RESEARCH ARTICLE

Back to the Roots: The Integration of a Constructed Wetland into a Recirculating Hatchery - A Case Study

Miloš Buřič1*, Josef Bláhovec2, Jan Kouřil1

1 University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vod any, Czech Republic, 2 Trout farm Mlýny, Stachy, Czech Republic

* buric@frov.jcu.cz

Abstract

Aquaculture is currently one of the fastest growing food-producing sectors, accounting for around 50% of the world’s food fish. Limited resources, together with climatic change, have stimulated the search for solutions to support and sustain the production of fish as a nutritious food. The integration of a constructed wetland (CW) into a recirculating hatchery (RHS) was evaluated with respect to its economic feasibility and environmental impact. The outcome of eight production cycles showed the potential of CW integration for expanded production without increased operation costs or environmental load. Concretely, the use of constructed wetland allows the rearing about 40% more fish biomass, resulting in higher production and profitability. The low requirements for space, fresh water, and energy enable the establishment of such systems almost anywhere. Constructed wetlands could enhance the productivity of existing small scale facilities, as well as larger systems, to address economic and environmental issues in aquaculture. Such systems have potential to be sustainable in the context of possible future climate change and resource limitations.

Introduction

The projected increase in global population to 8.3 billion by 2030 [1] will be accompanied by substantial growth in food, water, and energy demands. These factors are interlinked, and issues of supply and demand must be addressed within a context of future climate change [1,2]. The increased pressure on critical food and water resources are already observable. Aquaculture is an important source of high value food [3], [4], [5] and is the fastest growing animal-based food producing sector, outpacing population growth [2,6].

Aquaculture applies increasingly sophisticated approaches to obtain high production with low water usage and wastewater production [7,8,9,10]. The core of this practice is water re-use with complex systems for treatment of recirculating water and wastewater [11,12,13,14]. The development and expansion of new technologies usually go hand in hand with increasing initial investment and high energy demands during operation [7,10]. A scheme is needed to
balance economic feasibility and environmental sustainability. Currently, the emphasis is on simplification and streamlining of existing types of recirculating aquaculture systems (RAS) to expand production per surface unit while reducing initial investment, energy and water demands, and waste production and increasing waste utilization \[11,13,15\]. One potential solution may be the use of integrated systems \[16,17,18\] including constructed wetlands \[19,20,21\] or aquaponics \[22,23\] incorporated into the RAS or for effluent water treatment \[24,25,26\], or a combination of these approaches. Such an approach can maintain long-term environmental sustainability of aquaculture systems and production of a valuable food product \[3,4\].

Salmonids are one of the most important cultured fishes\[2\], with high nutritional and culinary value \[27,28\]. Among freshwater salmonids, the dominant culture species is rainbow trout (\textit{Oncorhynchus mykiss}, Walbaum 1752) \[2\]. Traditional culture is usually linked to clean headwaters or, in hatcheries, to a spring fed water supply \[29,30\]. With the progressive demand for trout leading to increased production, the environmental impact of traditional trout culture using flow-through systems is observable: Effluent waters are high in nutrients and organic matter \[26,30,31\], there is increased risk of disease transfer \[32,33\], and escaped fish pose a threat to indigenous fauna \[34,35\]. In addition, the potential locations for new flow-through facilities are limited, and certain governments have imposed restrictions on production levels of existing or future farms based on the volume and content of effluent water \[36,37\]. This has spurred development of new technologies for RAS that reduce fresh water usage, limit disease transfer, and decrease the possibility of environmental degradation \[11,38,39\]. Recirculating aquaculture systems, however, involve high initial investment and energy consumption, which determines economic viability. The move to recirculating systems has thus far not reached hatcheries to a significant extent. This is unfortunate, since in the areas where springs occur and where hatcheries are generally located can be vulnerable even to low volumes of effluent water and nutrient concentrations due to reduced flow and the occurrence of vulnerable organisms specific to oligotrophic waters \[15,30\].

Traditional recirculating hatcheries generally involve specialized technologies for water treatment, such as microsieve filtration, ozonization, and UV sterilization \[9,10,40\], raising energy consumption and requiring higher initial investment and operation costs. In contrast, a less sophisticated approach involving simple construction and low operation and investment costs can allow even low capacity hatcheries to be profitable \[15\]. Production capacity of such systems can be increased without additional cost by integration of a small constructed wetland into the recirculating hatchery which is an economical and environmentally sound practice. In consideration of the need for efficient space utilization, the primary focus of the present study was to establish the potential of a constructed wetland to increase fish production and expand profitability without additional energy demands, while decreasing the contamination of the environment by effluent water.

**Material and Methods**

**Study Site**

The study was conducted at a small trout farm in the Czech Republic (49°6’35”N, 13°45’10”E) where the simple recirculating hatchery system (RHS) with total energy consumption of 1.6 kW and overall fresh water demand of 0.05 L sec\(^{-1}\) was developed and tested in the past \[15\]. The RHS consisted of two separate systems in an area of \(\sim 65 \text{ m}^2\). The first system was used for egg incubation, hatching, and rearing through the change to exogenous feeding to a fish weight of \(\sim 0.50 \text{ g}\). This system was the source of fry that were used for evaluation of the second system with or without an incorporated constructed wetland (CW). This second system was equipped with seven circular tanks (\(\sim 0.7 \text{ m}^3\)); one biofiltration/sedimentation unit (\(\sim 2.2 \text{ m}^3\)) with 12
bioblocs (EXPO-NET A/S, Denmark); one retention tank (~3.5 m³); and a circulation pump (0.75 kW, Wilo SE, Germany). Six tanks containing 1.4 m³ of inert substrate (LIAFLOR, LIAS Vintirov, Czech Republic) planted with *Phalaris arundinacea* served as the body of the CW. The tanks were arranged horizontally with a cascading flow (Fig 1). A ball valve at the height of the inlet to the CW enabled operation of the system with or without the CW, so there was no need for additional pumps or supplemental power. During operation with the CW, one third (~ 6 L s⁻¹) of the total water flow was directed through the CW; hence the volume of water passing through the CW was 21.6 m³ h⁻¹, and the total volume of the system (10.6 m³) passed through the CW more than twice each hour. Source of fresh water was a borehole.

The study was carried out on private land with a permission of the owner, who is included among the article authors. No specific permissions were required for the locations and activities involved in this study. The study did not involve endangered or protected species. All experimental manipulations (rearing, capture and measurements) were conducted according to the principles of the Ethical Committee for the Protection of Animals in Research of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, based on the EU harmonized animal welfare act of Czech Republic. The above named Institutional Animal Care and Use Committee (IACUC) specifically approved this study. The principles of laboratory animal care and the national laws 246/1992 and regulations on animal welfare were followed (Ref. number 22761/2009-17210).

### Monitoring Physical and Chemical Conditions

Water samples from the RHS were collected bi-weekly throughout eight production cycles, four with CW and four without CW. Four sampling sites were monitored when CW was used: the inlet to fish tanks (IF), outlet from fish tanks (OF), outlet from the biofilter (OB), and outlet from CW (OCW). Samples were taken from the IF, OF, and OB when the CW was disconnected (Fig 1). All samples were analyzed in an accredited laboratory (Bioanalytika CZ, s.r.o., testing laboratory no. 1012) for ammonia (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) concentrations, biological oxygen demand (BOD), chemical oxygen demand (CODMn), alkalinity (acid-neutralizing capacity), chloride concentration (Cl⁻), and suspended solids. Oxygen saturation level (oximeter Oxi 3205 with CellOx 325, WTW Gmbh, Weilheim, Germany), pH (pH meter pH 330i with SenTix 41, WTW Gmbh, Weilheim, Germany), and water temperature (KM12 digital thermometer, Comark Instruments, Great Britain) were monitored daily.

### Fish and Production Cycles

The study used all-female rainbow trout delivered as eyed eggs (100 000 eggs per production cycle) from certified disease-free farms (Troutex ApS, Denmark). Eggs were raised in incubation units of the first system, and approximately four days post-hatching were moved to trays for rearing to the size required for the study (0.4–0.5 g). The stocked fry were fed 5–6 times per day on pelleted feed at 3.0–5.5% of fish biomass, according to water temperature, fish size, and appetite. During the production cycles, fish weight increments were measured bi-weekly. The amount of supplied feed and mortality were recorded daily. The feed conversion ratio (FCR) was calculated to assess the utilization of feed using following formula:

FCR = \( \frac{w_k}{w_p} \)

where \( w_k \) = amount of feed (kg) and \( w_p \) = obtained weight increment (kg).

The servicing was comparable for systems with and without CW and consisted of daily removal of faeces and dead fish and removal of sludge from biofiltration/sedimentation tank and cleaning of Bioblocs every second day. To prevent potentially high nitrite levels, sodium...
Fig 1. Schematic of recirculating hatchery system with the integrated constructed wetland: 1—fish tanks, 2—biofiltration/sedimentation unit, 3—circulation pump, 4—retention tank, 5—fresh water inlet, 6—ball valve, 7—constructed wetland (with the detail of water flow through tanks). Four water sampling sites are labelled: IF—inlet to fish tanks, OF—outlet from fish tanks, OB—outlet from biofilter, ICW—inlet to constructed wetland, OCW—outlet from constructed wetland, OP—outflow pipes, CP—connecting pipes for subsurface flow.

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chloride was added to maintain Cl⁻ content at ~100 mg L⁻¹. Trials were complete when mean fish weight reached 2 g. Eight production cycles were completed and monitored, four with CW realized throughout 2013 (rainbow trout eggs stocked at the end of December 2012, and in May, July, and September 2013) and four without the CW realized throughout 2012 (rainbow trout eggs stocked in January, April, June, and September 2012).

Data Analysis

Data were analysed using Statistica 9.0 (StatSoft, Inc.). Results were first examined for normal distribution (Kolmogorov–Smirnov test) and homoscedasticity (Levene’s test). One-way ANOVA with Tukey’s post hoc test was used to analyse the data from water analyses, the Mann–Whitney test was used for basic physical and chemical parameters, and the t-test for comparisons of fish growth and feed conversion ratio. The null hypothesis was rejected at α = 0.05. Data are presented as mean ± standard deviation.

Results

Physical and Chemical Parameters

Oxygen saturation fluctuated during the study in the range 87–100% and 79–97% at the inlet and outlet from fish tanks, respectively. There were no significant differences among trials. Water temperature ranged from 9.2 to 12.4°C throughout the study with no significant differences among trials and showed a maximum 24 h divergence of 0.3°C. The pH values in both trials remained near optimal values for fish rearing and biofilter function. Suspended solid concentration was higher when the CW was used, but with no observed negative impact on fish (Table 1).

The biofilter was adequate to deal with nitrous compounds both with and without CW integration. Significantly lower values were detected in mean ammonia concentration with CW use (Kruskal–Wallis test, H = 22.01, p = 0.0012), whereas the differences in mean nitrate

| Parameter             | O      | N      | Mean    | STD    | Min    | Max    |
|-----------------------|--------|--------|---------|--------|--------|--------|
| Biomass (kg)          | CW     | 21     | 131.7a  | 66.6   | 53.7   | 239.9  |
|                       | X      | 22     | 102.6a  | 60.7   | 24.7   | 199.7  |
| Water temperature (°C)| CW     | 310    | 10.2a   | 1.0    | 9.2    | 12.1   |
|                       | X      | 316    | 11.0a   | 1.0    | 9.9    | 12.4   |
| pH                    | CW     | 21     | 7.3a    | 0.3    | 6.8    | 7.8    |
|                       | X      | 22     | 7.4a    | 0.2    | 7.1    | 7.7    |
| Suspended solids (mg L⁻¹)| CW   | 21     | 6.3a    | 3.3    | 2.0    | 13.0   |
|                       | X      | 22     | 3.3a    | 1.5    | 2.0    | 5.0    |
| Chlorides (mg L⁻¹)    | CW     | 21     | 48.5a   | 22.7   | 23.6   | 77.2   |
|                       | X      | 22     | 50.0a   | 33.4   | 13.7   | 131.0  |
| Phosphorus (mg L⁻¹)   | CW     | 21     | 0.6a    | 0.3    | 0.2    | 1.1    |
|                       | X      | 22     | 0.6a    | 0.6    | 0.1    | 1.5    |
| Alkalinity (mmol L⁻¹) | CW     | 21     | 1.6b    | 0.3    | 1.1    | 1.9    |
|                       | X      | 22     | 1.9a    | 0.2    | 1.5    | 2.1    |

Data are presented as mean, standard deviation (STD), and minimum and maximum value obtained for n samples during hatchery operation (O) with constructed wetland (CW) and without constructed wetland (X).

Different alphabetic superscripts for the same parameter indicate significant differences at α = 0.05 (t-test; Mann–Whitney test).

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(Kruskal–Wallis test, $H = 4.28, p = 0.6393$) and nitrite (ANOVA, $H = 0.12, p = 0.9937$) were not significant, with levels varying over a wide range (Table 2). Nevertheless, the maximum values of nitrous compounds in the terminal phase of rearing, when total biomass had reached ~230 kg and ~200 kg with and without CW integration, respectively, showed large differences between trials, with potentially lethal concentrations of ammonia and nitrites observed when

### Table 2. Chemical values at different sampling sites (inlet to fish tanks—IF, outlet from fish tanks—OF, outlet from biofilter—OB, and outlet from CW—OCW).

| Parameter                               | O         | OM        | n  | Mean | STD  | Min  | Max  |
|-----------------------------------------|-----------|-----------|----|------|------|------|------|
| Total ammonia (mg L$^{-1}$)             | CW IF     | 21        | 0.5$^a$ | 0.2  | 0.2  | 0.7  |
|                                         | OF        | 21        | 0.6$^a$ | 0.2  | 0.3  | 0.9  |
|                                         | OB        | 21        | 0.5$^a$ | 0.2  | 0.2  | 0.8  |
|                                         | OCW       | 21        | 0.3$^a$ | 0.3  | 0.1  | 0.8  |
|                                         | X IF      | 22        | 1.0$^b$ | 0.7  | 0.1  | 2.2  |
|                                         | OF        | 22        | 1.1$^b$ | 0.7  | 0.2  | 2.3  |
|                                         | OB        | 22        | 1.0$^b$ | 0.7  | 0.1  | 2.3  |
| Nitrite (mg L$^{-1}$)                   | CW IF     | 21        | 0.3$^a$ | 0.2  | 0.1  | 0.8  |
|                                         | OF        | 21        | 0.3$^a$ | 0.3  | 0.1  | 1.1  |
|                                         | OB        | 21        | 0.4$^a$ | 0.2  | 0.1  | 0.9  |
|                                         | OCW       | 21        | 0.3$^a$ | 0.3  | 0.1  | 1.0  |
|                                         | X IF      | 22        | 1.0$^a$ | 1.5  | 0.1  | 4.9  |
|                                         | OF        | 22        | 0.9$^a$ | 1.3  | 0.1  | 4.3  |
|                                         | OB        | 22        | 1.0$^a$ | 1.3  | 0.1  | 4.5  |
| Nitrate (mg L$^{-1}$)                   | CW IF     | 21        | 25.2$^a$ | 12.6 | 7.3  | 41.9 |
|                                         | OF        | 21        | 24.6$^a$ | 14.3 | 7.9  | 49.8 |
|                                         | OB        | 21        | 25.5$^a$ | 14.1 | 7.6  | 51.7 |
|                                         | OCW       | 21        | 26.0$^a$ | 13.9 | 6.9  | 53.1 |
|                                         | X IF      | 22        | 21.5$^a$ | 24.3 | 5.0  | 73.8 |
|                                         | OF        | 22        | 21.5$^a$ | 24.3 | 5.0  | 72.9 |
|                                         | OB        | 22        | 21.6$^a$ | 24.8 | 5.0  | 74.0 |
| Biological oxygen demand (mg L$^{-1}$)  | CW IF     | 21        | *1.0$^a$ | 0.0  | *1.0 | *1.0 |
|                                         | OF        | 21        | *1.0$^a$ | 0.0  | *1.0 | *1.0 |
|                                         | OB        | 21        | *1.0$^a$ | 0.0  | *1.0 | *1.0 |
|                                         | OCW       | 21        | *1.0$^a$ | 0.0  | *1.0 | *1.0 |
|                                         | X IF      | 22        | 1.3$^b$ | 0.5  | *1.0 | 2.0  |
|                                         | OF        | 22        | 1.4$^b$ | 0.6  | *1.0 | 2.5  |
|                                         | OB        | 22        | 1.3$^b$ | 0.5  | *1.0 | 2.0  |
| Chemical oxygen demand (mg L$^{-1}$)    | CW IF     | 21        | 1.9$^a$ | 0.4  | 1.4  | 2.5  |
|                                         | OF        | 21        | 2.1$^a$ | 0.3  | 1.5  | 2.6  |
|                                         | OB        | 21        | 2.0$^a$ | 0.3  | 1.5  | 2.5  |
|                                         | OCW       | 21        | 2.0$^a$ | 0.4  | 1.1  | 2.4  |
|                                         | X IF      | 22        | 2.5$^a$ | 1.0  | 1.1  | 4.0  |
|                                         | OF        | 22        | 2.6$^a$ | 1.1  | 1.0  | 4.1  |
|                                         | OB        | 22        | 2.6$^a$ | 1.0  | 1.1  | 4.0  |

Data are presented as mean, standard deviation (STD), and minimum (Min) and maximum (Max) values of the number of samplings (n) during hatchery operation (O) with constructed wetland (CW) and without constructed wetland (X). Different alphabetic superscripts for the same parameter indicate significant differences at $\alpha = 0.05$ (ANOVA, Kruskal–Wallis test).

* The values of biological oxygen demand were below laboratory detection limits (1 mg L$^{-1}$).

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the CW was not used (Tables 2 and 3). Significantly lower BOD was observed with CW (Kruskal-Wallis test, $H = 15.55$, $p = 0.0164$), while the differences in COD were not significant (Kruskal-Wallis test, $H = 6.25$, $p = 0.3958$) (Table 2). The lower values of ammonia and nitrates, as well as of BOD and COD, obtained with the CW implied a potential final maximum biomass of approximately 40% over that obtained in the system without the CW (Table 3). There were no significant differences among system sampling sites in any measured value.

### Production Cycle and Feed Utilization

The length of the production cycle, mortality, and FCR did not differ significantly among trials (Table 4). The mean biomass was slightly higher in trials with CW, but the difference was not significant ($t$-test, $t = 1.02$, $p = 0.3220$, Table 1).

### Table 3. Ammonia and nitrite concentration and fresh water demand during operation of the recirculating hatchery system with and without constructed wetland (CW) for mean and maximum biomass, and an estimated potential maximum RHS capacity.

| Operational system | Without CW | With CW |
|--------------------|------------|---------|
| Parameter          | Mean biomass | Maximum biomass | Mean biomass | Maximum biomass |
| Biomass (kg)       | 102.6       | 199.7    | 131.7       | 239.4         |
| Ammonia (mg L$^{-1}$) | 1.0       | 2.2      | 0.5         | 0.7           |
| Nitrite (mg L$^{-1}$) | 1.0      | 4.9      | 0.3         | 0.8           |
| Water demand (L s$^{-1}$) | 0.05 |          | 0.06        |
| Potential RHS capacity (kg) | ~ 200 |         | ~ 280–290   |

### Table 4. Duration of Phase 1 (number of days from the beginning of egg incubation to weight ~0.50 g and Phase 2 (number of days to the weight of 2 g) and the production cycle length (number of days from the beginning of egg incubation to weight of 2 g); losses in Phase 1 (percent of dead and deformed specimens); losses in Phase 2 (the percent of dead specimens); total losses; and feed conversion ratio (FCR).

| Parameter                | O   | n | Mean   | STD |
|--------------------------|-----|---|--------|-----|
| Phase 1 length (days)    | CW  | 4 | 44.8$^a$| 2.4 |
| X                        | 4   |   | 44.1$^a$| 3.0 |
| Phase 2 length (days)    | CW  | 4 | 32.8$^a$| 2.9 |
| X                        | 4   |   | 35.0$^a$| 3.0 |
| Production cycle length (days) | CW  | 4 | 77.5$^a$| 3.6 |
| X                        | 4   |   | 79.0$^a$| 2.1 |
| Losses in phase 1 (%)    | CW  | 4 | 17.4$^a$| 2.7 |
| X                        | 4   |   | 18.0$^a$| 1.3 |
| Losses in phase 2 (%)    | CW  | 4 | 2.4$^a$ | 0.7 |
| X                        | 4   |   | 3.0$^a$ | 0.6 |
| Total losses (%)         | CW  | 4 | 19.9$^a$| 2.3 |
| X                        | 4   |   | 21.1$^a$| 2.1 |
| FCR                      | CW  | 4 | 0.63$^a$| 0.08|
| X                        | 4   |   | 0.61$^a$| 0.09|

Data are presented as mean and standard deviation (STD) of values observed at four samplings (n) during hatchery operation (O) with (CW) and without (X) a constructed wetland.

Different alphabetic superscripts for the same parameter indicate significant differences at $\alpha = 0.05$ ($t$-test).
Overall Annual Production

The production cycle length and construction of RHS enables the completion of at least four production cycles per year [15]. The calculation of potential annual production of the RHS with and without CW for two levels of initial stocked biomass is presented in Table 5. Potential final biomass with CW use was estimated to be 40% greater than without CW use.

Fresh Water Demand, Energy Consumption, and Labour

The fresh water necessary to replenish that lost through evaporation and tank cleaning, with and without CW was 2.33 and 2.01 m³ day⁻¹, respectively. The energy demand was similar in both cases, reaching an average of ~23 kWh day⁻¹. Labour was also comparable, with an additional ~4 h per year required for cutting of plants in the CW at the end of the growing season and composting the biomass (Table 6).

Discussion

The present study addressed the efficacy of a simple technology in aquaculture. Could the evaluated system potentially increase production and decrease pollution while maintaining low energy demands and provide an effective simplified approach counter to the current

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**Table 5. Economic value of constructed wetland (CW) use in a recirculating hatchery system compared to operation without CW with different numbers of eyed eggs as initial stock per production cycle (PC).**

| Operation               | Without CW | With CW |
|-------------------------|------------|---------|
| Initial stock (pcs)     | 90000      | 110000  |
| Total losses (%)        | 25         | 25      |
| Number of fingerlings per PC | 67500    | 82500   |
| Final biomass (kg)      | max 200    | max 200 |
| Mean weight (g)         | 2.96       | 2.42    |
| Price of fingerlings per PC ($)* | 13809    | 15858   |
| Price of fingerlings per year ($)* | 55236    | 63432   |

The mean final weight of fingerlings is based on the calculated possible maximum final biomass in the hatchery system. The price of fingerling is based on real prices at the study site. The annual price of fingerlings assumes four production cycles (PC) per year.

* Converted from real fingerling prices of trout farm at the study site.

**Table 6. Energy consumption (kWh), freshwater demand (m³), and labour (hr) per day during Phase 1 and Phase 2 of the production cycle and for the entire production cycle (PC) in the recirculating hatchery system with (CW) and without (X) use of integrated constructed wetland.**

| CW | days per PC | Energy consumption | Freshwater demand | Labour |
|----|-------------|--------------------|-------------------|--------|
|    |             | per day (kW)       | total per PC (kW) | per day (m³) | total per PC (m³) | per day (hr) | total per PC (hr) |
| Phase 1 | 44.8 | 20.9 | 936.3 | 0.86 | 38.53 | <4 | <179.2 |
| Phase 2 | 32.8 | 25.7 | 843.0 | 4.32 | 141.70 | <3 | <98.4 |
| PC | 77.5 | 23.0 | 1779.3 | 2.33 | 180.22 | <3.58 | <278.0 |
| X | days per PC | Energy consumption | Freshwater demand | Labour |
| Phase 1 | 44.1 | 20.9 | 921.7 | 0.86 | 37.93 | <4 | <176.4 |
| Phase 2 | 35 | 25.7 | 899.5 | 3.46 | 121.10 | <3 | <105.0 |
| PC | 80 | 23.1 | 1821.2 | 2.01 | 159.03 | <3.56 | <281.4 |

**Discussion**

The present study addressed the efficacy of a simple technology in aquaculture. Could the evaluated system potentially increase production and decrease pollution while maintaining low energy demands and provide an effective simplified approach counter to the current
technological boom? The development of technologically advanced systems overcomes former limitations on production, but is incompatible with minimizing demands on resources [1].

The use of a simple recirculating hatchery previously confirmed as effective [15] provided the opportunity to evaluate an added integrated CW under real operating conditions as a simple solution for environmentally sound culture intensification [16,41,42]. Water quality monitoring confirmed the effectiveness of the RHS, both with and without an integrated CW. The issue of primary importance for the operation of recirculating systems is the cycle of nitrous compounds [43,44,45], alterations of which can indicate malfunction of the RAS and risk to fish growth, health, and survival [46,47]. In general, biofilter function was adequate in both systems. Concentrations of ammonia and nitrites sufficiently high to affect fish stock [47] were detected only in the final phase of the production cycle when the total biomass in the RHS reached 170–200 kg in the system without CW. While the mean values of water parameters, except for BOD, did not differ, maximum concentrations with CW did not reach potentially dangerous levels as observed during operation without CW. In addition, with CW, less variation was detected for all monitored parameters except nitrates, indicating the use of CW as a tool for stabilizing conditions. This supports the feasibility of increasing biomass in systems with the CW. On the other hand, the toxic influence of high ammonia and nitrite levels was reduced by pH monitoring and adjustment, along with chloride application, enabling fish to withstand greater concentrations than those usually considered lethal [48,49].

Maintaining water quality gave consistently good production parameters, such as high growth rate and low FCR, resulting in a short production cycle. There were no observed positive or negative effects of CW use on fish growth and fitness. The shorter production cycle allowed at least four production cycles per year in both systems. The use of the CW offered the potential to increase biomass at the end of each production cycle either by rearing higher numbers of fish or by producing larger fingerlings. The latter is the preferred, because of the lower incidence of aggressive behaviour and cannibalism and better growth at lower densities [50,51,52]. Nevertheless, either approach could have important economic benefits, albeit at the possible expense of animal welfare (Table 5).

In most cases, increased production leads to higher environmental costs from effluents as well as higher water demands and energy consumption [1,30,53]. These issues, with a growing world population, form a nexus among food, water, and energy compounded by climate change [1]. Hence, new technologies and modifications for inland aquaculture are being developed to reduce its effect on freshwater ecosystems [29,39]. Large scale RAS systems have been developed that minimize fresh water demand and waste production, but with higher energy consumption [7,29]. Traditional flow-through farms, originally characterized by high fresh water demand and uncontrolled effluent discharge, currently may use lagoons [36], constructed wetlands, or aquaponic systems [22,26,54] to treat effluent water and decrease the amount of nutrients discharged into streams. Constructed wetlands and aquaponics are increasingly integrated into intensive RAS [16,42,55], but no such adaptations have been made in salmonid hatcheries, which are usually situated in spring areas with the potential to affect local oligotrophic ecosystems [30,56].

The evaluated small-scale hatchery has the potential to show a new direction of RAS simplification with integration of an environmentally sound approach for production increase. The use of a constructed wetland can provide a solution for larger systems, and can be modified to produce food plants in the CW [22,23]. It is possible that simplification of existing RAS and CW incorporation can conserve a considerable amount of energy. When considering the proportion of energy costs on the total costs, they covered only about 7% of total cost per production cycle, which can be classed as advantageous. This issue is naturally more accentuated in large systems for ongrowing phase. In addition, similar systems can be established almost anywhere.
The on-site production of fingerlings in simple systems can have positive effects on aquaculture facility economics (cheaper fingerlings, no shipping costs), zoohygiene (fingerlings from a known source without contact with surface waters), and the environment (no transportation of fingerlings, low energy consumption, low fresh water demands, low volume of effluent water, sludge management).

Waste from the RHS with CW integration can be better managed than in flow-through aquaculture systems. In traditional flow-through hatcheries the volume of waste water is substantially higher, and treatment options are limited. The volume of effluent from RHS was lower but more concentrated [15]. This allows sludge sedimentation and collection, the potential use of sludge as fertilizer or for composting [12,57], and treatment of the remaining wastewater through additional constructed wetlands [24,25] or aquaponics [22,31] to prevent the trophic effects of effluent on ecosystems [12,56,58]. The only added waste of CW use is the plant biomass, which can be composted [59] separately or along with collected sludge [57].

**Conclusions**

Constructed wetland integration has the potential to increase production of a recirculating hatchery without added operational or environmental costs. The use of a simple system with CW integration can be recommended for managing energy consumption and labour and increasing production of salmonid, or other fish species, fingerlings to supply RAS facilities and to support the development of this fast growing agriculture sector. Such systems can be sustainable, taking into account possible future climate change and increased pressure on resources.

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**Author Contributions**

Conceived and designed the experiments: MB JB JK. Performed the experiments: MB JB. Analyzed the data: MB. Contributed reagents/materials/analysis tools: JB JK. Wrote the paper: MB JK.

**References**

1. National Intelligence Council (2012) Global Trends 2030: Alternative worlds. Washington: Office of the Director of National Intelligence pp. 160.
2. Food and Agriculture Organization of United Nations (2013) FAO yearbook, Fishery and Aquaculture Statistics 2011. pp. 105.
3. Adarme-Vega TC, Thomas-Hall SR, Schenk PM (2014) Towards sustainable sources for omega-3 fatty acids production. Current Opinion in Biotechnology 26: 14–18. doi:10.1016/j.copbio.2013.08.003 PMID: 24607804
4. Godfray HCJ, Crute IR, Haddad L, Lawrence D, Muir JF, Nisbett N, et al. (2010) The future of the global food system. Philosophical Transactions of the Royal Society B-Biological Sciences 365: 2769–2777. doi:10.1098/rstb.2010.0180 PMID: 20713383
5. Blanchet C, Lucas M, Julien P, Morin R, Gingras S, Dewaily E (2005) Fatty acid composition of wild and farmed Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). Lipids 40: 529–531. PMID: 16094864
6. Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, et al. (2010) Aquaculture: global status and trends. Philosophical Transactions of the Royal Society B-Biological Sciences 365: 2897–2912. doi: 10.1098/rstb.2010.0170 PMID: 20713392

7. Wilfart A, Prudhomme J, Blancheton J-P, Aubin J (2013) LCA and emergy accounting of aquaculture systems: Towards ecological intensification. Journal of Environmental Management 121: 96–109. doi: 10.1016/j.jenvman.2013.01.031 PMID: 23531606

8. Davidson J, Good C, Welsh C, Summerfelt S (2011) The effects of ozone and water exchange rates on water quality and rainbow trout Oncorhynchus mykiss performance in replicated water recirculating systems. Aquacultural Engineering 44: 80–96.

9. Sharrer M, Rishel K, Taylor A, Vinci BJ, Summerfelt ST (2010) The cost and effectiveness of solids thickening technologies for treating backwash and recovering nutrients from intensive aquaculture systems. Bioresource Technology 101: 6630–6641. doi: 10.1016/j.biortech.2010.03.101 PMID: 20395138

10. Terjesen BF, Summerfelt ST, Nerland S, Ulgenes Y, Fjaera SO, Reiten BKM, et al. (2013) Design, dimensioning, and performance of a research facility for studies on the requirements of fish in RAS environments. Aquacultural Engineering 54: 49–63.

11. Martins CIM, Eding EH, Verdegem MCJ, Heinsbroek LTN, Schneider O, Blancheton JP, et al. (2010) New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquacultural Engineering 43: 83–93.

12. van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquacultural Engineering 53: 49–56.

13. Midilli A, Kucuk H, Dincer I (2012) Environmental and sustainability aspects of a recirculating aquaculture system. Environmental Progress & Sustainable Energy 31: 604–611.

14. Suhr KI, Pedersen PB (2010) Nitrification in moving bed and fixed bed biofilters treating effluent water from a large commercial outdoor rainbow trout RAS. Aquacultural Engineering 42: 31–37.

15. Buič M, Bláhovec J, Kouřil J. (2014) A simple and effective recirculating hatchery for salmonids. Journal of Aquaculture Research & Development 5: 271.

16. Zhong F, Liang W, Yu T, Cheng SP, He F, Wu ZB (2011) Removal efficiency and balance of nitrogen in a recirculating aquaculture system integrated with constructed wetlands. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 46: 789–794. doi: 10.1080/10934529.2012.667287 PMID: 22486661

17. Bunting SW, Shpigel M (2009) Evaluating the economic potential of horizontally integrated land-based marine aquaculture. Aquaculture 294: 43–51.

18. Zhang S-Y, Li G, Wu H-B, Liu X-G, Yao Y-H, Tao L, et al. (2011) An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. Aquacultural Engineering 45: 93–102.

19. Lin YF, Jing SR, Lee DY (2003) The potential use of constructed wetlands in a recirculating aquaculture system for shrimp culture. Environmental Pollution 123: 107–113. PMID: 12663210

20. Shi Y, Zhang G, Liu J, Zhu Y, Xu J (2011) Performance of a constructed wetland in treating brackish wastewater from commercial recirculating and super-intensive shrimp growout systems. Bioresource Technology 102: 9416–9424. doi: 10.1016/j.biortech.2011.07.058 PMID: 21852127

21. Zachritz WH II, Hanson AT, Saucedia JA, Fitzsimmons KM (2008) Evaluation of submerged surface flow (SSF) constructed wetlands for recirculating tilapia production systems. Aquacultural Engineering 39: 16–23.

22. Graber A, Junge R (2009) Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. Desalination 246: 147–156.

23. Endut A, Jusoh A, Ali N, Nik WNSW, Hassan A (2009) Effect of flow rate on water quality parameters and plant growth of water spinach (Ipomoea aquatica) in an aquaponic recirculating system. Desalination and Water Treatment 5: 19–28.

24. Flora C, Kroeger R (2014) Use of vegetated drainage ditches and low-grade weirs for aquaculture effluent mitigation: I. Nutrients. Aquacultural Engineering 60: 56–62.

25. Flora C, Kroeger R (2014) Use of vegetated drainage ditches and low-grade weirs for aquaculture effluent mitigation: II. Suspended sediment. Aquacultural Engineering 60: 68–72.

26. Sindiliaru P-D, Brinker A, Reiter R (2009) Factors influencing the efficiency of constructed wetlands used for the treatment of intensive trout farm effluent. Ecological Engineering 35: 711–722.

27. Dewailly E, Ayotte P, Lucas M, Blanchet C (2007) Risk and benefits from consuming salmon and trout: A Canadian perspective. Food and Chemical Toxicology 45: 1343–1348. PMID: 17343969
28. Weaver KL, Ivester P, Chilton JA, Wilson MD, Pandey P, Chilton FH (2008) The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. Journal of the American Dietetic Association 108: 1178–1185. doi: 10.1016/j.jada.2008.04.023 PMID: 18589026

29. d’Orbcastel ER, Blancheton J-P, Aubin J (2009) Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using Life Cycle Assessment. Aquacultural Engineering 40: 113–119.

30. Tello A, Corner RA, Telfer TC (2010) How do land-based salmonid farms affect stream ecology? Environmental Pollution 158: 1147–1158. doi: 10.1016/j.envpol.2009.11.029 PMID: 20036452

31. Turcios AE, Papenbrock J (2014) Sustainable Treatment of Aquaculture Effluents-What Can We Learn from the Past for the Future? Sustainability 6: 836–856.

32. Hastein I, Binde M, Hine M, Johnsen S, Lillehaug A, Olesen NJ, et al. (2008) National biosecurity approaches, plans and programmes in response to diseases in farmed aquatic animals: evolution, effectiveness and the way forward. Revue Scientifique Et Technique-Office International Des Epizooties 27: 125–145. PMID: 18666484

33. Salama NKG, Murray AG (2011) Farm size as a factor in hydrodynamic transmission of pathogens in aquaculture fish production. Aquaculture Environment Interactions 2: 61–74.

34. Arismendi I, Soto D, Penaluna B, Jara C, Leal C, Leon-Munoz J (2009) Aquaculture, non-native salmonid invasions and associated declines of native fishes in Northern Patagonian lakes. Freshwater Biology 54: 1135–1147.

35. Skaala O, Johnsen GH, Lo H, Borgstrom R, Wennevik V, Hansen MM, et al. (2014) A conservation plan for Atlantic salmon (Salmo salar) and anadromous brown trout (Salmo trutta) in a region with intensive industrial use of aquatic habitats, the Hardangerfjord, western Norway. Marine Biology Research 10: 308–322.

36. Yokumsen A, Svendsen LM (2010) Farming of freshwater rainbow trout in Denmark.: DTU Aqua, National Institute of Aquatic Resources, Denmark. 48 p.

37. Boyd CE (2003) Guidelines for aquaculture effluent management at the farm-level. Aquaculture 226: 101–112.

38. Muir J (2005) Managing to harvest? Perspectives on the potential of aquaculture. Philosophical Transactions of the Royal Society B-Biological Sciences 360: 191–218. PMID: 15713597

39. Klinger D, Naylor R (2012) Searching for Solutions in Aquaculture: Charting a Sustainable Course. Annual Review of Environment and Resources, Vol 37 37: 247–.

40. Good C, Davidson J, Welsh C, Snekvik K, Summerfelt S (2011) The effects of ozonation on performance, health and welfare of rainbow trout Oncorhynchus mykiss in low-exchange water recirculation aquaculture systems. Aquacultural Engineering 44: 97–102.

41. Jesus JM, Calheiros CSC, Castro PML, Borges MT (2014) Feasibility of Typha latifolia for high salinity effluent treatment in constructed wetlands for integration in resource management systems. International Journal of Phytoremediation 16: 334–346. PMID: 24912235

42. Idris SM, Jones PL, Salzman SA, Croatto G, Allinson G (2012) Evaluation of the giant reed (Arundo donax) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. Environmental Science and Pollution Research 19: 1159–1170. doi: 10.1007/s11356-011-0642-x PMID: 22006507

43. Pedersen L-F, Suhr KI, Dalsgaard J, Pedersen PB, Arvin E (2012) Effects of feed loading on nitrogen balances and fish performance in replicated recirculating aquaculture systems. Aquaculture 338: 237–245.

44. Malone RF, Beecher LE (2000) Use of floating bead filters to recondition recirculating waters in warm-water aquaculture production systems. Aquacultural Engineering 22: 57–73.

45. Hagopian DS, Riley JG (1998) A closer look at the bacteriology of nitrification. Aquacultural Engineering 18: 223–244.

46. Davidson J, Good C, Welsh C, Summerfelt ST (2014) Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. Aquacultural Engineering 59: 30–40.

47. Svbodova Z, Machova J, Polesczczuk G, Huda J, Hamackova J, Kruopova H (2005) Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. Acta Veterinaria Brno 74: 129–137.

48. Kozak P, Policar T, Fedotov VP, Kuznetsova TV, Buric M, Koub A, et al. (2011) Stress reaction in crayfish: chlorides help to withstand stress in high nitrite concentration conditions—preliminary study. Knowledge and Management of Aquatic Ecosystems.

49. Kruopova H, Machova J, Svbodova Z (2005) Nitrite influence on fish: a review. Veterinarni Medicina 50: 461–471.
50. Laursen DC, Andersson MA, Silva PIM, Petersson E, Hoglund E (2013) Utilising spatial distribution in two-tank systems to investigate the level of aversiveness to crowding in farmed rainbow trout Oncorhynchus mykiss. Applied Animal Behaviour Science 144: 163–170.

51. Barnes ME, Wipf MM, Domenici NR, Kummer WM, Hanten RP (2013) Decreased Hatchery Rearing Density Improves Poststocking Harvest and Return to Spawning of Landlocked Fall Chinook Salmon. North American Journal of Aquaculture 75: 244–250.

52. Manley CB, Rakocinski CF, Lee PG, Blaylock RB (2014) Stocking density effects on aggressive and cannibalistic behaviors in larval hatchery-reared spotted seatrout, Cynoscion nebulosus. Aquaculture 420: 89–94.

53. Cole DW, Cole R, Gaydos SJ, Gray J, Hyland G, Jacques ML, et al. (2009) Aquaculture: Environmental, toxicological, and health issues. International Journal of Hygiene and Environmental Health 212: 369–377. doi:10.1016/j.ijheh.2008.08.003 PMID: 18790671

54. Snow A, Anderson B, Wootton B (2012) Flow-through land-based aquaculture wastewater and its treatment in subsurface flow constructed wetlands. Environmental Reviews 20: 54–69.

55. Sindilariu P-D, Wolter C, Reiter R (2008) Constructed wetlands as a treatment method for effluents; from intensive trout farms. Aquaculture 277: 179–184.

56. Webb JA (2012) Effects of trout farms on stream macroinvertebrates: linking farm-scale disturbance to ecological impact. Aquaculture Environment Interactions 3: 23–32.

57. Bičič M, Bláhovec, J., Kouřil, J. (2014) Feasibility of open recirculating system in temperate climate—a case study. Aquaculture Research [Epub ahead of print].

58. Wang X, Olsen LM, Reitan KI, Olsen Y (2012) Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquaculture Environment Interactions 2: 267–283.

59. Vymazal J (2011) Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674: 133–156.