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Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system

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ARTICLE INFO

ABSTRACT

Over the past decade in the United States, there has been increased interest in the establishment and use of land-based, closed-containment systems [e.g., recirculating aquaculture system (RAS)] for salmonid culture. These culture systems have unique challenges compared to net pen culture of salmonids, including maintenance of pumps and filters as well as the potential growth of certain bacteria within the systems that can render fish off-flavored. The purpose of this study was to determine the impact of implementing a depuration process to purge the "earthy" and "musty" off-flavor compounds geosmin and 2-methylisoborneol (MIB) from Atlantic salmon fillets on fillet quality characteristics (e.g., lipid content, color). During two depuration trials, salmon were depurated without feed in a flow-through tank, a recently "cleaned" RAS system or the originally stocked grow-out tank for up to 20 days. Results from both trials determined that the salmon required depuration in odor-free water for 10–15 days in either a flow-through system or a recently cleaned RAS to obtain the lowest residual levels of geosmin and MIB in the fish flesh. In trial 1, after 20 days, fish had lost significantly more weight (5.8%) compared to day 5 (3.8%). In the second trial, lipid content of the fillet also significantly dropped from 8.2% to 5.1% and moisture content increased from 69.3% to 71.1%. Fillet color quality was not compromised during the 20-day depuration periods. In trial 1, MIB was the main off-flavor compound present in salmon fillets while geosmin was at higher levels than MIB in fish flesh in trial 2. During the second depuration study, three geosmin-producing species of actinomycetes were isolated from the recirculating system and were attributed as the likely sources of geosmin in the salmon fillets. Because fillet color quality was not compromised during the depuration periods used in these studies, the main fillet quality concerns for producers of RAS-cultured salmon are flavor, texture and lipid levels during the pre-harvest purging process.

Published by Elsevier B.V.

1. Introduction

For decades, the pond-raised channel catfish (*Ictalurus punctatus*) industry in the southeastern United States has been hampered by common off-flavor problems caused by the bioaccumulation of compounds in the catfish flesh that result in an "earthy" and/or "musty" off-flavor. Catfish that are determined to be off-flavor must be held in ponds until flavor quality improves. It has been estimated that 30% of potential revenue is lost annually by the pond-raised catfish industry due to off-flavor problems because of delays in harvest that result in additional feed costs, forfeiture of income from foregone sales because producers are forced to delay restocking ponds, and loss of catfish during the holding period from

disease, water quality deterioration, and bird depredation (Engle et al., 1995; Tucker, 2000; Smith et al., 2008). The compounds responsible for earthy and musty off-flavors are geosmin and 2-methylisoborneol (MIB), respectively (Tucker, 2000; Howgate, 2004). Geosmin and MIB are microbial metabolites produced by certain species of cyanobacteria (blue-green algae) growing in catfish aquaculture ponds (Tucker, 2000) and by certain species of actinomycetes found in recirculating aquaculture systems (RAS) (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010). These two compounds are believed to be produced by three different pathways including the 2-methylerythritol-4-phosphate (MEP) pathway, the mevalonate pathway, and/or the leucine pathway (Jüttner and Watson, 2007). Although geosmin and MIB-related off-flavors have not been reported in Atlantic salmon (*Salmo salar*) cultured in net pens or in ocean-caught Atlantic salmon, these compounds have been attributed as the cause of earthy and musty off-flavors in Atlantic salmon caught in freshwater rivers in

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Northern Ireland (Farmer et al., 1995). Other members of the salmonid family such as rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpines*) raised in recirculating aquaculture systems have been reported to possess earthy and musty off-flavors caused by geosmin and MIB (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010; Schrader et al., 2010; Houle et al., 2011).

Over the past decade in the United States, there has been increased interest in establishment and use of land-based, closed-containment systems (e.g., RAS) for salmonid culture, particularly for the culture of Atlantic salmon, and, unless the culture facility is located on the seashore, the salmon in these land-based systems would be cultured using fresh water rather than sea water. Although geosmin-related and MIB-related off-flavors can occur in aquatic animals from fresh and brackish water ecosystems, most of the previous reports involve aquatic animals from freshwater ecosystems, especially under circumstances in which the aquatic ecosystem has been subjected to high nutrient loading rates (Tucker, 2000). Therefore, management strategies need to be developed or refined for freshwater RAS to mitigate these common off-flavor problems. Until methods can be developed to reduce or prevent the growth of the microorganisms responsible for producing geosmin and MIB in RAS, the best management approach is purging these off-flavors from the fish by either moving the off-flavored fish to clean, odor-free water or removing geosmin and MIB from the culture water in situ to provide adequate conditions for depuration of off-flavors. During depuration, the reduction of geosmin and MIB levels in the flesh of channel catfish to provide an "on-flavor" product can take days, weeks, or even months in some cases and will depend upon a variety of factors including water temperature, adipose content of the flesh, and intensity of the initial off-flavor (Johnson and Lloyd, 1992; Perkins and Schlenk, 1997; Dionigi et al., 2000). Because the RAS culture of salmonids (e.g., Atlantic salmon) is still relatively new compared to pond-based systems of aquaculture, the depuration periods required for purging earthy and musty off-flavors from these types of fish raised in RAS have never been studied. In addition, the effects of depuration holding periods, often without feeding, on the fillet quality of Atlantic salmon is unknown. Previous research related to the fillet quality of Atlantic salmon grown in net pens determined that fillet gaping, texture and color are important characteristics that consumers evaluate before purchasing the product (Mørkøre and Rørvik, 2001; Kiessling et al., 2004; Johnston et al., 2006). However, the use of RAS for the growth of salmonids is still relatively new, and most of the research on the fillet quality of RAS-cultured salmon has been done.

The purpose of this study was to evaluate the effect of fillet quality as impacted by different holding periods required to reduce MIB and geosmin levels in the flesh of RAS-cultured Atlantic salmon in order to provide acceptable flavored fish. The effect of withholding feed during depuration on the fillet quality was also monitored. In addition, a preliminary assessment of utilizing granular activated carbon (GAC) for removal of geosmin from the culture water of the low-exchange rate RAS was performed.

2. Materials and methods

2.1. Fish culture, Trial 1

One hundred and eight Atlantic salmon were cultured at the National Cold Water Marine Aquaculture Center in one tank of a recirculating aquaculture system with a flow rate of 1514 lpm and fed a commercial diet, containing 44% protein, 29% lipid and 60 mg/kg astaxanthin for three weeks prior to the start of the study. The system was composed of four 36 m^3 tanks each receiving 379 lpm of recirculating water, each tank had an individual radial

flow clarifier, and the four tanks were connected to a common drum filter with a 60 μm mesh, a CycloBio fluidized-sand biological filter, gas stripping column and a low head oxygenator. Dissolved oxygen and temperature were monitored continually with stationary probes (Point Four Systems Inc., Coquitlam, BC, Canada) in the tanks and ammonia, nitrite, nitrate, carbon dioxide, and pH monitored weekly using a commercially available test kit (CEL/850 Aquaculture Lab Kit, Hach Company, Loveland, Colorado) to insure optimal water quality conditions. Water temperature ranged from 7.3 °C to 9.9 °C with an average of 9.0 °C. On day 0, twelve fish were sampled and the remaining 96 fish were taken off feed, with 48 being transferred to a clean flow-through system with a flow rate of 114 lpm and the other 48 remained in the same culture tank. Twelve fish from each system, flow-through and recirculating, were sampled on days 5, 10, 15 and 20.

2.2. Fish culture, Trial 2

One hundred and twenty Atlantic salmon were cultured in a recirculating aquaculture system and fed a commercial diet for three weeks prior to the start of the study. The system was the same system used in study 1. Dissolved oxygen and temperature were monitored continually and ammonia, nitrite, nitrate, carbon dioxide, and pH monitored weekly to insure optimal water quality conditions. On day 0, twelve fish were sampled and the remaining 108 fish were taken off feed, with 36 fish transferred to a clean flow-through system with the incoming water being filtered through a granular activated carbon filter (10.8 m^3), 36 fish remaining in the same culture tank (Fig. 1) and the final 36 fish being transferred to a clean RAS (9 m^3). The clean tank had been scrubbed to remove the film lining the tanks walls and then chlorinated (100 ppm chlorine for three hours) to disinfect the tank, the clean RAS system tank and pump sump were also scrubbed and then the entire system was chlorinated (100 ppm chlorine for three hours) to disinfect the entire system. Water temperature for the original system ranged from 8.7 °C to 9.6 °C with an average of 9.1 °C and for the clean RAS 9.1–10.2 °C with an average of 9.6 °C The water for the flow-through tank was filtered through a granular activated carbon filter (PF50C, Aqualogic, San Diego CA, with acid washed granular coal base activated carbon PS48AW, Prominent Systems Inc., City of Industry, CA) to remove contamination by geosmin or MIB that could be present in the incoming water. Twelve fish from each system, flow-through and the two recirculating systems, were sampled on days 5, 10, and 15 based on the previous experiment.

2.3. Water sampling and salmon fillet preparation

Water from each system was collected on every sampling day. The incoming water of the flow-through tank and recirculating tank was obtained by collecting tank incoming water in a 20-mL scintillation vial with a foil-lined cap. Sludge from the drum filter on the recirculating system was sampled every collection day using a spoon to collect sludge from the solids trough in the drum filter and the sludge was then placed into a 20-mL scintillation vial with a foil-lined cap. Water and biofloc samples were stored in a refrigerator (4 °C) until overnight shipment to the ARS-National Products Laboratory for processing and analysis.

On each sampling day, twelve fish from each system (24 fish total) were randomly collected and measured for total weight (carcass plus offal weight), carcass weight (head on gutted weight), and fillet weight. The fillets were then frozen until further analysis. One fillet from each sampled fish was sent by overnight shipment to the ARS-National Products Laboratory for analysis while the other fillet was thawed and scanned with a colorimeter (MiniScan XE, Hunter Labs, Reston, Virginia) and then a sample of the scanned fillet, approximately the first 2 inches closest to the head, was ground
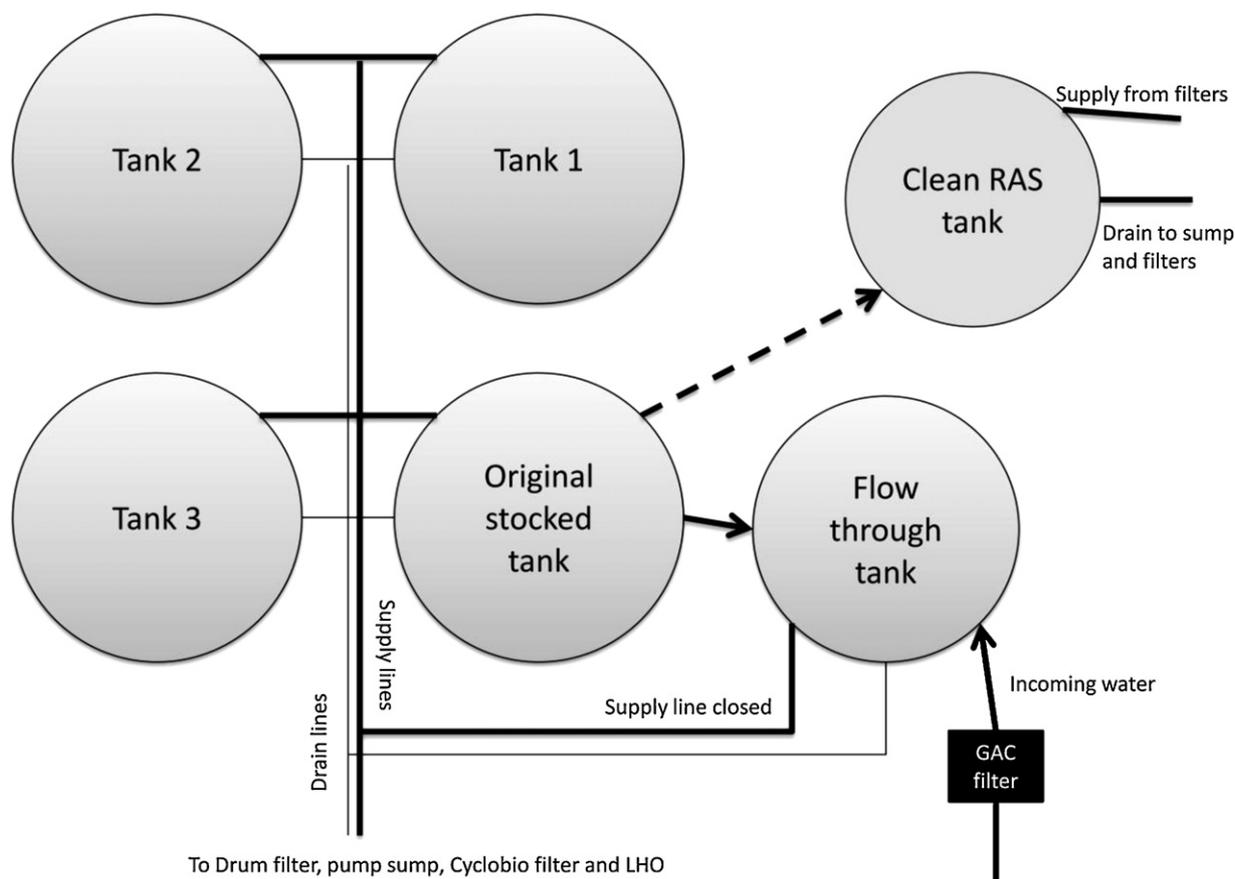
Fig. 1. Drawing of the original stocked system and the flow-through tank from trail 1 and 2. In trial 1, fish were either maintained in the original tank or stocked into a flow through tank (solid arrow). In trial 2, fish were either maintained in the original tank, stocked in a flow through tank (solid arrow), or transferred to a clean RAS system that was separate from the original system (dashed arrow). GAC filter was only used in trial 2.

and analyzed for crude lipid content using pressurized hot ether extraction (AOCS Am 5-04;XT-10, Ankom, Macedon, New York).

In Trial 2, the fillets were scanned with CR 400 colorimeter (Konica Minolta Sensing Americas INC, Ramsey, NJ). Sludge from the drum filter on the recirculating system was sampled every collection day using a spoon to collect sludge from the solids trough in the drum filter and the sludge was then placed into a 20-mL scintillation vial with a foil-lined cap. The biofloc samples were stored in a refrigerator (4 °C) until overnight shipment to the ARS-National Products Laboratory for processing and analysis. Sites inside and from the effluent of the drum filters were chosen as logical sampling sites in attempts to isolate odor-producing actinomycetes based upon previous studies (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010).

2.4. Analysis of geosmin and MIB in water samples

All water samples were maintained at 4 °C until prior to analysis. Water samples were processed prior to the determination of geosmin and MIB levels by micropipetting 0.6-mL aliquots into individual 2-mL glass crimp-top vials containing 0.3 g sodium chloride/vial. The method used to quantify levels of geosmin and MIB was similar to the SPME procedure by Lloyd et al. (1998) and as modified by Schrader et al. (2003). Specifically, the vials were heated at 40 °C for 20 min before the volatile compounds were absorbed onto a 100 μm polydimethyl siloxane solid-phase microextraction fiber (Supelco, Bellfonte, Pennsylvania). The original standards were obtained from Wako Chemicals USA, Inc., Richmond, Virginia., and were included at the beginning, middle, and end of each group of samples analyzed using a CombiPal

autosampler (LEAP Technologies, Inc., Carrboro, North Carolina). The fiber assembly was shaken for 10 min during the absorption period and desorbed for 2 min at 250 °C in the injection port of a HP 6890 gas chromatograph-mass spectrometer (Agilent, Palo Alto, California) with a 5973 mass selective detector operated in selected ion monitoring mode. The conditions of the gas chromatograph were as follows: (1) initial oven temperature was 60 °C for 0.5 min; (2) then ramp rate of 30 °C/min to 100 °C; (3) then ramp rate of 20 °C/min to 300 °C with an isotherm time of 2 min; and (4) the maintenance of flow pressure was at 18 lb/in^2 with helium used as a carrier gas. The ions monitored at m/z 168, 95, and 135 for MIB and at m/z 182, 112, and 126 for geosmin. A DB-5 capillary column (5%-phenyl-methylsiloxane, 30 m, 0.25 mm inside diameter, 0.25-μm film thickness; J&W Scientific, Folsom, California) was used. The retention time for geosmin was 6.8 min and, for MIB, 5.2 min. Standards for MIB and geosmin were prepared at 0.1, 0.5, 1.0, and 2.5 μg/L in deionized water. Each sample was run in triplicate.

2.5. Analysis of geosmin and MIB levels in salmon fillets

Salmon fillets were kept frozen until analysis could be performed. For each fillet, one 20-g portion (including the skin) was cut from the anterior end of the fillet and used to obtain distillate following the microwave distillation using the procedures of Lloyd and Grimm (1999). Aliquots (0.6-mL) of each distillate were micropipetted into individual 2-mL glass crimp-top vials containing 0.3 g sodium chloride/vial. The SPME-GC-MS method outlined previously to quantify levels of geosmin and MIB in water was used to determine levels of these compounds in salmon fillet samples.

2.6. Isolation and identification of geosmin-producing bacteria (actinomycetes)

The methods outlined in Schrader and Summerfelt (2010) were generally used, with some minor modifications. Biosolids samples were serially diluted in sterile 0.85% saline water and shaken 25 times in a back-and-forth motion within a distance of approximately 0.5 m and over a 10-s period before spread plating 0.1-mL aliquots on 1% yeast extract-1% dextrose (YD) agar (pH 7.5) plates and actinomycete isolation agar (AI) (Bacto^{TM}; Becton, Dickinson and Company, Sparks, Maryland) plates. Duplicate sets of inoculated plates were prepared, with one set incubated at 15 °C and the other set at 25 °C in order to aid in the isolation of actinomycetes.

At 7 days after incubation, colonies bearing resemblance to actinomycete colony morphology (e.g., chalky appearance, "biting" into agar surface) were streaked for isolation on to the same type of agar from which they were obtained, and these isolates were incubated at the temperature in which they had originally grown. After 7 days of incubation, plates were evaluated for odor production using olfaction. Isolates identified as producing typical odors associated with geosmin and MIB (e.g., earthy and musty) were chosen to perform analysis to detect production of those compounds. An isolated colony (2–5 mm in diameter) was aseptically removed from an agar plate and transferred to a 2-mL glass crimp-top vial containing 0.6 mL of ultrapure water (NANOpure® DIamond^{TM} UV/UF and reverse osmosis systems; Barnstead International, Dubuque, Iowa) (geosmin-free and MIB-free) and 0.3 g sodium chloride. These vials were immediately analyzed by SPME-GC-MS to detect and confirm geosmin and/or MIB production by the isolate.

Colony morphologies of the various actinomycete isolates were documented and used to group the isolates into supra-generic groups based upon similar colony morphology and pigment production. Genotypic identification of a representative of each subgroup of geosmin-producing isolates was performed by phylogenetic analysis (Accugenix, Inc.; Newark, Delaware) using comparative 16S rRNA gene sequencing (500 bp). For each representative of a subgroup, genomic DNA was extracted, purified, and target DNA (portion of 16S ribosomal gene) amplified using polymerase chain reaction (PCR) with the bacterial primers 531R (5′-TAC CGC GGC TGC TGG CAC -3′) and 005F (5′- TGG AGA GTT TGA TCC TGG CTC AG -3′). Each PCR product was purified and then sequenced using dye-terminator sequencing chemistry to fluorescently label each nucleotide of the PCR product. Fluorescently labeled product was analyzed on an automated fluorescent DNA sequencer to provide an electropherogram and sequence the sample, and each generated sequence was compared to the Accugenix, Inc., database. For each analyzed isolate, a phylogenetic tree was generated by neighbor joining and closest match methods, and final identification was made based upon genetic percent difference (distance measurement) and the phylogenetic tree. This similar polyphasic approach has been described for the identification of certain species of *Streptomyces* and *Nocardia* by Maldonado et al. (2000) and Anderson and Wellington (2001), respectively.

2.7. Data analysis

Means and standard deviations of geosmin and MIB levels in water were calculated and graphed. The means and standard errors were calculated and graphed for geosmin and MIB levels in the salmon fillets. For fillet data in each study, one-way repeated measures analysis of variance (ANOVA) was performed using SigmaPlot (Systat Software, Inc., Chicago, IL) version 11.0 software. The Holm-Sidak method was used for the multiple comparisons procedure ($\alpha = 0.05$).
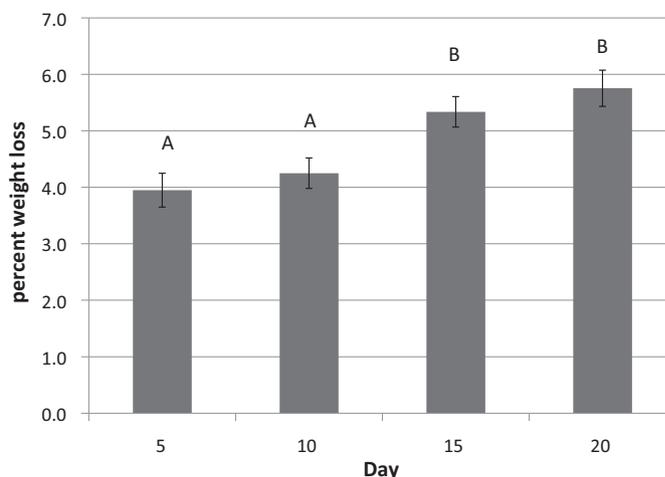
Fig. 2. Mean weight loss of Atlantic salmon from flow-through system and original culture tank depurated for 5, 10, 15, or 20 days (average ± 1 se).

2.8. Sensory analysis of salmon fillets

A panel consisting of three males and three females were trained over several sessions to identify and rate the intensity of flavors and off-flavors in the salmon fillets. Panelists were prescreened and selected based upon their abilities to detect specific off-flavors such as earthy and musty in channel catfish fillets. Frozen samples were thawed prior to conducting each training and evaluation session. The same salmon fillets used to quantify levels of geosmin and MIB were utilized for sensory analysis. For each fillet, an approximately 20-g portion was removed from the fillet next to the previously removed portion of the fillet that had been utilized for determining geosmin and MIB levels via microwave distillation and SPME-GC-MS. Each 20-g portion of fillet for sensory analysis was placed in a zip-lock bag and heated in the microwave (1100 W) for 45 s at 60% power setting. Each sample was presented to panelists for evaluation separately.

Panelists first evaluated the aroma of the steam dispersed as the bag was opened. Subsequent taste-testing of the sample was performed, and the panelists were asked to evaluate the sample for flavors and rate the intensity of any off-flavors that were detected using a hedonic scale. A separate paper plate and plastic fork was used for each sample. Between each sample that was tasted, panelists were provided with 0.03% (w/v) citric acid water and unsalted crackers in order to cleanse the palate.

3. Results

3.1. Trial 1

Weight loss, percent carcass weight, lipid levels, color score (a*), and fillet yield did not vary between the flow-through and the recirculating system. We have elected to combine the data from the two systems for analysis. Atlantic salmon had significant weight loss after 20 days (Table 1, Fig. 2). Atlantic salmon lost approximately 6% of their body weight after 20 days without food. Lipid levels in the flesh of salmon decreased over time, but this effect was not significant (Table 1, Fig. 3). The color of the fillets varied over time, but we did not detect a pattern to the variation in color score (Table 1, Fig. 4).

The mean levels of MIB in salmon flesh from both systems (flow-through and recirculating) generally decreased for the first 15 days of the depuration period (Figs. 5 and 6, respectively). In the flow-through system, the largest, significantly different ($P < 0.05$) decrease in mean MIB levels occurred between day 0 (mean MIB

Table 1
Physical and fillet quality parameters from trial 1.

| Day	n	Day 0, avg wt (g)	Total wt (g)	% weight loss	Carcass wt (g)	Fillet wt (g)	% carcass	% fillet (fillet wt/total wt)	% fillet (fillet wt/carcass wt)	Fat (%)	Color (a*)
5	24	2433.4 ± 61.3	2335.9 ± 57.0	3.9 ± 0.3	2127.0 ± 50.2	1285.2 ± 38.0	91.2 ± 0.5	54.9 ± 0.6	60.3 ± 0.9	6.5 ± 0.3	20.3 ± 0.5
10	24	2383.3 ± 67.1	2280.0 ± 62.4	4.3 ± 0.3	2090.0 ± 57.0	1273.5 ± 38.9	91.7 ± 0.4	55.6 ± 0.4	60.7 ± 0.4	6.3 ± 0.3	20.0 ± 0.5
15	24	2345.8 ± 70.1	2221.0 ± 63.4	5.3 ± 0.3	2066.7 ± 53.8	1221.0 ± 37.9	93.3 ± 0.3	55.1 ± 0.4	59.6 ± 0.4	6.2 ± 0.4	18.6 ± 0.7
20	24	2317.9 ± 79.3	2182.1 ± 72.4	5.8 ± 0.3	2014.0 ± 65.5	1192.7 ± 466	92.4 ± 0.6	54.5 ± 0.6	59.0 ± 0.4	5.3 ± 0.4	20.6 ± 0.5
P value				<0.0001			0.0702	0.4372	0.2038	0.0819	0.0574
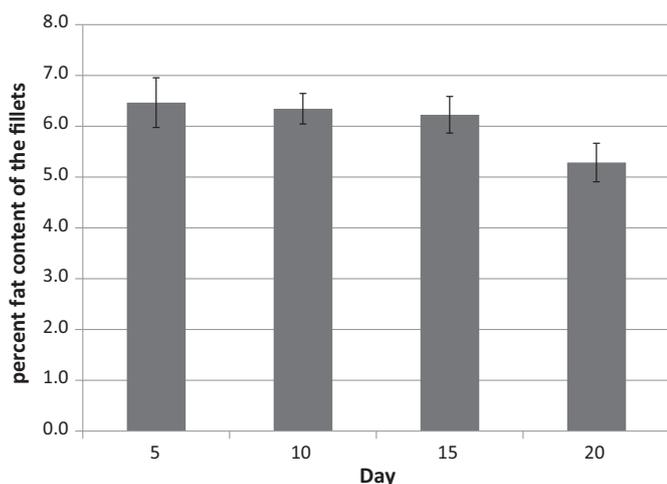
Fig. 3. Mean lipid content of Atlantic salmon fillets from flow-through system and original culture tank depurated for 5, 10, 15, or 20 days (average ± 1 se).

Fig. 5. Levels of 2-methylisoborneol in the flesh of Atlantic salmon collected from the flow-through system during the first depuration study. Means with the same letter do not differ significantly (*P* > 0.05).

of 94.2 ± 29.2 ng/kg) and 10 days (mean MIB of 15.0 ± 2.0 ng/kg) of depuration (Fig. 5). Mean MIB levels in the flesh of salmon maintained in the recirculating system during the depuration trial decreased in a similar manner, though the lowest, significantly different (*P* < 0.05) mean MIB level from day 0 was at 5 days (mean MIB of 27.9 ± 3.4 ng/kg) (Fig. 6). At day 20, there was an approximately two-fold increase in the mean MIB levels in the salmon fillet samples from both systems compared to day 15 results (Figs. 5 and 6). Overall, the patterns of MIB depuration from the salmon flesh were similar between the two systems.

Generally, the levels of MIB in the water were similar between the two systems and remained consistent in the flow-through system over the 20 days of the study (Fig. 7). However, MIB levels dropped approximately 50% in the recirculating system water from day 0 (mean MIB of 132.0 ± 13.2) to day 20 (mean MIB of
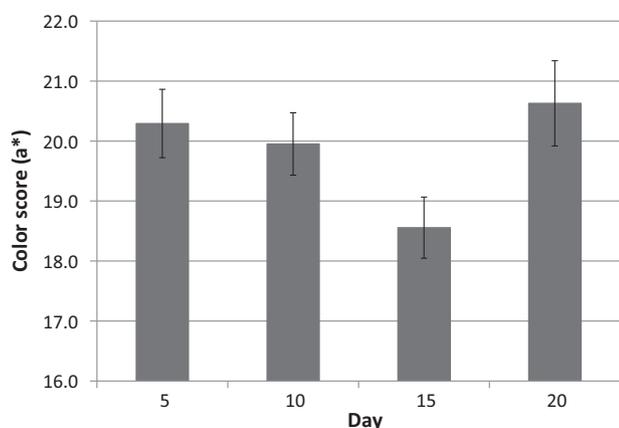
67.0 ± 17.4). Mean geosmin levels detected in the salmon flesh of most samples were generally below 10.0 ng/kg, though one fish sample contained geosmin at 26.1 ng/kg. The mean geosmin levels in salmon fillets were significantly lower than MIB levels throughout the course of the study, and these geosmin levels did not change dramatically during the study. Most salmon fillets contained MIB levels well above 10.0 ng/kg and several fillets contained MIB in the range of 100.0 to as high as 933.3 ng/kg. Mean geosmin levels in the water from both systems were very low (1.0–10.0 ng/L) compared to MIB levels during the study, and mean geosmin levels decreased to 1.0 ng/L in both systems by day 15 of the depuration period.

Fig. 4. Mean color score (a*) of Atlantic salmon fillets from flow-through system and original culture tank depurated for 5, 10, 15, or 20 days (average ± 1 se).
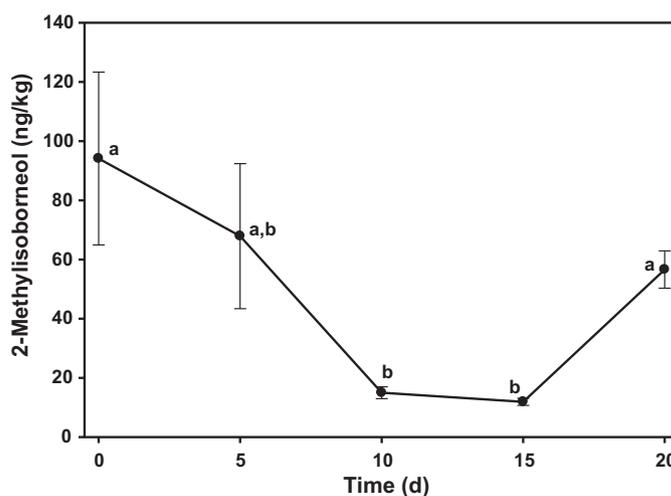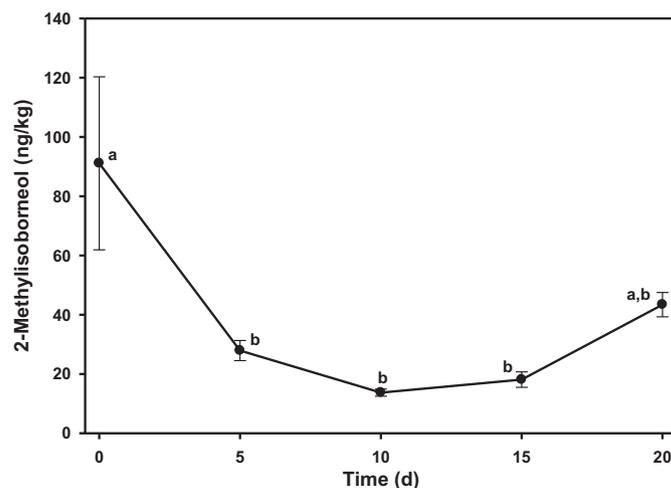

Fig. 6. Levels of 2-methylisoborneol in the flesh of Atlantic salmon collected from the original culture tank of a recirculating aquaculture system during the first depuration study. Means with the same letter do not differ significantly (*P* > 0.05).
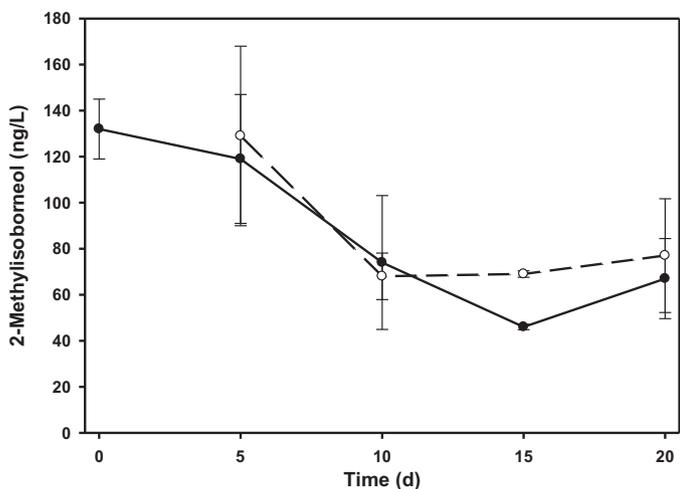
Fig. 7. Levels of 2-methylisoborneol in water collected from flow-through system (○) and recirculating aquaculture system (●) during the first depuration study.

Overall, MIB can be considered to be the more significant off-flavor compound in this first trial depuration study due to the higher MIB levels in the water and salmon fillets compared to geosmin levels.

Sensory analysis revealed only several fish to be slightly off-flavored (two of 12 fish at day 0 and two of 12 fish at day 5), and these fish were designated as possessing a "woody" off-flavor. None of the sampled fish were designated as having a musty off-flavor by the sensory panelists.

3.2. Trial 2

The percent carcass weight significantly increased from 89.8% to 92.7% after 15 days. The percent of the total weight contributed by the fillet increased from 53.5% to 55.6% after 15 days. The fillet fat content significantly decreased from 8.2% on day 0 to 5.1% on day 15 (Fig. 8). Fillet moisture significantly increased from 69.3% on day 0 to 71.1% on day 15 (Table 2).

During the second depuration study, mean geosmin levels in the muscle tissues of Atlantic salmon maintained in the flow-through tank (with GAC filtration of culture water) and in cleaned RAS decreased significantly ($P < 0.05$) from 197.8 ± 12.1 ng/kg to 12.1 ± 1.5 ng/kg and to 16.3 ± 2.3 ng/kg, respectively (Fig. 9).
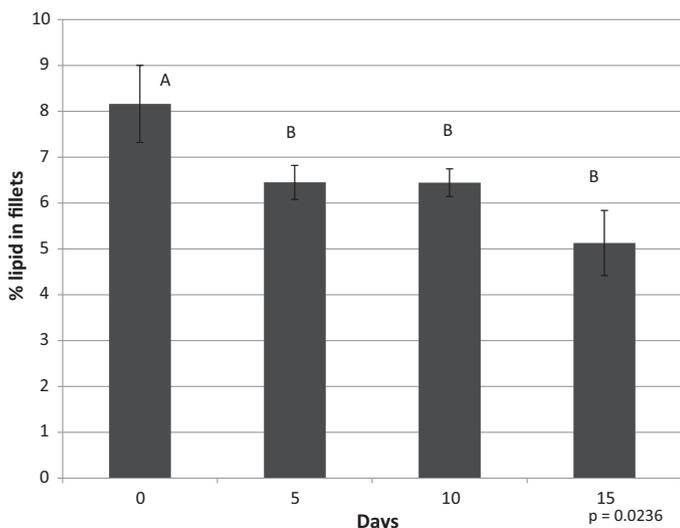

Fig. 8. Lipid content of Atlantic salmon fillets from fish depurated for 0, 5, 10, or 15 days during the second depuration study (average ± 1 se).
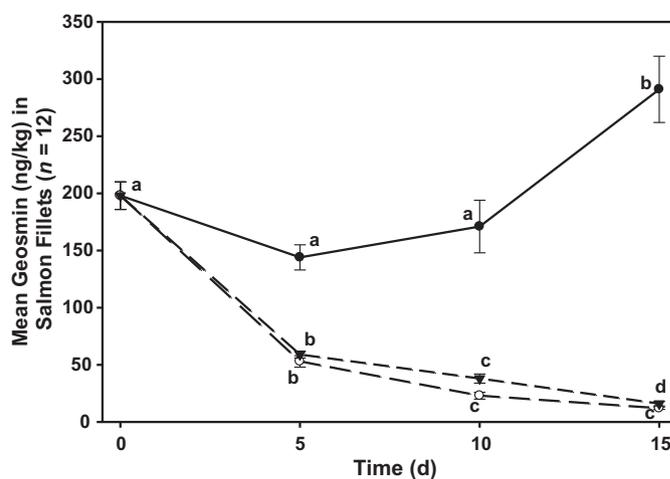

Fig. 9. Mean geosmin levels in the flesh of Atlantic salmon collected from two different recirculating aquaculture systems as follows: ● = system 1, on-grow culture tank 4; ○ = system 1, on-grow culture tank 5 (flow-through); ▼ = system 2. Means along the same plotted line with the same letter do not differ significantly ($P > 0.05$).

However, mean geosmin levels in the flesh of Atlantic salmon maintained in recirculating system without GAC filtration of culture water and cleaning prior to stocking increased significantly ($P < 0.05$) from 197.8 ± 12.1 ng/kg to 291.3 ± 29.0 ng/kg during the 15-day depuration period (Fig. 9). Overall, mean geosmin levels in the flesh of salmon from both systems averaged approximately one order of magnitude higher than mean MIB levels, and, therefore, geosmin was the more significant off-flavor compound of interest in the second depuration study. Sensory analysis revealed only two fish as possessing slight off-flavor, and the description was indicated as an earthy off-flavor.

Mean geosmin levels in the culture water from the flow-through tank decreased to below the instrument threshold detection level of 1 ng/L by day 10 of the depuration study (Fig. 10). Conversely, mean geosmin levels in water from the recirculating tank without GAC filtration increased steadily throughout the study to as high as 29 ng/L by day 15. Geosmin was not detected in any of the water samples collected from recirculating system 2.

Three different species of actinomycetes that were isolated from the biosolids samples obtained from the drum filter and drum filter effluent line were determined to be geosmin producers (Table 3).
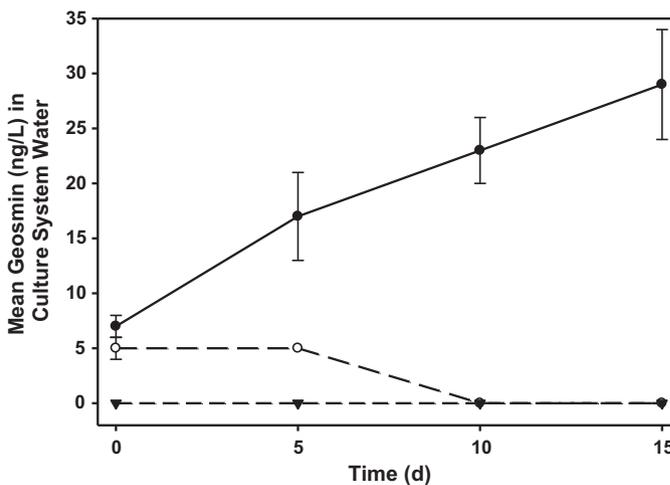

Fig. 10. Mean geosmin levels in water collected from two different recirculating aquaculture systems as follows: ● = system 1, on-grow culture tank 4; ○ = system 1, on-grow culture tank 5 (flow-through); ▼ = system 2.

Table 2

Physical and fillet quality parameters from trial 2.

Day	n	Total wt (g)	Carcass wt (g)	Fillet wt (g)	% carcass	% fillet (fillet wt/total wt)	% fillet (fillet wt/carcass wt)	Fat (%)	Color (a*)	Moisture (%)
0	12	1828.92 ± 138.32	1644.42 ± 126.00	982.00 ± 79.07	89.81 ± 0.29	53.5 ± 0.61	59.5 ± 0.61	8.16 ± 0.84	8.13 ± 0.48	69.3 ± 0.7
5	36	1894.81 ± 72.01	1727.86 ± 66.22	1039.22 ± 42.93	91.17 ± 0.17	54.6 ± 0.30	59.9 ± 0.33	6.45 ± 0.37	9.42 ± 0.41	70.2 ± 0.3
10	36	1664.22 ± 71.98	1545.42 ± 66.71	922.17 ± 42.67	92.88 ± 0.11	55.2 ± 0.32	59.4 ± 0.35	6.44 ± 0.30	8.24 ± 0.45	70.5 ± 0.4
15	36	1731.94 ± 78.50	1605.06 ± 72.56	969.31 ± 47.88	92.71 ± 0.14	55.6 ± 0.38	60.0 ± 0.42	5.13 ± 0.71	8.90 ± 0.38	71.1 ± 0.3
P value					<0.0001	0.0111	0.06356	0.0236	0.1573	0.0205

The only geosmin-producing species isolated from biosolids samples obtained from system 1 was *Nocardia salmonicida* while three geosmin-producing actinomycete species (*Nocardia* cf. *fluminea*, *Nocardia salmonicida*, and *Streptomyces cyaneofuscatus*) were isolated from system 2.

4. Discussion

The culture of salmonids in RAS offers many advantages, such as control of temperature, photoperiod and water quality. One of the major advantages of RAS systems is the removal and treatment of wastes. Fish waste is rapidly removed from the water and then sent for treatment and disposal. However, one dilemma with these systems is the potential to yield earthy-musty off-flavored fish due to the accumulation of geosmin and MIB in the fish flesh (Schrader et al., 2005, 2010; Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010; Houle et al., 2011). The most common management approach that producers utilize in dealing with earthy-musty off-flavor problems is purging of geosmin and MIB from the fish flesh prior to harvest. In the pond-raised channel catfish industry in the southeastern United States, producers may apply algicides such as copper sulfate and/or diuron [N'-(3,4-dichlorophenyl)-N,N-dimethylurea] to the ponds to reduce the abundance of the odor-producing cyanobacteria (Tucker, 2000; Schrader and Dennis, 2005). The outcome is a reduction in the levels of geosmin and MIB in the pond water that subsequently permits depuration of the earthy-musty taints from the fish flesh to provide an on-flavor crop of fish for harvest. Unfortunately, the application of compounds (e.g., bactericides) to reduce the abundance of actinomycetes in RAS is not practical because of the lack of an effective, selective antibacterial compound against actinomycetes in RAS and due to the adverse impact of the bactericide on the biofilter portion of the RAS. Currently, two options are available for depuration of earthy-musty off-flavors: (1) moving the tainted fish to a culture system containing geosmin-free and MIB-free water; or (2) adequately reduce levels of geosmin and MIB in the culture tank in which they are growing to provide suitable conditions for depuration.

Table 3

Geosmin-producing species of actinomycetes isolated from biosolids samples obtained from two recirculating aquaculture systems at Franklin, Maine, during second depuration study.

Biosolids sampling location[a]	Species
Drum filter effluent line, system 2 (0)	*Streptomyces cyaneofuscatus*
Drum filter effluent line, system 2 (5)	*Streptomyces cyaneofuscatus*
Drum filter effluent line, system 2 (10)	*Nocardia* cf. *fluminea*
	Nocardia salmonicida
	Streptomyces cyaneofuscatus
Drum filter effluent line, system 2 (15)	*Nocardia salmonicida*
	Streptomyces cyaneofuscatus
Inside drum filter, system 1 (0)	*Nocardia salmonicida*
Inside drum filter, system 1 (15)	*Nocardia salmonicida*

[a] System 1 was the on-grow recirculating system with an attached flow-through tank. The number in parentheses indicates the sampling day during the second depuration study.

Geosmin and MIB have been detected in other salmonids such as rainbow trout (Schrader et al., 2010; Zimba et al., 2012) and arctic charr cultured in recirculating systems (Houle et al., 2011), but the amount of time and the conditions required to reduce the levels of these compounds have not been investigated. Depuration studies related to geosmin and MIB off-flavors are lacking for many fish species including Atlantic salmon.

In our study, the first depuration trial cannot be considered the usual protocol that producers might follow when attempting to purge fish of earthy or musty off-flavors because the salmon, which contained relatively low levels of MIB at the start of the trial, were not moved to MIB-free water. Rather, fish were taken off feed and separated into two systems, which both still contained MIB in the culture water (Fig. 7). In the first depuration trial, the lowest levels of MIB in the salmon flesh were obtained at 10–15 days in fish kept in the flow-through system and in fish maintained in the original culture tank of a RAS (Figs. 5 and 6, respectively). The significant increase in mean MIB levels from day 15 to day 20 in salmon collected from the flow-through system was unexpected because levels of MIB remained relatively similar during this period (Fig. 7). There was also an increase in mean MIB levels in salmon flesh between day 15 and day 20 for salmon collected from the original culture tank of the RAS, though not a significant increase (Fig. 6). Generally, there was no difference between the depuration rates between fish held in the flow-through system and the fish held in the original culture tank. The decrease in mean MIB levels in fish flesh from both systems corresponds with the gradual decrease in MIB levels in the water from both systems (Fig. 7). The reduction in the lipid content of the fish flesh during the 20-day depuration period (see Fig. 3) likely also contributed to a reduction in mean MIB levels in the fish flesh. Increased lipid content of the fish will extend the depuration time (Howgate, 2004). Although the reduction of nutrient input due to cessation of feeding could have adversely impacted MIB production by bacteria within the RAS, geosmin levels increased in trial 2 when the fish were taken off feed, so feed cessation alone may not always provide suitable conditions for purging. Transfer of the salmon to a system containing MIB-free water would likely have aided and possibly increased the purging rate of MIB from the fish flesh.

Because there was a decrease in body weight (about 1%) and fillet color quality at 15 days compared to 10 days (Table 1) and no significant reduction in mean MIB levels ($P > 0.05$) in the flesh between days 10 and 15, a depuration period ending at 10 days would be more preferable to salmon producers utilizing RAS, when purging salmon with low levels of off flavor compounds. Fillet quality and appearance are imperative for producers of salmon because fillet quality characteristics such as gaping, texture, and color are important to consumers when they purchase fish at the local retailers (Mørkøre and Rørvik, 2001; Kiessling et al., 2004; Johnston et al., 2006). The fillet color is an indicator of the content of astaxanthin, an antioxidant acquired from the diet (Matthews et al., 2006).

For the first depuration study, sensory analysis confirmed that flavor quality improved after 10 and 15 days of purging. However, members of the sensory panel had difficulty in discerning the typical "musty" flavor characteristic with MIB presence in fish

flesh, due to the initial MIB concentration. Instead, taste-test members described the taint as "woody." This descriptor was used even for a fish sample containing MIB at a level of 933 ng/kg. Johnson and Lloyd (1992) estimated the sensory threshold level for MIB by average consumers to be 700 ng/kg. The assumed sensory detection level for MIB by the average trained flavor checker in channel catfish has been listed at 100–200 ng/kg (Grimm et al., 2004). In our study, none of the sampled fish were determined to be distinctly off-flavored due to MIB presence. The characteristic "fishy" and "oily" flavors of the Atlantic salmon may have masked detection or changed the characteristic musty off-flavor often described and associated with MIB presence in the flesh of fish such as channel catfish. Higher levels of MIB in the flesh of the Atlantic salmon (>933 ng/kg) may be required for detection of the characteristic musty off-flavor.

In the second depuration study, results of the mean geosmin levels in salmon flesh (Fig. 9) clearly demonstrate the benefits of transferring the geosmin off-flavored salmon to a system that contained water that was geosmin-free (i.e., clean RAS) (Fig. 10) and the benefits of utilizing GAC to remove geosmin from water entering the flow-through culture tank as demonstrated by the reduction of geosmin from 5 ng/L to below 1 ng/L. In the first trial, MIB levels were higher than geosmin levels while the opposite situation was encountered in the second trial. The abundance of MIB-producing and geosmin-producing actinomycetes can correlate with the levels of MIB and geosmin in RAS water, and, therefore, there was likely a change in the composition of the actinomycete communities between the periods when each trial was performed. This change was a natural shift in the microbial community and the authors did not attempt to alter the community associated with the system. The patterns and rates of reduction of mean geosmin levels in the flesh of salmon from the clean RAS and from the flow-through culture tank were very similar, and the most dramatic decrease in mean geosmin levels was within the first 5 days of the depuration period. Salmon fillets from on-grow culture tank 4 that contained some of the highest levels of geosmin (e.g., 406 ng/kg, 444 ng/kg) were designated as possessing slight earthy off-flavors; the detection of the earthy off-flavor was more discernible compared to detection of musty off-flavor in fillet samples containing MIB from the first depuration study.

Actinomycetes have been attributed as the major contributors to the presence of geosmin and MIB in recirculating aquaculture systems (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010). During the second depuration study, we isolated and identified novel species of geosmin-producing actinomycetes from RAS biosolids samples (Table 3). These isolates were obtained from different locations within each system and at various sampling times during the depuration trial. All three geosmin-producing species were isolated from the recently cleaned RAS. The isolates *Nocardia salmonicida* and *Streptomyces cyaneofuscatus* are novel additions to the species of actinomycetes that have previously been identified as geosmin producers over the past several decades. The other isolate that was identified genotypically as *Nocardia* cf. *fluminea* is very close on the phylogenetic tree to *N. salmonicida*, *N. fluminea*, and *N. cummidelens* (the latter two species were isolated from a RAS in West Virginia, USA; see Schrader and Summerfelt, 2010); the *N.* cf. *fluminea* isolate differs phenotypically from these other species due to production of a darker pink pigment in its colonies.

Results from our depuration studies indicate that 10–15 days in a flow-through or in a recently cleaned recirculating system are required to reduce the concentrations of geosmin and MIB to the lowest levels. Higher levels of geosmin and MIB in the salmon flesh than those encountered in these studies may require a longer depuration period than 10 days. Because most of the fish throughout the study (including at day 0) possessed geosmin and MIB in their flesh at levels below the sensory detection threshold of the sensory panelists, a definitive purging time required for improving flavor quality in strongly off-flavored salmon cannot be inferred from the current study. However, our studies provide guidance on the depuration period/days to centralize during future depuration studies.

Depuration rates have been studied in channel catfish (*Ictalurus punctatus*) (Johnson and Lloyd, 1992; Perkins and Schlenk, 1997; Dionigi et al., 2000), Nile tilapia (*Oreochromis niloticus*) (Yamprayoon and Noomhrom, 2000) and rainbow trout (*Oncorhynchus mykiss*) (Robertson et al., 2005). Tilapia were found to require 16 days of depuration in "clean" (odor-free) static water to eliminate geosmin from the muscle tissue (Yamprayoon and Noomhrom, 2000), but the authors did not determine if the elimination of geosmin would have been faster in odor-free water using a flow-through system. For channel catfish, depuration periods when using clean water systems have been determined to require 12 h to reduce MIB concentrations in fish that have had short exposure times and as much as 20 days for catfish with longer exposure times (Johnson and Lloyd, 1992; Perkins and Schlenk, 1997; Dionigi et al., 2000). In a depuration study by Dionigi et al. (2000), channel catfish that were allowed to naturally bioaccumulate geosmin and MIB over a growing season had maximum depuration periods of 13 days and 21 days for geosmin and MIB, respectively. However, other studies determined that exposure to geosmin and MIB for shorter periods of time (e.g., 24 h) prior to conducting depuration resulted in much less time for purging of these compounds from the fish flesh (Johnson and Lloyd, 1992; Perkins and Schlenk, 1997).

Purging studies involving rainbow trout that contained geosmin and MIB in their flesh have also been performed (Gobas and Mackay, 1987; Robertson et al., 2005). Results were similar to the catfish purging studies. After the trout were exposed to MIB for a limited time (less than 24 h), depuration time in clean water was relatively short (less than 3.5 days) (Gobas and Mackay, 1987). Robertson et al. (2005) determined that higher concentrations of geosmin in the trout flesh took longer to purge. Depuration times ranged from 2 days in fish exposed to 50 ng/L geosmin to 10 days in fish exposed to 200 ng/L geosmin (Robertson et al., 2005).

In addition, our results indicate that earthy and musty off-flavored Atlantic salmon containing lower concentrations of geosmin and MIB need to be placed into a system containing water that is both geosmin-free and MIB-free, either recirculating or flow through, for at least 10–15 days to achieve the greatest reduction of the bioaccumulated off-flavor compounds in the fish flesh. Also, this study is the first to determine that a decrease of percent lipids occurs in salmon flesh during this short depuration time. The increase in moisture content of the fillets needs to be minimized in order to maintain fillet an acceptable fillet texture. However, a study by Einen et al. (1998) to determine the effects of starvation on fillet quality determined that fillet fat levels did not vary during the first 30 days. The salmon used in the study by Einen et al. (1998) were twice the size of the fish used in our study, and the fish may have used protein as an energy source. Our results for the percent carcass weight and the fillet yield from carcass weight were similar to those results observed by Einen et al. (1998). The visceral weight decreased as feed was withheld and the fillet yield remained constant during the course of our study.

In conclusion, 10–15 days were required for the greatest reduction of geosmin and MIB in the flesh of RAS-cultured Atlantic salmon used in our study. The approach of taking salmon off feed prior to harvest cannot be relied on as the sole reliable method for reducing levels of geosmin and MIB in the fish flesh. Fillet color quality was not compromised during the depuration periods used in this study, so the main fillet quality concerns should be related to flavor, texture and lipid levels.

Acknowledgments

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