fillet. Major producing countries are: Germany, France, and Finland where it is mostly bred in ponds, extensive and semi-intensive systems based on co-culture with common carp (Good et al., 2010; Szkudlarek and Zakęś, 2007). Currently, there is a high demand for this fish species for human consumption and restocking programs but the available sources (ponds and natural habitats) cannot sustain production. Therefore, research in RAS for sustainable production of pikeperch larvae and fry is underway to optimize favorable growth conditions required by the species for better survival and growth under intensive rearing (Hermelink et al., 2013).

Scophthalmids

Turbot (*Scophthalmus maximus*) is a relatively large species of flatfish belonging to the family scophthalmidae. Turbot is a ground-dwelling fish native to marine and brackish waters of the North-East Atlantic, Baltic Sea, and the Mediterranean Sea. China is the largest producer of turbot and is followed by Europe. This species is mainly reared in RAS (Wang S. et al., 2019) as well as flow-through systems (Jia et al., 2016). In 2015, the global turbot aquaculture production peaked at 6.5 thousand tons of which China produced 85% of the total production (Wu et al., 2019, 2020).

Moronids and sparids

European seabass (*Dicentrarchus labrax*) belongs to family Moronidae of teleost fishes commonly found throughout the estuaries of the Mediterranean and the coasts of the Atlantic Ocean (Kır et al., 2019). The do-

mestication of this species started recently in the early 2000s with its hatcheries being typically inland, in temperature-controlled systems. Intensive farming of this species is mainly through flow-through land-based systems and sea cages although research on seabass RAS for intensive cultivation is underway. Major producing countries are: Greece, Turkey, Spain, and Egypt (Vandeputte et al., 2019). By 2016, seabass production was 191 thousand tons valued at USD 1089 million (Llorente et al., 2020). Unlike other important fish species like carp and trout, European seabass can be marketed whole and fresh with only limited value-addition. Other economically important seabass include: Asian seabass (*Lates calcarifer*), white seabass (*Atractoscion nobilis*), and wild black seabass (*Centropristis striata*).

Among the sparids, the gilthead seabream (*Sparus aurata*) is the most economically important fish species around the world. By 2016, seabream production was 186 thousand tons valued at USD 977 million. Together with seabass, their total aquaculture production in 2016 stood at 376,984 tons valued at USD 2066 million (Llorente et al., 2020).

Serrasalminids

Tambaqui (*Colossoma macropomum*) (Cuvier, 1818) belonging to the family Serrasalminidae is a freshwater fish species native to the Amazon river basin and is currently the most commercially reared native species in Brazil. This species is also widely cultured in South America and certain parts of Asia (Santos et al., 2021). Traditionally, this fish species has been extensively and semi-intensively cultured in earthen ponds (de Farias Lima et al., 2019). However, previous studies on RAS have indicated that cultivating this species at stocking densities of 1.4 and 2.5 kg/m³ does not affect performance thus studies with higher stocking densities to reach the optimal densities in RAS are warranted (Santos et al., 2021).

Penaeids and ostreids

Crustacean aquaculture production is a global industry with a large economic and commercial significance. In particular, marine shrimp dominate crustacean aquaculture, contributing 7 million tons annually with a 60 percent production attributed to penaeid shrimp. Many countries in the Americas and Asia rely on the production of such shrimp and thus, it is the fastest-growing crustacean aquaculture sector. Whiteleg shrimp (*Litopenaeus vannamei*) is the most widely reared shrimp species due to its hardness to diseases, high stocking densities, tolerance to a wide range of water quality parameters (salinity, temperature, NO₃⁻), and improved growth performance compared to the tiger shrimp (*Penaeus monodon* Fabricius) (Bardera et al., 2019). A previous study by Otoshi et al. (2003) showed comparable production capacities of broodstock shrimp (*Litopenaeus vannamei*) reared in both RAS and flow-through earthen ponds. Moreover, shrimp co-culture with other aquatic species such as red strain Nile tilapia (*O. niloticus*) in RAS has been report-

ed to result in better survival and growth performance of both species hence leading to higher economic benefits (Sharawy et al., 2017).

Pacific oysters (*Crassostrea gigas*) belonging to the family Ostreidae are bivalve species of great economic importance due to their high productivity and tolerance to biotic and abiotic stress. Hence, this species has been introduced in several countries to boost up the economic growth of their aquaculture industries (Ramos et al., 2021). According to FAO (2020), the annual production of *Crassostrea gigas* by 2018 stood at 643,000 tons, thus making a significant contribution to aquaculture production worldwide. As such, there is an urgent need to farm this species using advanced aquaculture production systems such as RAS for hatchery seed production.

Stocking density and its effect on behavior, growth performance, feed utilization, and immunity of several species reared in RAS

In all aquaculture food production systems, stress measurement is a useful way to assess the health status of reared species. Generally, fish and crustaceans ex-

perience stress at any stage of production but chronic exposure to stress often deteriorates their health status thus resulting in poor growth performance, reduction in feed utilization, and weakening of their immunity which makes them more susceptible to disease and thus death. Generally, aquaculture producers stock larger quantities of fish per rearing unit to maximize production and profit. However, overcrowding easily suppresses growth and production as a result of a decline of water quality hence certain reared species never reach their marketable size. On the other hand, stocking densities below the optimum levels would not only reduce productivity due to unused available space but also result in size heterogeneity of cultured animals which is considered as one of the major challenges in aquaculture. As such, huge financial losses are incurred. It is therefore imperative to determine the optimum stocking densities of reared species in RAS for increased sustainable production of food and maintenance of good water quality. Table 1 summarizes the desirable stocking densities of different fish and crustacean species reared in RAS.

Table 1. Optimum stocking densities of different fish and crustacean species reared in RAS under specific culturing conditions

Species	Optimum stocking density	Observations and culturing conditions	Reference
1	2	3	4
<i>Cyprinus carpio</i> larvae	200 larvae/L	↑ Survival, final total length, daily total length increments	Jelkic et al. (2012)
<i>Cyprinus carpio</i>	32 kg/m ³	↑ SGR ↓ FCR	Enache et al. (2011)
<i>Cyprinus carpio</i> var. koi co-cultured with <i>Carassius auratus</i>	<i>Cyprinus carpio</i> var. koi : <i>Carassius auratus</i> 0.26:0.54 kg/m ³	↑ Growth	Nuwansi et al. (2017)
<i>Oreochromis niloticus</i> L. fry	5 fry/L	↑ Growth rates and survival (larval test diet 40%, and feeding rate 30% per day)	El-Sayed and Kawanna (2007)
<i>Oreochromis niloticus</i>	10 fish/m ³	↑ Daily growth rate	Arredondo-Figueroa et al. (2015)
<i>Oreochromis niloticus</i> fingerlings	400 and 500 fish/m ³ hyper intensive culture	↑ Growth rate	Gullian-Klanian and Arámburu-Adame (2013 b)
<i>Salmo salar</i>	< 50 kg/m ³	↑ Survival, growth, performance, immunity	Liu et al. (2017) Wang et al. (2019) Liu et al. (2014)
<i>Oncorhynchus mykiss</i>	≤ 100 kg/m ³	↑ Survival, immunity, growth ↓ FCR	d'Orbcastel et al. (2009) Good et al. (2009) Roque d'Orbcastel et al. (2009)
<i>Heterobranchus longifilis</i> , Valenciennes 1840	15 larvae/L	↑ Survival (provided larvae are fed on beef brain)	Atse et al. (2009)
<i>Rhamdia quelen</i>	16–32 kg/m ³	↑ Liver weights, glycogen levels, plasma triglyceride levels, ↔ glucose levels, LDH activity, pituitary PRL expression	Menezes et al. (2015)
<i>Clarias gariepinus</i> Burchell	200–500 kg/m ³	↑ Growth, survival, ↓ agnostic behavioral patterns (depending on body size)	Palm et al. (2018) van de Nieuwegies-sen et al. (2009)
<i>Sander lucioperca</i> larvae	100 larvae/L	↑ Survival, growth (4- to 18-day post-hatch)	Szkudlarek and Zakęś (2007)
	15 larvae/L	↑ Survival, growth (post-19-day period)	

Table 1 – contd.

1	2	3	4
<i>Sander lucioperca</i> fry	9 and 12 fry/L	↑ Growth performance	Grozea et al. (2010)
<i>Scophthalmus maximus</i> juveniles	≤ 22 kg/m ³	↑ Growth, performance, immunity	Liu et al. (2017) Liu et al. (2016) Li et al. (2016)
<i>Scophthalmus maximus</i> adults	9.3 and 13.6 kg/m ²	↑ Growth performance	Jia et al. (2016)
<i>Sparus aurata</i>	65–120 kg/m ³	↑ Growth performance	Arechavala-Lopez et al. (2020) Seginer and Ben-Asher (2011)
<i>Dicentrarchus labrax</i>	< 80 kg/m ³	↑ Growth performance, immunity	Sammouth et al. (2009) Santos et al. (2010) Marco et al. (2008)
<i>Centropristis striata</i>	3.48–27.2 kg/m ³	↑ Growth performance, survival	Copeland et al. (2003)
<i>Colossoma macropomum</i> larvae	50 larvae/L	↑ Larval weight, length, and specific growth rate (slightly saline water; 2.01 ± 0.41 g of salt L ⁻¹)	Santos et al. (2020)
<i>Colossoma macropomum</i> juveniles	3–5 animals/L	↑ Growth performance	de Souza e Silva et al. (2021)
<i>Colossoma macropomum</i>	0.5 and 1.5 kg/m ³	↑ Growth performance (depending on size)	Santos et al. (2021)
<i>Litopenaeus vannamei</i> juveniles	500–9000 animals/m ³	↑ Growth performance (depending on water salinity and stage)	Esparza-Leal et al. (2015) Rodríguez-Olague et al. (2021) Suantika et al. (2018)
<i>Penaeus monodon</i> (Fabricius 1798)	10 shrimp/m ²	↑ Growth performance (water salinity of 20–23 g L ⁻¹)	Nguyen Duy et al. (2012)
<i>Crassostrea gigas</i>	50 larvae/ml	↑ Growth rates and larval yield	Ramos et al. (2021)

(↑): Increase; (↓): decrease; (↔): no change; SGR: specific growth rate; FCR: feed conversion ratio; LDH: lactate dehydrogenase; PRL: prolactin.

Carp

Jelkic et al. (2012) experimented on post-embryonic carp larvae (*Cyprinus carpio*) reared in a closed RAS at two stocking densities (200 larvae/L, and 400 larvae/L) and control which consisted of 800,000 larvae/ha in a pond. Based on survival and growth performance results, the authors recommended a stocking density of 200 larvae/L in RAS. In adult carp, Enache et al. (2011) recorded the best growth performance (SGR) and feed utilization (FCR) at a stocking density of 32 kg/m³. Polyculture is one of the strategies used by aquacultural producers to maximize the production of different species within the same rearing environment. Nuwansi et al. (2017) found better growth of Koi carp (*Cyprinus carpio* var. koi) co-cultured with goldfish (*Carassius auratus*) at an optimum stocking density ratio of 0.26:0.54 kg/m³.

Oreochromis sp.

El-Sayed (2002) showed that rearing Nile tilapia fry (*Oreochromis niloticus* L.) at different stocking densities (3, 10, 15, and 20 fry/L; 20 L fiberglass tanks) and fed on a larval test diet (40% crude protein) resulted in better growth performance at 5 fry/L compared to other stocking densities. Moreover, a stocking density of 5 fry/L and a feeding level of 30% per day enhances growth rates and

survival. Likewise, Arredondo-Figueroa et al. (2015) observed a better daily growth rate (3.6 g/day) at a stocking density of 10 fish/m³ (3000 L tanks) compared to 30 and 75 fish/m³ (0.9 g/day and 0.4 g/day respectively).

Gullian-Klanian and Arámburu-Adame (2013 b), investigated the effect of hyper-sensitive stocking density (400, 500, and 600 fish/m³; 12000 L tank) with limited water exchange (2.52 × 10² L/day) on the growth performance of Nile tilapia fingerlings (2.07 ± 0.04 g). The authors observed a high growth rate of 0.96 g/day; 5.01% per day at 400 fish/m³ and 0.92 g/day; 4.95% per day at 500 fish/m³ which was significantly higher compared to 0.83 g/day; 4.80% per day at 600 fish/m³. Thus, the authors recommend 400 and 500 fish/m³ for a better growth rate of Nile tilapia fingerlings provided the biomass does not exceed 37 kg/m³ a stage at which fish can be transferred to grow-out facilities.

Salmo salar and *Oncorhynchus mykiss*

Studies on Atlantic salmon (*Salmo salar*) have shown that stocking densities not exceeding 50 kg/m³ promote better fish survival, growth, and growth performance. For example, Liu et al. (2017) have shown that stocking densities above 50 kg/m³ lead to deterioration of water quality thus should be avoided when rearing At-

lantic salmon in RAS. A previous report by Wang et al. (2019) has indicated that a maximum stocking density of 30 kg/m³ should be attained in RAS since it does not negatively affect growth and fish non-specific immunity. However, Liu et al. (2014) found that Atlantic salmon grows well at 40 kg/m³ provided the water qualities are maintained within the desirable range of this fish species.

Roque d'Orbcastel et al. (2009) compared the growth and welfare of rainbow trout (*O. mykiss*) reared in RAS and flow-through rearing systems. It was found that a stocking density of 100 kg/m³ (70 m³ total volume) in RAS is desirable for maintenance of water quality parameters and without detrimental effects on fish performance, dorsal and pectoral fins. Fish reared in flow-through systems (FTS), however, exhibited poor survival at similar stocking densities due to high accumulation of CO₂. The maximum stocking density of FTS was 85 kg/m³ thus indicating that RAS can accommodate higher stocking densities than FTS. As shown in Table 1, a stocking density of ≤ 100 kg/m³ is desirable for better survival and growth of rainbow trout provided that water quality parameters are within the optimum range for this species (Good et al., 2009; Roque d'Orbcastel et al., 2009). Moreover, Docan et al. (2011) have shown that stocking densities of 7.12 and 9.42 kg/m³ (0.320 m³ tank volume) increase the fish's adaptability to stressful conditions as evident from increased hematocrit and hemoglobin values. An increase in hematocrit and hemoglobin levels enhances the oxygen-carrying capacity of blood under high-energy conditions such as long-term stress (Srivastava and Sahai, 1987).

Catfish

Atse et al. (2009) studied the effect of different stocking densities on the growth and survival of African catfish larvae (*Heterobranchus longifilis*, Valenciennes 1840). The authors recommend a high stocking density of 15 larvae/L provided that the beef brain is used as a feed alternative to *Artemia* due to increased survival rates of larvae fed on the beef brain.

Menezes et al. (2015) found that a stocking density of 16–32 kg/m³ (250 L tank volume) does not negatively affect the wellbeing of silver catfish (*Rhamdia quelen*). Palm et al. (2018) reported that a stocking density of up to 200 kg/m³ does not suppress the growth or growth performance of African catfish (*Clarias gariepinus* Burchell, 1822) (275 g initial weight). Likewise, van de Nieuwegiessen et al. (2009), reported that African catfish (*Clarias gariepinus* Burchell) (1044.6 g initial weight) reared at a high stocking density of 500 kg/m³ can still survive well under RAS conditions hence indicating that African catfish can be intensively reared at 200–500 kg/m³ without negatively impacting their growth and performance, provided water quality parameters are maintained within the recommended range of the species and initial weights (size) of the fish are considered.

Sander lucioperca

In pikeperch (*Sander lucioperca*), rearing larvae is the most challenging and labor-intensive stage during production. One major challenge of high stocking densities in pikeperch larvae is cannibalism. Likewise, very high stocking densities could result in size heterogeneity and lower growth hence optimizing initial stocking densities is key for maximum production of fish. Szkudlarek and Zakęś (2007) studied the effects of different stocking densities (25, 50, and 100 larvae/L) on growth performance and survival of pikeperch larvae reared in 200-L cylindro-conical tanks in a closed, recirculating system. The larvae were fed on a mixed feed for 14 consecutive days and in the second experiment fed on exclusively artificial feed (trout starter) but at lower stocking densities (6, 10, and 15 larvae/L). Based on the survival and growth performance results of their study, the authors recommended an initial stocking density of up to 100 larvae/L for the 4- to 18-day post-hatch and 15 individuals/L for the post-19-day period due to the correlation of fish biomass gain with the initial stocking density.

Grozea et al. (2010) investigated the effect of stocking density (9 and 12 fry/L) of pikeperch fry reared until 40 days post-hatch in RAS. It was found that pikeperch fry can be reared at stocking densities of 9 and 12 fry/L without negatively affecting the growth performance of fish. Moreover, the authors report that a high stocking density led to better use of feed and space.

Scophthalmus maximus

Liu et al. (2017) investigated the influence of different stocking densities (low: ~ 0.21 – 5.31 kg/m² (initial to final density), medium: ~ 0.42 – 10.81 kg/m², high: ~ 0.63 – 14.27 kg/m²) on growth and stress response of juvenile turbot (*Scophthalmus maximus*) (initial weight to final weight ~ 3 – 75 g) reared in land-based RAS for 120 days. Results indicated no difference in survival and Fulton's condition factor (K) of turbot among all stocking densities. However, the authors recommend a stocking density of up to 11.7 kg/m² since no observable negative effects in survival, growth performance, and immune response were recorded at this stocking density. However, Liu et al. (2016), observed no significant negative effects in survival, growth, and immune response in juvenile turbot reared at a stocking density of 22.38 kg/m² (30 m³ tank volume) for 60 days in land-based RAS. Likewise, Li et al. (2016) found that a stocking density below 17.47 kg/m² (550 L tank volume) is suitable for juvenile turbot culture in RAS provided that multi-level feeding devices are installed to reduce feeding competition. Therefore, in juvenile turbot, stocking densities should not exceed 22 kg/m². In adult turbot, Jia et al. (2016) found that initial stocking densities of 9.3 and 13.6 kg/m² (30 m³ tank volume) do not cause any stress in adult turbot reared in RAS for 120 days.

Sparus aurata*, *Dicentrarchus labrax*, and *Centropristis striata

Seginar and Ben-Asher (2011) have designed a model for determining the optimal harvest size of seabream (*Sparus aurata*) in continuous-production aquaculture, RAS. In larger fish, the authors recommend a stocking density of 80 and 120 kg/m³ for maximum profits. However, larger rearing units will be required to accommodate this high stocking density. Arechavala-Lopez et al. (2020) investigated the effect of different stocking densities (low: 3–15 kg/m³ and high: 11–65 kg/m³; 400 L tank volume) and feeding strategies on social and individual stress response in juvenile gilthead seabream. No significant differences in weight gain were noted between the stocking densities. However, higher stocking densities promoted a schooling behavior in contrast to low stocking densities.

Research on European seabass (*Dicentrarchus labrax*) has indicated that the best stocking density of this species should be less than 80 kg/m³ provided that water quality parameters are within the desirable range. For example, Sammouth et al. (2009) found that rearing European seabass under a stocking density of up to 70 kg/m³ (1 m³ tank volume) did not cause any significant effect on FI and SGR of fish compared to those reared at 100 kg/m³. Lupatsch et al. (2010) reported that stocking European seabass at densities up to 60 kg/m³ (200 L tank volume) does not negatively affect their FI and feed efficiency (FE) or cause any negative metabolic shifts. Santos et al. (2010) also found that stocking densities of 25.2 and 50.5 kg/m³ (200 L tank volume) had no significant negative effect on FCR. Moreover, fish exhibited higher growth compared to those reared at 8.1 kg/m³. This is because fish reared at a low stocking density had reduced nutrient digestibility despite their high feed intake. Marco et al. (2008) have shown that rearing European seabass at 45 kg/m³ (5 m³ tank volume) for 6 weeks does not affect their ability to cope up with multiple stressors as evident from a stress recovery (a decline of blood cortisol and non-esterified fatty acids to normal levels) after 24–48 h. A previous study on captive wild black seabass (*Centropristis striata*) has shown that a stocking density of 3.48–27.2 kg/m³ (2660 L tank volume) does not cause any impairment in growth, survival, and feed utilization despite a slight reduction in water quality at this stocking density (Copeland et al., 2003).

Colossoma macropomum

Santos et al. (2020) investigated the influence of different stocking densities (10, 30, and 50 larvae/L) on the survival and growth performance of *C. macropomum* larvae reared in slightly saline RAS for 30 days. The authors recommended that larvae of *C. macropomum* can be successfully reared in slightly saline RAS at stocking densities of up to 50 larvae/L.

In another study, de Souza e Silva et al. (2021) found better survival, growth, and stress resistance in *C. macropomum* juveniles (initial weight 0.35 ± 0.10 g) at

stocking densities of between 3 and 5 animals/L. However, under different experimental conditions (bigger size), Santos et al. (2021) recommended 0.5 kg/m³; the initial weight of 34.88 ± 0.60 g and 1.5 kg/m³; the initial weight of 150.61 ± 0.58 g thus indicating that optimum stocking densities correlate with the size of the stocked animals.

Shrimp

Super-intensive production of shrimp is gaining increasing attention around the globe as a potential strategy to enhance aquaculture production. In the nursery phase, producers rear the animals at higher stocking densities to maximize production but this has previously been reported to increase cannibalism due to increased competition for available feed, deteriorating water quality, and stress (Arnold et al., 2009; Esparza-Leal et al., 2015; Nga et al., 2005).

Esparza-Leal et al. (2015) observed no significant differences in survival, final weight, and SGR in *Litopenaeus vannamei* postlarvae reared in clear water RAS at four stocking densities: 1500, 3000, 6000, and 9000 animals/m³. Likewise, Rodríguez-Olague et al. (2021) did not observe any significant difference in survival of *Litopenaeus vannamei* juveniles reared in clear water RAS at three stocking densities: 500, 1000, and 1500 animals/m³. However, Suantika et al. (2018) observed better growth performance (SGR, survival) and feed utilization (FCR) of *Litopenaeus vannamei* reared for grow-out production at 500 PL/m³ compared to those reared at 750 and 1000 PL/m³. The discrepancy in results could be attributed to the difference in rearing conditions such as water salinity. A reduction in water salinity tends to favor improved survival and growth performance only under low stocking densities. For example, Nguyen Duy et al. (2012) studied the interactive effect of stocking densities, water salinity and water exchange rate on the growth and survival of *Penaeus monodon* (Fabricius, 1798) reared in two production systems (a sand-based recirculation system with low water exchange; and a sand-based system with high rates of flow-through water). It was observed that a low stocking density of 10 shrimp/m² and water salinity of 20–23 g/L in a sand-based recirculation system produced the best growth rate and survival during the grow-out phase of *P. monodon*.

Crassostrea gigas

Ramos et al. (2021) has recently investigated the effect of different stocking densities (50, 75, and 100 larvae/ml) on the growth and survival of *Crassostrea gigas* reared under RAS. Growth and survival were significantly affected by larval densities and authors recommended a stocking density of 50 larvae/ml as evident from higher growth rates and larval yield. However, Asmani et al. (2017) had recorded poor survival and performance of *Crassostrea gigas* larvae reared at a stocking density of 50 larvae/ml. The discrepancy in results could be attributed to differences in rearing conditions.

Conclusions

Recirculating aquaculture systems are indeed one of the most elegant aquacultural food production systems designed for environmentally friendly and sustainable intensive aquaculture production and profitability. This is achieved through intensive rearing of aquatic species, maintenance of good water quality, limited water exchange, and minimized wastewater discharge to the environment. However, stocking density is one of the most vital parameters that should be put into serious consideration for sustained survival and performance of reared species. Stocking densities below or above the recommended optimal levels negatively affect the behavior, survival, growth, performance, and immunity of animals. It is imperative to note that optimum stocking densities in RAS systems closely depend on rearing conditions such as feeding regimes, feed quality, water salinity, water flow rates, animal size, and stage of the animals' life cycle among others. However, most studies in the literature have reported optimum stocking densities for a single species under specific culturing conditions in RAS and few studies have tackled the influence of several levels of stocking density on two species co-cultured in RAS. More studies tailored in this direction are thus warranted.

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Received: 12 XI 2021

Accepted: 4 I 2022