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Rainbow trout larvae production in an airlift-based recirculating system

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ABSTRACT

Replacement of traditional flow-through (FT) systems with recirculating aquaculture systems (RAS) can reduce fresh water demand and environmental effects of aquaculture practices. Thus, we investigated the utility of a simple airlift-based recirculating egg incubation system with low initial construction costs and low demand for fresh water to incubate rainbow trout (*Oncorhynchus mykiss*) eggs and produce advanced larvae. An airlift-based recirculating system, consisting of a trough with four trays was designed, and a flow-through California trough with four trays was used as control. In each tray of the flow-through and recirculating systems, 5000 fertilized eggs were introduced. Water flow rates were 6–7 l/m in both systems. Physicochemical conditions, quality of eggs, and number of yolk-sac larvae were surveyed during the 42-day experimental period. In both systems, eyed eggs occurred at 14 days after fertilization. In the FT system and the RAS, all of the fertilized eggs hatched at 28 and 29 days after fertilization, respectively, and yolk-sac depletion occurred at 41–43 days after fertilization. In the FT system and RAS, percentages of eyed eggs were 79.75% and 79.01% respectively, and hatching rates were 70.31% and 63.65%, respectively. The survival rate of fry was 68.26% in the FT system and 61.07% in the RAS. There were no significant differences between two systems in terms of wet weight or dry weight at hatching time ($p > .05$), while final length (25.53 mm), wet weight (161.19 mg) and dry weight (30.78 mg) of larvae in the FT system were significantly higher than in the RAS (25.09 mm, 149.99 mg and 29.37 mg, respectively). In conclusion, the simple airlift-based system could be used effectively in the egg incubation period, while growth performance of larvae was little affected by this system as compared with the FT system.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most popular cultured fishes, accounting for about 2% of total aquaculture production worldwide (FAO, 2018). Major countries producing this species have been Chile with 30.3%, Iran with 12.6%, Turkey with 11.7%, and Norway with 7.5% of total production (FAO, 2011).

Low water consumption, low land requirements and minimum environmental impacts have been the main reasons compelling aquaculturists to use recirculating aquaculture systems (RASs) for producing fish and shellfish (Irani and Agh, 2019). In addition, culturists might want to practice stronger biosecurity against pathogens, and they can have fuller control over water quality in recirculating systems. Reusing wastewater from rearing tanks after purification (physical and biological filtering) generally conserves > 90% of the water compared to traditional systems and decreases environmental impacts of aquaculture practices as well (Timmons et al., 2001). Recirculating aquaculture systems have been used to culture many warm-water, cold-water, fresh water, and marine fish species during recent years (Irani and Agh, 2019; Summerfelt and Sharrer, 2004). Rainbow trout is one of the most popular fishes produced in RAS (Jokumsen et al., 2009; Roque d'Orbcastel et al., 2009).

Circulation of water, commonly performed by centrifugal pumps, besides generation of oxygen, is among the expensive practices in any recirculating system, affecting both purchase and operational costs. Therefore, airlift systems, as a more cost-effective approach, have been used to provide water circulation and to increase the oxygen concentration of water in systems ranging from small aquaria to large waste treatment facilities (Neori et al., 2004). The most common type of airlift consists of an open-ended tube or pipe that is partially submerged in fluid into which air is injected (Gephardt et al., 2009). Water flow is generated using a rising column of air. Operation of airlift pumps is due to the difference in specific gravity between the fluid on the outside and the air-fluid mixture on the inside of the tube (Wheaton, 1977).

Wastewater in RAS includes many particles such as residual feed, feces, and suspended particulates; it also contains toxic compounds of nitrogen (ammonia and nitrite) and a large number of heterotrophic and nitrifying bacteria (Chen et al., 1993). These harmful substances and microbes can seriously affect the health and welfare of fish. Thus, water treatment facilities are required to remove these particles and

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substances (Xiao et al., 2018). However, in hatchery systems, during the egg incubation and yolk-sac larvae stages (before the beginning of exogenous feeding), most of these particulate matters and harmful substances are not present. Therefore, in this research, we designed a simple airlift-based recirculating egg incubator with low construction costs and low needs for energy and fresh water. We evaluated the utility of this system for incubating rainbow trout eggs and producing advanced larvae. We also investigated egg quality, fish performance, and main water quality factors during the egg incubation and larval rearing period. Because of recent droughts, some of the wells, rivers, and wetlands in Iran have dried (Mousaei Sanjerehei and Rundel, 2017). In this situation, replacement of traditional flow-through hatchery systems with recirculating systems can be helpful in the development of the aquaculture industry.

2. Material and methods

2.1. Hatching and rearing system

This research was conducted in the Artemia & Aquaculture Research Institute of Urmia University, Urmia, Iran. Two egg incubation systems were used. An airlift-based recirculating system consisting of a trough with four trays was designed. An airlift pump consisting of a PVC pipe (diameter: 2.5 cm, height: 33 cm, submergence: 25 cm and lift: 8 cm) and an aerating tube (diameter: 0.8 cm) provided the water recirculation and oxygen requirements of the system (Fig. 1). Because the study was performed in the egg incubation and yolk-sac larvae stages, without feeding and producing solids, there were no mechanical and biological filters in this system. A flow-through California trough with four trays was used as control. Fertilized rainbow trout eggs were transported to the Institute; after 2 h, the eggs became water-hardened and were distributed between the two experimental systems. Each tray of flow-through and recirculating system received 5000 eggs. Water flow rates were 6–7 l/m in both units. Water exchange in the recirculating system was performed at intervals of three days, two days, and one day during the first four weeks, fifth, and final weeks, respectively. Incoming water was disinfected by a UV instrument before entering the systems. During the incubation, eggs were disinfected with 500-ppm formalin for 20 min at three-day intervals to prevent saprolegniasis.

2.2. Sampling and measurements

Temperature, pH, dissolved oxygen, and conductivity (EC) were measured daily during the study period with a portable multimeter (WTW, Multi 3630 IDS, Weilheim, Germany). Total solids (by evaporation method) and carbon dioxide (by titration) were measured weekly. Biological oxygen demand (BOD_5) was measured using a BOD meter (Hach, BODTrak, Loveland, USA), Total ammonia nitrogen (TAN), nitrite (NO_2^--N), nitrate (NO_3^--N), alkalinity, and orthophosphate (PO_4^{2-}) were measured weekly using a photometer (Hach, DR 2800, Loveland, USA) and commercial kits. Water samples were taken from inlet, outlet, and inside of each system.

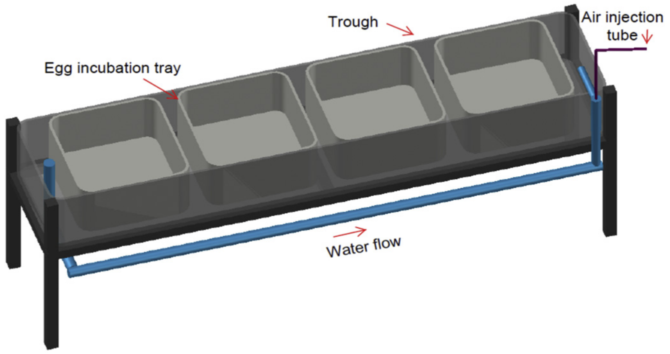

Fig. 1. Designed airlift pump system installed on the California trough to drive recirculation in a rainbow trout egg incubation and larval rearing system.

To compare the egg quality and larval performance in the two systems, the percentage of eyed eggs (number of eyed eggs × 100/total number of eggs), hatching rate (number of larvae × 100/total number of eggs), percentage of malformation (number of deformities × 100/total number of larvae), and survival rate (number of advanced larvae × 100/total number of eggs) were calculated. After hatching and at the end of the experimental period, 20 fish in four replicates were taken from both systems, and the total length, total weight, and dry weight of larvae were measured.

2.3. Statistical analysis

Excel 2016 was used for processing the data and creating the charts. Statistical analyses were carried out using SPSS 22. Homogeneity of variances and normality of distribution were tested using the Levene's test and Shapiro-Wilk test, respectively. Independent-samples *t*-test ($\alpha = 0.05$) was used to compare the mean values of parameters for the two systems. Kinetics of data during the experimental period (weekly) was analyzed using repeated measures ANOVA. Once there were significant differences, one-way ANOVA and Tukey's post-hoc test were used to compare data. All analyses was performed at $\alpha = 0.05$.

3. Results

In both systems, eyed eggs occurred at 14 days after fertilization (DAF). In the FT system, hatching began at 25 DAF. At 26 DAF, > 50%, and at 28 DAF, all of the fertilized eggs hatched. In the RAS, hatching began at 26 DAF. At 27 DAF, < 30% and at 29 DAF all of the fertilized eggs hatched. Freely swimming larvae were observed at about 37–38 DAF. At 42 DAF, most larvae in the FT system and > 50% of larvae in the RAS absorbed the majority of their yolk sac and were able to swim freely.

3.1. Physicochemical conditions

There were significant differences ($p < .05$) between the two units in terms of physiochemical conditions. In the flow-through and recirculating systems, the values of water temperature ranged from 11.36–13.51 and 10.83–12.58 °C, respectively (Fig. 2A), values of pH ranged from 7.84–8.07 and 8.24–8.60, respectively (Fig. 2B), values of dissolved oxygen ranged from 7.77–8.93 and 6.86–9.10 mg/l, respectively (Fig. 2C), and values of conductivity ranged from 1126.86–1137.14 and 1085.29–1119.14 µS/cm, respectively (Fig. 2D). Results of repeated measures ANOVA testing (Table 1) for these factors indicated that the interactions between time and treatments were significantly different ($p < .05$), and the inlet and outlet values of temperature in the FT system were significantly higher than in the RAS except at weeks 1 and 3. The values of pH and EC in the FT system during the study period were significantly lower and higher than in the RAS, respectively ($p < .05$).

There were no significant differences during the incubation period (first four weeks) in terms of dissolved oxygen between two units, except for the inlet of the RAS at weeks 2 and 4 that were significantly higher than in the FT system. During the larval rearing period (weeks 5 and 6), the values of DO at the RAS outlet were significantly lower compared than of the RAS inlet, and at the inlet and outlet of the FT system.

Repeated measures ANOVA testing for the all measured physicochemical factors during the study period showed that there were significant interactions ($p < .05$) between time and the treatment effect (Table 1), suggesting that the dynamics of these factors were influenced by the type of system. Except for the first week, dissolved oxygen

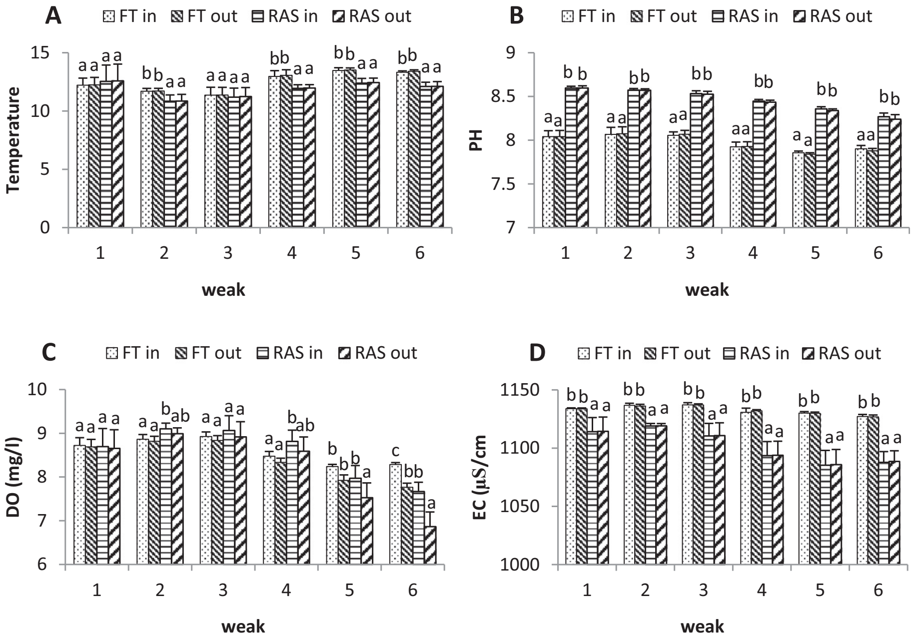

Fig. 2. Dynamics of temperature (A), pH (B), DO (C) and conductivity (D) at the inlet and outlet of the flow-through (FT) and recirculating (RAS) systems during the study period. Values are mean of each week ± SD. Different superscripts indicate significant differences (one-way ANOVA, α = 0.05) in the same week.

consumption was significantly higher in the RAS than in the FT system ($p < .05$). The values increased with development of the embryos and growth of the larvae during the experimental period in both systems (Fig. 3A). DO consumption rates in the FT system and RAS in the first week were 0.0068 and 0.0087 µg/l/egg, respectively, while at the last week they were 0.1523 and 0.2629 µg/l/egg, respectively. The values at week 4 were significantly higher than in the first two weeks, and in the last two weeks were significantly higher than in other weeks ($p < .05$).

The alkalinity was relatively high in both systems during the study period. The values ranged from 302.5–320 mg/l in the FT system and 300–325 mg/l in the RAS (Fig. 3B). The total solids were low in both systems. The values in the FT system and RAS ranged from 615 to 795.5 and 615.5–818 mg/l, respectively. There were no significant differences ($p < .05$) between the two systems, except at 28 and 35 DAF, when the values in the RAS were significantly higher (Fig. 3C).

The values of biological oxygen demand (BOD_5) ranged from 1.05–1.6 mg/l in the FT system and 1.65–2.15 mg/l in the RAS. The values in the RAS were significantly higher than in the FT system. During the incubation period, there were no significant differences ($p > .05$) in the FT system, while in the RAS, the highest value was observed at 28 DAF, which was significantly different from other sampling times (Fig. 3D).

The values of total ammonia nitrogen (TAN) in the RAS were significantly higher than in the FT system ($p < .05$) during the whole study period. The differences between two systems increased from 28 DAF onwards, when the values in the FT system and RAS were 0.07 and 1.37 mg/l, respectively (Fig. 3E). Likewise, the values of nitrite nitrogen (NO_2^--N) in the RAS were significantly higher than in the FT system ($p < .05$) during the whole study period. The values increased with developing embryos and growing larvae in both systems, and ranged from 0.0064–0.0091 mg/l and 0.014–0.2891 mg/l in the FT system and the RAS, respectively (Fig. 3F). The values of nitrate-nitrogen (NO_3^--N) in the FT system and RAS ranged from 3.69–4.66 mg/l and 3.71–5.19 mg/l, respectively. Except for 1 and 14 DAF, there were significant differences ($p < .05$) between the two systems (Fig. 3G).

The values of orthophosphate (PO_4^{2-}) in the FT system and RAS ranged from 0.045–0.165 mg/l and 0.12–0.36 mg/l, respectively. The RAS had significantly higher values than the FT system during the study period (Fig. 3H). Carbon dioxide in the RAS was maintained at zero during the experimental period, while in the FT system the values ranged from 4.25–5.50 mg/l (Fig. 3I).

3.2. Eggs and larvae

In the FT system and RAS, percentages of eyed eggs were 79.75 and 79.01, respectively, and hatching rates were 70.31% and 63.65%, respectively. The survival rate of fry (number of yolk sac-depleted larvae and total eggs ratio) was 68.26% in the FT and 61.07% in the RAS. Malformation was 0.81% in the FT and 0.80% in the RAS. Wet weight and dry weight at hatching time were 85.09 mg and 30.75 mg respectively in the FT, and 84.64 mg and 29.39 mg in the RAS. There were no significant differences ($p > .05$) between the two systems in terms of these parameters (Table 2), while final length (25.53 mm), wet weight (161.19 mg) and dry weight (30.78 mg) of larvae in the FT were significantly higher than in the RAS (25.09 mm, 149.99 mg and 29.37 mg, respectively).

4. Discussion

Air-lift pumps have been successfully utilized in recirculating systems due to their circulation, aeration, and degasification capabilities (Loyless and Malone, 1998). The amount of lift and submergence is crucial to air-lift design and operation. Lift height represents the vertical distance above the water surface to the set discharge point (centerline of the headpiece). Submergence is the distance below the water source to the point of air injection. Together, submergence and lift represent the airlift pump height. Percent submergence (submergence to total airlift pump height ratio) is also helpful when designing air-lifts. Air-lifts are said to be most efficient in applications of the between 65 and 75% submergence (Wheaton, 1977). However, maximum pumping efficiencies have been reported to be at the higher end, about 80%, in the more recent literature (Timmons et al., 2001). In this study, the percentage of submergence was about 76% and showed efficient water circulation, oxygenation, and degasification capabilities.

Recirculating aquaculture systems generally conserve > 90% of the water compared to traditional systems (Timmons et al., 2001). The overall freshwater requirement of the airlift-based recirculating system

Table 1
Repeated measures ANOVA testing for the physicochemical factors during the study period.

Source		df	Mean Squere	*F*-value	*P*-value	Partial Eta Squared
Temperature	Time	2.46	30.31	37.16	0.000	0.608
	Error (Time)	58.97	0.82			
	Treatment	3	6.47	24.86	0.000	0.757
	Error (Treatment)	24	0.26			
	Time * Treatment	7.37	1.88	2.31	0.035	0.224
pH	Time	2.65	0.67	180.44	0.000	0.883
	Error (Time)	63.70	0.004			
	Treatment	3	3.28	2181.82	0.000	0.996
	Error (Treatment)	24	0.002			
	Time * Treatment	7.96	0.019	5.22	0.000	0.395
O_2	Time	2.45	16.91	156.81	0.000	0.867
	Error (Time)	58.86	0.108			
	Treatment	3	0.969	15.30	0.000	0.657
	Error (Treatment)	24	0.063			
	Time * Treatment	7.36	1.03	9.55	0.000	0.544
EC	Time	2.4	4720.06	42.67	0.000	0.640
	Error (Time)	57.65	110.62			
	Treatment	3	13,208.29	168.86	0.000	0.955
	Error (Treatment)	24	15,231.27			
	Time * Treatment	7.21	607.65	5.49	0.000	0.407
O_2 Consumption	Time	4.35	0.91	233.05	0.000	0.951
	Error (Time)	52.21	0.004			
	Treatment	1	0.233	21.19	0.001	0.638
	Error (Treatment)	12	0.011			
	Time * Treatment	4.35	0.037	9.38	0.000	0.439
NH_4 - N	Time	1.94	1.81	10,614.91	0.000	1.00
	Error (Time)	7.77	0.00			
	Treatment	1	5.78	14,043.15	0.000	1.00
	Error (Treatment)	4	0.00			
	Time * Treatment	1.94	1.71	10,011.15	0.000	1.00
NO_2 - N	Time	2.46	0.049	5671.73	0.000	0.999
	Error (Time)	9.84	8.57			
	Treatment	1	0.12	3984.72	0.000	0.999
	Error (Treatment)	4	0.00003			
	Time * Treatment	2.46	0.048	5559.48	0.000	0.999
NO_3 - N	Time	2.99	2.00	103.01	0.000	0.963
	Error (Time)	11.98	0.019			
	Treatment	1	3.07	25.73	0.007	0.865
	Error (Treatment)	4	0.119			
	Time * Treatment	2.99	0.345	17.79	0.000	0.816
PO_4	Time	2.39	0.047	78	0.000	0.951
	Error (Time)	9.58	0.001			
	Treatment	1	0.29	1021.34	0.000	0.982
	Error (Treatment)	4	0.001			
	Time * Treatment	2.39	0.023	38.27	0.000	0.905
CO_2	Time	1.33	1.14	177.50	0.000	0.978
	Error (Time)	5.33	0.006			
	Treatment	1	224.95	19,683.0	0.000	1.00
	Error (Treatment)	4	0.011			
	Time * Treatment	1.33	1.14	177.50	0.000	0.978
Alkalinity	Time	2.37	419.68	11.13	0.003	0.736
	Error (Time)	9.47	37.71			
	Treatment	1	133.93	30.00	0.005	0.882
	Error (Treatment)	4	4.46			
	Time * Treatment	2.37	584.84	15.51	0.001	0.795
BOD_5	Time	3.34	0.203	32.84	0.000	0.891
	Error (Time)	13.37	0.006			
	Treatment	1	2.06	70.32	0.001	0.946
	Error (Treatment)	4	0.029			
	Time * Treatment	3.34	0.158	25.5	0.000	0.864
TS	Time	1.73	129,977.32	493.04	0.000	0.992
	Error (Time)	6.92	263.62			
	Treatment	1	180.21	5.16	0.085	0.564
	Error (Treatment)	4	34.89			
	Time * Treatment	1.73	744.62	2.82	0.13	0.414

during the study period was approximately 3.6 m^3 (0.06 l/m), about 0.92% of that for the flow-through system, which needed about 393 m^3 (6.5 l/m).

In this research, the time of eyed egg and hatching duration (from observing first larvae to hatching of all fertilized eggs) were statistically the same. Hatching time lasted one day more in the RAS. Physicochemical conditions during the experimental period were not in the harmful range (Irani and Agh, 2019) for performance, health and welfare of developing embryos and larvae, except that TAN concentrations after hatch in the recirculating system exceeded 1 mg/l.

It was unmanageable to provide exactly the same physicochemical conditions in the flow-through and recirculating systems. The water

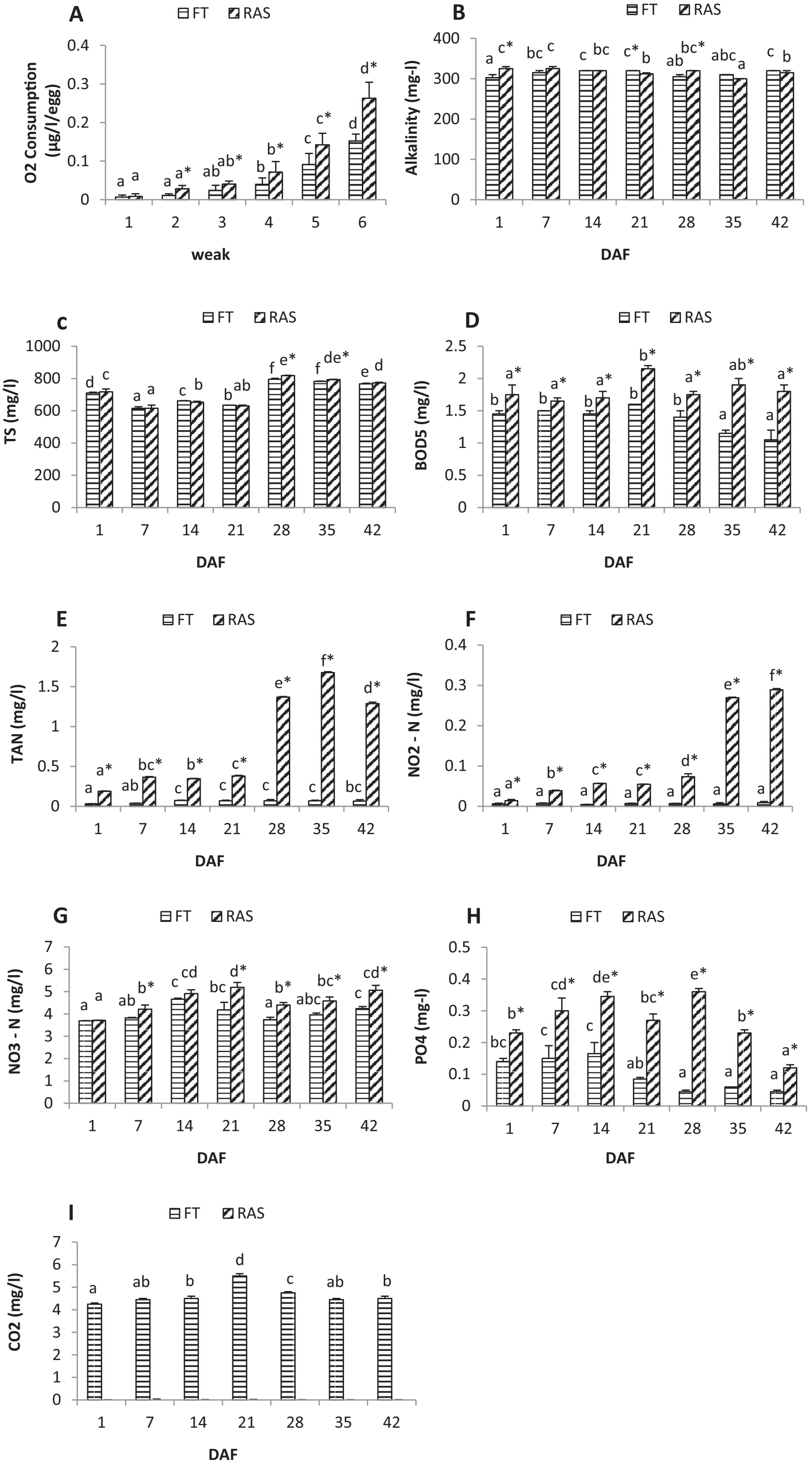

Fig. 3. Dissolved oxygen consumption (A), Alkalinity (B), Total solids (C), Biological oxygen demand (D), Total ammonia nitrogen (E), Nitrite nitrogen (F), Nitrate nitrogen (G), Orthophosphate (H) and Carbon dioxide (I) in flow-through (FT) and recirculating (RAS) system during the study period. Values are mean (inlet, outlet, and inside of each trough) ± SD. DAF means days after fertilization. * indicates significant differences between treatments (independent-samples *t*-test, α = 0.05) on the same week/day. Different letters indicate significant differences (one-way ANOVA, α = 0.05) in each system during the study period.

Table 2
Measured and calculated parameters of eggs and larvae in the FT system and RAS.

Parameter	Flow-through system	Recirculating system
Eyed egg (%)	79.75 ± 5.59^a	79.01 ± 2.05^a
Hatching rate (%)	70.31 ± 5.08^a	63.65 ± 3.59^a
Survival of fry (%)	68.26 ± 5.13^a	61.07 ± 3.98^a
Malformation (%)	0.81 ± 0.1^a	0.80 ± 0.05^a
Length 1 (mm)	16.96 ± 0.19^a	16.35 ± 0.2^a
Length 14 (mm)	25.53 ± 0.16^b	25.09 ± 0.23^a
Wet weight 1 (mg)	85.09 ± 2.87^a	84.64 ± 4.04^a
Wet weight 14 (mg)	161.19 ± 3.54^b	149.99 ± 1.62^a
Dry weight 1 (mg)	30.75 ± 0.91^a	29.39 ± 0.75^a
Dry weight 14 (mg)	30.78 ± 0.55^b	29.37 ± 0.78^a

Values are mean ± SD. Different superscripts across rows indicate significant differences (independent-samples *t*-test, $\alpha = 0.05$) between two systems. 1: hatching time, 14: time of yolk-sac depletion.

supply of the FT system was a well subject to outdoor conditions, while the RAS water recycled so many times under indoor conditions; therefore, water temperature in the RAS was 0.2–1.2 °C lower than in the FT system. These differences in water temperature could be a major reason for the hatching time lasting one day more in the recirculating system.

During the study period, pH values in the RAS were about 0.4–0.55 units higher than in the FT system, which could be because of the airlift pump degassing function, as carbon dioxide was zero in this system during the study period. Dissolved oxygen was not a limiting factor for developing eggs and larvae, indicating good performance of designed airlift pump in providing the oxygen requirements of the system, as DO values in the RAS were even higher than in the FT system during the egg incubation stage. However, DO concentrations in outlet water of RAS were significantly lower than in the FT system during the larval stage, indicating higher oxygen requirements of larvae. The oxygen consumptions in the last week were 22 (in the FT system) and 30 (in the RAS) times more than during the first week. The higher values of oxygen consumption in the RAS could be because of stressful conditions (e.g., higher concentrations of nitrogen compounds), activities of heterotrophic and nitrifying bacteria, and accumulation of metabolites (causing higher BOD values). Although the values of BOD_5 in the RAS were significantly higher than in the FT system, as expected (due to delivering no feed), the maximum level (2.15 mg/l) was much lower than the criterion reported for BOD_5 in the literature for recirculating systems (Figueroa and Silverstein, 1992).

The values of total ammonia nitrogen were relatively low during the first three weeks in both systems, while the values in the RAS increased from the hatching time (28 DAF) onwards to the point that it could be harmful to growing larvae (Brinkman et al., 2009; Pillay and Kutty, 2005). Therefore, water exchange was performed at three-day intervals during the first four weeks, at two-day intervals during the fifth week, and every day in the last week. In spite of the significant difference between two systems, overall nitrite values did not reach a toxic level (Pillay and Kutty, 2005; Williams and Eddy, 1989) in both systems. Increasing values of nitrite from 35 DAF onwards (one week after increasing values of TAN) may relate to establishment and activities of nitrifying bacteria converting ammonia to nitrite (Irani et al., 2016).

Fluctuation in the values of nitrate and orthophosphate probably depended on the source of entering water (outdoor well), because the values varied significantly in the influent during the study period. However, the recirculating system had significantly higher values of orthophosphate than the flow-through system, suggesting accumulation of this factor in the RAS.

5. Conclusion

This recirculating system was designed for rainbow trout egg incubation, hatching, and larval rearing to yolk-sac depletion and onset of exogenous feeding. The design and construction were based on the simplicity and effectiveness of the system. Without using special or expensive units (e.g., oxygen generator, biofilter, centrifugal pumps, etc.) that are common in RASs, > 12,000 advanced larvae were produced with only 3.6 m^3 fresh water. There was no negative impact on the developing embryos and negligible effects on the yolk-sac larvae. However, increasing levels of nitrogen compounds and decreasing dissolved oxygen were observed at the end of the study period. Hence, we suggest that the designed system is effective for rainbow trout egg incubation and rearing of yolk-sac larvae. Thus, advanced larvae (after depletion of yolk sac) should be transferred to systems equipped with water purification units and extra aeration/oxygenation structures.

Declaration of Competing Interest

None.

Acknowledgment

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