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Growth and Depuration of Off-Flavors in Rainbow Trout *Oncorhynchus Mykiss* in a Partial Recirculating Aquaculture System (PRAS)

Petra Camilla Lindholm-Lehto[1] | Tapio Kiuru[1,2]

[1]Aquatic Production Systems, Natural Resources Institute Finland (Luke), Jyväskylä, Finland | [2]Paras Aqua Oy, Jyväskylä, Finland

Correspondence: Petra Camilla Lindholm-Lehto (petra.lindholm-lehto@luke.fi)

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ABSTRACT

In recirculating aquaculture systems (RAS), off-flavours accumulated in fish muscle tissue can be problematic in terms of consumer acceptance and the reputation of farmed fish products. Off-flavours often give fish earthy, muddy, or other undesirable flavours. Typically, off-flavours are removed during a depuration period in which fish are fasted and held in clean water. Unfortunately, this causes additional costs and delayed sales, while fish lose weight and show a decrease in lipid content. First, we studied fish growth in a partial RAS (PRAS) where the conditions are very similar to those in depuration with a water exchange rate of 4000 L kg^{-1} feed, compared to RAS with a 650-L water kg^{-1} feed. Rainbow trout (*Oncorhynchus mykiss*) was reared in both systems. Our aim was to combine the benefits of a higher water exchange rate: the lack of need for biofilters and a lower accumulation of off-flavours while obtaining stable rearing conditions. Additionally, we studied the effects of moderate feeding and H_2O_2 addition during depuration. The fish grew faster in a PRAS than in a RAS when fed ad libitum. Thirteen off-flavour compounds were found in the fish flesh and 11 in the circulating water. The H_2O_2 addition led to decreased levels of off-flavours in the tank water and in fish muscle. The results showed no significant differences in off-flavours between the fed and not-fed systems, showing that moderate feeding did not prevent a good depuration result. However, the lipid content and the overall fish weight were higher in the fed systems, which suggests more effective depuration. Increased depuration efficiency can be an important tool when considering ways to improve the profitability of production.

1 | Introduction

In an aquacultural system, the percentage of water recirculation or water volume per kilogram feed is used to describe the systems' intensity. A typical recirculating aquaculture system (RAS) uses 500–1000 L of clean water per kilogram feed, while in a flow-through system, the water renewal rate can be 50,000 L kg^{-1} feed (Vielma, Kankainen, and Setälä 2022). In a partial RAS (PRAS), the water renewal rate lies in between; the aim is to combine the benefits of both systems. The higher water exchange rate is designed to ensure sufficient water quality and rearing conditions.

Biofilters and drum filters often contain a lot of organic matter which can support the growth of off-flavour-producing microbes, such as *Streptomyces* (Podduturi et al. 2020). RAS farms have had difficulties with profitability in Northern Europe and in the United States (Engle, Kumar, and van Senten 2020; Vielma, Kankainen, and Setälä 2022). To improve profitability and mitigate off-flavour-related problems, the development of the PRAS aims to remove the expensive equipment which can harbour unwanted microbes. A PRAS is a recirculating system, applied in an outdoor facility, or indoors with temperature regulation in northern latitudes and colder outside temperatures. Bottom-drained tanks are designed to act as particle matter removal systems, but expensive equipment with a maintenance requirement, such as drum filters, are discarded. Additionally, biofilters are not required due to less intensive water recirculation.

Total ammonia nitrogen (TAN) occurs in water as the sum of ammonia (NH_3) and ammonium ion (NH_4^+) which vary based on pH, temperature, and salinity (Kolarevic et al. 2013). The toxicity of TAN increases with increasing NH_3 content which is considered damaging for fish. Interestingly, Wood (2004) showed that long-term exposure to low levels of ammonia can promote growth of rainbow trout (*Oncorhynchus mykiss*) and therefore the fish growth was monitored in this study. Wicks and Randall (2002) and later Zimmer, Nawata, and Wood (2010) reported that feeding protected juvenile rainbow trout from ammonia toxicity, while fasting exacerbated this effect. In PRAS, biofilters for ammonia transformation to nitrate are not used and therefore increased concentrations of TAN (or ammonia) may be formed.

In aquaculture systems with a low water renewal rate, off-flavours can be formed due to microbial activity in aquaculture water and accumulate in fish muscle tissue (Auffret et al. 2011). Off-flavours are typically produced as metabolic by-products of a variety of microbial species, such as *Cyanobacteria*, *Actinobacteria*, *Myxobacteria*, and *Sorangium* (Lukassen et al. 2017; Mahmoud and Magdy 2021). Off-flavours perceived in fish are often described as musty and earthy flavours and odours that fish consumers find objectionable. These flavours are typically induced by the terpenoid compounds geosmin (GSM) and 2-methylisoborneol (MIB) (Gerber 1968, 1969), although a wide variety of other compounds are also known to cause unwanted flavours in fish, such as alcohols, aldehydes, carboxylic acids, pyrazines, and terpenes (Lindholm-Lehto 2022; Mahmoud and Buettner 2017; Podduturi et al. 2017).

In depuration, the fish are kept in clean water until their off-flavour concentrations are below consumers' sensory detection limits. Different depuration times have been reported for salmonids: 10–15 days for Atlantic salmon (*Salmo salar*; Burr et al. 2012; Davidson et al. 2020), 16 days for European whitefish (*Coregonus lavaretus*; Lindholm-Lehto et al. 2019), 15 days for pike perch (*Sander lucioperca*; Podduturi et al. 2021), and 7–15 days for rainbow trout (*Oncorhynchus mykiss*; Robertson et al. 2005; Lindholm-Lehto 2022). The required time depends on the initial off-flavour concentrations in fish, process arrangements in depuration, properties of depuration water, water temperature, and the rate of fish metabolism (Davidson et al. 2020; Howgate 2004; Lindholm-Lehto et al. 2019). The depuration water must be clean and free of off-flavours and the system pre-disinfected to avoid the growth of biofilm (Davidson et al. 2014; Houle et al. 2011; Lindholm-Lehto and Vielma 2019). As a rule of thumb, the concentrations of off-flavours should be below 10 ng L^{-1} in water for successful depuration (Davidson et al. 2020; Petersen et al. 2011).

Traditionally, fish are not fed during depuration, and the feeding is stopped hours beforehand (48 h before; Podduturi et al. 2021) to ensure the best possible water quality and depuration results (Davidson et al. 2021). However, fish often lose weight and their lipid content decreases (Burr et al. 2012; Lindholm-Lehto et al. 2019) which can induce unwanted changes in the fatty acid profile of fish (Lindholm-Lehto et al. 2022). Feed cost is a large proportion of total production costs in intensive aquaculture ($>50\%$, Rana, Siriwardena, and Hasan 2009; 63%, Arru et al. 2019). It is therefore important to understand feeding efficiency and aim for cost-effectiveness (H. Li et al. 2022).

The gut microbial community of fish is affected by factors that include age, nutrition, and water quality (Dehler, Secombes, and Martin 2017; Shastry and Rekha 2021). Starvation affects the nutrition, health status, and intestinal microbiota of fish (e.g., Sakyi et al. 2020), causing changes in microbial-mediated metabolism (Xia et al. 2014). The digestive system of fish can harbour off-flavour-producing bacteria (Lukassen et al. 2019). For example, decreased concentrations of GSM and MIB have been observed in largemouth bass (*Micropterus salmoides*) flesh during starvation (Zou et al. 2022).

Fish feed has also been suggested as a source of unwanted taste and flavours (Mahmoud et al. 2018). Lipid and protein compounds of feed ingredients can contain off-flavours, while other compounds such as pyrazines can originate in thermal treatment in feed pellet formation (Mahmoud and Buettner 2017). Once formed, off-flavour compounds are relatively stable against chemical and biological degradation (Mahmoud and Magdy 2021).

Off-flavour excretion requires the compounds to be transported from the tissues via the circulatory system to the gills and the water. However, fasting may negatively affect the transportation and excretion function. Schram et al. (2021) reported that depuration and elimination of GSM were faster when rainbow trout were fed during the depuration period. This was based on the suggested effects on gill ventilation and blood lipids. The lipid fraction of plasma may be involved in lipophilic geosmin (K_{ow}: 3.57) transportation via the circulatory system, but this has not been sufficiently studied or confirmed.

In this study, the design of a pilot-scale PRAS was presented and compared with a RAS of a similar size. Our aim was (1) first to test the PRAS assembly and follow the fish growth (rainbow trout, *Oncorhynchus mykiss*) in a PRAS, which combines the benefits of a RAS and flow-through systems, although increased TAN concentrations are possible. In the second part of the study, (2) the goal was to monitor the depuration of off-flavours in a PRAS and a RAS and the effect of H_2O_2 addition on off-flavour concentrations. Our hypothesis was that the depuration would be enhanced in PRAS with lower recirculation rate compared to RAS. Additionally, H_2O_2 addition would further enhance the depuration. Finally, (3) the goal was to test if any differences occurred during the depuration period between the RAS and PRAS when the fish were fed or not fed. Based on our hypothesis, low feeding would reduce the weight loss in fish but still lead to a good depuration result.

2 | Materials and Methods

2.1 | Experimental Design

The recirculating systems were located on the Laukaa fish farm of Natural Resources Institute Finland (Luke). The experimental design was performed in six individual systems. Each system included a 600-L bottom-drained tank with a water volume of 500 L. The Cornell-type tanks contained a dual-drain outflow, but in this experiment, water was removed from the tank only via the bottom drain. The side-drain flow was pumped at 2.5 L s^{-1} with an air-lift pump, which worked as an aeration unit, one for each tank. The air was supplied to all six airlifts from one side channel blower (FPZ K04 MS, 0.75–0.90 kW).

Solid particles were removed with a closed radial-flow settler coupled to the outflow drain. The circulating water of systems 3–6 was led via moving-bed bioreactors (MBBR). Even the outflow water (systems 3–6) was led via biofilters after the closed radial-flow settler. There were two bioreactors coupled in a series with a total of 1200 L and filled with 600 L of plastic media (RK-Plastiks, surface area 750 m^3 m^{-3}). The biofilters were fully matured for 93 days with the tank outflow water before the experiment was started.

The tank was designed to act like a swirl separator and remove particle matter via the bottom drain, and no separate equipment for particle removal was therefore required. Additionally, no biofilter was applied (systems 1–2) to avoid any hotspots of off-flavour formation and accumulation in fish. The accumulation of toxic nitrogen species was avoided by a high water exchange rate and the addition of hydrogen peroxide (H_2O_2) during depuration, which oxidizes ammonia to nitrite and nitrate. Additionally, no oxygen addition was required.

The pH of the circulating water in the RAS was adjusted with $NaHCO_3$ (Solvay Chemicals International SA, Brussels, Belgium). The temperature of the water was maintained at 13.3 ± 0.4°C by adjusting the inlet water source and by using a plate heat exchanger and an electric heater (Alfa Laval 3• 3 kW). Water temperatures were measured online in tanks 1 and 6 as the water circulated via tanks 1–2 in PRAS and via tanks 3–6 in RAS. Water temperatures were measured daily with a hand-held thermometer (Traceable, VWR International), and similar temperatures were confirmed in the tanks of RAS and PRAS. The pH was maintained at 6.7–7.0. CO_2 content was kept at 12.4 ± 2.6 mg L^{-1}, and dissolved oxygen in the rearing tanks was kept at 8.0–10.0 mg L^{-1}.

The inlet water, a 1:1 mixture of surface water (depth of 3 m) and from the aphotic layer (depth of 8 m), was led from the oligotrophic Lake Peurunka (62.44886, 25.85201, area 694 ha, 59 600 m^3, average pH 6.7). The mean annual temperature in the area was 4°C, and the annual precipitation was 600 mm (Finnish Meteorological Institute, 2023). The inlet water was led via a heat-exchanger and an electrical resistance chamber, but it was otherwise untreated.

For the PRASs (systems 1–2), the water exchange rate was kept at 4000 L kg^{-1} feed. For the RASs (systems 3–6), the water exchange rate was 650 L kg^{-1} feed. The aerated make-up water was led directly in PRASs (systems 1–2) and into bioreactor chamber in RASs (systems 3–6).

Water quality was monitored regularly and included weekly water quality tests of the total ammonia nitrogen (TAN), nitrite, and nitrate, using quick spectrophotometric tests (Procedure 8038 Nessler, LCK341/342, LCK340, LCK349, respectively, and DS 3900, Hach, USA). Additionally, dissolved oxygen (Handheld YSI Pro20; Yellow Springs, USA), CO_2 content, and temperature were measured with hand-held sensors (S/N 1638; Franatech, Germany)., Water pH (ProMinent, Germany), circulating water-flow rate (Fluxus F501; Flexim, Germany), and the flow-rate of inlet water were adjusted manually once a week.

The selected off-flavour compounds (Table 1; Tables S1–S3) were quantified using the method reported in Lindholm-Lehto (2022).

2.2 | Experimental Setup

2.2.1 | Part I

The first part of the experiment was conducted between 9 December 2021 and 11 January 2022 and lasted 33 days to monitor the fish growth and function of the newly built systems. There were 75–90 fish (rainbow trout, *Oncorhynchus mykiss*) per tank, with an average weight of 236 ± 17 g and a tank density of 65 kg m^{-3}. The fish were fed a restricted diet of 1.44%. Systems 1 and 2 (PRAS) were run with a water exchange rate of 4000 L kg^{-1} feed, and systems 4–6 (RAS) with 650 L kg^{-1} feed.

2.2.2 | Part II

In Part II, the fish (rainbow trout, *Oncorhynchus mykiss*) were on average 390 ± 17 g in size with a final tank density of 105 kg m^{-3} and fed ad libitum. Part II lasted 40 days (11 January 2022 to 20 February 2022) until the beginning of the depuration period. The water exchange rates were kept at 4000 L kg^{-1} (PRAS1-2) and 650 L kg^{-1} feed (RAS4-6).

2.2.3 | Depuration

The depuration was conducted between 22 February 2022 and 22 March 2022. The inlet water was pumped into all the systems at 2.4 L min^{-1} (hydraulic retention time, HRT 3.5 h). The feeding was withheld in systems 3 and 6, while systems 1–2 and 4–5 were fed with 400 g day^{-1}, and the water exchange rate was kept at 8600 L kg^{-1} feed. Depuration occurred in the rearing tanks without transferring the fish, cleaning, or disinfection of tanks.

First, H_2O_2 solution (50% solution Bang & Bonsomer Group Oy) was manually added to all the systems for a week (7 March–14 March). H_2O_2 was added directly to the fish tanks near the source of the inlet water flow to ensure rapid distribution to the entire water volume and added three times per day at 5 mg L^{-1}. After 90 min, the concentration decreased to 1.0 mg L^{-1} and below

TABLE 1 | Selected off-flavour compounds, their densities (g $\mathrm{mL^{-1}}$), aroma descriptions, sensory threshold values in different mediums, and references.

Compound	Density (g $\mathrm{mL^{-1}}$)	Aroma	Sensory threshold	Medium	Reference
Acetoin/3-hydroxy-butan-2-one/	1.013	Buttery	150 mg $\mathrm{L^{-1}}$	Wine	Ehsani et al. 2009
Caproic acid/hexanoic acid	0.927	Goat-like	1.8–3.6 mg $\mathrm{L^{-1}}$	Tea	Van Gemert 2003; Zhu et al. 2017
Caproic aldehyde/hexanal	0.815	Grass	0.3–14 µg $\mathrm{L^{-1}}$	Water	Culleré, Ferreira, and Cacho 2011
Caprylic acid/octanoic acid	0.910	Fruity-acid, irritating	0.16–1.9 mg $\mathrm{L^{-1}}$	Tea	Van Gemert 2003; Zhu et al. 2017
Caprylic aldehyde/octanal	0.821	Fruit-like	1.75 µg $\mathrm{L^{-1}}$ 0.7 µg $\mathrm{L^{-1}}$	Wine Water	Culleré, Ferreira, and Cacho 2011
Geosmin/dimethyl-8 -hydronaphtalen	10^{-4}	Musty	250–900 ng $\mathrm{kg^{-1}}$ 15 ng $\mathrm{L^{-1}}$	Fish Water	Grimm, Lloyd, and Zimba 2004; Robertson et al. 2005; Howgate 2004; Lindholm-Lehto and Vielma 2019
3-Isobutyl-2-methoxy-pyrazine	0.990	Undesirable, musty	1–2 ng $\mathrm{L^{-1}}$ 2 ng $\mathrm{L^{-1}}$	Wine Water	Sala et al. 2004; Godelmann, Limmert, and Kuballa 2008; X. Li et al. 2016
2-Isopropyl-3-methoxypyrazine	0.996	Undesirable, musty	2 ng $\mathrm{L^{-1}}$ 2 ng $\mathrm{L^{-1}}$	Wine Water	Sala et al. 2004; Godelmann, Limmert, and Kuballa 2008; X. Li et al. 2016
2-Methylisoborneol	0.968	Earthy	100–700 ng $\mathrm{kg^{-1}}$ 15 ng $\mathrm{L^{-1}}$	Fish Water	Grimm, Lloyd, and Zimba 2004; Robertson et al. 2005; Howgate 2004; Lindholm-Lehto and Vielma 2019
3-(Methylthio)propionaldehyde	1.052	Onion-like, meat-like	0.5 µg $\mathrm{L^{-1}}$ 0.2 µg $\mathrm{L^{-1}}$	Beer Water	Soares da Costa et al. 2004
Phenylacetaldehyde	1,075	Sweet, rose, flowery	1.0 µg $\mathrm{L^{-1}}$	Water	Soares da Costa et al. 2004
α-Terpineol	0.930	Terpenic	330–350 µg $\mathrm{L^{-1}}$	Water	Podduturi et al. 2017
2,4,6-Trichloroanisole	solid	Medicinal, phenolic, iodine-like	1–10 ng $\mathrm{L^{-1}}$ 7 ng $\mathrm{L^{-1}}$	Water	Cravero et al. 2015; X. Li et al. 2016
Vanillin/4-hydroxy-3-methoxybenzaldehyde	1.056	Vanilla, sweet	20 µg $\mathrm{L^{-1}}$	Water	Sobolev

the limit of detection (LOD) after 4 h. The residual H_2O_2 in the water was monitored using colorimetric peroxide test strips (1–25 mg L^{-1}; MQuant, Merck). This raised a concern if the addition had a sufficient effect on off-flavours. Therefore, H_2O_2 addition was changed to continuous mode (a week, 14 March–22 March) to reach a more long-term effect. H_2O_2 was constantly pumped (Milton Roy Model PO 743-822S2) into the inlet water at a rate of 23.6 mL h^{-1}, resulting in 13.66 mg H_2O_2 L^{-1} in the inlet flow and 5 mg H_2O_2 L^{-1} in the rearing tanks.

2.3 | Fish and Feeding

In the experiment, we studied rainbow trout (*Oncorhynchus mykiss*) which originated in the Hanka-Taimen facility.

The fish were fed by an automated feeding system (T Drum 2000, Arvo-Tec, Finland) with a commercial fish feed (BioMar Orbit, 4.5 mm) containing crude protein (42.4%), crude fat (31.5%), ash (6.9%), crude fibre (1.2%), calcium (0.92%), phosphorous 0.9%, and sodium (0.48%) as given by the manufacturer. The fish were visually inspected on a daily basis. In Part II, the fish were fed ad libitum. At the end of the feeding, the fish were considered sated when no eating behaviour was observed for 1 min.

The fish were sampled from the rearing tanks before the depuration period (22 February 2022) and once a week during the depuration (7 March 2022; 14 March 2022; 22 March 2022). In each sampling, the fish were randomly selected and humanely euthanized. The fish were weighed, gutted, filleted, and sampled from the lateral part of the fillet as reported by Hathurusingha and Davey (2016). They were stored at − 22°C until used for the analyses. For the analyses, three fish were taken from each tank. One fillet of each fish was used for the off-flavour analyses, the other fillet for the lipid content determination. The fish fillets (of a tank) were pooled, and two replicate measurements were made from the pooled samples to study the tank replicates.

Water samples were collected from the rearing tanks (22 February 2022; 7 March 2022; 14 March 2022; 22 March 2022) in 250-mL HDPE bottles and stored at − 22°C before the analysis.

The study followed the protocols approved by the Luke Animal Care Committee, Helsinki, Finland, and EU Directive 2010/63/EU for animal experiments.

2.4 | Lipid Content

Total fat was determined by an accredited in-house method JOK3008 which is based on the AOAC Official Method 920.39 Fat (Crude) or Ether extract in animal feed and the acid hydrolysis method (AOAC Method 954.02, Association of Official Analytical Chemists, USA), and the AACC method 30-25 Crude fat in wheat, corn, and soy flour, feeds, and mixed feeds.

A Foss Soxtec/Hydrotec 8000 System was used for total fat analysis, consisting of Soxtec 8000 extraction unit and Hydrotec hydrolysis unit (FOSS Analytical, Denmark).

2.5 | Statistical Analyses

Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 27.0.1.0 (Armonk, NY: IBM Corporation, released 2020). First, normality of the collected data was tested with a Shapiro–Wilk test. Based on this (non-normal data), a Mann–Whitney *U* test was used to study the statistical significance of the off-flavour compounds between systems where the fish were fed or not fed during the depuration. Additionally, a Spearman's correlation test was conducted to evaluate the relationship between fish growth (g) and concentrations of TAN (mg L^{-1}). The confidence interval was set at 95%.

3 | Results

3.1 | Growth and Water Quality

During the experiment (Part I, restricted feeding), the fish grew to an average weight of 390 ± 16 g (Figure 1; Table 2) and a biomass of 31.3 ± 1.2 kg per tank. They achieved an average growth of 12.9 ± 0.9 kg per tank with 10.0 ± 0.7 kg feed per tank, resulting in a feed conversion ratio (FCR) of 0.80 and SGR of 1.48 (Table 2). Overall, the number of mortalities was low in both system types.

In Part I, no statistically significant difference ($p = 0.414$) was observed in the growth of rainbow trout or in SGR ($p = 0.196$) between the PRAS and RAS.

In the second part of the experiment (Part II, feeding ad libitum), the fish were on average 695 ± 60 g in size with an average density of 105 kg m^{-3}, and SGR of 1.51 (Figure 1; Table 2). However, the fish in the PRAS were on average 764 ± 51 g, while only 660 ± 20 g in RAS, showing significant difference between the growths of the two groups ($p = 0.042$). In part II, feeding ad libitum led to loss of feed because all the unfed feed particles were not possible to collect. Therefore, FCR was not possible to be determined correctly for Part II.

Water quality parameters, including temperature, pH, CO_2, O_2 content, ammonia, nitrite-N, and nitrate-N, were monitored during the experiment (Figure 2; Table S4). The pH was on average at 6.7 and slightly lower in the PRAS than in the RAS (pH 7.0) due to the somewhat lower natural pH and low alkalinity of the lake water which was used as the inlet water. Increased concentrations of TAN and decreased NO_3-N were found in the PRAS with increased water exchange rate compared to the RAS (Figure 2). The TAN concentrations were significantly different in PRAS than in RAS ($p = 0.001$). However, the relationship between fish growth (g) and concentrations of TAN (mg L^{-1}) was not significant ($p = 0.145$).

The average fish weight after the depuration was 716.5 ± 99.6 g (Table 3). The fed fish increased in weight during depuration on average 53.7 ± 17.6 g, while the fish in not-fed systems decreased in weight on average 42.5 ± 15.4 g.

3.2 | Off-Flavours

The off-flavour concentrations ranged from below the LOD (acetoin, methional, TCA) to 47 ng L^{-1} (MIB) in the circulating

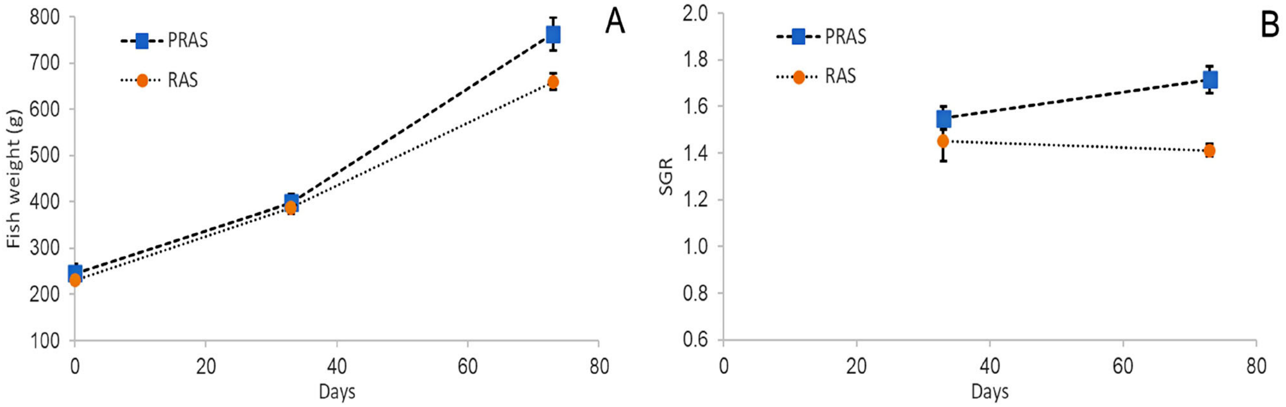

FIGURE 1 | Change in fish weight (g) during the experiment (A, 33 days, Part I; 40 days Part II) and SGR (B, Part II) in the PRAS and RAS. There was restricted feeding in Part I (1.44%) and feeding ad libitum in Part II.

TABLE 2 | Average fish weight (g), rearing densities (kg m^{-3}), mortalities (%), and specific growth rates (SGR) in the PRAS and RAS. Part I: First 33 days with restricted diet (1.44%); Part II: The next 40 days feeding ad libitum.

Treatment	Average weight (g)	Density (kg m^{-3})	Mortalities %/nbr	SGR	FCR
Part I					
PRAS1	225→380	67.4	1.12/3	1.6	0.78
PRAS2	266→416	65.2	1.28/3	1.5	0.79
RAS3	244→395	65.8	1.23/3	1.5	0.78
RAS4	227→392	65.3	1.23/3	1.5	0.81
RAS5	223→390	65.9	2.41/3	1.5	0.80
RAS6	229→368	60.7	1.22/4	1.3	0.84
Part II					
PRAS1	380→727	114.9	7.95	1.7	nd
PRAS2	416→800	110.4	9.10	1.7	nd
RAS3	395→663	94.7	3.00	1.4	nd
RAS4	392→674	106.5	1.25	1.4	nd
RAS5	390→673	105.1	3.70	1.4	nd
RAS6	368→631	98.5	3.00	1.4	nd

Note: FCR was not possible to be determined correctly due to feed loss when feeding ad libitum.
Abbreviation: nd, not determined.

TABLE 3 | Average weights calculated based on tank biomass and number of individuals in the PRAS and RAS. Treatment (depuration in fed and not-fed systems), final average fish weight (g) after Part II (40 days feeding ad libitum), final average fish weight (g) after the depuration, and changes in average weight (g and %) after the depuration.

System	Treatment	Final weight ad libitum (g)	Final weight, depuration (g)	Change (g)	Change (%)
PRAS1	Fed	727.3	802.0	74.7	10.3
PRAS2	Fed	800.0	846.7	46.7	5.8
RAS3	Not-fed	663.4	610.0	– 53.4	– 8.0
RAS4	Fed	674.2	707.7	33.5	5.0
RAS5	Fed	673.0	732.8	59.8	8.9
RAS6	Not-fed	631.2	599.6	– 31.6	– 5.0

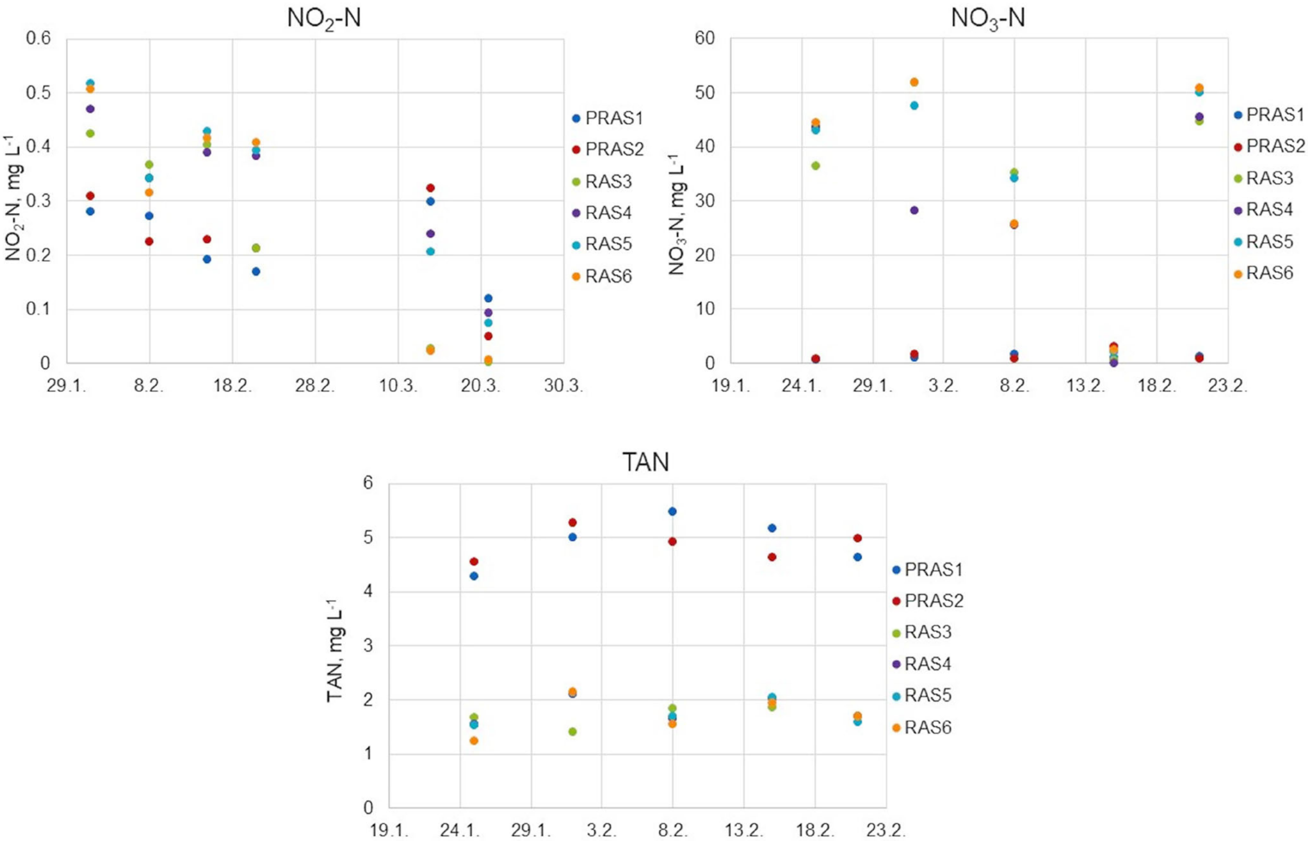

FIGURE 2 | Nitrite (NO$_2$-N, mg L^{-1}), nitrate (NO$_3$-N, mg L^{-1}), and total ammonia nitrogen (TAN, mg L^{-1}) concentrations in circulating water of PRAS1-2 and RAS3-6 during the experiment (Part II) and depuration. Concentrations of 13 off-flavour compounds in rainbow trout muscle tissue (ng kg^{-1}, ± SD; IBMP and hexanoic acid < LOD) and 11 off-flavour compounds in the circulating water (ng L^{-1}, ± SD; IBMP, methional, and TCA < LOD) in fed (PRAS1-2, RAS4-5, $n = 4$) and not-fed (RAS3,6, $n = 2$) systems. Concentrations of 13 off-flavour compounds in rainbow trout muscle tissue (ng kg^{-1}, ± SD; IBMP and hexanoic acid < LOD) and 11 off-flavour compounds in the circulating water (ng L^{-1}, ± SD; IBMP, methional, and TCA < LOD) in fed (PRAS1-2, RAS4-5, $n = 4$) and not-fed (RAS3,6, $n = 2$) systems. Abbreviations: GSM, geosmin; IBMP, 3-isobutyl-2-methoxypyrazine; IPMP, 3-isopropyl-2-methoxypyrazine; MIB, 2-methylisoborneol; TCA, 2,4,6-trichloroanisole.

water (Figure 2). In the fish muscle tissue, concentrations of off-flavour compounds ranged from below the LOD (hexanoic acid, IBMP, octanal, TCA) to 2200 ng kg^{-1} (GSM).

In the inlet water from Lake Peurunka, hexanal (0.9–1.4 ng L^{-1}), 3-isopropyl-2-methoxypyrazine (IPMP) (0.16–0.19 ng L^{-1}), octanoic acid (7.5–9.5 ng L^{-1}), GSM (1.2-3.3 ng L^{-1}), and MIB (12.4–15.4 ng L^{-1}) were found, while others remained below the LOD.

Overall, the concentrations fluctuated over time, but for most compounds, the concentrations in the circulating water decreased after the H$_2$O$_2$ addition was started. For vanillin, GSM, methional, hexanal, and phenylacetic acid decreased concentrations were observed. The effect was less substantial in fish muscle.

Generally, the off-flavour concentrations in the water were somewhat lower in systems where the feeding was withheld (Figure 2). However, a statistically significant difference ($p < 0.05$) was not observed between fed and not-fed systems neither in water nor in fish muscle (Tables S5 and S6).

3.3 | Lipid Content

The average lipid content in fish was lower in the systems where feeding was withheld during depuration than in the system in which the fish were fed (400 g day^{-1}). In the not-fed group, the lipid content was at a constant level throughout the observation period, and the total weight of fish also remained at the same level, but they increased in the fed systems (Figure 3).

In Figure 3, the concentrations of lipophilic GSM and MIB, the most commonly studied off-flavour compounds, have been calculated and shown based on lipid content. The results showed that in the PRAS (fed 400 g day^{-1}), the fish grew during depuration, while in RAS (without feed) their weight remained at the same level (Figure 3). This was the case for the fish individuals sampled for the off-flavour analysis. The fed fish increased in weight, but the fish decreased in weight when not-fed. The overall concentrations of GSM and MIB per lipid content decreased during the depuration similarly, and no significant difference (GSM $p = 0.689$, MIB $p = 0.118$) was observed between the concentrations in the PRAS and RAS.

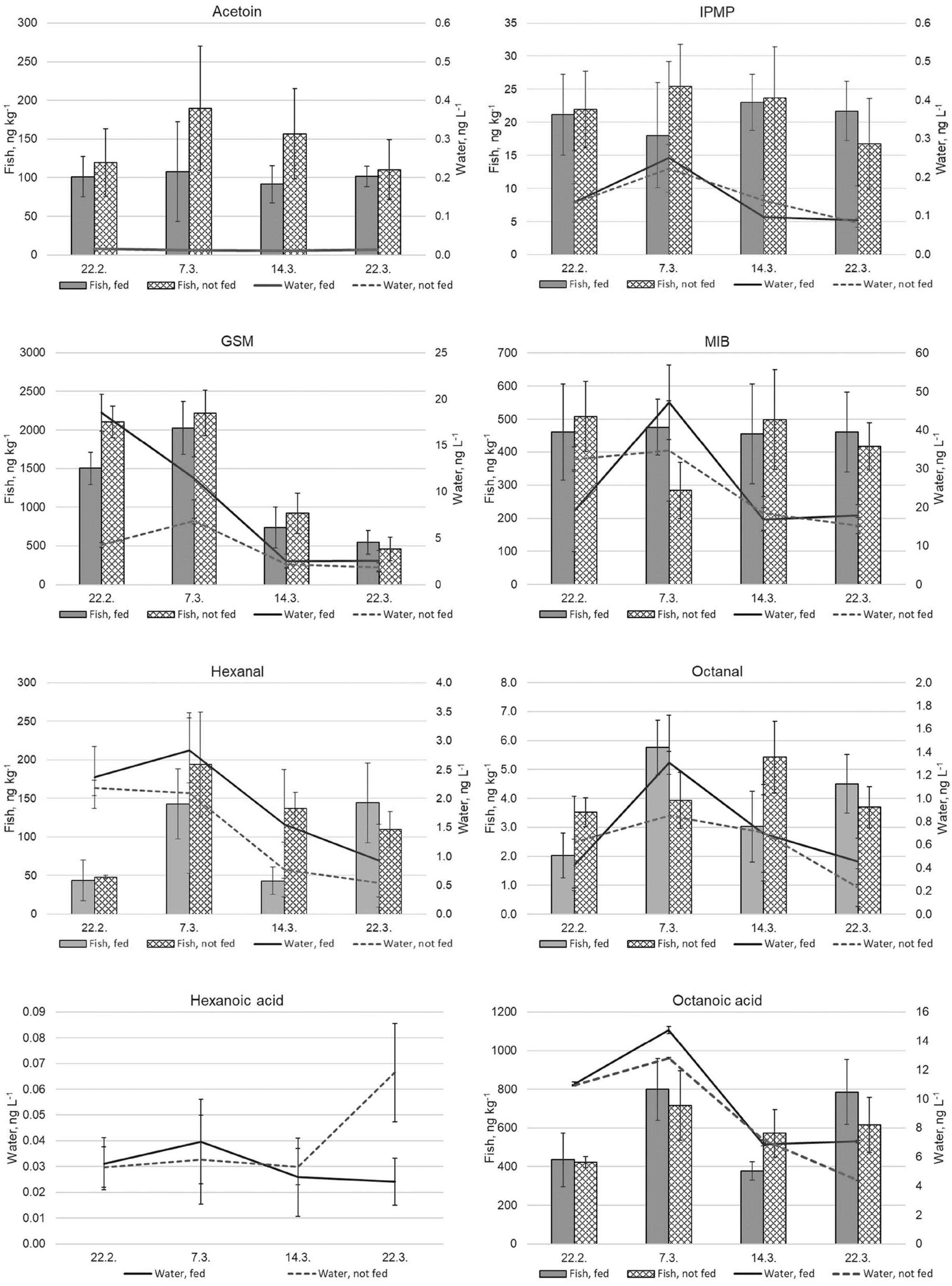

FIGURE 3 | The average concentrations of GSM and MIB per lipid content (ng kg^{-1} lipid, ± SD) and the average weight of fish (g) in fed (PRAS1-2) and not-fed systems (RAS3,6).

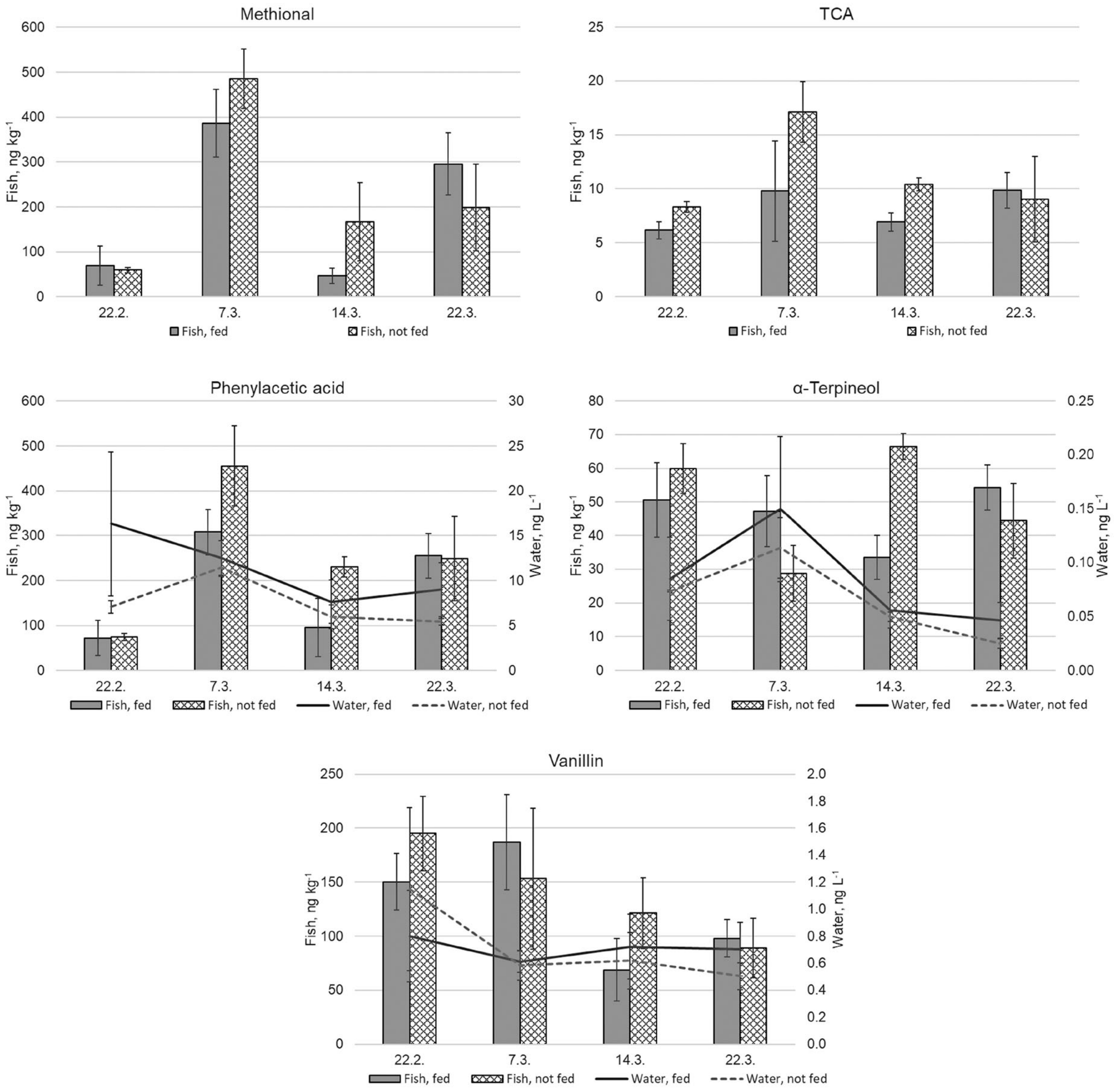

FIGURE 3 | (Continued)

4 | Discussion

4.1 | Growth and Lipid Content in Depuration

Traditionally, feed has been withheld during depuration to ensure good water quality and reduce the depuration period. Starvation decreases the off-flavour-producing microbes and off-flavour concentrations in fish gut (Zou et al. 2022). In a study by Zou et al. (2022), GSM and MIB concentrations decreased during starvation and were strongly correlated with an abundance of actinobacteria such as *Microbacterium* and *Nocardioides*, which are known to produce off-flavours in largemouth bass (*Micropterus salmoides*). However, opposite observations have also been made. Schram et al. (2021) reported a faster decrease in GSM concentrations in the muscle of Nile tilapia (*Oreochromis niloticus*) if the fish were fed (1.1% day^{-1}) during depuration. This may be related to starvation which affects blood lipid content and composition (Figueiredo-Silva et al. 2013; Sheridan 1988), probably affecting geosmin transportation and subsequent elimination.

Fish can lose weight and decrease the lipid content substantially during depuration (Lindholm-Lehto et al. 2019) or change the fatty acid content in fish flesh. Fortunately, studies suggest that decreased lipid content can alter the lipid components toward polyunsaturated fatty acids (PUFA) and n-3, which are considered health-promoting groups of fatty acids (Lindholm-Lehto et al. 2022). The off-flavour compounds are typically lipophilic, and higher concentrations are therefore often found in fish with a high lipid content.

Schram et al. (2021) studied if the feeding of fish can promote the elimination of geosmin. This was based on the suggested effects on gill ventilation and blood lipids. They observed that the depuration and elimination of GSM were faster when the fish were fed during depuration. Feeding increased gill ventilation to meet the increased oxygen demand. In our study, no significant difference ($p < 0.05$) was observed for GSM (or other off-flavours) between fed and not-fed systems, and the depuration was successful in both cases. However, the lipid content and the overall fish weight were higher in fed systems, and the removal of lipophilic GSM can be considered more effective. This can be an important factor in terms of the profitability of production.

When off-flavour concentrations were calculated based on lipid content, no significant difference was observed between the fed and not-fed groups. In both groups, the concentrations decreased during depuration to below the known sensory limits of GSM and MIB (Robertson et al. 2005). This suggests that the duration procedure was more efficient in fish with a higher lipid content. All kinds of methods to enhance depuration have been widely tested in recent years (Davidson et al. 2020, 2021; Kropp et al. 2022). Our results seem promising in terms of decreased weight loss and decreased off-flavour concentrations.

4.2 | Water Use and Nitrogen Species

The results of this study showed increased fish growth in the PRAS compared to the RAS. Similarly, Wood (2004) showed that low concentrations of ammonia (70 µmol L^{-1}) could serve as a growth stimulant in rainbow trout and increase the SGR, while Kolarevic et al. (2013) did not observe effects of ammonia on feed utilization (0.1–25 mg L^{-1} TAN, Atlantic salmon parr). Wood (2004) suggested that ammonia might stimulate amino acid and protein synthesis or reduce metabolic costs, and at a theoretical level, exogenous ammonia can stimulate protein synthesis and growth if it is incorporated into amino acids (Randall and Tsui 2002). In our study, we observed 5 mg L^{-1} TAN in PRAS, meaning 0.52–1.10 µmol L^{-1} ammonia in the experimental conditions. We observed that the fish growth and concentrations of TAN were correlated, but this was not with statistical significance. This was a very small concentration of ammonia and although it may have been one of the factors inducing the increased growth, further studies are required to show this with statistical significance.

Previous studies have suggested that fish cultured in recirculating aquaculture systems (RAS) grew more slowly than those with a higher water exchange rate. For example, Martins et al. (2009) reported that a low water exchange rate (30 L kg^{-1} feed day^{-1}) retarded the growth of larger (300 g) fish, while small fish (80 g) grew better in such conditions. This is supported by the results of our study. We observed an increased growth in the PRAS (4000 L kg^{-1} feed) compared to the RAS (650 L kg^{-1} feed) when fed ad libitum. However, we used larger fish (630–800 g) in our experiment.

Nitrate and nitrite levels were higher in RASs than in PRASs, but even in the RAS, the levels were below those known to induce harmful effects (Davidson et al. 2014; Timmons, Guerdat, and Vinci 2018). On the other hand, nitrite levels were above the recommendations in some cases, but this was the case in both the PRAS and the RAS. All in all, our results cannot confirm the reason for the increased growth, but this is definitely worth further study and is promising for PRAS users because increased growth would also be beneficial in terms of profitability.

4.3 | Off-Flavours

All surfaces are likely to harbour the growth of biofilm and bacteria (Davidson et al. 2020). Biological filters and other components in the RAS can be hotspots for microbes which produce off-flavours (Podduturi et al. 2020). Systems managed with a minimum amount of water treatment equipment are therefore less likely to include hotspots for off-flavour formation, and equipment with a large surface area should be replaced with less problematic equipment or eliminated from the depuration cycle (Podduturi et al. 2017). In this study, the overall water quality remained good in all systems, although slightly elevated concentrations of TAN were observed in the PRASs. However, relatively high off-flavour concentrations (e.g., GSM) were observed in water and in fish before the depuration. It seems that off-flavour-related issues or depuration stage cannot be avoided simply by discarding a biofilter. Additionally, the size of the tank affects the surface area available for the biofilter formation. In this experiment, 500-L tanks were used, resulting smaller water volume per surface area (4.75 m^2 per 1 m^3 water). In a full-scale commercial systems, larger tanks (e.g., rearing tank of 500 m^3, Ø 11 m, 5.3 m) are used and surface area per water volume is smaller (0.56 m^2 per 1 m^3). It is possible that the formation of off-flavours would therefore remain lower in full-scale systems.

The concentrations of the off-flavour compounds GSM and MIB were relatively high in the first two samplings (22 February and 7 March) and significantly above the human sensory thresholds (0.7 ng kg^{-1} for MIB, 0.9 ng kg^{-1} for GSM; Robertson et al. 2005). However, the concentrations decreased after the addition of H_2O_2 was started. In the final sampling on March 23rd, the concentrations were below 500 ng kg^{-1} in all systems.

Off-flavours are absorbed passively from the water, mostly across gill membranes (From and Hølyck 1984), but minor amounts may be absorbed across the skin or lining of the stomach and intestines as water is swallowed while feeding (Tucker and Schrader 2020). Once in the bloodstream, the compounds are eventually concentrated in lipid-rich tissues (Johnsen and Lloyd 1992). Fatter fish (> 2.5% muscle lipid) accumulate nearly three times more 2-MIB than lean fish (< 2% muscle lipid) (Tucker and Schrader 2020). In this study, the fish (average weight of 630–800 g) had high lipid content of 28%–32% and accumulated high concentrations at the beginning of the experiment. However, the concentrations decreased after the beginning of the depuration and H_2O_2 addition to below the known sensory threshold values of GSM and MIB.

Unlike most other studied off-flavour compounds, the concentrations of IPMP remained fairly constant in the fish throughout the experiment (Figure 2). After the H_2O_2 addition, there was a slight decrease in the water, but the effect was not seen in the fish muscle (Figure 2). The IBMP and IPMP may be formed in the thermal treatment in feed pellet formation (Mahmoud and Buettner 2017), suggesting a possibility of a difference between fed and not-fed systems. According to some studies, feed containing high levels of marine fish meal or fish oil is more likely to induce unwanted flavours (Tucker and Schrader 2020), and current high-quality fish feeds contain grain, which is less likely to induce these unwanted flavours (Schrader 2023). However, no statistically significant difference in IPMP concentrations was found between fed and not-fed systems ($p > 0.05$, Figure 2; Tables S5 and S6). The overall concentrations of IPMP were very small and below the LOD for IBMP and thus unlikely to cause off-flavour sensation. However, sensory detection limits for IPMP and IBMP in fish have not been determined (Li et al. 2016).

Small concentrations of GSM and MIB are typically found in surface water during the summer and produced by cyanobacteria (Wang et al. 2015). Concentrations above 10–15 ng L^{-1} GSM and MIB in water can induce off-flavours in fish (Howgate 2004; Petersen et al. 2011), but even very low levels have been suspected as the source of problems. The sensory threshold values for GSM and MIB are very low, and concentrations of 1.3–4.0 ng L^{-1} and 6.3–15 ng L^{-1} in water (Watson 2004; Young et al. 1996) and 250–900 ng kg^{-1} (GSM), 700 ng kg^{-1} (MIB) in fish muscle have been reported (Grimm, Lloyd, and Zimba 2004; Persson 1980).

5 | Conclusions

In this study, the PRAS was applied to rearing rainbow trout. With the restricted diet, fish growth and feed use was similar in the PRAS and the RAS, but the growth increased in the PRAS when fish were fed ad libitum. Additionally, selected off-flavour compounds were quantified. Similar concentrations were observed in the RAS and PRAS in fed and not-fed systems. Later in the experiment, H_2O_2 was added which decreased the off-flavour concentrations in the circulating water and in fish muscle. The results suggested that low feeding was appropriate during depuration and it did not induce an additional accumulation of off-flavours. Interestingly, when considering the increased growth and lipid contents in fed systems (PRAS), the depuration seemed more efficient than in not-fed systems. The results are encouraging when considering methods to enhance depuration, reduce fish weight loss, and improve the profitability of production.

Author Contributions

The experiment was planned and conducted by T.K. P.L.L. performed the chemical analyses and drafted the manuscript. T.K. carefully examined the manuscript.

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Ethics Statement

Ethical approval of this study was obtained from the Finnish Food Authority, and the experiment was performed in accordance with the guidelines of Directive 2010/63/EU (Directive 2010/63/EU on the protection of animals used for scientific purposes).

Conflicts of interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.