Aquaculture 530 (2021) 735754

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Case study on depuration of RAS-produced pikeperch (*Sander lucioperca*) for removal of geosmin and other volatile organic compounds (VOCs) and its impact on sensory quality

Raju Podduturi[a], Mikael Agerlin Petersen[b], Martin Vestergaard[c], Grethe Hyldig[d], Niels O.G. Jorgensen[a,*]

[a] Microbial Ecology and Biotechnology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark
[b] Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark
[c] Aquapri Denmark, Haugårdsvej 4B, 6600 Vejen, Denmark
[d] National Food Institute, Technical University of Denmark, Kemitorvet 204, 2800 Kgs. Lyngby, Denmark

ARTICLE INFO

Keywords:
Pikeperch production in RAS
Off-flavor
Geosmin removal
Depuration

ABSTRACT

Effect of depuration on content of geosmin in pikeperch (*Sander lucioperca*) produced in a commercial RAS farm was examined during a 15-day period. Concentrations of geosmin in the fish were related to geosmin content in the water. For depuration, half of the water volume in a 230 m^3 production tank was replaced daily with geosmin-free water. After 8 days of depuration and absence of feeding, content of geosmin in the fish was reduced from 710 ± 245 ng/kg to 165 ± 50 ng/kg (mean ± SD, $p < .01$). Additional depuration for 7 days only reduced the geosmin content to 135 ± 24 ng/kg. Geosmin concentration in the water was initially 34 ng/L but declined to 10 ng/L after 15 days. Changes in geosmin concentrations in water of the depuration tank indicated that geosmin was released by the fish during the depuration. In addition to removal of geosmin, the depuration also decreased concentrations of 28 different volatile compounds from the fish. Sensory analysis showed decrease in intensity of geosmin flavor upon depuration and improved the overall sensory quality of the fish after 2 weeks of depuration. Our study shows that geosmin and other off-flavors in pikeperch from RAS production can efficiently be removed to be a level that is below the threshold to most consumers.

1. Introduction

Fish production in closed-containment systems, such as recirculated aquaculture systems (RAS), has been increasingly applied in recent years due to advantages with respect to a high production volume at a low water consumption and controllable environmental parameters to minimize mortality. Even though RAS farms facilitate the production of high-quality fish, occurrence of off-flavors has become a major concern for RAS-farmed fish, as well as for fish produced in other types of freshwater systems, e.g. in ponds or in cages in rivers and reservoirs (Houle et al., 2011; Petersen et al., 2011; Schrader et al., 2005; Schrader and Summerfelt, 2010). Presence of off-flavors in fish deteriorates the sensory quality and may cause significant financial loss to the fish producers (Badiola et al., 2012). The financial loss incurred by the US channel catfish industry was reported to be 30% due to off-flavor in the fish (Engle et al., 1995).

Two commonly reported off-flavor compounds in water in RAS and in other freshwater aquaculture systems are geosmin and 2-MIB (2-methylisoborneol), which attribute earthy and musty flavor to the cultured fish. Geosmin and 2-MIB are terpenoid alcohols that mainly are produced by actinobacteria (large group of Gram-positive bacteria) and myxobacteria (also known as slime bacteria and belonging to the Gram-negative class of Deltaproteobacteria) (Auffret et al., 2011; Guttman and van Rijn, 2008; Lukassen et al., 2016). Cyanobacteria can also cause off-flavor formation along with the other two groups in outdoor RAS, when light is present (Houle et al., 2011).

Fish take up geosmin and 2-MIB from the water mainly through gills but a fraction could also be through skin and gut (Howgate, 2004). The uptake mechanism is diffusion, and the diffusion process depends on partition coefficient of the compounds and their concentration gradient. After uptake, the compounds accumulate in the flesh and fat tissue. Since diffusion is a reversible process, depuration of off-flavor compounds can be conducted to remove the compounds by placing off-flavor-tainted fish in clean, off-flavor free water (Howgate, 2004;

* Corresponding author.
E-mail address: nogj@plen.ku.dk (N.O.G. Jorgensen).

https://doi.org/10.1016/j.aquaculture.2020.735754
Received 9 June 2020; Received in revised form 20 July 2020; Accepted 20 July 2020
Available online 24 July 2020

Schram et al., 2017). However, geosmin uptake in fish is significantly faster (saturation is completed within hours) than removal by depuration (typically lasts several days) (Howgate, 2004; Robertson et al., 2005). The rate of depuration in fish is influenced by several parameters, including initial concentration of the compound, fat content of the tissue, temperature, and physical activity of fish (Howgate, 2004; Johnsen et al., 1996; Schram et al., 2016).

Pikeperch (*Sander lucioperca*) is a relatively new species in the RAS industry in Europe, and Denmark is one of the Nordic countries pioneering large scale commercial breeding of this high-value fish (Dalsgaard et al., 2013). Pikeperch is mainly sold as a luxury fish and due to the high market price, it is essential to produce pikeperch without off-flavors to ensure economical sustainability. Even though geosmin occurs at relatively low concentrations in rearing water of RAS fish production tanks (typical concentrations range from 10 to 20 ng/L; Podduturi et al. (2020)), the concentration level in RAS has been shown to cause an unpleasant off-flavor tainting of rainbow trout produced in RAS (Petersen et al., 2011). For pikeperch, Alexi et al. (2018) conducted a sensory and consumer analysis of aquaculture-produced fish and observed that pikeperch fillets (cultured in France) had a negative consumer acceptability due to a characteristic earthy odor and flavor. Depuration of the fish proved to have a positive effect on improvement of the taste.

Even though geosmin and 2-MIB unarguably are two major compounds responsible for tainting of cultured fish, there could be several other compounds occurring in fish, either from pre-harvest uptake or from post-harvest handling, and they could potentially deteriorate the sensory quality of cultured fish. Sensory analyses indicate that off-flavor characteristics in fish are not limited to muddy and musty flavors. There are several other undesirable flavors that may occur in fish, e.g. woody, piney, rancid, metallic, sulfury and chemical (diesel/petroleum like) flavors (Podduturi et al., 2017; Tucker, 2000; Van Der Ploeg, 1991). Terpenes, e.g. β-caryophyllene, α-humulene and β-ionone, are mainly responsible for woody and piney odors, while aldehydes, e.g. 2-nonenal, can impart rancid flavor to fish, and aromatic compounds could typically be responsible for petroleum and metallic flavors (Podduturi et al., 2017; Selli et al., 2006). These undesirable flavors can be of microbial, biochemical, dietary origin, or may be environmental pollutants. Effects of depuration of off-flavor compounds other than geosmin and 2-MIB in farmed fish have only been examined in very few studies (Lv et al., 2018; Palmeri et al., 2008), despite knowledge on depuration effects to the total volatile composition is essential to understand and improve the sensory quality of fish.

While a positive effect on fish quality due to depuration has been observed for some fish species cultured in RAS (Davidson et al., 2014; Petersen et al., 2011; Robertson et al., 2005), the depuration process adds extra costs to the production (Azaria and van Rijn, 2018). Thus, the depuration requires a high consumption of clean water and causes weight loss of the fish (feeding is avoided to reduce microbial growth in the water). Wet weight loss during depuration (duration of up to 2 weeks) was reported to make up 6% in Atlantic salmon (Burr et al., 2012) and 4% in murray cod (Palmeri et al., 2008), and was assumed to be 5% in RAS-farmed pikeperch (Kamstra, 2003). As of now, depuration is only reliable option for the production of taint-free fish, and therefore optimal conditions for the depuration process are needed to help the fish farmer in minimizing the costs of production.

Effects of depuration of pikeperch in commercial RAS farms have so far not been determined. Therefore, the objective of the present study was to determine efficiency of depuration on marketable pikeperch, cultured in commercial, indoor RAS, and to establish optimal depuration conditions for effective removal of geosmin or at least removal to below the sensory threshold. Concentrations of geosmin in water of a depuration tank and levels of geosmin in fish from the tank were followed before and during a 15-day depuration period. In addition to geosmin, impact of depuration on composition of other volatile compounds (aldehydes, alcohols, ketones and terpenes) in the fish flesh was also examined. Flavor of the fish was subsequently characterized by a trained sensory panel.

2. Materials and methods

2.1. RAS overview

This study was conducted in the RAS breeding facility for commercial indoor production of pikeperch (*Sander lucioperca*), located in Vejen in southern Denmark. Overview and operation of the system are described in Podduturi et al. (2020). Briefly, the RAS facility consists of eight 230 m^3 grow-out with tanks with diameter of 9.2 m and depth of 3 m. Each tank has an individual water inlet and outlet, and retention time of water in each tank is 30 min. Water treatment includes drumfilter, UV exposure, biofilter units and an inline denitrification unit. The total fish biomass in the 8 tanks is 60–80 t and the average feeding rate is 0.6% of the biomass.

2.2. Depuration process

The depuration process was conducted in the same 230 m^3 tanks as used for fish production. When the fish reach marketable size (individual weight of 0.95–1.8 kg, corresponding to 15,000 kg biomass in each tank), feeding was stopped 48 h prior to depuration. During this process, the tank remains connected to the system and water circulates through as in normal production process. At the time of depuration, the tank is disconnected from the system and around 115 m^3 (half of total volume) of tank water is replaced by fresh groundwater (free of geosmin). Water in the depuration tank was replaced as continuous dilution process with a constant flow of incoming water of 78 L/min. This exchanged half of the 230 m^3 water in the tank every day. It might have been more effective to replace all water in a single operation, but it was not possible for practical reasons. The temperature of incoming water was 8–10 °C and the average temperature of water inside the depuration tank throughout the process was about 13 °C.

The depuration process continues for 15 days and every day half the tank water is exchanged by fresh water. For analysis of geosmin concentrations in the water, 20 mL water samples were collected every day from the depuration tank outlet and preserved with 5% NaCl and kept refrigerated until analysis. Every day from stop of the feeding to end of the 15-day depuration period (except for Day 10 and 11), 5 fish were collected (total of 75 fish for the sampling period). Fish were processed and filleted on site and kept frozen (−20 °C) until the analysis. Weight of the processed fillets ranged from 160 to 380 g. Chemical and sensory analyses were performed on the same fillets.

2.3. Analysis of geosmin and 2-MIB content in water

Geosmin content in the water was analyzed by stir bar sorptive extraction (SBSE) – Gas chromatography mass spectrometry(GC- MS) as described in Podduturi et al. (2020). A commercial stir bar of 1.00 cm length (Twister®, Gerstel GmbH, Germany) coated with polydimethylsiloxane (PDMS; thickness of 1.00 mm) was added to 10 mL of water in a 10 mL glass vial. SBSE was carried out at room temperature by stirring at 1000 rpm for 120 min. After extraction, the twisters were removed with forceps, rinsed with water, dried with lint-free tissue and transferred to thermal desorption tubes. A calibration curve was prepared from GC grade mixture of geosmin and 2-MIB pure compounds (Sigma-Aldrich) at dilution series of 1, 10, 50, and 100 ng/L (triplicates) in water and used for quantification. The limit of detection (LOD) and quantification (LOQ) of the method used for geosmin and 2-MIB are 1 ng/L. As described in Podduturi et al. (2020), variation between replicates are less than 5%. The water analysis was carried out on single samples due to the low RSDs of geosmin concentrations and limited resources for GC–MS analysis.

2.4. Volatile composition analysis of fish fillets by dynamic headspace extraction

For determination of volatile organic compounds, including also geosmin and 2-MIB in fish flesh, a dynamic headspace analysis was used as explained in Podduturi et al. (2017). Briefly, 10 g fish flesh was homogenized in 30 ml of water in a 250 ml gas-washing bottle, using an Ultra Turrax homogenizer (Ika, Germany). Volatile compounds in the homogenates were collected on Tenax TA traps by purging with N_2 for 60 min at flow rate of 150 ml/min at 50 °C. For quantification of geosmin, fish meat samples were spiked with geosmin at 100, 250, 500, and 1000 ng/kg prior to homogenization. After volatile extraction, the traps were further purged with N_2 at 100 mL/min for 10 min to remove water from the traps. The LOQ of geosmin and 2-MIB in fish flesh was 100 ng/kg.

2.5. GC–MS analysis

Volatiles adsorbed on Twisters and Tenax TA traps were desorbed in two stages using an automatic thermal desorption unit (TurboMatrix 350, Perkin Elmer, Shelton, USA). Primary desorption was carried out by heating the trap/twister to 250 °C with a flow of carrier gas (50 mL/min H_2) for 15 min. The stripped volatiles were trapped in a Tenax TA cold trap (30 mg held at 5 °C), which was subsequently heated at 300 °C for 4 min (secondary desorption; outlet split 1:10). This allowed for rapid transfer of volatiles to a gas chromatograph–mass spectrometer (GC–MS, 7890A GC-system interfaced with a 5975C VL MSD with Triple-Axis detector from Agilent Technologies, Palo Alto, California) through a heated (225 °C) transfer line.

Separation of the volatiles was carried out on a DB-Wax capillary column (30 m length × 0.25 mm internal diameter and 0.5 μm film thickness) using H_2 as carrier gas with an initial flow rate of 1.4 mL/min. The GC oven temperature program started at 40 °C for 1 min, then raised to 97 °C at the rate of 8 °C/min followed by a hold for 10 min, and finally increased from 97 °C to 240 °C at the rate of 8 °C/min with hold for 10 min at 240 °C. The mass spectrometer was subjected to electron ionization mode at 70 eV. Mass-to-charge (m/z) ratio between 15 and 300 were scanned. Simultaneously, data were collected in selected ion monitoring mode, monitoring mass 95 and 107 for 2-MIB and mass 112 for geosmin.

2.6. GC–MS data processing

For quantification of geosmin and 2-MIB in water and fish samples, chromatograms were processed using MSD Chemstation software (v. E.02.00, Agilent Technologies). Peak area of ions 112 and 95 were collected at corresponding retention of time of geosmin and 2-MIB standard chromatograms. Calibration curves were calculated and used to interpret the concentration of detected compounds in the fish. For the analysis of total volatile profile, peak areas and mass spectra were extracted from the chromatograms using the PARAFAC2 based software PARADISe (Johnsen et al., 2017) and mass spectra were identified using the NIST05 database. Peak areas were used as relative measures of concentration. Retention indices (RI) were calculated for all detected compounds by running an alkane standard mixture (C_5–C_{22}, Supelco; www.sigmaaldrich.com).

2.7. Sensory profiling

Fish sample from each day of depuration and prior to depuration were examined by sensory profiling (n = 17). The sensory panel consisted of four assessors that all were selected, tested, and trained in descriptive analysis according to ISO 11035. The vocabulary was developed in the first session. The following sessions were quantitative to train evaluation of the descriptors on a line scale. Each descriptor was evaluated on an unstructured 15 cm scale anchored 1.5 cm from both ends with 0 = none and 15 = strong intensity of the descriptor. The anchor points were placed 1.5 and 13.5 cm from 0 on the scale and marked with “little” and “much” of the attribute intensity (Meilgaard et al., 1999). For odor (O), the sensory panel used the descriptors Sweet, Warm milk, Green, Mushroom/mould, Geosmin and 2-MIB; for flavor (F), the descriptors were Green, Sweet, Sourish, Metalllic, Mushroom/Mould, Geosmin and 2-MIB; and for taste (T), the descriptors were Burning and Astringency.

The sensory analysis was performed in separated booths under normal daylight and at ambient temperature (ISO 8589). The assessors used water and flat bread to clean the palate between samples. Data were collected using a computer system (FIZZ Network version 2.0, Biosystems, France). Prior to the sensory analysis, the samples were minced and placed in individual porcelain bowls and covered with porcelain lids with three-digit codes before being heated in a pre-warmed convection oven (Rational Combi-Dämpfer CCM; www.gastrodax.de) with air circulation to a core temperature of 70 °C. After heat treatment, the samples were immediately served to the panel. The samples were served one by one in random order. A sample without any sensory detectable 2-MIB and geosmin was used as reference.

2.8. Statistical analysis

All the statistical data analyses and graphic visualization were performed using JMP®, Version 14, SAS Institute Inc., Cary, NC, 1989–2019. Geosmin concentrations in fish flesh during depuration were illustrated with a box plot analysis. A linear mixed model fitting, restricted maximum likelihood (REML) variance was calculated to test significance of depuration days on geosmin concentration. Linear regression analysis was used to calculate the relationship between fish weight and geosmin content. To assess the significance of depuration on sensory variables, a Student's t-test was used.

3. Results

3.1. Geosmin content in water

After stop of feeding two days before start of the depuration, geosmin concentration in water from the selected depuration tank was about 34 ng/L but increased slightly to 36 ng/L 24 h later (Fig. 1). On the first day of depuration (Day 1), when half of the tank volume was replaced by fresh groundwater, the geosmin level was reduced by dilution effect from 36 to 21 ng/L. After additional 24 h of depuration (Day 2), the geosmin content had increased by 7 ng/L, after which the geosmin concentration began declining to 10 ng/L on Day 15. If content of geosmin in the tank was only controlled by dilution due to the daily water exchange, a different decline in geosmin would have been predicted, as shown in Fig. 1. There were no detectable concentrations of 2-MIB in neither the system water nor the depuration tank water. The inlet water used for depuration was tested for presence of geosmin and 2-MIB, but no traces of these compounds were found.

3.2. Geosmin and 2-MIB content in fish

Geosmin concentration in fillets from 75 fish, collected from 2 days prior to depuration and during the 15 days of depuration, showed an increase from the day feeding ended (600 ± 25 ng/kg) to the subsequent day, when the geosmin content had increased up to 920 ng/kg (mean of 710 ± 245 ng/kg) (Fig. 2). A simultaneous increase of geosmin was measured in the tank water between Day −2 and Day −1, as mentioned above.

When depuration started at Day 1, mean geosmin content in the fish was 650 ± 130 ng/kg (average of 650 ng/kg), after which the concentration began declining to reach a minimum of 100 ng/kg (average of 135 ± 25 ng/kg) at Day 15 when the depuration ended. Mean

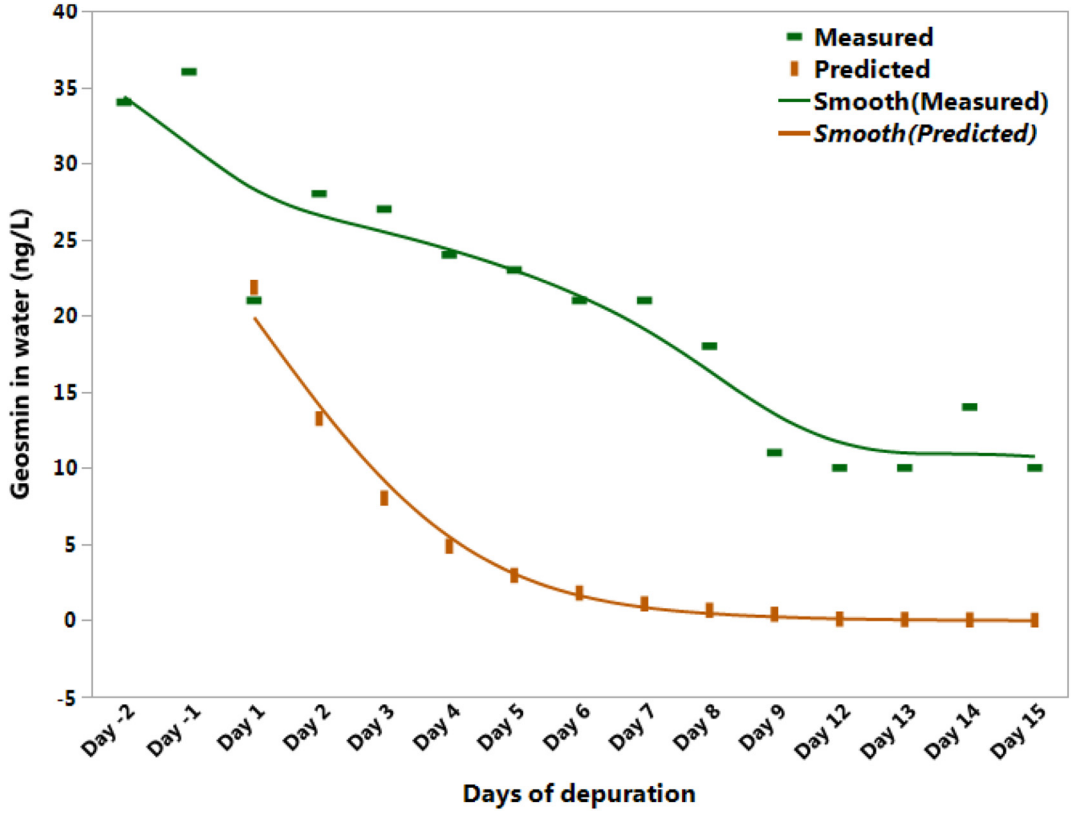

Fig. 1. Geosmin concentration in water from the depuration tank during depuration of pikeperch over 15 days. Days before start of depuration are indicated as Day −2 and Day −1. Green markers show measured geosmin level in water, while the orange markers indicate the predicted geosmin level, calculated from the water exchange rate. Single analysis of geosmin was conducted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

geosmin levels in fish between Day 3 and Day 15 of the depuration period were significantly lower ($p < .01$) than the geosmin in the fish prior to depuration (Day −2). The average geosmin content in the fish declined only until 8 days of depuration, when an unexpected increase was observed on Day 9 (rise from 165 ± 25 ng/kg to 232 ± 58 ng/kg). After that, the decrease in geosmin continued for the following days to reach the level of 135 ± 25 ng/kg at Day 15 (close to the level of 165 ng/kg on Day 8). There was no significant difference between geosmin content of fish from Day 8 to Day 15 of the depuration. The geosmin removal rate in the fish did not appear to be a simple diffusion process, as the daily geosmin removal rate ranged from 0.7 to 32% (average of 17%) during 8 days of depuration. Linear regression analysis between weights of fish to its geosmin content indicated a significant and positive correlation during Day 1 to Day 3 of depuration ($R^2 = 0.679$, $p < .01$, Fig. 3). No relationship was observed between weight and geosmin content of the fish from Day 4 to Day6 of depuration and Day 7 to Day 15 of depuration.

The off-flavor compound 2-MIB was only detected in the fish on Day −2 and Day −1 and at concentrations below 50 ng/kg, or less than the analytical limit of quantification (LOQ) (no data shown). Due to the low concentration, no depuration effects on 2-MIB removal were seen.

3.3. Volatile composition of the fish

A total of 52 volatile compounds were detected in the pikeperch fillets. Among those were aldehydes (12), alcohols (9), ketones (7), terpenes (4), sulfur compounds (2), benzene compounds (17) and one acid. Along with geosmin, there were around 28 other volatile compounds decreased significantly after 1 or 2 weeks of depuration ($p < .05$, Table 1). Depuration had no statistically significant influence on the levels of around 21 volatile compounds in the fish.

Low-molecular and early-eluting aldehydes from C_3 to C_5 and their methylated forms decreased upon depuration, while levels of C_6 to C_9 aldehydes were not affected. The depuration also seemed to lower the content of simple alcoholic volatiles; both straight chained (e.g. propanol, pentanol and hexanol) and branched (2-pentenol) compounds, while there was no influence on methylated and ethylated alcoholic compounds. Four out of seven detected ketones were reduced after depuration, including 2-butanone, 2,3-pentanedione, 6-methyl-5-hepten-2-one and acetophenone. Interestingly, the level of one of the ketones (acetoin) increased in the fish after depuration. Along with geosmin, another terpene compound (caryophyllene oxide) was also reduced upon depuration, while levels of limonene and carvone remained unchanged. Two sulfur compounds, 2-aminoethyl hydrogen sulfate and dimethyl disulfide, were detected in the fish, and both compounds had declined after depuration. Among the detected benzene compounds, nine compounds decreased during depuration, while six compounds were not affected, and levels of two compounds had increased.

3.4. Sensory profile of fish

Sensory analysis of pikeperch sampled before and after depuration by the assessor panel showed that the intensity of geosmin, 2-MIB and metallic flavors was significantly lower in the fillets after 8–15 days of depuration, as compared to before depuration ($p < .05$, Fig. 4). In contrast, the intensity of green and sweet flavors had significantly increased after 8–15 days of depuration, relative to fish without depuration ($p < .05$). Depuration did not influence sourish or mushroom flavors of fish. The astringent taste was reduced significantly ($p < .05$) by depuration for 8–15 days. The weak burning taste was not affected by the depuration.

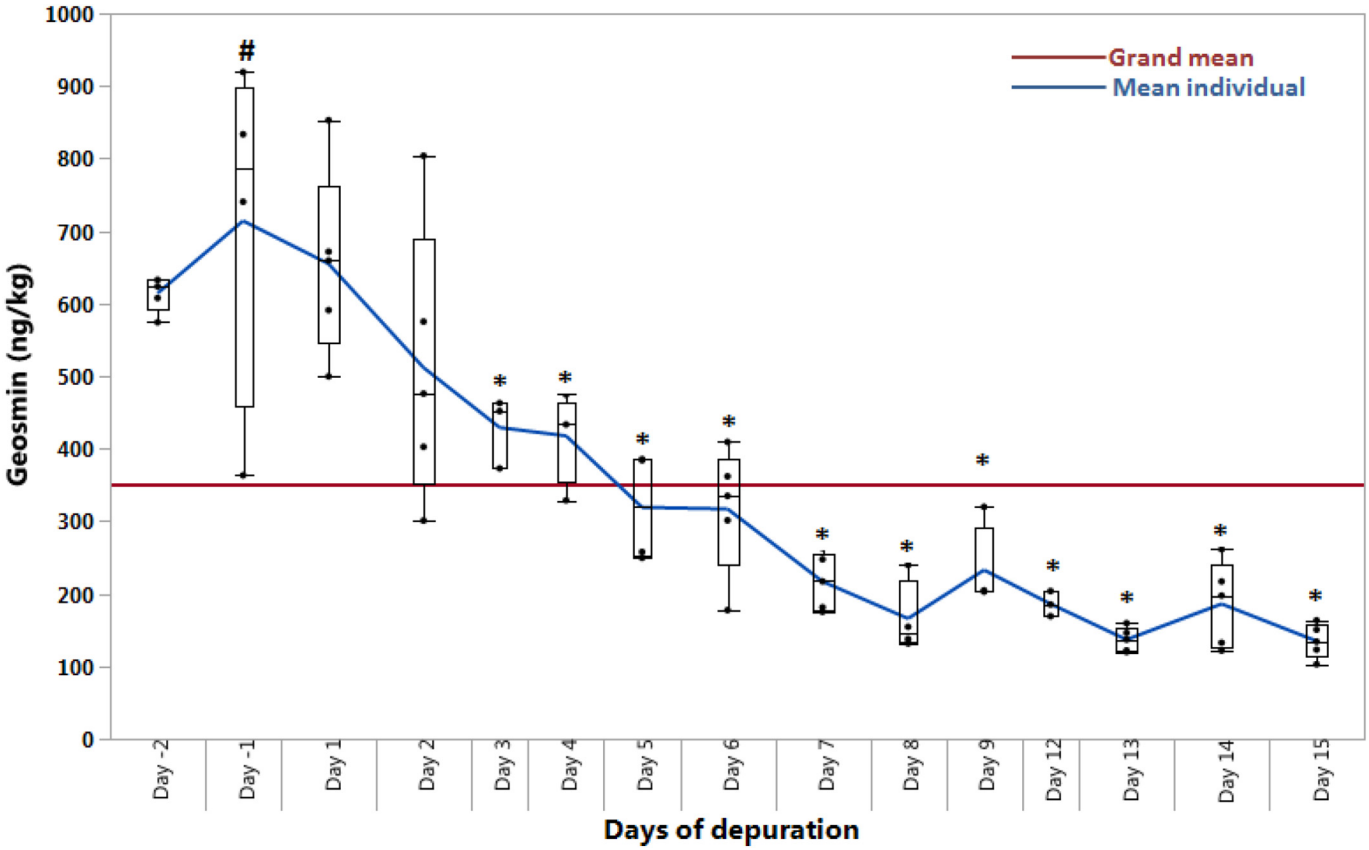

Fig. 2. Geosmin concentration of pikeperch fillets during depuration over 15 days ($n = 75$, five fish from each day). Days before start of depuration are indicated as Day −2 and Day −1. Geosmin content is shown as quantile box plots. Boxes show 25th to 75th percentile; error bars show 90th and 10th percentile. A linear mixed model fitting, restricted maximum likelihood (REML) variance estimate showed significant effect of depuration days on geosmin concentration.'#' indicates geosmin levels prior to depuration on Day −1, *represents the days after depuration that are significantly different ($p < .01$) as compared to the Day −1.

Although not statistically significant, changes of the odor attributes appeared to follow similar trends as observed for the flavor attributes, i.e., increase of green and sweet odors and decrease of geosmin and 2-MIB odors in the fish from 8 to 15 days of depuration, as compared to fish without depuration.

4. Discussion

4.1. Geosmin content in water

Geosmin concentrations in the tank water were slightly higher than concentrations measured in other RAS production tanks, e.g. in outdoor rearing tanks for rainbow trout (Petersen et al., 2011; Sarker et al., 2014), but were below levels measured in a subtropical outdoor RAS for tilapia production and an indoor pilot tank for rainbow trout (up to 75 ng geosmin/L measured; Guttman and van Rijn (2008) and Schrader et al. (2013)).

Daily replacement of half of the tank water with geosmin-free water was expected to infer a daily 50% reduction in geosmin concentration. Based on the dilution factor and the initial concentration of geosmin in the water, a geosmin concentration less than 1 ng/L was expected by Day 7 (Fig. 1). However, the actual geosmin concentration in the water at Day 7 was 21 ng/L, or 20-fold higher than the expected concentration. The likely source of this increase in geosmin is release by the fish. The continuous decline of geosmin content of the fish flesh (see later), substantiates that the geosmin in the water originated from release by the fish.

4.2. Geosmin content in fish

The increase in geosmin concentration in fish between Day −2, when the feeding stopped, to Day −1 was unexpected. A minor increase in geosmin in the water also occurred. On Day −2 and Day −1, the depuration tank was still connected to the entire water system, meaning that water in the depuration tank was identical to water circling between the remaining seven tanks. Thus, an elevated geosmin in the water appeared not to cause the geosmin increase in the fish. The large variation in geosmin content among the fish on Day −1, as compared to the rather homogeneous content on Day −2, may speculatively indicate that the fish reacted to the lack of feeding, but the exact reasons behind the enhanced geosmin level are not known.

Our findings of geosmin removal in pikeperch by depuration agree well with previous depuration studies of fish produced in RAS, e.g. in Atlantic salmon (Burr et al., 2012; Davidson et al., 2014; Ruan et al., 2013), rainbow trout (Petersen et al., 2011; Robertson et al., 2005) and European eel (Schram et al., 2017). However, the number days required to remove geosmin to below sensory threshold, as well as depuration rates, varied between the different studies. Optimum conditions for depuration are probably system-specific and depend on construction type, fish species and initial geosmin concentration, but access to geosmin-free water is also critical. In most studies it has been suggested that 10–15 days of depuration are required for removal of geosmin from fish, at least to reach below sensory threshold (Burr et al., 2012; Davidson et al., 2014; Lindholm-Lehto et al., 2019).

For pikeperch, we observed a large variation in geosmin content between individual fish, particularly at beginning of the depuration, with fish-to-fish variations from 500 to 850 ng/kg on Day 1, and 300 to 800 ng/kg on Day 2. The depuration process seemed to reduce the

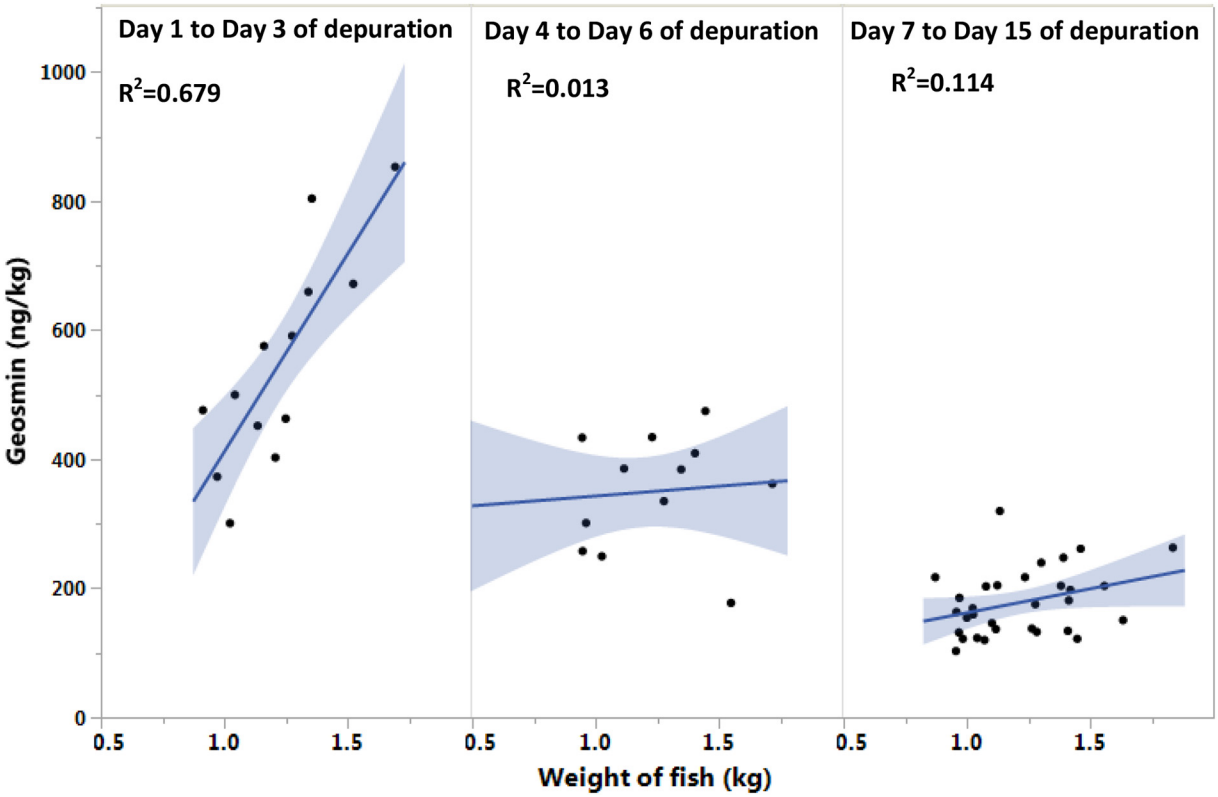

Fig. 3. Linear regression analysis of geosmin concentration in the fish relative to weight of the fish during depuration. The depuration period is divided in three groups: Day 1 to Day 3 ($n = 15$), Day 4 to Day 6 ($n = 15$) and Day 7 to Day 15 ($n = 30$). The relationship between geosmin content and weight of the fish was only significant during Day 1 to Day 3 of the depuration (ANOVA, $p < .01$).

biological variation of geosmin levels in fish, as seen from a geosmin content of 120 to 160 ng/kg on Day 13 and 100 to 160 ng/kg on Day 15. Similar observations of initially large variations in off-flavor compounds (geosmin and 2-MIB) were reported for individual Atlantic salmon at beginning of the depuration but after 10 days of depuration, the variation in off-flavor content among the fish was reduced (Davidson et al., 2014).

The accumulation rate of off-flavors in fish has been suggested to depend on the concentration of compounds in the water, temperature, fat content and biomass of the fish (Johnsen et al., 1996; Johnsen and Lloyd, 1992). Among these parameters, only fat content and fish biomass might have influenced the individual variation in geosmin content among the pikeperches, since all fish were exposed to similar geosmin concentrations and temperature in the production tanks. Fat content of cultured pikeperch is low, only about 1.1% of the wet weight (Linhartová et al., 2018), suggesting that fat only was a minor component in controlling the geosmin content of the fish. Regarding fish biomass, we observed a positive correlation between fish weight and geosmin content (larger fish had a higher geosmin content) between Day 1 to Day 3 during the depuration. For fish with a low geosmin content, a similar correlation between weight and geosmin content was not found (tested for fish between Day 4 and Day 15). This geosmin-weight relation might also have influenced the loss of geosmin by diffusion across the gills and other surfaces (Howgate, 2004). Smaller fish have a relatively larger surface area than larger fish, and this might speculatively imply a higher diffusion from a smaller fish. However, Schram et al. (2017) showed that geosmin depuration in European eel was not influenced by the exchange rate of water in the depuration tank, suggesting that diffusion was not a major process in controlling geosmin content in the fish. Other reasons for a positive correlation between geosmin content and fish size might be related to activity and metabolism, since a high respiration rate implies a more intense exposure to water (with geosmin) for uptake of oxygen. However, more studies are needed to confirm this.

4.3. Sensory analysis

The sensory threshold concentration for geosmin in pikeperch is yet unknown. The sensory panel in this study was able taste geosmin in fish fillets with lowest geosmin concentration of 135 ng/kg (Day 15 of depuration). Sensory threshold values of geosmin have only been published for few fish species. For rainbow trout, the threshold of geosmin is reported to range from 250 ng/kg (Petersen et al., 2011) to 900 ng/kg (Robertson et al., 2005; Robin et al., 2006). Sensory studies of channel catfish suggested that geosmin in the concentration range 250–500 ng/kg did not taint the fish (Grimm et al., 2004). However, a direct comparison of threshold values between different fish species might not be accurate, as sensory threshold values also depend on other flavor compounds, and presence of these other flavors may, for example, be affected by fat content in the fish. In most studies, geosmin content above 200 ng/kg is suggested to cause tainting of the fish with muddy flavor, while geosmin content below 200 ng/kg is less likely to cause tainting of the fish (Grimm et al., 2004; Petersen et al., 2011). In our study, we found that geosmin concentration in pikeperch was 165 ng/kg after 8 days of depuration and the sensory panel could still taste geosmin at this level. The sensory panel in this study is highly trained for geosmin tasting, and many consumers may not taste geosmin at such low levels. If assuming an acceptable sensory level for geosmin at 200 ng/kg, we can conclude that 8 days of depuration is required to reduce the geosmin level to be satisfactory for pikeperch in the studied RAS.

Table 1
Effect of depuration on the volatile composition of pikeperch fillets and its odor/flavor description (n = 75, five fish from each day).

RI calculated	RI standard	RI literature[a]	Proposed compound	Odor/Flavor[b]	Effect of depuration[c]
Aldehydes (12)					
804	801		Propanal	Solvent, pungent	Decreased†
821	812		2-Methylpropanal	Green, malty	Decreased†
887	877		Butanal	Pungent, green	Decreased†
923	913		2-Methylbutanal	Musty, nutty	Decreased†
927	916		3-Methylbutanal	green, nutty	Decreased†
990	983		Pentanal	Almond	Decreased††
1094	1089		Hexanal	Grassy	No effect
1199	1192		Heptanal	Green	No effect
1291	1306		Octanal	Green, fruity	No effect
1407	1405		Nonanal	Green	No effect
1470	1475		Furfural	Sweet, woody	No effect
1532	1531		Benzaldehyde	Almond	No effect
Alcohols (9)					
1053	1048		1-Propanol	Alcoholic, earthy	Decreased†
1218		1217	2-Methyl-1-Butanol	Roasted	No effect
1219	1222		3-Methyl-1-Butanol	Whiskey, malt	No effect
1259	1273		1-Pentanol	Balsamic	Decreased†
1326		1325	2-Penten-1-ol	Green	Decreased†
1361	1359		1-Hexanol	Green	Decreased†
1502			3,5-Octadien-2-ol		Decreased†
1506	1504		2-Ethyl-1-hexanol	Roasty, Earthy	No effect
1626		1617	1,2-Propanediol	Sweet	No effect
Ketones (7)					
825	817		Acetone		No effect
911	907		2-Butanone	Fruity, camphor	Decreased††
991	985		2,3-Butanedione	Buttery	No effect
1071	1073		2,3-Pentanedione	Buttery	Decreased†
1290	1300		Acetoin	Sweet	Increased††
1343	1353		6-Methyl-5-hepten-2-one	Green, musty	Decreased††
1667		1667	Acetophenone	Floral, bitter	Decreased†
Terpenes (4)					
1208	1206		D-Limonene	Citrus	No effect
1747		1748	(−)-Carvone	Mint, caraway	No effect
1844	1844		Geosmin	Earthy	Decreased†
1878			Caryophyllene oxide	Woody	Decreased††
Sulfur (2)					
855			2-Aminoethyl hydrogen sulfate		Decreased††
1087		1086	Dimethyldisulfide	Sulfury, garlic	Decreased††
Acid (1)					
1459	1462		Acetic acid	Vinegar-like	No effect
Benzenes (17)					
949		948	Benzene	Aromatic	No effect
1051		1051	Toluene	Paint	Decreased†
1139		1139	Ethylbenzene		Decreased†
1185		1185	Iso-propylbenzene	(Cumene)	Decreased††
1209			2-Methyldecalin		Increased†
1219		1219	Propylbenzene		Decreased†
1238			Methyl-octahydro Naphthalene		No effect
1263		1263	Styrene	Balsamic, gasoline	Decreased††
1299			1-Methyldecalin		Increased†
1334		1333	α-Methylstyrene		Decreased††
1778			Methylstyrene		Decreased†
1846			1-Ethylidene-7a-methyloctahydro-1H-indene		Decreased††
1933		1904	4,6-di-tert-Butyl-m-cresol		Decreased†
1933		1927	Butylated Hydroxytoluene	Phenolic, camphor	No effect
2024		2024	Phenol	Phenolic, medicinal, leather-like	No effect
2124			Butyl-m-cresol		No effect
2237			1,1,3-Trimethyl-3-phenylindan		No effect

†Denotes significant effect after 1 week of depuration and ††denotes significant effect after 2 weeks of depuration. The depuration effects mentioned in the table are statistically significant (ANOVA, $p < .05$).

[a] Retention Indices (RI) values were compared to the databases; Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider (http://www.chemspider.com/).

[b] Odor/Flavor descriptors are referred from data bases; Flavornet (http://www.flavornet.org/) and The Good Scents Company Database (http://www.thegoodscentscompany.com/).

[c] Effect of depuration on the levels of individual volatile organic compounds was compared between no depuration and depuration.

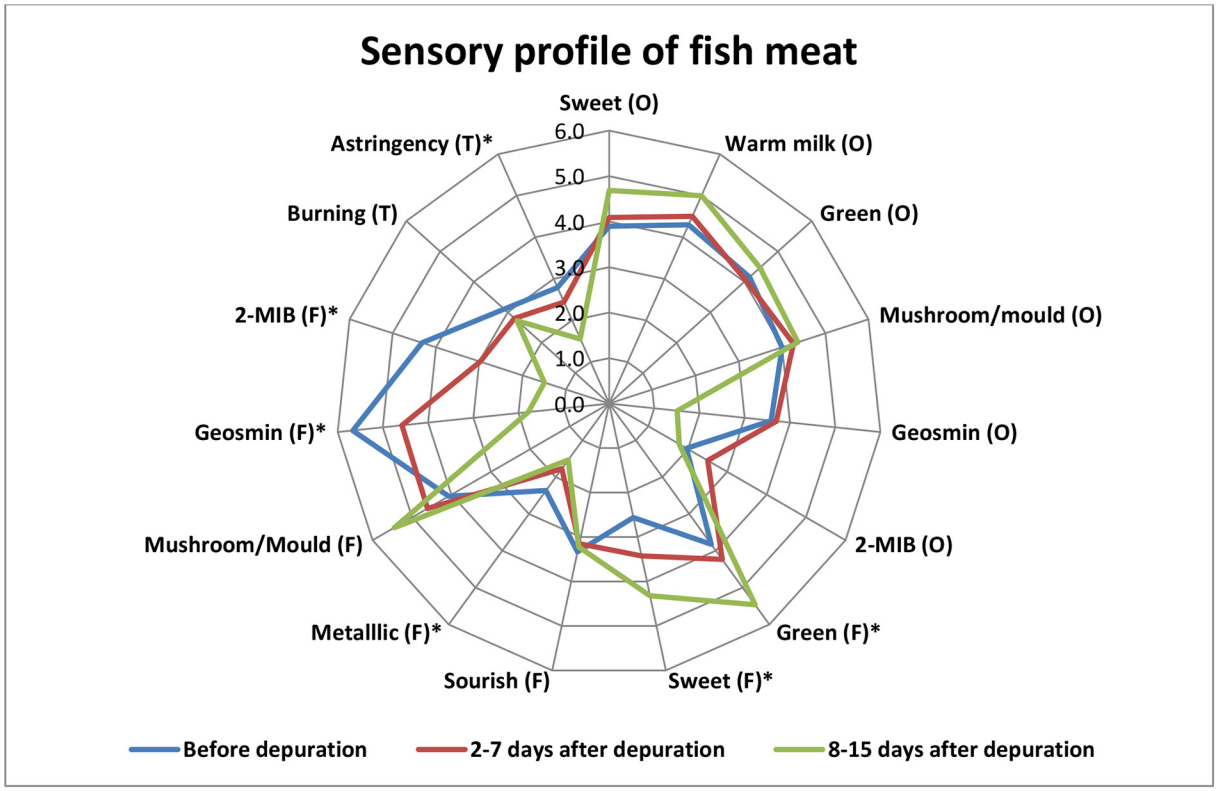

Fig. 4. Influence of depuration on sensory profile of pikeperch fillets. Data represent mean intensity scores (by 4 assessors) of sensory attributes of fish collected before depuration ($n = 3$, Day-2 to Day 1), 2–7 days after depuration ($n = 6$) and 8–15 days after depuration ($n = 6$). Attributes marked with * are the attributes with $p < .05$ (t-test, each pair comparison) and indicates a significant influence of depuration. The letters O, F and T represent odor, flavor and taste.

4.4. 2-MIB and musty flavor of fish

No depuration effects on removal of 2-MIB could be detected due to the low concentration in the fish (levels below the LOQ of 50 ng/kg were observed but only on Days −2 and − 1). Presence of 2-MIB in water and fish in RAS farms has been reported in few studies (Davidson et al., 2014; Guttman and van Rijn, 2008; Ruan et al., 2013). In the pikeperch facility, only geosmin appeared to be responsible for tainting of the fish, as also confirmed by Podduturi et al. (2020) in a study of this facility. The finding of geosmin as the dominant off-flavor and 2-MIB as insignificant in the pikeperch production is supported by Auffret et al. (2013) for rainbow trout in a Canadian RAS farm.

Although 2-MIB concentrations in the pikeperches were very low, the sensory analysis indicated presence of 2-MIB flavor and odor in fish fillets. This impression of musty flavor and odor was most likely caused by other compounds that the sensory panel associated with the 2-MIB attribute. Various forms of chloroanisoles and chlorophenols were previously reported to be causative agents for musty odors in water and damp buildings (Lian et al., 2019; Lorentzen et al., 2020). Whatever compound caused the musty flavor, the mustiness was significantly reduced after depuration of the pikeperches.

4.5. Total volatile composition and sensory quality of pikeperch

The knowledge on effects of depuration on total volatile composition and the impact of sensory quality of cultured fish is scarce. Here, it was shown that apart from common off-flavors like geosmin and 2-MIB, levels of several other volatile compounds can be reduced in fish by depuration. A similar observation was made for cultured cod in which the content of aldehydes decreased after depuration for 2 and 4 weeks (Palmeri et al., 2008).

The sensory analysis of pikeperch flesh indicated that intensity of a sweet flavor and odor increased in the fish after depuration. This observation matches the higher level of acetoin in the fish after depuration. Acetoin has a characteristic sweet and buttery flavor that may have contributed to the fish flavor. The depuration also significantly increased the intensity of green flavor and odor after 15 days of depuration, relative to fish from either 1-week depuration or prior to depuration. Aldehydes like hexanal, heptanal, octanal and nonanal have a characteristic green flavor and odor, but the volatile profile did not indicate effects of depuration on the content of these aldehydes. This indicates that when the intensity of strong flavors e.g. geosmin (earthy/muddy), 2-MIB (musty) and metallic in fish decrease, there could be an increased perception of other and milder flavors.

Changes in composition of volatile compounds during depuration, as observed in our study, were also observed in depuration of grass carp, but abundance of the most common volatiles, e.g. hexanal, nonanal and D-limonene, appeared unpredictable (Lv et al., 2018). As for pikeperch, depuration of the carps also improved the sensory quality.

5. Conclusion

Depuration of geosmin in pikeperch (*Sander lucioperca*) from the commercial RAS farm efficiently reduced the geosmin content in the flesh to a level expected to be below detection threshold by most consumers and it also improved overall sensory quality of the fish. Release of geosmin by the fish was mirrored in a higher geosmin concentration in the depuration tank water than predicted from the daily water exchange. Chemical analysis indicated that the applied 15-day depuration period could be shortened to about 8 days, since only a minor reduction of geosmin was observed from Day 7 to Day 15. However, prolonged purging for up to 15 days decreased the intensity of strong and undesirable flavors, such as geosmin, 2-MIB and metallic flavors, and enhanced the intensity of milder and possible more desirable green and sweet flavors. Although a depuration period for 2 weeks appears to achieve the highest sensory quality, a 1 week duration period might

represent a trade-off with respect to depuration costs and animal welfare, since this can reduce the major off-flavor compound geosmin to a level expected to be close to or below the human sensory threshold concentration.

Declaration of competing interest

None.

Acknowledgements

We wish to thank staff at the AquaPri facility for help and advice during the sample collection. This study was funded by GUDP-COFASP, grant 34009-15-1059.

References

Alexi, N., Byrne, D.V., Nanou, E., Grigorakis, K., 2018. Investigation of sensory profiles and hedonic drivers of emerging aquaculture fish species. J. Sci. Food Agric. 98, 1179–1187. https://doi.org/10.1002/jsfa.8571.

Auffret, M., Pilote, A., Proulx, É., Proulx, D., Vandenberg, G., Villemur, R., 2011. Establishment of a real-time PCR method for quantification of geosmin-producing *Streptomyces* spp. in recirculating aquaculture systems. Water Res. 45, 6753–6762. https://doi.org/10.1016/j.watres.2011.10.020.

Auffret, M., Yergeau, É., Pilote, A., Proulx, É., Proulx, D., Greer, C.W., Vandenberg, G., Villemur, R., 2013. Impact of water quality on the bacterial populations and off-flavours in recirculating aquaculture systems. FEMS Microbiol. Ecol. 84, 235–247. https://doi.org/10.1111/1574-6941.12053.

Azaria, S., van Rijn, J., 2018. Off-flavor compounds in recirculating aquaculture systems (RAS): production and removal processes. Aquac. Eng. 83, 57–64. https://doi.org/10.1016/j.aquaeng.2018.09.004.

Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: main issues on management and future challenges. Aquac. Eng. 51, 26–35. https://doi.org/10.1016/j.aquaeng.2012.07.004.

Burr, G.S., Wolters, W.R., Schrader, K.K., Summerfelt, S.T., 2012. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system. Aquac. Eng. 50, 28–36. https://doi.org/10.1016/j.aquaeng.2012.03.002.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., Pedersen, P.B., 2013. Farming different species in RAS in Nordic countries: current status and future perspectives. Aquac. Eng. 53, 2–13. https://doi.org/10.1016/j.aquaeng.2012.11.008.

Davidson, J., Schrader, K., Ruan, E., Swift, B., Aalhus, J., Juarez, M., Wolters, W., Burr, G., Good, C., Summerfelt, S.T., 2014. Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems. Aquac. Eng. 61, 27–34. https://doi.org/10.1016/j.aquaeng.2014.05.006.

Engle, C.R., Pounds, G.L., Ploeg, M., 1995. The cost of off-flavor. J. World Aquacult. Soc. 26, 297–306. https://doi.org/10.1111/j.1749-7345.1995.tb00258.x.

Grimm, C.C., Lloyd, S.W., Zimba, P.V., 2004. Instrumental versus sensory detection of off-flavors in farm-raised channel catfish. Aquaculture 236, 309–319. https://doi.org/10.1016/j.aquaculture.2004.02.020.

Guttman, L., van Rijn, J., 2008. Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. Aquaculture 279, 85–91. https://doi.org/10.1016/j.aquaculture.2008.03.047.

Houle, S., Schrader, K.K., Le François, N.R., Comeau, Y., Kharoune, M., Summerfelt, S.T., Savoie, A., Vandenberg, G.W., 2011. Geosmin causes off-flavour in arctic charr in recirculating aquaculture systems. Aquac. Res. 42, 360–365. https://doi.org/10.1111/j.1365-2109.2010.02630.x.

Howgate, P., 2004. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: a review of sensory aspects and of uptake/depuration. Aquaculture 234, 155–181. https://doi.org/10.1016/j.aquaculture.2003.09.032.

Johnsen, P.B., Lloyd, S.W., 1992. Influence of fat content on uptake and depuration of the off-flavor 2-methylisoborneol by channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 49, 2406–2411. https://doi.org/10.1139/f92-266.

Johnsen, P.B., Lloyd, S.W., Vinyard, B.T., Dionioi, C.P., 1996. Effects of temperature on the uptake and depuration of 2-methylisoborneol (MIB) in channel catfish *Ictalurus punctatus*. J. World Aquacult. Soc. 27, 15–20. https://doi.org/10.1111/j.1749-7345.1996.tb00589.x.

Johnsen, L.G., Skou, P.B., Khakimov, B., Bro, R., 2017. Gas chromatography – mass spectrometry data processing made easy. J. Chromatogr. A 1503, 57–64. https://doi.org/10.1016/j.chroma.2017.04.052.

Kamstra, A., 2003. A Bio-Economic Model for Commercial on-Growing of Pikeperch.

Lian, H., Lin, Q., Sun, G., 2019. Automated ultratrace determination of musty odiferous compounds from environmental waters by online purge and trap (P&T) gas chromatography–mass spectrometry (GC–MS). Instrum. Sci. Technol. 47, 278–291. https://doi.org/10.1080/10739149.2018.1527774.

Lindholm-Lehto, P.C., Vielma, J., Pakkanen, H., Alén, R., 2019. Depuration of geosmin- and 2-methylisoborneol-induced off-flavors in recirculating aquaculture system (RAS) farmed European whitefish *Coregonus lavaretus*. J. Food Sci. Technol. 56, 4585–4594. https://doi.org/10.1007/s13197-019-03910-7.

Linhartová, Z., Krejsa, J., Zajíc, T., 2018. Proximate and fatty acid composition of 13 important freshwater fish species in Central Europe. Aquac. Int. 26, 695–711. https://doi.org/10.1007/s10499-018-0243-5.

Lorentzen, J.C., Juran, S.A., Ernstgård, L., Olsson, M.J., Johanson, G., 2020. Chloroanisoles and chlorophenols explain mold odor but their impact on the Swedish population is attributed to dampness and mold. Int. J. Environ. Res. Public Health 17, 930. https://doi.org/10.3390/ijerph17030930.

Lukassen, M.B., Saunders, A.M., Sindilariu, P.D., Nielsen, J.L., 2016. Novel Diversity of Putative Geosmin-Producing Bacteria in Freshwater Aquaculture. pp. 1–11.

Lv, H., Hu, W., Xiong, S., You, J., Fan, Q., 2018. Depuration and starvation improves flesh quality of grass carp (*Ctenopharyngodon idella*). Aquac. Res. 49, 3196–3206. https://doi.org/10.1111/are.13784.

Meilgaard, M., Vance Civille, G., Thomas Carr, B., 1999. Sensory Evaluation Techniques, Third edition. CRC Presshttps://doi.org/10.1201/9781439832271.

Palmeri, G., Turchini, G.M., Caprino, F., Keast, R., Moretti, V.M., De Silva, S.S., 2008. Biometric, nutritional and sensory changes in intensively farmed Murray cod (*Maccullochella peelii peelii*, Mitchell) following different purging times. Food Chem. 107, 1605–1615. https://doi.org/10.1016/j.foodchem.2007.09.079.

Petersen, M.A., Hyldig, G., Strobel, B.W., Henriksen, N.H., Jørgensen, N.O.G., 2011. Chemical and sensory quantification of geosmin and 2-methylisoborneol in rainbow trout (*Oncorhynchus mykiss*) from recirculated aquacultures in relation to concentrations in basin water. J. Agric. Food Chem. 59, 12561–12568. https://doi.org/10.1021/jf2033494.

Podduturi, Raju, Petersen, M.A., Mahmud, S., Rahman, M.M., Jørgensen, N.O.G., 2017. Potential contribution of fish feed and phytoplankton to the content of volatile terpenes in cultured Pangasius (*Pangasianodon hypophthalmus*) and Tilapia (*Oreochromis niloticus*). J. Agric. Food Chem. 65, 3730–3736. https://doi.org/10.1021/acs.jafc.7b00497.

Podduturi, R., Petersen, M.A., Vestergaard, M., Jørgensen, N.O.G., 2020. Geosmin fluctuations and potential hotspots for elevated levels in recirculated aquaculture system (RAS): a case study from pikeperch (*Stizostedion lucioperca*) production in Denmark. Aquaculture 514, 734501. https://doi.org/10.1016/j.aquaculture.2019.734501.

Robertson, R.F., Jauncey, K., Beveridge, M.C.M., Lawton, L.A., 2005. Depuration rates and the sensory threshold concentration of geosmin responsible for earthy-musty taint in rainbow trout, *Onchorhynchus mykiss*. Aquaculture 245, 89–99. https://doi.org/10.1016/j.aquaculture.2004.11.045.

Robin, J., Cravedi, J.-P., Hillenweck, A., Deshayes, C., Vallod, D., 2006. Off flavor characterization and origin in French trout farming. Aquaculture 260, 128–138. https://doi.org/10.1016/j.aquaculture.2006.05.058.

Ruan, E.D., Aalhus, J.L., Summerfelt, S.T., Davidson, J., Swift, B., Juárez, M., 2013. Determination of off-flavor compounds, 2-methylisoborneol and geosmin, in salmon fillets using stir bar sorptive extraction-thermal desorption coupled with gas chromatography-mass spectrometry. J. Chromatogr. A 1321, 133–136. https://doi.org/10.1016/j.chroma.2013.10.038.

Sarker, P., Pilote, A., Auffret, M., Proulx, É., Villemur, R., Deschamps, M., Vandenberg, G., 2014. Reducing geosmin off-flavor compounds and waste outputs through dietary phosphorus management in rainbow trout aquaculture. Aquac. Environ. Interact. 6, 105–117. https://doi.org/10.3354/aei00119.

Schrader, K.K., Summerfelt, S.T., 2010. Distribution of off-flavor compounds and isolation of geosmin-producing bacteria in a series of water recirculating systems for rainbow trout culture. N. Am. J. Aquac. 72, 1–9. https://doi.org/10.1577/A09-009.1.

Schrader, K.K., Rubio, S.A., Piedrahita, R.H., Rimando, A.M., 2005. Geosmin and 2-methylisoborneol as the cause of the earthy/musty off-flavors in cultured largemouth bass (*Micropterus salmoides*) and white sturgeon (*Acipenser transmontanus*) reared in recirculating-water system. North Am. J. Aquacult 67, 177–180. https://doi.org/10.1577/A04-070.1.

Schrader, K.K., Davidson, J.W., Summerfelt, S.T., 2013. Evaluation of the impact of nitrate-nitrogen levels in recirculating aquaculture systems on concentrations of the off-flavor compounds geosmin and 2-methylisoborneol in water and rainbow trout (*Oncorhynchus mykiss*). Aquac. Eng. 57, 126–130. https://doi.org/10.1016/j.aquaeng.2013.07.002.

Schram, E., Schrama, J., Kusters, K., Kwadijk, C., Palstra, A., 2016. Effects of exercise and temperature on geosmin excretion by European eel (*Anguilla anguilla*). Aquaculture 451, 390–395. https://doi.org/10.1016/j.aquaculture.2015.09.032.

Schram, E., van Kooten, T., van de Heul, J.W., Schrama, J.W., Verreth, J.A.J., Murk, A.J., 2017. Geosmin depuration from European eel (*Anguilla anguilla*) is not affected by the water renewal rate of depuration tanks. Aquac. Res. 48, 4646–4655. https://doi.org/10.1111/are.13287.

Selli, S., Rannou, C., Prost, C., Robin, J., Serot, T., 2006. Characterization of aroma-active compounds in rainbow trout (*Oncorhynchus mykiss*) eliciting an off-odor. J. Agric. Food Chem. 54, 9496–9502. https://doi.org/10.1021/jf0619582.

Tucker, C.S., 2000. Off-flavor problems in aquaculture. Rev. Fish. Sci. 8, 45–88. https://doi.org/10.1080/10641260091129170.

Van Der Ploeg, M., 1991. Testing flavor quality of preharvest channel catfish. South. Reg. Aquac. Cent. 431, 1–8.