

## PHYSICOCHEMICAL WATER PARAMETERS - LIMITING FACTORS ON THE RAINBOW TROUT GROWTH IN RECIRCULATING AQUACULTURE SYSTEMS

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### *Abstract*

*The rainbow trout raised in the recirculating system is dependent on the water quality, which must have certain specific parameters. Using specialized literature, the current research aimed at improving the rainbow trout growth conditions in different situations was approached and analyzed. The water parameters differ depending on the season, feed, photoperiod, filter system. The obtained results showed that the most important limiting factors of the physicochemical parameters of water are: temperature, dissolved oxygen, ammonia, nitrites, pH and turbidity. Trout farming in recirculating systems involves high energy consumption and high equipment cost. By optimizing, monitoring and strictly complying with the water physicochemical parameters in RAS, it is possible to improve water quality and obtain large productions of fish, which can quickly amortize the initial investments and profit gain.*

**Key words:** aquaculture, limits, management, RAS, water chemistry.

### INTRODUCTION

In recirculating aquaculture systems, the growth rate of trout is a complicated process that depends on external and internal factors affecting the trout. External environmental conditions such as feed quantity and quality, water physicochemical parameters and the way the system is designed affect the growth rate and welfare of the fish. The quality of fish production in aquaculture is closely linked to the well-being of fish (Broom, 1998; Southgate & Wall, 2001). Growth rate and well-being is also closely related to the internal physiological state of the trout, such as health, stress and reproductive system. In order to avoid stress and increase the well-being of the fish, care must be taken that the physicochemical parameters are interconnected according to the physiological needs of the trout.

All these processes of physicochemical parameters interconnection take place in the water. As a result, water is one of the limiting factors of crucial importance, and the physicochemical processes occurring within the water tip the balance between failure and

success in a recirculating rainbow trout rearing system.

In recirculating systems, the need for make-up water is approximately 1000 L kg<sup>-1</sup> forage that is 10<sup>2</sup> times less than the classic system (Blancheton et al., 2007).

The growth, development and reproduction of salmonids involves knowing the minimum and maximum values of the parameters, specific to the species of interest, as well as the application of effective management regarding production (Cocan et al., 2018).

In order to maintain the quality of the water, the quality parameters must be permanently checked. In general, water samples are collected manually, to be sent to the laboratory where they are analyzed, but the lost time does not allow rapid intervention on fish (Pasika & Gandla, 2020). The researchers differ on the frequency of parameter control. Therefore, some researchers think that the turbidity and the pH can be measured weekly, while temperature and oxygen is good to be measured every day (Ferreira et al., 2011).

Wi-fi equipment and sensors come to the aid of aquaculture so the parameters of the pools can

be tracked even if you are not present near the pools (Jamroen et al., 2023; Lindholm-Lehto, 2023). It is very important to follow the parameters of the water qualitatively for trout and to intervene in time when the water degrades and endangers the life of fish.

## MATERIALS AND METHODS

In order to write this article, papers were studied in which various physicochemical parameters of water were monitored. The study tried to discover and synthesize some of the most recent research on the physicochemical parameters of water from the specialized bibliography.

This study can form a basis for future research aimed at growing rainbow trout in recirculating systems.

## RESULTS AND DISCUSSIONS

### Temperature, Dissolved Oxygen and Ozone

Water temperature and dissolved oxygen content are closely related and can have a negative influence on fish growth (Matthews & Berg, 1997; Uiuu et al., 2020). When temperature approaches the limit value, the oxygen absorption capacity in the trout's body decreases (Pörtner & Giomi, 2013).

Jiang et al. (2021), investigated the increase in antioxidant capacity to stress, how amylase, prosthesis and digestive lipase act, physiologic growth parameters, but also the growth factor similar to insulin in the liver, in conditions of temperature changes and dissolved oxygen. In this study trout were bred with 4.2 mg kg<sup>-1</sup> and 9.6 mg kg<sup>-1</sup> dissolved oxygen, at 21°C, 17°C, 13°C. The results showed that the increase in relation to the initial weight and use of the feed, decreased to 21°C and increased to 17°C.

The problems with rising temperatures were moderate in the presence of increased dissolved oxygen content. When the dose of dissolved oxygen was enlarged, survival increased, but 4 mg kg<sup>-1</sup> of dissolved oxygen is the lowest survival level for trout. Regarding trout growth indicators, were decreased to 21°C and raised to 17°C.

According to specialized literature, trout feel best at 16-17°C, but Cocan (2008), in his research, concluded that the comfort

temperature for trout is 12-14°C. Also, according to Mishra et al. (2020; 2021), trout grown at 14-16°C showed the best specific growth rate value.

Dissolved oxygen plays a key role in recirculating systems. (Ebeling & Timmons, 2012). Low oxygen reduces weight gain and decreases feed conversion (Chabot & Dutil, 2005; Tran-Duy et al., 2005). The lack of dissolved oxygen increases ammonia toxicity in rainbow trout (Thurston et al., 1981).

Water turbidity, NH<sub>2</sub>, lack of oxygen, water color and dissolved or total organic carbon can be improved with ozone (Goel et al., 1995; Tango & Gagnon, 2003; Summerfelt, 2003). Other research has shown that using ozone can also be effective in fighting algae and some heavy metals (Langlais et al., 1991; Plummer & Edzwald, 2002). The reintroduction of ozone promotes the formation of dissolved oxygen (Summerfelt, 2003).

When ozone is used longer than necessary, there is a risk of increased mortality (Summerfelt et al., 2004).

The application of ozone in RAS could solve some problems regarding optimization of press parameters and fish growth in recirculating systems (Davidson et al., 2011). Ozone reduces total suspended solids, therefore increasing water transparency solar penetration is favored. Davidson et al. (2011), propose the use of ozone in recirculating systems due to the increase of water quality.

Recirculating systems are subject to an increased risk of disease and parasite infestation. The most common method of disease control consists in the use of ultraviolet rays and ozone (Cocan, 2008). In general, ozone positively influences fish survival.

### Heavy Metals

Heavy metals such as aluminum become increasingly bioavailable as pH decreases (<6.0) and the increase of free ionic calcium. Therefore, toxicity occurs at a low pH than at neutral or high pH (Playle & Wood, 1989). Therefore, fish diseases occur which can be attributed both to the decrease in pH and to the increase in the level of heavy metals which can cause the appearance of white spots on the mouth, gills and tegument.

Hunn & Schnick (1990) observed that a decrease in pH combined with heavy metals increases hyperexcitability and attempts by fish to get out of the water (swim to shore).

Majlesi et al. (2019), did research tracking mercury, cadmium and lead levels in muscle tissue in rainbow trout. The determinations showed concentrations of: mercury 0.22 mg/kg, cadmium 0.105 mg/kg and lead 1.070 mg/kg. The level of mercury and cadmium fell within the maximum levels allowed by the WHO (World Health Organization), but lead did not. Consumption of fish in the study area had no negative effects on human health. The release of wastewater, pollutants from human activities and certain feeds increases the level of heavy metals in trout (Majlesi et al., 2019).

### Water pH and Nitrogen-based Compounds

The Directive of the Council of Europe of 06.08.2006 regarding the quality of fresh waters, which must be protected or improved in order to maintain fish life, stipulates that the pH should fall between 6 and 9, non-ionized ammonia in salmonid and cyprinid waters can have in concentrations of 0.005 mg/L

(indicative) and 0.025 mg/L (mandatory), total ammonium ( $\text{NH}_3 + \text{NH}_4$ )  $\leq 0.04 \text{ mg L}^{-1}$  and mandatorily  $\leq 1 \text{ mg L}^{-1}$  and  $\text{NO}_2 \leq 0.01 \text{ mg L}^{-1}$  (<https://eur-lex.europa.eu>).

The factor that determines the ratio of ammonia to ammonium in water is the pH. The activity of ammonia is influenced by the ionic strength and the temperature of the solution (<https://ro.hach.com/parameters/ammonia>).

Ammonia and ammonium ( $\text{NH}_3$  and  $\text{NH}_4$ ) concentrations are expressed in mg/L or PPM of N. From a chemical point of view, the relationship between ammonia and ammonium is:  $\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^-$ .

When pH decreases ammonium increases and ammonia decreases, and when pH increases ammonium decreases and ammonia increases. The activity of ammonia in water is much lower at low temperatures. As pH and temperature increase, so does ammonia. Knowing the temperature and pH, the percentage of ammonia ( $\text{NH}_3$ ) in the total ammonia can be determined according to Table 1 (Emerson et al., 1975).

Table 1. The percentage of non-ionized ammonia according to pH and temperature (after Emerson et al., 1975)

T (°C)	Ph																
	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0	10.2
4	0.11	0.18	0.29	0.45	0.72	1.13	1.79	2.80	4.37	6.75	10.30	15.39	22.38	31.36	42.00	53.44	64.53
6	0.13	0.21	0.34	0.53	0.84	1.33	2.10	3.28	5.10	7.85	11.90	17.63	25.33	4.96	46.00	57.45	68.15
8	0.16	0.25	0.40	0.63	0.99	1.56	2.45	3.83	5.93	9.09	13.68	20.08	28.47	38.38	50.00	61.31	71.52
10	0.18	0.29	0.46	0.73	1.16	1.82	2.86	4.45	6.88	10.48	15.65	22.73	31.80	42.49	53.94	64.98	74.63
12	0.22	0.34	0.54	0.86	1.35	2.12	3.32	5.17	7.95	12.04	17.82	25.58	35.26	46.33	57.78	68.44	77.46
14	0.25	0.40	0.63	1.00	1.57	2.47	3.85	5.97	9.14	13.76	20.18	28.61	38.84	50.16	61.47	71.66	80.03
16	0.29	0.46	0.73	1.16	1.82	2.86	4.45	6.88	10.48	15.66	22.73	31.80	42.49	53.94	64.99	74.63	82.34
18	0.34	0.54	0.85	1.34	2.11	3.30	5.14	7.90	11.97	17.73	25.46	35.12	46.18	57.62	68.31	77.35	84.41
20	0.39	0.62	0.98	1.55	2.44	3.81	5.90	9.04	13.61	19.98	28.36	38.55	49.85	61.17	71.40	79.83	86.25
22	0.46	0.82	1.14	1.79	2.81	4.38	6.76	10.31	15.41	22.41	31.40	42.04	53.48	64.56	74.28	82.07	87.88
24	0.52	0.83	1.31	2.06	3.22	5.02	7.72	11.71	17.37	25.00	34.56	45.57	57.02	67.77	76.92	84.08	89.33
26	0.60	0.96	1.50	2.36	3.70	5.74	8.80	13.26	19.50	27.74	37.83	49.09	60.45	70.78	79.33	85.88	90.60
28	0.69	1.10	1.73	2.71	4.23	6.54	9.98	14.95	21.78	30.62	41.16	52.58	63.73	73.58	81.53	87.49	91.73
30	0.80	1.26	1.98	3.10	4.82	7.43	11.29	16.78	24.22	33.62	44.53	55.99	66.85	76.17	83.51	88.92	92.71
32	0.91	1.44	2.26	3.53	5.48	8.42	12.72	18.77	26.80	36.72	47.91	59.31	69.79	78.55	85.30	90.19	93.58

High ammonia values are associated with a deficiency in biological filtration for the simple reason that the bacteria in the filter should consume the ammonia and convert it to nitrite. There is evidence that nitrite can grow a lot ( $100\text{-}700 \text{ mg L}^{-1}$ ) in the recirculating system with low and almost zero

water exchanges. Thus, in the recirculating system the denitrification unit must be cleaned (depending on the load of excrement and food scraps) to discharge the big concentration of nitrite (Van Rijn et al., 2006).

Nitrite concentrations in the recirculating system can be monitored by partially replacing the

water, washing the filters and adding denitrifying bacteria (Camargo et al., 2005; Martins et al., 2010; Schipper et al., 2010; Pulkkinen et al., 2021). There is evidence to suggest that relatively low concentrations of  $\text{NO}_3\text{-N}$ , once thought to be harmless (Wedemeyer & Schild, 1996; Colt, 2006; Lekang, 2013), can influence the toxicity for some of the fish raised in RAS.

The harmful effect on ammonia has negative consequences when the pH rises and a low pH needs very little ammonia to increase the harmfulness (WHO, 1986; Wurts 2003). Elevated ammonia levels in water favor accumulation in the body of ammonia fish that causes death (Randall & Tsui, 2002). Toxic chemicals such as fenix and cyanide ion are becoming more toxic when the pH drops (Könemann, 1986). Hydrocyanic acid is present at low pH, being even more toxic than cyanide ion (Rand, 1995).

In general, research evaluating the chronic toxicity of  $\text{NO}_3\text{-N}$  to crop species at different life stages is limited. Lin & Randall (1990), added caustic soda to the water to raise the pH to  $9.91 \pm 0.02$  for 90 min, and in another tank, they added hydrochloric acid to lower the pH of the tested solution at  $3.88 \pm 0.02$ , for 90 min.

At high pH level, the water exhaled by the fish was acidified as it passed through the gills, and at low pH, the exhaled water was alkalized rather than acidified. Lowering the ammonia in the water could lower the ammonia in the blood. However, blood ammonia increased when the fish were exposed to acidic conditions. Exhaled water pH ranged from 3.88 to 9.91, but exhaled pH only ranged from 4.33 to 7.10, the microenvironment of the fish's fragile gill epithelium managing to maintain a pH balance.

Becke et al. (2017) have studied what happens when rainbow trout is kept in cloudy water, with an increased level of non-ionized ammonia in a RAS over a period of three months and a week. The survived rate was over 99%, without be affected too much of the high turbidity of the water ( $\text{TSS} > 25 \text{ mg L}^{-1}$ ). Ammonia at level of  $0.05 \text{ mg L}^{-1}$  had no significant negative influence on physiology and trout growth. Also, at load of total

suspended solids  $> 10 \text{ mg L}^{-1}$ , it was a bacterial growth, which did not affect the fish growth and health.

Davidson et al. (2014) conducted a study on rainbow trout to investigate the effects of nitrate ( $\text{NO}_3$ ) concentrations on fish health. They stocked equal numbers of trout fry in six RAS, maintaining three systems at low  $\text{NO}_3$  concentrations (30 mg/L) and three at high  $\text{NO}_3$  concentrations (91 mg/L). Trout growth was not affected by mean  $\text{NO}_3$  concentrations, but survival was lower where ammonia was added. In addition, lateral swimming and health problems were observed in trout exposed to high concentrations of  $\text{NO}_3$ .

Anaerobic oxidation of ammonium (anammox process) may be used in recirculating systems as a biological alternative for ammonia removal (Van Rijn et al., 2006). This oxidation of ammonium is very important for RAS. Oxidation allows complete removal of autotroph nitrogen, unlike the traditional mode with nitrification biofilters and heterotrophic denitrification systems that require the addition of organic carbon. Therefore, the oxidation process uses half of the ammonia that fish produce and the rest is converted anaerobically into nitrogen gas along with the produced nitrite. Anaerobic oxidation can reduce the consumption of electricity and the excessive use of dissolved oxygen (Van Rijn et al., 2006).

### **Carbon Dioxide**

Carbon dioxide ( $\text{CO}_2$ ) is toxic to fish and has a limiting effect on RAS. The accumulation of  $\text{CO}_2$  is favored by the high density of fish. A high level of  $\text{CO}_2$  in water leads to acidification of the blood and reduced oxygen absorption (Molleda, 2007).

At lower alkalinity levels ( $10 \text{ mg L}^{-1}$ ),  $\text{CO}_2$  removal is significantly higher compared to higher alkalinity (70 and  $200 \text{ mg L}^{-1} \text{ CaCO}_3$ ). When assessing total inorganic carbon loss from the RAS, daily loss was found to be roughly equal at 10 and  $70 \text{ mg L}^{-1}$ , but highest at  $200 \text{ mg L}^{-1}$  alkalinity. Additionally, pH records indicated that the  $10 \text{ mg L}^{-1}$  alkalinity treatment led to the lowest system pH (Summerfelt et al., 2014).

At trout, the excretion of  $\text{CO}_2$  is about  $1\text{-}2 \text{ mg CO}_2 \text{ kg}^{-1} \text{ min}^{-1}$ , and it can bear a maximum  $20 \text{ mg CO}_2 \text{ L}^{-1}$  in water (Timmons et al., 2018). Elimination of carbon dioxide occurs with the

help of compliant (gassing tunnels) (Summerfelt et al., 2003); by injecting very small air bubbles that favor atmospheric air intake and CO<sub>2</sub> removal (Barrut et al., 2012); air transport pump with CO<sub>2</sub> removal that can remove 13-20 g CO<sub>2</sub> kW h<sup>-1</sup> (Loyless & Malone, 1998); aeration devices that can remove 1.2 kg CO<sub>2</sub> kW h<sup>-1</sup> (Eshchar et al., 2003).

### **Photoperiod**

Ma, et al. (2023) have researched how different types of lights influence the juvenile rainbow trout. Spectra of blue-violet-red light, blue-violet red light, blue light, and red light alternated at 300, 900, and 1200 lx. At the end of the research, they noticed better results with the help of red violet blue light that showed a significant increase in lipase, a better digestion process in the stomach, and increased feed consumption.

Red light has been suggested for intensive rearing of rainbow trout also by Karakatsouli et al. (2008). Good results can be achieved with spectra of light trying to copy light from nature.

### **Salinity**

Some fish like rainbow trout have a higher salt tolerance, which gives them an advantage for growth in RAS (Tian et al., 2022).

Bordignon et al. (2024) wanted to see if three levels of water salinity in an aquaponic system (low: 0.5‰; average: 3.0‰; high: 6.0‰) may influence the growth performance and characteristics of the trout housing. The addition of salt to the water did not affect the weight and the trout filet, also salmastra water with up to 6‰ reduced the consumption of freshwater and did not affect the growth of fish and leafy vegetables or the overall balance of the aquaponic system. After 21 days, considered adaptation period, salt was added little by little to 3‰ in systems with average salinity and 6‰ in high salinity systems observing a slight salinity effect water on microbial communities in water, namely the measured bacterial ecosystem diversity, has decreased to salinity of 6‰, observed by other researchers in other saline environments such as mud (Ya el al., 2023), and coastal lakes (Lew et al., 2022).

Microbial analysis has shown that there are no significant changes between salinity doses used.

### **Alkalinity and Hardness of Water**

Reaction between different types of alkali ions such as: hydroxide; carbonate; hydrogen carbonate and water pH can cause total alkalinity (Boyd et al., 2016; Lindholm-Lehto, 2023).

Alkali ions HCO<sub>3</sub><sup>-</sup>, CO<sub>2</sub><sup>-</sup> and OH<sup>-</sup> are found naturally in water (Boyd et al., 2016). Due to the calcium and magnesium salts present in the water alkalinity is measured in mg CaCO<sub>3</sub> L<sup>-1</sup>. Due to the limestone present in the water, it may have a certain hardness, the water hardness is measured in mg L<sup>-1</sup> or German degrees. Sweet water hardness is considered between 5 mg L<sup>-1</sup> and over 500 mg L<sup>-1</sup> (Timmons et al., 2018).

The addition of acidic or basic solutions in RAS water can change the level of alkalinity or acidity of the pH in the water (Boyd et al., 2016). Also, temperature can influence together with alkalinity and pH carbon dioxide dissolved in water (Timmons et al., 2018).

Chen et al. (2006), consider that an alkalinity value of 200 mg CaCO<sub>3</sub> L<sup>-1</sup> is very good for the nitrification unit, but Timmons et al. (2018), found it is better to use a value between 50-300 mg CaCO<sub>3</sub> L<sup>-1</sup>.

The energy consumed for osmoregulation is lower in hard water than fine (Klontz, 1991).

Sudden and extreme changes in water hardness, even in combination with increased water temperature, do not cause trout mortality (Huysman et al., 2022).

### **Turbidity**

Total suspended solids (TSS) directly influence water turbidity, which can impede fish vision and ultimately compromise their life cycle. At 160 nephelometric turbidity units (NTU), i.e. approximately 54.4 mg L<sup>-1</sup> (TSS), trout feeding is not affected (Greer et al., 2015).

In a RAS, about 25% of the water turbidity comes from fish feed (Cripps & Bergheim, 2000).

Suspended solid particles can cause stress reactions and endanger the health of aquatic animals (Alabaster & Lloyd, 2013). An increase in concentration (TSS) can lead to increased oxygen consumption, and poor biofilter performance (Michaud et al., 2006).

## CONCLUSIONS

The optimum temperature for growing rainbow trout can fluctuate between 12°C and 17°C, and higher or lower temperatures have adverse effects influencing growth, viability, appetite, digestive capacity and antioxidant capacity.

Dissolved oxygen can improve the health of fish, but it cannot eliminate the causes.

Using ozone with caution helps maintaining water parameters for fish.

Improper use of ozone can cause high mortality among fish.

Ultraviolet light kills most parasites and bacteria and should be installed after the biological filter of the recirculating system. Heavy metals can influence the pH, and its decrease raises a question mark about their existence in the water.

Water pH in recirculating trout rearing systems is crucial to trout health and rearing performance. An optimum pH for trout in such systems is generally between 6 and 9. Failure to meet these pH limits can affect nutrient uptake, metabolism and fish stress. Regular pH monitoring and adjustment are essential to maintaining an optimal balance in recirculating trout rearing systems. Higher than normal pH values can cause high mortalities among fish, especially in the initial stages of development. Biological effects of high or low pH can affect the development of fish. Following certain symptoms of fish, including eye exophthalmos, skin or gill injuries, can suspect an unfavorable pH that can even cause death.

pH can influence the biology and chemistry of water, so it is important to carefully observe the complex interactions between pH, dissolved oxygen, ionic strength and ammonia concentration in the damaged environment

In the aquatic environment is an important complex interaction between pH, dissolved oxygen, ionic strength and ammonia concentration.

Ammonia (NH<sub>3</sub>) is harmful to trout, but ammonium ion (NH<sub>4</sub>) is practically harmless. High concentrations of nitrite are harmful to fish life and can increase mortality.

Carbon dioxide is a limiting factor in recirculating systems and the maximum concentration allowed for rainbow trout is 20 mg L<sup>-1</sup>.

Artificial light spectrums that mimic natural light are beneficial and can be used in recirculating systems. Red and blue light can ensure a well-being of fish.

The alkalinity can fluctuate around the value of 50-300 mg of CaCO<sub>3</sub>/L, and at a value above 6‰ the salinity destroys the balance of the nitrifying and denitrifying bacteria in the biological filter.

Turbidity is closely related to the size of the space, the density of the fish, the food and the efficiency of the filtering biosystem, and it is preferable that the water be as clean as possible.

The subject being so vast and important for the growth of trout in recirculating systems, certainly the research in the field will expand, and the discovery of new technologies will lead to the adjustment of the limiting parameters that will ensure an optimal aquatic environment.

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