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Research Article

Distribution of Odorant Compounds in Recirculating Aquaculture Systems Used in Sturgeon Farming

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This study investigated the distribution of odorant compounds in water collected from the tank, drum filter, biological filter, and UV system outlets and the sludge inside the drum filter across two sturgeon RAS farms. A combinatory sensory-analytical approach was applied to assess the aroma profiles via descriptive sensory analysis and the odorant composition by gas chromatography–olfactometry and gas chromatography–mass spectrometry. All water samples were described as fishy, moldy, earthy, algae-like, and sea-water-like notes. Eighty-six odorants were detected olfactorily, of which 44 were identified. Notably, several compounds are reported here for the first time in RAS water, including 1-butanol, myrcene, 1-tetradecene, γ-crotonolactone, 2,6-di-tert-butyl-4-methylphenol, 2-phenoxyethanol, ethyl palmitate, methyl stearate, and methyl dihydrojasmonate. Geosmin and 2-MIB were quantified in all samples. Their concentrations varied from 85 to 432 and from 40 to 43 ng/L for RAS 1 and from 70 to 178 and from 60 to 82 ng/L for RAS 2, respectively. The concentration of both compounds did not vary through the compartments due to water recirculation, and a high concentration was only detected in water from the sludge.

Keywords: γ-crotonolactone; 2-phenoxyethanol; aroma extract dilution analysis; smell; solvent-assisted flavor evaporation

1. Introduction

In 2020, 88 million tons of aquatic animals were produced from aquaculture worldwide, and its contribution to human nutrition is increasing [1]. In this context, recirculating aquaculture systems (RASs) bring advantages in terms of water control and upcycled waste streams, for example, predigested ingredients for animals can be made from the sludge recycled from RAS, thus advancing toward a circular bioeconomy [2]. Comparatively low water consumption highlights RAS as a potential sustainable solution to fish production at scale [2, 3]. To achieve these goals, RAS require specialized technological infrastructure, including biological and drum filters, disinfection systems using ultraviolet lamps and ozone, as well as pumping and aeration equipment [3]. Combining these technologies maintains water quality at optimum levels for healthy fish growth and maximizes production efficiency.

Nevertheless, maintaining water quality is a crucial aspect of ensuring a good environment for healthy fish growth and high product quality. To this end, constant monitoring of some parameters, for example pH, carbon dioxide (CO_2), and dissolved oxygen (DO), is needed, and less frequent assessment may be required regarding off-flavors, trace elements, and metals [4]. Concerning off-flavors, some crucial points that can influence the production of undesirable substances must be considered, such as the farm environment, water quality, fish feed, contaminants, and postharvest conditions [5]. For example, Lukassen et al. [6] explored the relation of water quality and geosmin producers in RAS, highlighting the importance of monitoring water quality parameters to control the

increase in geosmin-producing microorganisms. More recently, oxidative treatment applying ozone and hydrogen peroxide was reported as a potential method to improve water quality by reducing the quantities of dissolved organic carbon and off-flavor compounds in RAS water [7].

From an economic perspective, sturgeon fish holds significant value as a valuable source for caviar and meat production [8]. Accordingly, several sturgeon companies have based their production on combined rearing technologies and intensive systems, for example, RAS, flow-through/RAS, RAS/cages, and RAS/ponds, which collectively represented 32.1% of sturgeon farming facilities in 2016 [8]. More recent study suggests that the rearing system of Russian sturgeon (*Acipenser gueldenstaedtii*) significantly influences the quality, growth, and maturity of caviar, with RAS being preferred for sturgeon meat production. Furthermore, the combination of RAS and cages is recommended for caviar production to avoid the undesirable flavors detected in caviar produced only in RAS [9]. In this context, a common challenge in RAS is the bioaccumulation of earthy and musty off-flavors, a phenomenon that is even more problematic in caviar production due to its reputation as an exquisite delicacy [10].

Many researches related to off-flavors in RAS has focused mainly on geosmin and 2-methylisoborneol (2-MIB) [11–13]. Geosmin and 2-MIB are sesquiterpenoid and monoterpenoid alcohols, respectively, considered responsible for earthy and moldy off-flavors in water [11]. These compounds were identified in several RAS production systems, including those of different fish species, namely Nile tilapia (*Oreochromis niloticus*) [14], Rainbow trout (*Oncorhynchus mykiss*) [15], and Pikeperch (*Stizostedion lucioperca*) [13, 16]. They are associated with the production of organic matter in high amounts in the RAS, which favors an increase of microorganisms, including those that produce off-flavor [11]. In this context, some studies were conducted to investigate the association and levels of both compounds in RAS. For example, Lukassen et al. [12] showed that the concentration of geosmin and the amount of microorganisms that might produce this compound were not directly related to each other in different compartments of a RAS, and no potential geosmin hotspots were identified. Moreover, fish off-flavors have been associated with a wide range of odor-active compounds that had previously been overlooked [5]. In this regard, many undesirable compounds have been linked to aquacultural farming stemming both from exogenous (from microorganisms and feed) and endogenous sources and comprising a range of substances, such as carboxylic acids, alcohols, and aldehydes, amongst others [5]. Therefore, a deeper investigation to the study of RAS water is necessary to bring further strategies to improve the management of smells in the production process.

Odor monitoring is thus an important tool for ensuring the economic and environmental viability of a RAS systems. In this context, previous studies were conducted to investigate volatile organic compounds (VOCs) and their effects on sensory quality in RAS by stir bar sorptive extraction and gas chromatography–mass spectrometry (SBSE–GC–MS) [12, 13, 16] or solid-phase microextraction gas chromatography–mass spectrometry (SPME–GC–MS) [7]. Furthermore, the concentration of geosmin and 2-MIB has been studied in pilot-scale systems in some compartments of a RAS in the case of Tilapia (*Oreochromis niloticus* × *Oreochromis aureus*), comprising sampling at the tank outlet, of water inside the drum filter, of water within the digestion basin, and at the inlet and outlet trickling filter [11]. Additionally, the sand filter and biofilters [6], as well as the production and depuration pond of a Pikeperch (*Stizostedion lucioperca*) culture were investigated [16]. More recently, the short-term dynamics and concentration of geosmin were measured in tanks, at the denitrification unit and at the biofilters (inlet and outlet), for Pikeperch (*Stizostedion lucioperca*) cultured in a RAS, in which it was observed that the denitrification unit and biofilter cleaning might increase the geosmin content [13].

Our previous work investigated aroma-active compounds in Russian Sturgeon [17] and Nile Tilapia [14] raised in RAS through a combinatory sensory and analytical approach. However, the quantitative data and sensory parameters have not been examined carefully in RAS water in sturgeon culture. The aim of this study was to investigate the odorant composition of water in two independent RAS facilities across different compartments to understand the odorant fluctuation and relate these observations to management practices and potential sources. First, the aroma profile of water from five different compartments, namely the tank, drum filter, biological filter, UV system outlets, and sludge, was determined by a trained panel. Next, odorant compounds were analyzed in all water samples by gas chromatography–olfactometry (GC–O) and GC–MS, and ranked via aroma extract dilution analysis (AEDA). In addition, the well-known off-flavor compounds geosmin and 2-MIB were quantified in all water samples by SBSE–GC–MS in order to observe potential hotspots.

2. Materials and Methods

2.1. Chemicals. Dichloromethane (Merck, Darmstadt, Germany); hexanal 98%, 1,4-xylene 99.5%, butan-1-ol (1-butanol) 99.9%, 1,3-xylene 99.5%, 3,7,7-trimethylbicyclo [4.1.0]hept-3-ene (3-carene) 90%, (*E*)-pent-3-en-2-ol 96%, 7-methyl-3-methylideneocta-1,6-diene (myrcene) analytical standard, (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene ((*R*)-limonene) 97%, heptanal > 92%, 1,2,4-trimethylbenzene (pseudocumene) 98%, oct-1-en-3-one 50%, 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (1,8-cineole) 99%, heptan-2-ol ≥ 97%, 6-methylhept-5-en-2-one 99%, 3,7-dimethyloctan-3-ol (tetrahydrolinalool) ≥ 97, 3-methylsulfanylpropanal (methional), (*E*)-pent-2-en-1-ol 95%, acetic acid 99%, benzaldehyde > 99.5%, (*E*)-non-2-enal > 97%, undecanal 97%, decanal, (2*E*,6*E*)-nona-2,6-dienal 95%, (2*E*,4*E*)-nona-2,4-dienal 85%, dodecanal 92%, (4*S*,4a*S*,8a*R*)-4,8a-dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-4a-ol (geosmin) ≥ 97%, phenylmethanol (benzyl alcohol) 99%, 4-methylphenol (p-cresol) 98%, 5-pentyloxolan-2-one (γ-nonalactone) > 98%, (*E*)-3-phenylprop-2-enal ((*E*)-cinnamaldehyde) 99%, 2,6-di-tert-butyl-4-methylphenol (BHT) > 99%, 1-(4,5-dihydro-1,3-thiazol-2-yl)ethanone (2-acetyl-2-thiazoline) > 98%, ethyl hexadecanoate (ethyl palmitate) > 97%, non-3-yn-1-ol > 99%, hexadecan-1-ol > 99%, methyl octadecanoate (methyl stearate) 99%, methyl 2-(3-oxo-2-

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pentylcyclopentyl)acetate (methyl dihydrojasmonate) 96%, 2-phenoxyethanol 99%, 2H-furan-5-one (γ-crotonolactone) 98%, (1R,2R,4R)-1,2,7,7-tetramethylbicyclo [2.2.1]heptan-2-ol (2-MIB) 98%, nonan-2-one ≥ 99% (Aldrich, Steinheim, Germany); 2-ethylhexan-1-ol ≥ 99%, octan-1-ol > 99.5%, oct-1-en-3-ol 98%, nonanal 95% (Fluka, Steinheim, Germany); tetradec-1-ene (1-tetradecene) > 99.5%; cumene > 99%, 1,3,5-tribromo-2-methoxybenzene (2,4,6-tribromoanisole) > 98.0% (TCI Europe); octanal 99% (Thermo Fischer, Kandel, Germany); (5*Z*)-octa-1,5-dien-3-one ((*Z*)-1,5-octadien-3-one) 99%, 3-(3-pentyloxiran-2-yl)prop-2-enal (*tr*-4,5-epoxy-(*E*)-2-decenal) 97% (AromaLab, Planegg, Germany), 5-butyloxolan-2-one (γ-octalactone) (EGA Chemie, Steinheim, Germany). Commercial Sniffin Stick test pen (16) (Burghart Messtechnik GmbH, Holm, Germany). The retention indices were calculated using a solution of a homologous series of the n-alkanes (C6-C31; all supplied from Sigma–Aldrich, Steinheim, Germany) in pentane.

2.2. Samples. In order to represent the variety and dynamics found in a commercial RAS site, we set out to collect data under typical working settings. Therefore, the results shown in the manuscript represent the odorant composition, fluctuation, and off-flavor concentrations found in a commercial production farm. All water samples were obtained from a commercial RAS farm in France in May and July 2023. On that farm, sturgeons were raised in two independent RAS with the same water inlet from a reservoir, in six 35 m^3 tanks for RAS 1 and four 55 m^3 for RAS 2, each with an individual water inlet and outlet. *Acipenser gueldenstaedtii* and *Acipenser baerii* of different sizes and ages (3.5–8 kg) were raised concomitantly in RAS 1, each batch from a species and an age class in its own tank (average individual weight of 6.72 kg corresponding to 14,860 kg biomass in production tanks). *Acipenser gueldenstaedtii* were raised in RAS 2 from 2.5 months of age (15 g of individual weight and 203 kg of biomass in the tank) to 5.5 months of age (177 g of individual weight and 897 kg of biomass in the tank). The outlets from all grow out tanks from an RAS merged into one pipe before passing through a drum filter with a 63-μm membrane. After the drum filter, filtered water passed to a bed biofilter unit. Then, water was exposed to UV light and returned to the tanks. Each biofilter consisted of floating bed media (1 cm size). The average hourly renewal rate per tank was approximately 1.57% in RAS 1% and 1.69% in RAS 2. Water samples (300 mL) were collected from five different compartments for each independent RAS (RAS 1 and RAS 2), namely, one sample from each of the tank outlet, drum filter outlet, biological filter outlet, UV system outlet, and sludge inside the drum filter. To guarantee the optimal conditions, some parameters were monitored regularly at the RAS farm: levels of nitrite, ammonium, nitrate, and phosphate were examined using commercial kits from Hanna Instruments France, while O_2, pH, and temperature were monitored using online probes from OxyGuard International A/S (data not shown). All water samples were collected and frozen at −20°C until transport on ice blocks. Then, all samples were stored at −80°C (for a maximum period of 3 months). The layout of RAS 1 and RAS 2 is shown in Supporting Information 1: Figure S1.

2.3. Solvent Extraction and Solvent-Assisted Flavor Evaporation (SAFE). The procedure for extraction of the odorant compounds from water was adapted from Mahmoud and Buettner [18] and the methods description partly reproduces their wording. Briefly, 300 mL of each water sample was added to 100 mL dichloromethane and stirring per 30 min at room temperature. After that, the dichloromethane phases in the mixture were collected using a separatory funnel. This procedure was performed in triplicate (300 mL per sample). Then, the extract was distilled using SAFE at 50°C. Next, the distillate extract was dried over anhydrous Na_2SO_4. Subsequently, the volume of the distillate extract was reduced to approximately100 μL via Vigreux distillation and micro distillation at 50°C. An equivalent volume of DCM was extracted in parallel as a control sample.

2.4. Odorant Identification. For odorant identification, a combined sensory and analytical analysis method was adapted from our previous work on fish aroma [14, 17].

2.4.1. Gas Chromatography–Olfactometry/Flame Ionization Detection (GC–O/FID) and Comparative Aroma Extract Dilution Analysis (cAEDA). A TRACE GC Ultra (Thermo Fischer Scientific, USA) was used for GC–O analysis of the concentrated distillate samples. A DB–FFAP (30 m x 0.32 mm fuzed silica capillary FFAP, 0.25 μm; Agilent J&W GC Columns, USA) or DB-5 (30 m × 0.32 mm fuzed silica capillary DB-5 0.25 μm; Agilent J&W GC Columns, USA) capillaries were installed. The initial temperature of 40°C was maintained for 2 min in both columns. Following that, the temperature was increased by 8°C/min to 250 or 240°C and maintained for 10 or 8 min (for DB-5 and FFAP, respectively). A total of 2 μL of the sample was inject into the GC system using a cold on-column technique (40°C). The helium carrier gas flowed at a rate of 2.2 mL/min. A Y-splitter was used at the end of the capillary to split the GC effluent 1:1 to the sniffing port and the flame ionization detector (FID) by using two 0.5 m × 0.2 mm deactivated, uncoated fuzed-silica capillaries. The sniffing port and FID were kept at 270°C and 250°C, respectively. Two trained panelists evaluated the concentrated distillate (FD = 1), with sniffing durations of 38 min on DB-5 and 35 min on FFAP. The panelists were selected from a trained sensory panel (Chair of Aroma and Smell Research, Friedrich–Alexander-Universität Erlangen–Nürnberg, Germany). Each odorant compound was identified by the linear retention index calculated using a reference set of homologous alkanes (C6–C31).

Comparative aroma extract dilution analysis (cAEDA) [19] was performed by one of the two trained panelists, using the DB-FFAP column, to rank the odorants based on their relative smell intensities in the distillates extracted from tank (T) water samples for each RAS (RAS 1 and RAS 2). To this aim, flavor dilution (FD) factors were obtained from a sequence of dilutions: the undiluted distillate (FD = 1) was gradually diluted with DCM (ratio 1:3; v/v), resulting six solutions for each sample with FD factors ranging from FD 3 to FD 729. Each FD factor is expressed by the final solution in which the odor-active compound could be detected by GC–O. Therefore, no odor was perceived during GC–O at the highest dilution (FD 729).

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2.4.2. Gas Chromatography–Mass Spectrometry (GC–MS). The GC–MS consisted of a GC 7890A system connected to an MSD 5975C (Agilent Technologies, Santa Clara, CA, USA), equipped with a CIS4 injection system and MPS 2xL Autosampler (Gerstel GmbH & Co. KG). An uncoated and deactivated fuzed silica capillary (2.5–5 m length × 0.53 mm diameter) either DB-FFAP column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent J&W GC Columns) or DB-5 (30 m × 0.25 mm fuzed silica capillary DB-5 0.25 μm; Agilent J&W GC Columns, USA) was installed into the GC system. The same temperature programs for the GC oven previously described were applied (subsection 2.4.1). Helium was used as the carrier gas and the analysis was carried out in splitless mode at a flow rate of 1.0 mL/min. The injection volume was 1.0 μL, and it was performed using an MPS 2 XL autosampler (Gerstel GmbH & Co. KG). The ionization energy was set to 70 eV, and the EI mass spectra were recorded in scan mode (40–400 m/z). The odor quality, retention indices on two capillaries (DB-FFAP and DB-5) and mass spectra were compared with those of standard compounds in order to identify the odorant molecules. The retention index for each odor-active compound was determined using a reference series of homologous alkanes (C6–C31).

2.5. Geosmin and 2-MIB Quantification. Geosmin and 2-MIB were quantified in the 10 samples, in triplicate, from both RAS (RAS 1 and RAS 2). The sampling from the different sites of the RAS system was performed only once (see above). Geosmin and 2-MIB amounts were evaluated by stir bar sorptive extraction–gas chromatography–mass spectrometry (SBSE–GC–MS) using a method adapted from Podduturi et al. [13]. Briefly, a commercial stir bar (Twister Gerstel GmbH, Germany) coated with polydimethylsiloxane (PDMS; length 2.00 cm and thickness of 1.00 mm) was added to 20 mL of water in a 50-mL glass vial and then covered with a plastic lid. At room temperature, the extraction procedure was carried out by stirring the stir bars for 120 min at 1000 rpm. After that, forceps were used to remove the twisters and they were then rinsed with distilled water, dried with tissue free of lint, and transferred to thermal desorption tubes. A GC-grade mixture (1:1 v/v) of geosmin and 2-MIB in a dilution series of 0, 10, 50, 100, 200, 600, and 1200 ng/L in water was prepared to create an external calibration curve. All analyses were performed in triplicate for both the sample and the calibration curve points. Reproducibility and linearity were considered satisfactory (0.5%–8.8% RSD for geosmin and 0.2%–2.8% for 2-MIB; $R^2 = 0.99$). Limit of detections (LODs) were calculated as three times of the standard deviation of the blank sample and limit of quantifications (LOQs) were determined as 10 times. The LOD and LOQ values of GSM and 2-MIB were approximately 1 and 6ng/L. The raw data for the calibration curves and merit parameters of the applied method are given in Supporting Information 1: Figure S2 and Table S1, respectively. The VOCs adsorbed on the stir bars were desorbed through a two-step procedure by a thermal desorption unit TurboMatrix 350 (Perkin Elmer, Shelton, USA). First, the stir bar was heated to 240°C for 15 min with a carrier gas flow of 50 mL H_2/min to perform primary desorption. Second, the desorbed volatiles were subsequently trapped in a Tenax TA trap that was first kept at 1°C and then heated to 280°C for 4 min. This resulted in the quick transfer of volatiles from the thermal desorption unit to a gas chromatograph–mass spectrometer (8890, GC-system, coupled with a 5977B MSD from Agilent Technologies, Palo Alto, California) through a temperature-controlled transfer line held at 225°C. A ZB-Wax capillary column was used to separate the transferred volatiles (30 m × 0.25 mm × 0.5 μm) using H_2 as the carrier gas at an initial flow rate of 1.4 mL/min. The oven program was set as follows: isothermal for the first 10 min at 35°C, then raised to 240°C at a rate of 8°C/min and held per 10 min. Mass spectra acquisition were obtained after standard electron ionization conditions (70 eV) and detected through QMS, where mass acquisition was set in a hybrid version combining both SCAN and SIM modes. The SCAN mode scanned m/z values ranging from 15 to 300, while the selected ion monitoring mode (SIM) specifically targeted masses 95 and 107 for 2-MIB and 112 for geosmin for enhanced sensitivity. After data acquisition, chromatograms were evaluated by the MSD ChemStation software (v.E.02.00, Agilent Technologies). The retention times of both compounds were determined through the calibration curve points, and peaks were integrated based on the SIM chromatograms. In this way, linear regression models for both compounds were calculated in water, and their concentration in the samples was subsequently predicted.

2.6. Sensory Evaluation

2.6.1. Panelists. Seven to eight participants (six to seven females and one male) with ages ranging from 22 to 34 years old formed up the trained panel. All panelists were recruited from the Chair of Aroma and Smell Research at Friedrich–Alexander-Universität Erlangen–Nürnberg, Germany. All panelists had normal olfactory function and no known illness at the time of the examination. Prior to the experiments, the panelists were trained on how to perceive, describe, and rate the odor qualities and intensities of roughly 150 odorants, including common odorants from the food and nonfood sector.

2.6.2. Aroma Profile Analysis. A 120 mL Weck glass (WECK GmbH u. CO KG, Germany) containing around 50 mL of each water sample was used for the sensory evaluation. The sensory evaluation was carried out according to ISO 13299:2016–09 and complemented by the intensity of the overall smell. Two sessions of sensory analyses were conducted in a well-lit and ventilated sensory panel room at 21°C. Prior to the orthosanal evaluation by panelists, the samples were coded with a random three-digit number. First, the panelists were asked to note their individual odor impression perceived of each water sample. Then, the listed odor qualities were discussed within the panel and selected the attributes with an agreement of > 50%. Second, each panelist scored the intensities of each of these attributes for each water sample on a scale from 0 (no perception) to 10 (strong perception). Additionally, the intensity of each descriptor could not exceed the overall odor intensity of the sample, which were scored on a scale from 0 (no perception) to 10 (strong perception). A spider web diagram was created by plotting the average values of these ratings. A commercial Sniffin' Sticks test pen 16 (Burghart Messtechnik GmbH, Germany) and one smell pen containing 2-MIB were used as references

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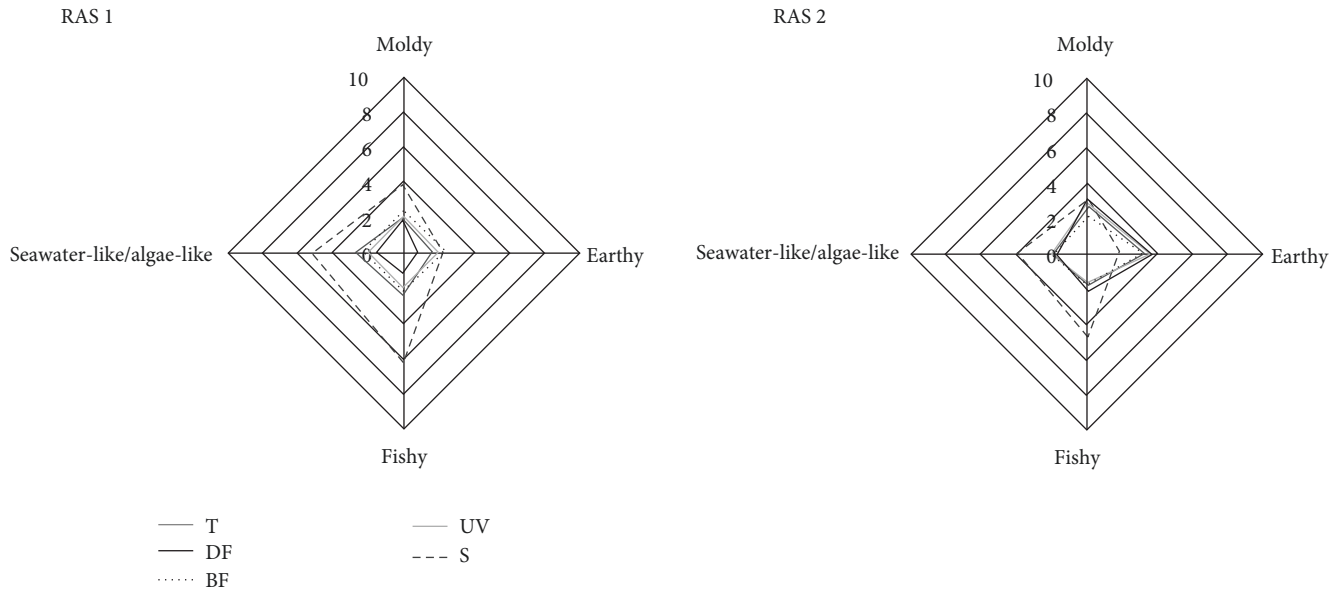


FIGURE 1: Aroma profiles of water samples from different compartments of RAS 1 and RAS 2: T – tank outlet, DF – drum filter outlet, BF – biological filter outlet, UV – ultraviolet system outlet, S – sludge inside the drum filter. Average intensity ratings on a range of 0 (no perception) to 10 (strong perception) for all panelists ($n = 7$–8).

for the assessments of the olfactory qualities fishy and moldy, respectively. For the attribute earthy, 50 mL of an aqueous geosmin solution (0.2 µL/mL) was used as a reference. The sea water-like/algae-like attribute was rated solely on the basis of the concept, which each panelist was familiar with, and no references were used.

2.7. Statistical Analysis. Excel 2016 was used to plot the average intensities values of the sensory analysis results on spider web diagrams. IBM SPSS Statistics 29.0 (IBM SPSS, Armonk, NY, United States) was used to statistically evaluate the assessments of the individual odor descriptors, overall odor intensity, and the concentration of geosmin and 2-MIB in water. The nonparametric Kruskal–Wallis test was applied to evaluate the differences across and within RAS compartments. When significant differences were observed, pairwise differences were identified applying Dunn–Bonferroni post hoc testing. For all analyses, $p < 0.05$ was the significance criterion.

3. Results

3.1. Aroma Profile. In the first session, four odor qualities were selected to characterize the RAS water samples. Figure 1 shows the average intensities perceived for each odor descriptor. Supporting Information 1: Table S2 displays the raw data for the aroma profile and overall odor intensity. All water samples were mainly characterized by the odor attributes moldy, fishy, earthy, and sea water-like/algae-like. For both RAS, the average intensity ratings for the odor descriptors ranged from 1.1 to 6.4. Fishy was the dominant odor attribute in samples from RAS 1 and RAS 2 collected in the water from the sludge, scored with average values of 6.4 and 4.7, respectively. For RAS 1 and RAS 2, the average intensity values for the moldy, earthy, and sea water-like/algae-like odor notes ranged from 0.8 to 5.0 and 1.7 to 4.0, respectively.

There were minor differences between the samples for fishy and seawater-like/algae-like perceptions when comparing RAS 1 and RAS 2, while the other attributes (moldy and earthy) were similar. Significant differences in the comparison within each compartment from RAS 1 vs. RAS 2 were observed only for the earthy descriptor (chi-square = 7.307, $p = 0.004$). Following post hoc tests (Dunn–Bonferroni) showed that water from the drum filter from RAS 1 differed significantly from RAS 2 ($z = 2.224$, $p = 0.007$): drum filter outlet water from RAS 2 had a more intense earthy note. In addition, when compartments within the same RAS were compared, significant differences were observed only for the fishy descriptor (chi-square = 17.871, $p = 0.001$) in RAS 1. Following post hoc tests (Dunn–Bonferroni) showed that sludge water differed significantly from drum filter ($z = -3.947$, $p = 0.001$), UV system ($z = -3.142$, $p = 0.017$), and biological filter ($z = -2.878$, $p = 0.040$) water: sludge water had a more intense fishy note.

Figure 2 displays the overall odor intensity. The average rating of the water samples was between 3.1–7.3 and 4.0–6.1 for RAS 1 and RAS 2, respectively. A statistically significant difference between the compartments was observed for RAS 1 (chi-square = 17.756, $p = 0.001$). Post hoc tests (Dunn–Bonferroni) showed that water from sludge differed significantly from drum filter ($z = -3.853$, $p = 0.001$), UV system ($z = -3.165$, $p = 0.016$), and biological filter ($z = -2.847$, $p = 0.044$) water. Water from sludge had a higher overall odor intensity. For RAS 2, there was no statistically significant difference between the compartments (chi-square = 7.114, $p = 0.130$).

3.2. Identification of Odor-Active Compounds Across Two Independent RAS. The undiluted extracts exhibited the odors of the 10 water samples collected from two RAS (RAS 1 and RAS 2; five different sampling sites each). Table 1 shows the 86 odorant compounds detected in the cAEDA with FD values ranging from 1 to 729. By comparing the retention indices,

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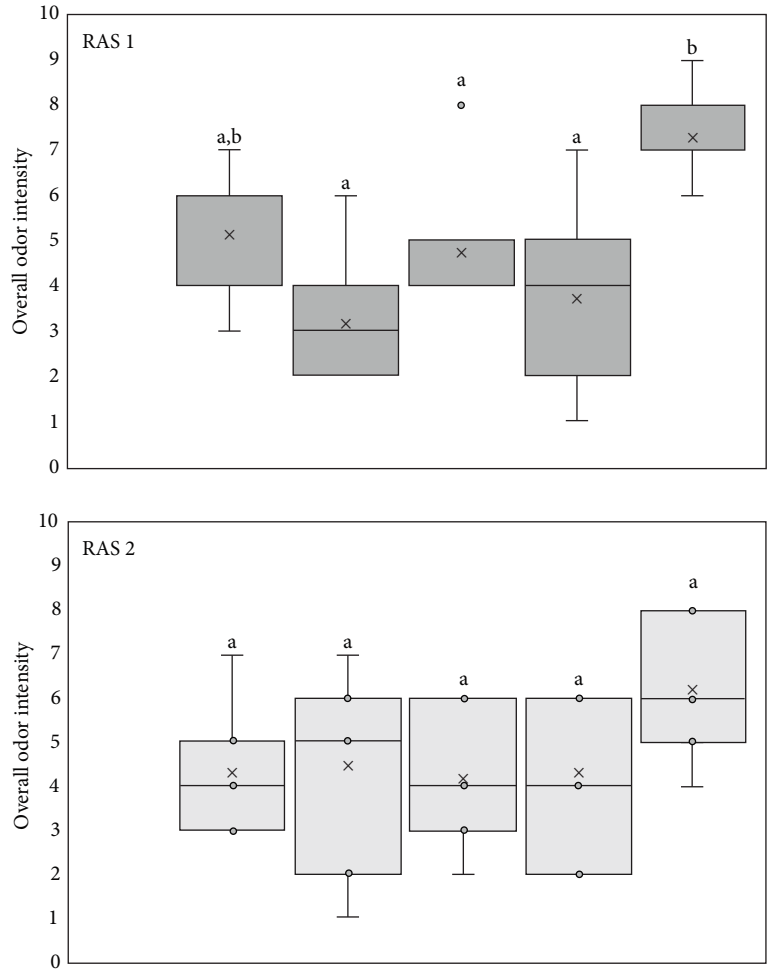


FIGURE 2: Overall odor intensity of samples of different compartments from RAS 1 and RAS 2: T – tank outlet, DF – drum filter outlet, BF – biological filter outlet, UV – ultraviolet system outlet, S – sludge inside the drum filter. Scale range: 0 (no perception) to 10 (strong perception). Sensory evaluation average values are presented ($n = 7–8$) ± standard deviation. Significant differences between samples with $p < 0.05$ (Kruskal–Wallis test) are shown for each graph by distinct uppercase letters.

mass spectra, and odor quality of these compounds with their respective standard compounds, 44 of them were identified; however, 33 compounds could not be identified because of their low concentration. Moreover, nine compound were tentatively identified comparing the retention indices and odor qualities with the corresponding standard compounds. Tank, drum filter, biological filter, UV system outlets, and sludge from RAS 1 and RAS 2 showed a high qualitative similarity because most of the odorants were detected in all samples. Overall, 82 and 77 compounds were detected olfactorily in RAS 1 and RAS 2, respectively. Six unknown odorants (36, 44, 47, 59, 60, 62), (2*E*,4*E*)-nona-2,4-dienal (46), γ-nonalactone (66), and methyl dihydrojasmonate (83) were solely perceived in RAS 1. The compounds myrcene (10), limonene (12), cumene (15), and phenyl acetaldehyde (45) were solely detected in RAS 2. Furthermore, the Table 1 displays the 39 odorant compounds identified in the present study that were previously reported in Russian sturgeon fillets raised in RAS [17].

Overall, 11 odorants were detected with FD factors ≥ 81 in the samples from the tank (T) in both RAS. The odorants with higher FD factor in the distillates from both RAS were the earthy-smelling compound geosmin (54), detected with FD 729 and 243 in RAS 1 and RAS 2, respectively. The compound methyl dihydrojasmonate (83) was detected only in RAS 1 with FD 729. The compounds (*Z*)-1,5-octadien-3-one (popcorn-like, roasty; FD = 27 and 243 in RAS 1 and RAS 2, respectively), geosmin (earthy, moldy; FD = 729 and 243 in RAS 1 and RAS 2, respectively) and *tr*-4,5-epoxy-(*E*)-2-decenal (earthy, moldy; FD = 243 and 81 in RAS 1 and RAS 2, respectively) were detected with FD factors = 243 in one of the RAS samples from the tank (T). Seven odorant compounds had FD factors = 81 in at least one of the RAS samples: oct-1-en-3-one (mushroom; FD = 9 and 81 in RAS 1 and RAS 2, respectively), nonan-2-one (fruity, soapy; FD = 3 and 81 in RAS 1 and RAS 2, respectively), methional (cooked potato; FD = 81 in RAS 1 and RAS 2), 2-acetyl-2-thiazoline (popcorn, roasty; FD = 81 and 9 in RAS 1 and RAS 2, respectively), benzyl alcohol (marzipan, FD = 81 and 27 in RAS 1 and RAS 2, respectively), 2,4,6-tribromoanisole (cork-like; FD = 81 and 1 in RAS 1 and RAS 2, respectively), and unknown (rubber; FD = 81 and

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Table 1: Odor compounds identified in different compartments of two RAS.

No[a]	Odorant	Odor attribute[b]	RI on[c]		FD-factor[d]		Identification method[e]	RAS 1[f]	RAS 2[f]
			FFAP	DB-5	RAS 1	RAS 2			
1	Unknown	Butter-like[g]	1006	—	3	3	—	T, BF, S, DF	T, BF
2	Unknown	Green[g]	1020	—	9	27	—	T, BF, UV, S, DF	T, BF, UV, S, DF
3	Unknown	Green[g]	1031	—	9	n.d.	—	T, BF, UV, S, DF	BF, UV, DF
4	Unknown	Fatty, soapy[g]	1051	—	3	n.d.	—	T, BF, UV, S	UV, S
5	Hexanal[h]	Grassy	1086	808	1	9	O, RI, MS, RC	T, BF, UV, DF, S	T, BF, DF, S
6	1,4-Xylene[h]	Green[g]	1121	867	3	3	O, RI, MS, RC	T, UV, S, DF	T, UV
7	1-Butanol	Malty	1128	—	n.d.	n.d.	O, RI, MS, RC	BF, S, DF	BF
8	1,3-Xylene[h]	Green, fruity[g]	1141	867	3	3	O, RI, MS, RC	T	T, S
9	3-Carene[h]	Citrus-like, eucalyptus-like	1148	1231	n.d.	1	O, RI, MS, RC	DF	T
10	Myrcene	Earthy, metallic, geranium leaf-like	1162	991	n.d.	1	O, RI, MS, RC	—	T
11	(*E*)-Pent-3-en-2-ol[h]	Nutty	1177	715	3	1	O, RI, MS, RC	T, BF, UV, S	T, BF, UV
12	Limonene[h]	Orange peel-like, lemon peel-like	1183	—	n.d.	1	O, RI, MS, RC	—	T, UV
13	Heptanal[h]	Soapy, fatty	1190	905	1	1	O, RI, MS, RC	T, BF, UV, DF	T, BF, S
14	1,8-Cineole[h]	Eucalyptus-like	1208	1037	1	1	O, RI, MS, RC	DF	T, BF
15	Cumene[h]	Nutty, green, glue[g]	1213	920	n.d.	1	O, RI, MS, RC	—	T
16	Unknown	Popcorn-like, soapy, moldy[g]	1243	—	9	1	—	T, BF, DF	T, BF, UV, S, DF
17	Pseudocumene[h]	Green, soapy, fish[g]	1258	1002	9	9	O, RI, MS, RC	T	T S
18	Octanal[h]	Citrus-like, soapy	1290	1000	3	1	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, S, DF
19	Heptan-2-ol[h]	Soapy, citrus-like	1308	898	9	1	O, RI, MS, RC	T	T
20	Oct-1-en-3-one[h]	Mushroom-like	1323	979	9	81	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, S, DF
21	(*E*)-Pent-2-en-1-ol[h]	Fruity, green apple-like, cheesy[g]	1325	—	n.d.	27	O, RI, MS, RC	BF S, DF	T, BF, UV, S
22	6-Methylhept-5-en-2-one[h]	Fruity	1393	1000	9	n.d.	O, RI, MS, RC	T, UV, S, DF	DF
23	Unknown	Potato-like, green[g]	1349	—	9	n.d.	—	T, BF, S	BF, S
24	(*Z*)-1,5-Octadien-3-one[h]	Geranium leaf-like, green, metallic	1369	977	27	243	O, RI, RC	T, BF, UV, S, DF	T, BF, UV, S
25	Nonanal[h]	Soapy, citrus-like	1390	1103	3	1	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
26	Nonan-2-one[h]	Fruity, soapy	1400	1070	3	81	O, RI, RC	T, BF, UV, S	T, BF, UV, S
27	Tetrahydrolinalool	Flowery, soapy	1427	—	9	3	O, RI, MS, RC	T, BF, UV, DF	T, S
28	1-Tetradecene	Moldy[g]	1434	1395	1	3	O, RI, MS, RC	T	T
29	Acetic acid[h]	Vinegar-like	1438	—	3	n.d.	O, RI, MS, RC	T, S	DF
30	Oct-1-en-3-ol[h]	Mushroom-like	1443	975	1	3	O, RI, MS, RC	T, DF	T, UV, S
31	Methional[h]	Cooked potato-like	1457	905	81	81	O, RI, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
32	Unknown	Fatty[g]	1468	—	3	n.d.	—	T, BF, UV	BF, S
33	2-Ethylhexan-1-ol[h]	Eucalyptus-like, band-aid-like	1484	1018	27	1	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
34	Decanal[h]	Soapy	1503	1206	3	3	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
35	Benzaldehyde[h]	Marzipan-like, almond-like	1514	979	3	9	O, RI, MS, RC	T, BF	T, BF, S
36	Unknown	Coriander[g]	1523	—	3	n.d.	—	T, BF	—
37	(*E*)-Non-2-enal[h]	Fatty, cucumber, cardboard-like	1537	1161	1	n.d.	O, RI, MS, RC	T, UV, S, DF	UV
38	Octan-1-ol	Soapy, citrus-like, fatty	1566	1076	1	1	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, S, DF
39	(2*E*,6*E*)-Nona-2,6-dienal[h]	Fatty, cucumber-like	1571	1158	n.d.	n.d.	O, RI, RC	S, DF	S, DF

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Table 1: Continued.

No[a]	Odorant	Odor attribute[b]	RI on[c]		FD-factor[d]		Identification method[e]	RAS 1[f]	RAS 2[f]
			FFAP	DB-5	RAS 1	RAS 2			
40	Undecanal[h]	Coriander-like, soapy, citrus-like	1583	1303	3	3	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S
41	2-MIB[h]	Earthy, musty	1591	1189	9	27	O, RI, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
42	Unknown	Popcorn-like, soapy, fatty[g]	1609	—	9	3	—	T, BF, DF	T, DF
43	Menthol	Eucalyptus-like	1633	1189	1	n.d.	O, RI, MS, RC	T, BF, UV, DF, S	BF, S
44	Unknown	Popcorn-like, fatty[g]	1662	—	1	n.d.	—	T	—
45	Phenyl acetaldehyde	Beeswax-like, rape seed-like, flowery	1667	1058	—	n.d.	O, RI, MS, RC	—	S
46	(2*E*,4*E*)-Nona-2,4-dienal[h]	Fatty, nutty	1697	1207	1	n.d.	O, RI, RC	T, S, DF	—
47	Unknown	Earthy[g]	1706	—	1	n.d.	—	T	—
48	Dodecanal[h]	Soapy, coriander-like	1713	1420	1	n.d.	O, RI, MS, RC	BF	UV, DF
49	γ-Crotonolactone[h]	Fruity	1719	914	1	n.d.	O, RI, MS, RC	T, BF, UV, DF, S	S
50	Non-3-yn-1-ol[h]	Fatty, geranium leaf-like	1750	1189	1	n.d.	O, RI, MS, RC	T, S, DF	—
51	2-Acetyl-2-thiazoline[h]	Popcorn-like, roasty	1759	1103	81	9	O, RI, RC	T, BF, UV, S, DF	T, S
52	Unknown	Green[g]	1781	—	1	n.d.	—	T, BF, UV, S, DF	UV
53	Unknown	Soapy[g]	1800	—	1	n.d.	—	T, BF, UV, S, DF	BF, UV, S, DF
54	Geosmin[h]	Earthy, moldy	1816	1410	729	243	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
55	Unknown	Metallic, plastic[g]	1855	—	1	*	—	T, BF	DF
56	Benzyl alcohol[h]	Marzipan-like	1877	1032	81	27	O, RI, MS, RC	T, S, DF	T, BF, S
57	BHT	Waxy, pepper-like	1900	1500	1	1	O, RI, MS, RC	T, UV, S, DF	T, S
58	γ-Octalactone[h]	Coconut-like	1904	1279	3	1	O, RI, MS, RC	T, BF	BF
59	Unknown	Green, fecal[g]	1917	—	1	n.d.	—	T	—
60	Unknown	Earthy, metallic, fish[g]	1943	—	3	n.d.	—	T	—
61	Unknown	Plastic[g]	1963	N.D	1	1	—	T, BF, S	T, BF, S
62	Unknown	Cheesy[g]	1973	—	1	n.d.	—	T	—
63	*tr*-4,5-Epoxy-(*E*)-2-decenal[h]	Metallic	2007	1382	243	81	O, RI, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
64	Unknown	Soapy[g]	2025	—	3	3	—	T, BF, DF	T, BF
65	(*E*)-Cinnamaldehyde[h]	Cinnamon-like	2031	1276	3	3	O, RI, MS, RC	T, S, DF	T, S
66	γ-Nonalactone[h]	Coconut-like	2039	1352	9	3	O, RI, MS, RC	T, BF	—
67	Unknown	Bitter almond, fruity, rubber-like[g]	2057	—	3	n.d.	—	T	UV
68	*p*-Cresol[h]	Horse stable-like, fecal	2082	1089	3	9	O, RI, RC	T, BF, UV, S, DF	T, BF, UV, DF
69	2-Phenoxyethanol	Flowery, soapy	2104	1220	3	1	O, RI, MS, RC	T, BF, UV, S, DF	T, BF, UV, S, DF
70	Unknown	Rubber-like, perfume, flowery[g]	2152	—	1	n.d.	—	T, BF, UV, S, DF	BF, S, DF
71	Unknown	Rubber-like, fruity[g]	2170	—	3	n.d.	—	T, BF, UV, S, DF	BF, S
72	Unknown	Rubber-like, flowery[g]	2252	—	1	1	—	T, BF, UV, S	T, BF, UV, S
73	2,4,6-Tribromoanisole	Cork-like	2274	1669	81	1	O, RI, RC	T, BF, S	T, BF, S
74	Ethyl palmitate	Waxy	2292	1996	n.d.	n.d.	O, RI, MS, RC	BF, UV, S, DF	S
75	Unknown	Plastic[g]	2304	—	1	n.d.	—	T	BF
76	Hexadecan-1-ol	Waxy	2372	1876	1	n.d.	O, RI, MS, RC	T, BF, UV, S, DF	BF, UV, S
77	Methyl stearate	Waxy[g]	2408	2118	3	n.d.	O, RI, MS, RC	T, BF, UV, S, DF	BF, S
78	Unknown	Rubber-like, metallic, fecal[g]	2442	—	3	3	—	T, BF, UV, DF	T, DF
79	Unknown	Rubber, fecal[g]	2454	—	3	3	—	T, BF, UV	T, DF

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Table 1: Continued.

No[a]	Odorant	Odor attribute[b]	RI on[c]		FD-factor[d]		Identification method[e]	RAS 1[f]	RAS 2[f]
			FFAP	DB-5	RAS 1	RAS 2			
80	Unknown	Rubber-like, flowery[g]	2504	—	1	9	—	T, BF, UV, S, DF	T, BF, UV, S, DF
81	Unknown	Rubber-like, plastic-like[g]	2525	—	81	3	—	T, BF, UV, S, DF	T, BF, UV, S
82	Unknown	Fecal[g]	2542	—	3	n.d.	—	T, BF	BF
83	Methyl dihydrojasmonate	Flowery, fruity, soapy	2625	—	729	1	O, RI, MS, RC	T, BF, S, DF	—
84	Unknown	Fruity, green[g]	2691	—	9	1	—	T, BF, UV, S, DF	T, DF
85	Unknown	Flowery, plastic-like[g]	2871	—	3	3	—	T, BF, UV, S, DF	T
86	Unknown	Flowery, plastic-like, sweaty[g]	3004	—	9	1	—	T, BF, UV, DF	BF, DF

Note: The FD factors of the odorants obtained by cAEDA, the respective retention indices (RI), the identification criteria, and the compartments where each compound was detected at FD1 are displayed.
[a]Numbering according to elution on DB-FFAP capillary.
[b]Odor quality described by the Chair of Aroma and Smell Research in the FAU database.
[c]Linear retention index (RI) on DB-FFAP and DB-5 capillaries.
[d]Flavor dilution (FD) factor on DB-FFAP capillary from 1 to 729, where 1 means the nondiluted sample. Determined in T samples from RAS 1 and RAS 2.
[e]Identification based on comparison of retention indices (RI), odor quality (O), mass spectrum (MS-EI) obtained by GC–MS analysis (MS), and reference compound (RC).
[f]Compartments detected in RAS 1 and RAS 2: T, tank outlet, DF, drum filter outlet; BF, biological filter outlet; UV, ultraviolet system outlet; S, sludge inside drum filter.
[g]Odor quality determined by one trained panelist at the sniffing port.
[h]Compounds previously identified in Russian Sturgeon raised in RAS [17].

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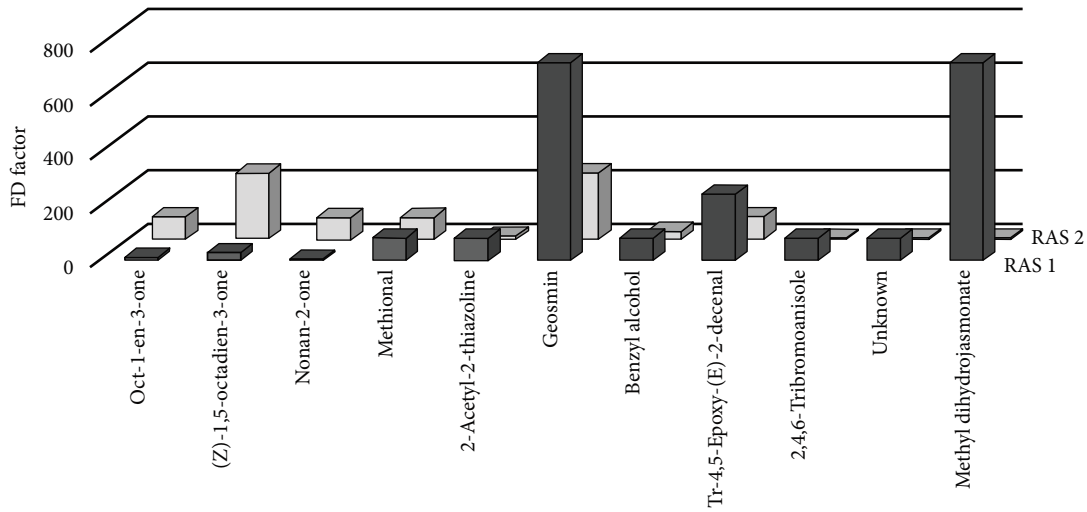


FIGURE 3: Aroma-active compounds with their respective FD factors determined in the tank from RAS 1 – grey and RAS 2 – striped grey. Only odorants with FD ≥ 81 are included.

3 in RAS 1 and RAS 2, respectively). Figure 3 displays the odorant compounds identified with the highest FD factors (≥81) in at least one of the tank (T) samples.

Overall, the reported odorants can be categorized in different chemical classes (Supporting Information 1: Figure S3). These include aldehydes, followed by alcohols, terpenes, ketones, lactones, fatty acid esters, and sulfur-containing compounds. Other compounds could also be identified, such as 1,4-xylene, 1,3-xylene, cumene, pseudocumene, 1-tetradecene, BHT, methyl dihydrojasmonate, acetic acid, 2-phenoxyethanol, and 2,4,6-tribromoanisole (Table 1).

3.3. Geosmin and 2-MIB Distribution Across Two Independent RAS. Geosmin and 2-MIB concentrations were measured in water samples to identify possible hotspots in the two RAS facilities. The levels of geosmin in the water samples ranged from 84.7 to 432.1 ng/L (RAS 1) and 70.4–177.7 ng/L (RAS 2) (Figure 4). The geosmin concentration was two–five times higher in the water from the sludge inside the drum filter than in the other compartments for the same RAS farm. The higher levels of geosmin in the sludge water within the same RAS farm were statistically significant for RAS 1 (chi-square = 12.900, $p = 0.012$) and RAS 2 (chi-square = 13.257, $p = 0.010$). Following post hoc tests (Dunn–Bonferroni) showed that the geosmin concentration was higher in the sludge water and differed significantly from all other compartments within the same RAS ($p < 0.05$).

For 2-MIB, the concentration ranged from 40.6 to 43.3 (RAS 1) ng/L and from 60.0 to 81.9 ng/L (RAS 2). Within the same RAS farm, there were significant difference in the 2-MIB concentration between the compartments in RAS 1 (chi-square = 9.867, $p = 0.043$) and RAS 2 (chi-square = 12.005, $p = 0.017$). Following post hoc analyses (Dunn–Bonferroni) in RAS 1 and RAS 2 revealed that the 2-MIB concentrations in the water from the sludge inside the drum filter differed significantly from those of all other compartments ($p < 0.05$). Additionally, the drum filter outlet water was lower and statistically different from the tank, biological filter, and UV system outlet water within the same RAS farm ($p < 0.05$). The outcomes are summarized in Figure 3, while the raw data are provided in Supporting Information 1: Tables S3 and S4.

4. Discussion

4.1. Sensory and Analytical Results. The odor qualities of the identified odor-active compounds corresponded very well with the odor descriptors reported by the panelists in the aroma profile analysis. Thereby, the moldy descriptor, which was rated with an intensity of 1.8–3.8 in both RAS farms, may be related to the identification of many compounds described as moldy, namely 1-tetradecene, geosmin, and 2-MIB. The attribute earthy, detected with a higher intensity in RAS 1 than in RAS 2, might be related to geosmin. This result agrees with cAEDA analysis, in which geosmin had a higher FD factor in RAS 1 compared to RAS 2 (Table 1). In addition, myrcene (10) and two unknown compounds (47 and 60) with an earthy smell were detected. Even with a low FD factor, such compounds might contribute to a given attribute via additive or synergistic effects. Geosmin and 2-MIB have been studied extensively as the main undesirable compounds in RAS, but other compounds might be associated with off-flavor notes and lead to perceptual interaction effects [5].

Fishy and seawater-like/algae-like odor notes were higher in RAS 1 compared to RAS 2 for the water from the sludge inside the drum filter. For these characteristics of odor, no specific compounds were found. Nevertheless, these impressions are known to be caused by a mixture of odorants in fish [20]. In smell research, complex combinations of odorants frequently produce odor perceptions that are not able to be conveyed by any of their individual substances. The overall aroma impression of complex odorant combinations can be modulated by additive and synergistic effects [21]. For example, heptanal and (*E*,*Z*)-3,5-octadien-2-one, two lipid oxidation products, can contribute to a fishy malodor by enhancing the perception of the unpleasant fishy odor [21]. In another study,

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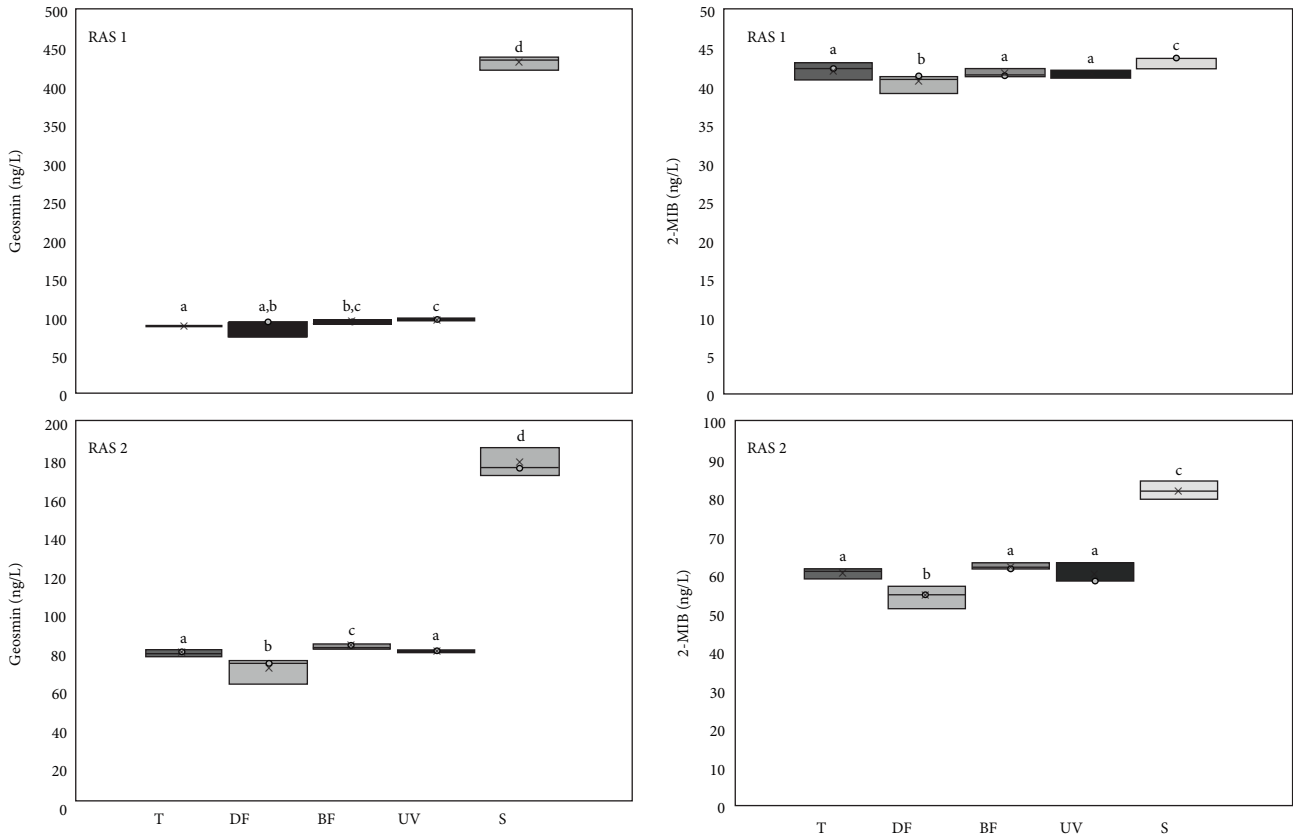


FIGURE 4: Concentration of geosmin and 2-MIB in water (ng·L^{-1}) across different compartments from RAS 1 and RAS 2: T – tank outlet, DF – drum filter outlet, BF – biological filter outlet, UV – ultraviolet system outlet, S – sludge inside drum filter. For each graph, distinct uppercase letters indicate significant differences between the samples for geosmin and 2-MIB amount, as determined by the Kruskal–Wallis test $p \leq 0.05$. Observe the different scaling of the *y*-axis. All samples were collected once at the same time, and extractions were performed in triplicate for quantification.

a fishy and metallic off-odor was detected in human milk, whereas the odorants generated were mostly oxidation products of long-chain (poly)unsaturated fatty acids, such as (Z)-1,5-octadien-3-one, 1-octen-3-one, tr-4,5-epoxy-(E)-2-decenal, and (Z)-3-hexenal [22]. Additionally, any odorant described as fishy was identified, and the representation of the fishy odor was attributed to a combination of the above mentioned compounds [22]. Nevertheless, to comprehend the connection between the perceived fishy aroma and its odorant composition, as well as any synergistic or masking effects, more research would be necessary. This would include, for instance, odorant quantification, aroma recombination, and omission tests. This, however, was not the objective of this study; instead, it was to identify odorants and compare their scent profiles using water from RAS.

4.2. Odorant Compounds Through RAS. Saturated and unsaturated aldehydes, followed by alcohols, terpenes, and ketones, were the most prevalent odorants found in this work. In the aldehyde group, only *tr*-4,5-epoxy-(*E*)-2-decenal (63) had a high FD factor in RAS 2 (FD 243). This compound has previously been identified in fish, feed, and water from earthen ponds [18, 23]. Regarding alcohols, geosmin (54) was detected as the major odor-active compound in both RAS, with FD factor 729 and 243 in RAS 1 and RAS 2, respectively, while 2-MIB (41) was detected with a low FD factor. Geosmin and 2-MIB are well-known off-flavor compounds in fish farming, and the increase in these compounds in RAS has been associated with the organic-rich aerobic parts in RAS [11]. In addition, the odorant benzyl alcohol (56) was detected with a high FD factor (FD 81) in RAS 1. This compound was also identified in red mullet by AEDA but was not described as an odor-active compound [24].

Among the terpene groups, six compounds with a low FD factor were identified. The possible sources of these secondary metabolites are fish feed, water, and microorganisms such as cyanobacteria [25]. Concerning ketones, the mushroom and metallic-smelling compounds oct-1-en-3-one (20) and (*Z*)-1,5-octadien-3-one (24) were detected with a high FD factor only in RAS 1, with FD 81 and 243, respectively. These substances have been linked to off-flavors in freshwater fish, which could lead to a negative effect on the sensory quality of the seafood [5]. This is the first time that both substances have been reported in water from RAS. Additionally, it should be pointed out that other compounds with earthy or metallic nuances were detected in the present study within the terpene, ketone, and aldehyde groups, namely myrcene (10), (*Z*)-1,5-octadien-3-one (24) and *tr*-4,5-epoxy-(*E*)-2-decenal (63), which might enhance these off-flavor notes in water.

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Sulfur-containing compounds were reported with a high FD factor (FD ≥ 81), namely, methional (31) and 2-acetyl-2-thiazoline (51). These substances have been reported as odorant compounds in cooked shrimp, which enhances the desired flavor and adds to the distinctive aroma [26]. Methional was detected with a high FD factor in both RAS. This substance has a low odor threshold (0.45 μg/L in water) [27] and its formation is related to the Strecker degradation of methionine from raw materials [28]. 2-Acetyl-2-thiazoline was detected with a high FD factor only in RAS 1. This compound may be derived from amino acids, such as cysteine [29].

Lactones and fatty acid esters were identified as minor odorant groups and detected with a low FD factor in water samples. Among the lactones, γ-crotonolactone, octalactone, and nonalactone were detected in both RAS. γ-Crotonolactone has been used to treat and prevent aeromoniasis, and its effectiveness has been proven to stimulate an increase in the speed of mass accumulation in sturgeon fish reared in RAS [30]. Moreover, ethyl palmitate and methyl stearate were detected in the fatty-acid ester group. The presence of these compounds is well known in fish and might contribute to the aroma profile of sturgeon meat [31].

Other substances with smells ranging from flowery (methyl dihydrojasmonate and 2-phenoxyethanol) to waxy (BHT) were identified, and their presence is likely related to external influences in RAS. For example, methyl dihydrojasmonate was reported to have a high FD factor (FD ≥ 81) only in RAS 1. This compound is commonly used in personal care products and has been previously reported as an environmental contaminant in water [32], which should be taken as a warning to fish producers and water environmental protection agencies. In addition, the compounds 2-phenoxyethanol and BHT were detected with a low FD factor in both RAS. Regarding 2-phenoxyethanol, this compound is widely used as anesthetic in aquaculture to reduce the stress caused by procedures in fish, namely the handling, sorting, transportation, and administration of vaccines [33]. The synthetic antioxidant BHT was previously reported in wild and farmed fish, and the possible sources may be derived from fish feed and environmental contamination [34]. Moreover, due to the low concentration, an unknown compound (81) smelling of rubber and plastic with an FD factor ≥ 81 could not be identified.

In this study, the odorant compounds 1-butanol (7), myrcene (10), 1-tetradecene (28), γ-crotonolactone (49), BHT (57), 2-phenoxyethanol (69), ethyl palmitate (74), methyl stearate (77), and methyl dihydrojasmonate (83) are reported for the first time in water from RAS. Nevertheless, the substances 7, 10, 57, and 69 have been reported in farmed and wild seafood production from different sources, for example, feed, water, and phytoplankton [15, 23–25, 34].

Overall, the odor descriptors used in the aroma profile analysis and most of the compounds identified in RAS water in sturgeon culture are in accordance with our previous findings in Russian Sturgeon fillets raised in RAS [17].

4.3. Geosmin and 2-MIB Fluctuation. Geosmin and 2-MIB exist as (+) and (−) enantiomers, but off-flavor issues are caused by (−) enantiomers [35]. The low human detection threshold for geosmin and 2-MIB in water (3.8 and 15 ng/L, respectively) leads to an easy perception of these compounds [36, 37]. Due to their common occurrence in RAS, we quantified these compounds in water samples from different compartments.

Geosmin and 2-MIB were reported in water from all compartments at concentrations above the odor threshold (Figure 4). Overall, RAS 1 had a higher amount of geosmin than RAS 2, while RAS 2 had a higher amount of 2-MIB than RAS 1. The highest concentration of geosmin and 2-MIB in both RAS was detected in the sludge inside the drum filter; while the lowest concentration of these compounds was found after the drum filter. This result is in line with the literature for Tilapia production, in which the highest amount of these substances was also detected in the sludge at the drum filter [11]. Similarly, geosmin fluctuation was monitored in Pikeperch production and it was observed that the denitrification unit increased the concentration of geosmin while the biofilters had no effect [13]. Biofilters are frequently associated as possible hotspots for off-flavor compounds, like geosmin and 2-MIB, due to their microbial communities. According to this study the biofilters did not serve as a major source of off-flavor in these particular commercial RAS. The overall flow dynamics and water renewal rates of the systems may have decreased the growth of off-odor producing microorganisms in the biofilter compartments, as well as efficient biofilter management techniques (e.g., routine cleaning or design elements that limit the biofilm build-up of odor-producing microorganisms) are possible explanations [3, 13]. Therefore, water circulation may complicate geosmin and 2-MIB production hotspots detection. Accordingly, except for sludge inside the drum filter, the concentrations of these substances were comparable. Previous studies, where the geosmin concentration did not vary in various compartments within an RAS, and water recirculation balanced the concentration of off-flavors within the system, supported these findings [12, 16]. Methods to reduce geosmin and 2-MIB concentrations in RAS require more investigation, especially in the latter phases of production when fish get close to market size, to guarantee effectiveness and viability of the production. Nevertheless, reducing off-flavors concentrations was not the goal of this study, as we focused on sturgeon at an early growth stage.

It has been reported that geosmin and 2-MIB amount in water is influenced by the fish species, water temperature, fish fat content, fish biomass, production method, and system outlook [13, 38, 39]. Therefore, differences between RAS 1 and RAS 2 are probably caused due to a number of factors that affect water quality, microbial activity, and the presence of off-flavor, such as fish biomass, size, age, feed load, system design, and flow dynamics [3, 6, 38]. These variables probably influenced the variations in sensory characteristics and compound concentrations that were noted. For example, the highest concentration across different compartments in water from a Rainbow trout RAS farm was 350 ng/L for geosmin and 35 ng/L for 2-MIB [40], while on a hybrid tilapia RAS farm, the highest concentration was 47 ng/L for geosmin and 260 ngL^{-1} for 2-MIB [11]. Therefore, this study reports for the first time the concentration of geosmin and 2-MIB in RAS water from a

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Sturgeon farm. Additionally, the study reported here was conducted on a commercial RAS farm, which can provide more information on the challenges related to off-flavors encountered on commercial farms. Furthermore, the concentration of geosmin and 2-MIB across different compartments and after each treatment combination can give useful information for large-scale operation optimization. Although numerous interesting odorants were found, the quantification of those would require more effort and this was not the main goal of the investigation. Nevertheless, future quantitative research is necessary to better understand the contribution of odorant compounds and their contribution to off-flavors in RAS.

5. Conclusion

The overall aroma profile and potential odorants, as well as the concentration of geosmin and 2-MIB across five compartments in two independent RAS facilities are reported for the first time in this work. Through a sensory-analytical approach, 86 odorants were olfactorily reported, and 44 substances were identified. A significant degree of similarity was observed qualitatively among the samples from four compartments (tank, drum filter, biological filter, and UV system outlet) of RAS 1 and RAS 2. Additionally, the aroma profile of the water samples agreed with the instrumental analysis results. Apart from geosmin and 2-MIB, other compounds with earthy or moldy notes were reported, for example 1-tetradecene and myrcene, potentially contributing to the off-odor notes in water.

Many odorant compounds were identified for the first time in RAS water, namely 1-butanol, myrcene, 1-tetradecene, γ-crotonolactone, BHT, 2-phenoxyethanol, ethyl palmitate, methyl stearate, and methyl dihydrojasmonate. Furthermore, the distribution of geosmin and 2-MIB in different compartments of the two RAS facilities revealed that the only potential source of off-flavors was the sludge from the drum filter, whereas the other compartments were comparable.

Finally, adequate information for monitoring odorous compounds through combined production techniques is not fully available. Therefore, sensory evaluation in RAS water can serve as a tool to assess off-flavor issues in commercial farms and anticipate consumer acceptance of RAS fish, as well as monitor water quality from environmental perspective. Accordingly, the characterization of the compounds can be used as valuable information for further quantitative comparisons in RAS, such as variations between species or RAS farm designs. However, further research is required, especially on combined techniques like flow-through/RAS, RAS/cages, and RAS/ponds.

Data Availability Statement

This study's supporting data are available from the corresponding author upon appropriate request.

Disclosure

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Mariana Rodrigues da Silva: conceptualization, investigation, methodology, validation, formal analysis, data curation, writing – original draft, visualization. **Pedro Martínez Noguera:** investigation, methodology, review and editing. **Bastien Debeuf:** sampling, conceptualization, methodology, writing – review. **Mikael A. Petersen:** methodology, review and editing. **Helene M. Loos:** conceptualization, methodology, supervision, writing – review and editing. **Andrea Buettner:** conceptualization, writing – review and editing.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) Figure S1: Plan of RAS with overview of the multiple compartments and water flow (). BF, biofilter; DF, drumfilter; UV, ultraviolet. Sampling points marked with 1–5: 1 = outlet tank; 2 = outlet drumfilter; 3 = biofilter outlet; 4 = ultraviolet outlet, and 5 = sludge. The blue arrow indicates the oxygenation system (jet platform). Same water inlet from a reservoir, in six 35 m^3 tanks for RAS 1 and four 55 m^3 for RAS 2, each with an individual water inlet and outlet. Figure S2: Calibration curves used for the quantification of geosmin and 2-methlisoborneol in water (SBSE–GC–MS). Figure S3: Chemical classes of odorants identified in all water samples. The value represents the number of compounds detected within each chemical family. Table S1: Merit parameters of the methods used for quantification of geosmin and 2-methylisoborneol in water (SBSE–GC–MS). Table S2: Descriptive odor analysis of water from production RAS 1 and RAS 2. Displayed are the average intensity ratings (±standard deviation) provided by all panelists ($n = 7–8$) on a scale from 0 (no perception) to 10 (strong perception). Compartments: BF, biological filter; DF, drum filter; S, sludge; T, tank; UV, ultraviolet. Table S3: Total amount of geosmin and 2-MIB (ng/L) in water samples across different compartments from RAS 1 and RAS 2 determined by GC–MS analysis. Average ±standard deviation (triplicate). Compartments: BF, biological filter; DF, drum filter; S, sludge; T, tank; UV, ultraviolet. Table S4: Results of the statistical analysis regarding geosmin and 2-MIB concentrations in water samples across different compartments from RAS 1 and RAS 2,

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comprising the Chi^2, p-value of the Kruskal–Wallis test as well as the test statistic (z) and significance (p) of Dunn–Bonferroni test. Compartments: BF, biological filter; DF, drum filter; S, sludge; T, tank; UV, ultraviolet.

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