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Chemical and Sensory Quantification of Geosmin and 2-Methylisoborneol in Rainbow Trout (*Oncorhynchus mykiss*) from Recirculated Aquacultures in Relation to Concentrations in Basin Water

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ABSTRACT: Globally, aquaculture systems with water recirculation experience increasing problems with microbial taste and odor compounds (TOCs) such as geosmin and 2-methylisoborneol (MIB). This study investigated the content of geosmin and MIB in water and the flesh of 200 rainbow trouts from eight recirculated aquaculture systems in Denmark. TOC content in the fish flesh was measured by a dynamic headspace extraction method and was evaluated by a sensory panel. The results showed significant correlations between TOC content in water and fish and between chemical analysis and sensory perception. When geosmin exceeded 20 ng/L in the water, 96% of the fish had an intense muddy flavor, but below 10 ng geosmin/L, 18% of the fish (only 3% in special depuration ponds) had an intense muddy flavor. The results indicate that TOC levels <10 ng/L will ensure that a negligible portion of the fish obtains an unpalatable taste and flavor due to TOCs.

KEYWORDS: geosmin, MIB, rainbow trout, recirculated aquaculture systems, sensory perception, dynamic headspace extraction

INTRODUCTION

Globally, production of taste and odor compounds (TOCs) in freshwater aquacultures causes tainting of fish and lowers the market value of fish products.[1] In the United States, TOCs are assumed to decrease sales of channel catfish by 30%,[2] and in Europe, tainting of fish by TOCs has been reported in several countries. In the United Kingdom up to 20% of trout farmers have experienced seasonal problems,[3] and one of four rainbow trouts from French aquaculture systems has been characterized as tainted or strongly tainted by TOCs.[4]

The most common TOCs in fish and water of freshwater aquaculture systems are geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (*exo*-1,2,7,7-tetramethyl[2.2.1]-heptan-2-ol, MIB), which are produced by different microorganisms,[5] but several other unpalatable organic compounds have been detected in farmed fish as well.[6,7] Tainting of fish by TOCs has been known to occur in traditional flow-through systems, but off-flavor episodes caused by TOCs appear more pronounced in systems with water recirculation.[8] Replacement of traditional systems by water recirculation systems has been enforced due to growing restrictions on water consumption in many European countries due to the EU Water Framework Directive (Directive 2000/60/EC). A consequence of the reduced water consumption has been up to 8-fold higher levels of geosmin in water of recirculated systems, relative to the traditional pond systems, as observed in systems in Denmark.[9] TOCs appear to be only slowly degradable in biofilters of recirculated systems, and this leads to accumulation in the water.[10]

The content of TOCs in fish has been measured or estimated by both chemical and sensory detection. Using chemical extraction, concentrations of geosmin of 0.5−6.5 μg/kg flesh have been reported for rainbow trout and arctic char.[4,8,11] Sensory detection of the same fish allowed grouping of the flesh as ranging from “not tainted” to “grossly tainted”, but the sensory evaluation appears inconstant when related to the geosmin content. As an example, different sensory panels characterized fish flesh with the same content of geosmin (1.3 μg/kg) as “very slightly tainted”[4] or (clearly) “tainted”.[3] This discrepancy suggests that there is a need for standardization of sensory evaluation of fish tainted by TOCs as well as of the methods used for chemical analysis.

To predict TOC episodes, fish breeders are interested in knowing if there is a relationship between contents of TOC in water and in fish. Experimental data and modeling by Howgate[10] suggest such a relationship for geosmin and MIB in water and in fish flesh, and empirical observations of arctic char seem to

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confirm this relationship.[8] Concentrations of TOCs in water can be determined at medium costs by commercial companies, whereas analysis of TOCs in fish flesh requires advanced analytical facilities that are available only at research institutes. If the fish breeder can predict the content of TOCs in fish from analysis of a water sample, this would allow the breeder to deliver palatable fish with no or minimum tainting from geosmin or MIB.

The aim of this study was to quantify and relate contents of geosmin and MIB in fish flesh of rainbow trout (*Oncorhynchus mykiss*) and in water of aquaculture systems with water recirculation in Denmark. Some of the systems had depuration facilities to reduce the content of TOCs before delivery for slaughtering. A dynamic headspace sampling method was optimized to quantify geosmin and MIB in fish flesh. To relate the content of TOCs in the fish flesh to palatability, the fish were evaluated for taste, odor, and texture attributes by a trained sensory panel. The results were expected to reveal relationships between content of TOCs in fish flesh and water and between chemical analysis and sensory detection of TOCs in fish flesh. On the basis of the obtained results, recommendations regarding acceptable levels of geosmin and MIB in rainbow trout grown in recirculated systems are given.

MATERIALS AND METHODS

Sampling. Rainbow trout and water samples were collected in September and October 2009 in eight aquaculture facilities with water recirculation in Jutland, Denmark. Seven of the facilities were located in the open terrain, whereas one facility was partly an indoor tank system. The facilities received stream, well, or spring water and consisted of two to four production basins, each of about 1000 m^3. After passage in the production ponds, water was treated by filtration in drum filters (removal of particles above approximately 50 μm) and biofilter tanks. The degree of water recirculation varied from 85 to 95%, meaning that 5−15% of the water was replaced daily, and flow rates were typically 500 L/s in the basins. Water temperature during sampling was 8−15 °C. The fish were fed dried pellets at a daily ration of about 1% of the body weight, depending on the size of the fish and water temperature. Composition of the trout feed varied, but typical ingredients were (based on the Enviro 921 feed by Biomar; www.biomar.com) approximately 47% protein and 26% oil (both originating from fish and plants), 12.7% carbohydrates (from plants), 7.5% ashes, and 0.8% wood fibers. Seven of the facilities had depuration basins in which the water was replaced regularly by TOC-free water from stream, well, or spring. The density of fish in the systems was 50−70 kg/m^3. The fish were kept for about 1 week without feeding in the depuration ponds to purge TOCs before delivery to the processing factories. In each aquaculture facility, 12 fish from production ponds and 12 fish from depuration ponds were collected. The fish were typically 9 months old and weighed 308 ± 72 g (mean ± SD). The fish were slaughtered on site and allowed to bleed before being transported to the laboratory in coolers at 4 °C.

Water samples for analysis of geosmin and MIB were collected in 250 mL bottles that were filled completely and capped before transport to the laboratory in a cooler. Triplicate subsamples of 40 mL of water were transferred to 100 mL serum bottles to which were added 12 g of NaCl (to increase the volatility of the TOCs) and a stir bar. The serum bottles were capped with a silicone−PTFE seal and kept at 3 °C until analysis.

Preparation of Fish and Lipid Analysis. In the laboratory, each fish was weighed, skinned, and analyzed for content of fat by the Fatmeter technique (www.distell.com; model FM 692 calibrated for trout flesh) by taking the average of eight consecutive readings with a Fatmeter probe. Excess water on the fish was gently removed with paper towels before the Fatmeter measurements. The probe was held under the dorsal fin of the fish, directly over the lateral line. The Fatmeter sensor transmits microwave radiation at 2 ± 20 GHz, measuring the dielectric permittivity of tissues. The fat content is estimated from the inverse relationship between water and lipid in the tissue. The fillets were vacuum-packed and frozen at −80 °C until chemical and sensory analysis.

Analysis of Geosmin and MIB in Water. Geosmin and MIB were extracted from water samples by solid phase microextraction (SPME) using a 2 cm 50/30 μm StableFlex fiber (part no. 57348-U) in a manual fiber holder (part no. 57330-U), both from Supelco (Sigma-Aldrich). The 40 mL water samples with added NaCl were placed in a 60 °C water bath with a magnetic stirrer. The SPME fiber was exposed to the headspace of the flask for 15 min to collect the TOCs. Vaporization of the TOCs was increased by vigorously rotating the stir bar. After the absorption, TOCs on the fiber were desorbed for 3 min at 260 °C in the splitless injector of a gas chromatograph−mass spectrometer (GC-MS) (TRACE GC with Polaris GCQ, Thermo-Finnigan, USA). The GC column was 30 m × 0.25 mm internal diameter, 0.25 μm film thickness Rtx-5MS (Restek, USA) with helium as carrier gas at a constant flow of 1.2 mL/min. The temperature program was 45 °C (3 min), raised at 30 °C/min to 250 °C and at 100 °C/min to 300 °C (2 min). The temperatures of the transfer line and ion source were 275 and 200 °C, respectively, and the scan range was m/z 30−200. Quantification of geosmin and MIB was made by selected ion monitoring of the m/z 112 and 95 fragments, respectively. Linearity and detection limit of the GC-MS procedure was tested using a standard series of MIB and geosmin of 10−100 ng/L. The analytical lower detection limit was estimated to 0.2 ng/L, and the precision for concentrations <30 ng/L was 6−9%. For capacity reasons most samples were run as a single analysis, but measurements of triplicate samples were done for every five to six samples.

Analysis of Geosmin and MIB in Fish. From each of the eight facilities, between 7 and 20 fish representing both production ponds and depuration ponds (in total, 96 fish) were analyzed. All of these were also sensory profiled. Geosmin and MIB were extracted using an optimized dynamic headspace sampling method. Twenty grams of fish meat was transferred to a gas washing bottle, 25 mL of water and 100 μL of internal standard solution (5 mg/L 4-methyl-1-pentanol in water) were added, and the content was homogenized for 30 s at 13500 rpm using an Ultra Turrax (IKA T25 digital, Staufen, Germany). An additional 10 mL of water was used to rinse the Ultra Turrax, and immediately thereafter the gas washing bottle was closed with a purge head. The bottle was placed in a water bath at 50 °C, and after temperature equilibration, it was purged with 100 mL of N_2/min for 60 min; geosmin and MIB were collected on a Tenax TA trap. After headspace sampling, the traps were dried with a flow 100 mL/min of nitrogen for 15 min. The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin-Elmer, Norwalk, CT). Primary desorption was carried out by heating the trap to 250 °C with a flow (60 mL/min) of carrier gas (He) for 15.0 min. The stripped volatiles were trapped on 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 a Triple-Axis detector from Agilent Technologies, Palo Alto, CA) through a heated (225 °C) transfer line.

Separation of volatiles was carried out on a DB-Wax capillary column, 30 m × 0.25 mm internal diameter and 0.25 μm film thickness. The column flow rate was 1 mL/min using helium as carrier gas. The column temperature program was 40 °C (10 min) and 8 °C/min until 240 °C (5 min). The mass spectrometer was operating in the electron ionization mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned, and simultaneously data were collected in selected ion monitoring mode, monitoring mass 69 for internal standard, mass 95 for MIB, and mass 112 for geosmin. The identity of peaks was confirmed by probability-based matching of their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A) and by comparison with retention times of authentic standards. The software program MSD Chemstation (version E.02.00, Agilent Technologies) was used for data analysis.

To carry out an absolute quantification, known amounts of geosmin and MIB (0, 1, 5, and 25 μg/kg) were added to meat from six selected fish during homogenization. The relationship between peak area and concentration was shown to be linear, and the slopes of the obtained calibration curves were used to convert peak areas to concentrations. The method's limit of detection was below 0.1 μg/kg for both compounds (signal-to-noise ratio above 10).

To determine the method's reproducibility, four samples of meat were taken from each of three fish and analyzed. For the geosmin analysis, the average coefficient of variation was 10% in fish with a representative geosmin content (1−2 μg geosmin/kg). Unfortunately, the selected fish had a low MIB content, and the reproducibility could not be determined. It is, however, expected to be in the same range as for geosmin.

Sensory Profiling of Fish. Between 15 and 22 fish from each facility (representing both production pond and depuration pond, 149 in total) were examined by sensory profiling. The sensory panel consisted of eight assessors (five females and three males; average age, 56 years) that were all selected, tested, and specifically trained in descriptive analysis of rainbow trout.[13,14] The vocabulary was developed in a preliminary study consisting of four sessions, the first session being qualitative; here the aim was to develop a list of descriptors for odor and flavor of rainbow trout.[15,16] The following three sessions were quantitative; here the assessors were trained to evaluate the descriptors on a line scale. Trout with a known content of geosmin and MIB was minced and mixed for specific scale training of the attributes "moldy/fusty" odor and taste and "muddy" odor and taste. 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.[17] The descriptors are shown in Table 1.

The sensory analysis was performed in separated booths under normal daylight and at ambient temperature.[18] Data were collected using a computer system (FIZZ Network version 2.0, Biosystems, France). The samples were placed in individual porcelain bowls and covered with porcelain lids with three-digit codes. The fillets were cut into six pieces, and each fillet was served in replicate to the same assessor, meaning that one fillet was assessed by three assessors in total. The fish samples were heated in a prewarmed convection oven (Rational Combi-Dämpfer CCM; www.gastrodax.de) with air circulation to a core temperature of 70 °C. After heat treatment, the samples, then having a temperature of 50 °C, were immediately served to the panel. The samples were served one by one in random order. The assessors used water and flat bread to clean the palate between samples. A sample without any sensory detectable MIB and geosmin was used as reference.

Statistical Methods. Multivariate statistics (principal component analysis, PCA) was carried out using the software package LatentiX ver. 2.00 (Latent5, Copenhagen, Denmark) to obtain an overview of all sensory parameters in all samples. PCA is a technique to condense information from many (often correlated) variables into a few uncorre-

Table 1. Descriptors Used in the Sensory Profiling

odor	flavor
moldy/fusty (O)	moldy/fusty (F)
earthy (O)	earthy/mushroom (F)
muddy (O)	muddy (F)
cooked potato (O)	cooked potato (F)
sourish (O)	sourish (F)
green (O)	smoke (F)
warm milk (O)	bitter (F)
	astringent

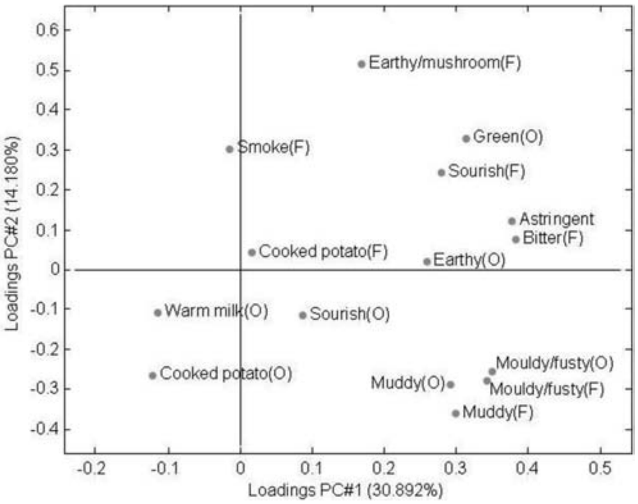


Figure 1. Loadings plot from PCA on sensory descriptors showing relationships between the descriptors used. The PCA includes data of 149 fish. F, flavor; O, odor.

Table 2. Background Data on Pond Systems and Water Parameters

					geosmin (ng/L)		MIB (ng/L)	
pond facility	type of system[a]	production of fish (tons/year)	degree of recirculation (%/day)	water temperature (°C)	PP[b]	DP[c]	PP	DP
1	O	1200	95	14.0	6.6	13.7	3.2	3.1
2	O	1150	95	13.0	27.1	8.4	4.3	5.4
3	O	200	70	12.0	17.7	17.8	6.1	5.3
4	O	550	95	13.2	36.1	26.1	28.5	10.6
5	I	500	95	15/8[d]	7.9	5.3[d]	4.7	4.6[d]
6	O	220	70	8.7	10.1	11.2	3.8	4.3
7	O	450	95	9.0	12.4	1.2	4.2	1.2
8	O	400	95	9.9	16.7	14.6[e]	4.2	6.4[e]

[a] Open terrain (O) or indoor (I). [b] Production ponds (PP). [c] Depuration ponds (DP). [d] Outdoor depuration ponds. [e] Production pond used as depuration pond (increased water exchange).

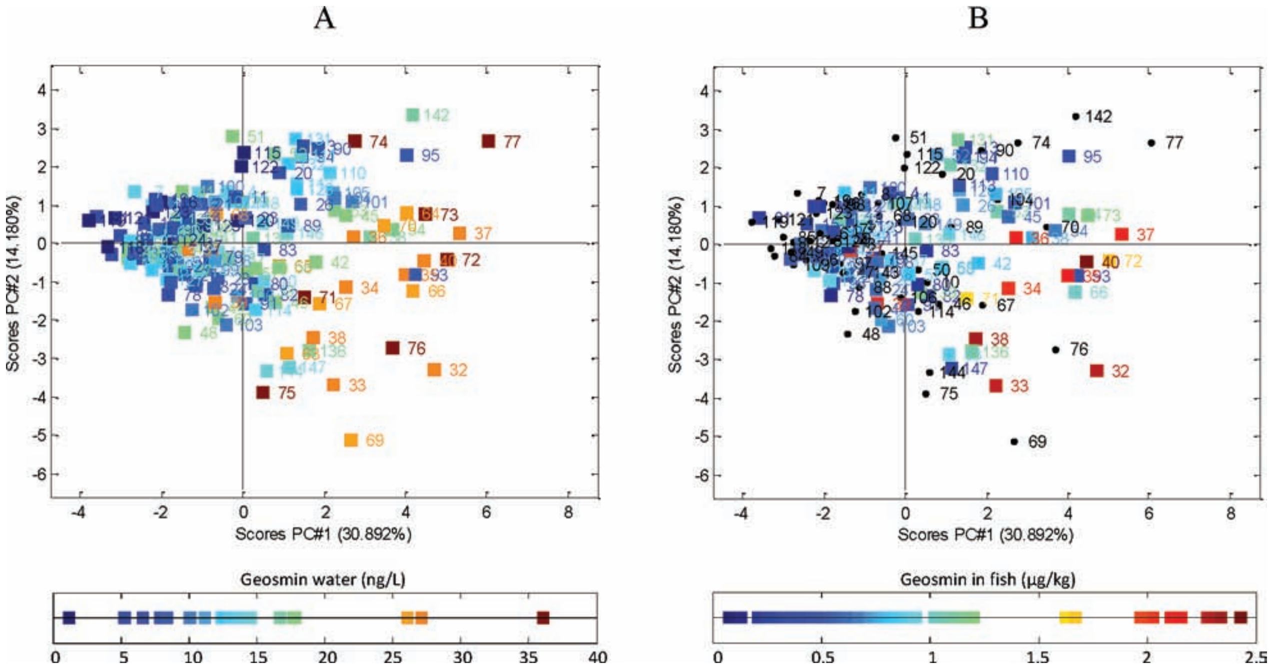


Figure 2. Score plot from PCA on sensory descriptors of 149 fish (same fish as were used for PCA in Figure 1). The two plots are identical, except that in plot A each point is colored according to the concentration of geosmin (GSM) in the water from which the fish was taken, and in plot B each point is colored according to the concentration of geosmin in the fish. Color indicating concentration of geosmin is shown in the lower panel.

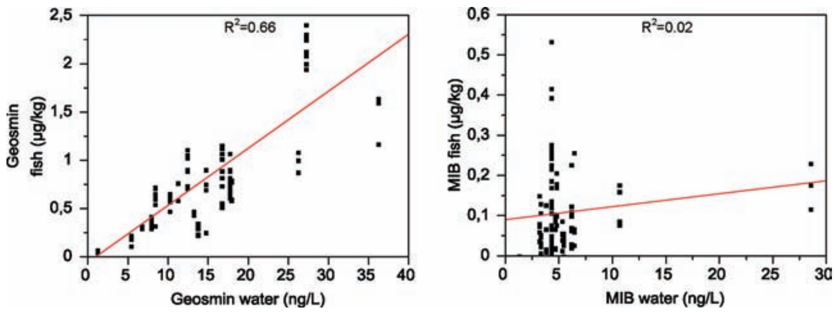


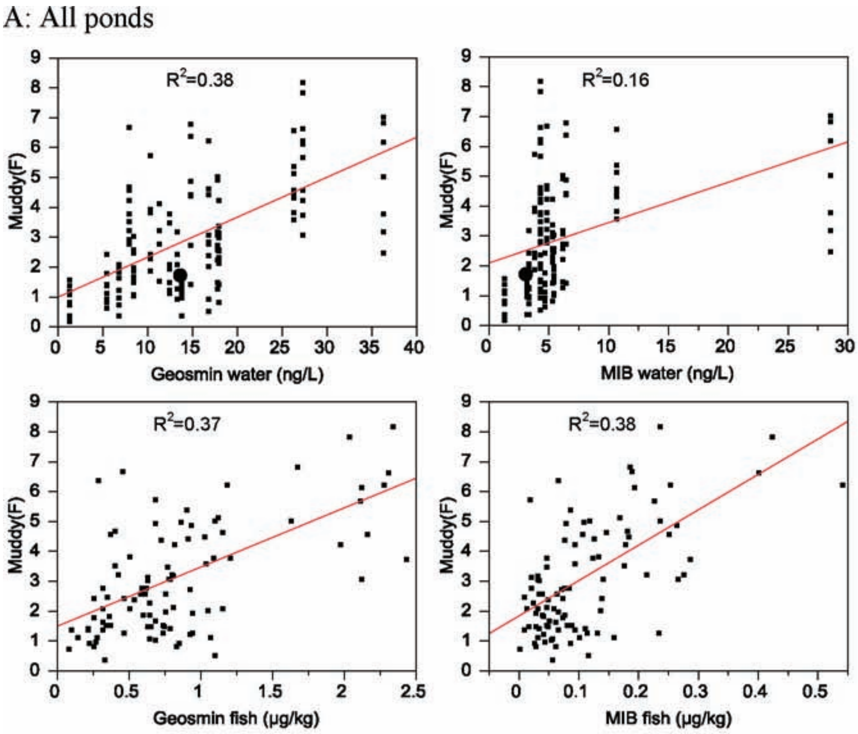
Figure 3. Relationship between concentrations in water and fish of geosmin and MIB. Data of 96 fish are shown.

lated principal components (latent variables). The main outputs from the PCA are the loadings plot showing relations between the measured variables and the score plot that shows relationships between samples. Univariate statistics (correlation coefficients, ANOVA, and chi-square test) were carried out using the software package JMP ver. 8.0 (SAS, Cary, NC). ANOVA was used to test whether observed differences in individual variables were significant, and the chi-square test was used to test whether distributions of a categorical response variable (fish with or without distinct muddy flavor) were significantly different conditioned by the values of a categorical factor (high, intermediate, or low concentration of geosmin in water).

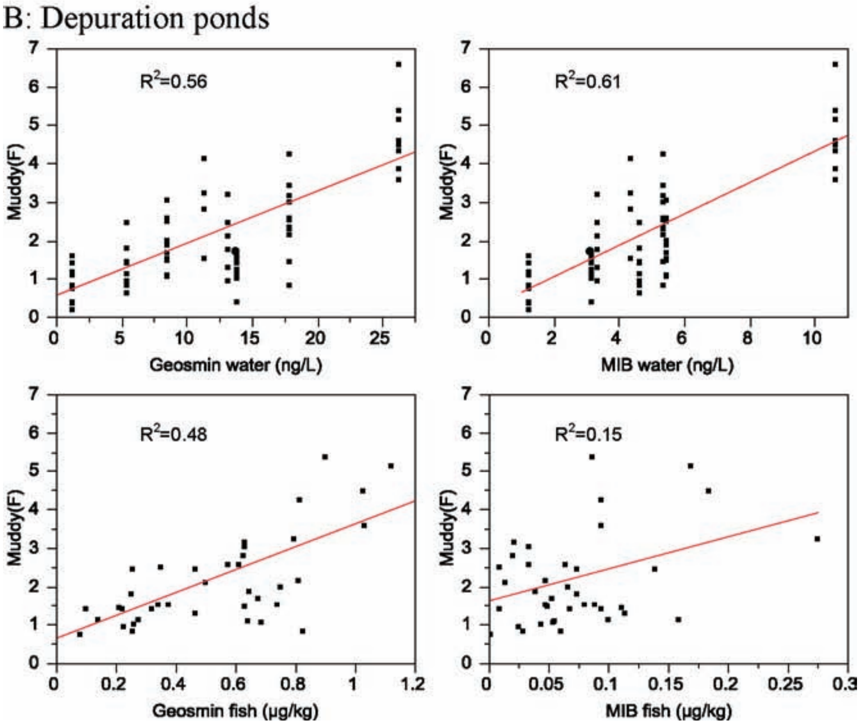
■ RESULTS AND DISCUSSION

Concentration of Geosmin and MIB in Pond Water. Concentrations of geosmin and MIB in water of the production ponds were 6.6−36.1 and 3.2−28.5 ng/L, respectively (Table 2). In the depuration ponds, concentrations of 1.2−26.1 ng/L (geosmin) and 1.2−10.6 ng/L (MIB) were measured. There were rather large differences between the individual ponds and facilities, especially for concentrations of geosmin. A large variation in concentrations of geosmin between pond systems has also been found in other studies, and even larger variations than observed here were reported in some cases.[3,5,8,19] The depuration ponds were expected to have lower levels of geosmin and MIB than the production ponds due to a higher frequency of water replacement, but a lower level of off-flavors was not always found in the depuration ponds, as discussed later.

Chemical Detection and Sensory Profiling of TOC in Fish: Overview of Data Using Multivariate Statistics. A PCA was carried out based on the 15 sensory descriptors and included data from 149 fish. The PCA loadings plot shows that the descriptors are well distributed, meaning that many different sensory properties are described consistently by the assessors (Figure 1). Also, the descriptors “moldy/fusty” and “muddy” (both odor and flavor) correlate closely. The PCA is based solely on the sensory variables, but in the corresponding score plot (Figure 2) each point, representing an individual fish, is colored according to the concentration of geosmin in the water from which the fish was taken (Figure 2A). By comparing Figures 1 and 2A it is seen that fish with higher intensity of “muddy” and “moldy/fusty” odor and flavor and “earthy” odor generally originated from water with the highest



A: All ponds



B: Depuration ponds

Figure 4. Relationship between muddy flavor (F) and concentration of geosmin and MIB in water and fish from all ponds (A) and depuration ponds (B). In the upper panels in each part of the figure, concentrations of geosmin and MIB in water of 149 fish are shown; in the lower panels, contents of geosmin and MIB of 96 fish are shown.

concentration of geosmin. In Figure 2, plot B is identical to plot A except that each fish is colored according to the concentration of geosmin (GSM) in the fish flesh. The plot shows that fish with higher intensity of "muddy" and "moldy/fusty" odor and flavor and with "earthy" odor also have a higher content of geosmin in the flesh. Similar correlations were obtained for MIB (not shown). These observations confirm that fish take up and accumulate geosmin and MIB from the water, as also documented in other studies,[12] and that the odor character for geosmin can be described as earthy[20] and as musty, mold-like, and earthy for MIB.[21]

Table 3. Geosmin in Water and Score of Muddy Flavor in Fish from the Ponds Examined.[a]

	geosmin in water (ng/L)		muddy (F)		
pond facility	PP	DP	PP	DP	
1	6.6	13.7	1.3 ± 0.7	1.4 ± 0.4	ns
2	27.1	8.4	5.8 ± 1.7	2.0 ± 0.7	***
3	17.7	17.8	3.1 ± 1.4	2.7 ± 0.9	ns
4	36.1	26.1	5.0 ± 1.8	4.9 ± 1.0	ns
5	7.9	5.3	4.2 ± 1.2	1.3 ± 0.7	***
6	10.1	11.2	2.9 ± 1.7	2.5 ± 1.0	ns
7	12.4	1.2	2.3 ± 1.1	1.0 ± 0.4	***
8	16.7	14.6	3.7 ± 1.9		

[a] Values are the mean of all fish from each pond. PP, production ponds; DP, depuration ponds. "ns" indicates no significant difference and "***" indicates significant ($p < 0.001$) difference between muddy (F) in PP and DP (ANOVA).

Univariate Correlations of TOCs in Fish and Water. The relationship between concentration of geosmin in water and fish demonstrates a rather high correlation ($R^2 = 0.66$), but a similar relationship is not seen for MIB (Figure 3). Presumably, the small variation in MIB concentrations in the water, relative to the concentration in the fish, prevents a water—fish relationship for MIB. In fact, 13 of the 16 examined ponds had concentrations of MIB between 3.1 and 6.4 ng/L.

The PCA plots demonstrated that the descriptors "muddy odor" and "muddy flavor" and "moldy/fusty odor" and "moldy/fusty flavor" had a high correlation, and therefore only one of these descriptors is characterized in the following. "Muddy flavor" was chosen because it generally gave the highest correlations. Figure 4A shows that "muddy flavor" correlated with geosmin concentration in water and fish and with MIB concentration in fish. The observed correlation coefficients (R^2) of 0.16—0.38 are intermediate but significantly different from 0. The lowest correlation was found between "muddy flavor" and concentration of MIB in water. Possibly, accumulation of MIB in rainbow trouts is more variable than accumulation of geosmin as indicated by Zimba et al.,[22] who found a 4-fold variation in content of MIB, as compared to only a 2-fold variation in geosmin, in trout flesh from fish of comparable size. Our results indicate that the content of geosmin (and MIB) is rather variable in farmed fish, and this notion is supported by other studies of both rainbow trout and arctic char (*Salvelinus alpinus*).[4,8,11] The reason for these individual differences may be related to feeding patterns, physiology, activity, and growth rate, but more studies are needed to confirm this.

The data presented above originate from both production and depuration ponds. However, from a farmer's perspective, conditions in the depuration ponds are most relevant because fish for the consumer market are delivered from these ponds. A comparison of "muddy flavor" versus content of geosmin and MIB in fish from depuration ponds shows a higher correlation for geosmin ($R^2 = 0.48$) than for MIB ($R^2 = 0.15$) (Figure 4B). However, because there was no clear relationship between concentration of MIB in water and fish, a clear relationship between MIB in water and "muddy flavor" would not be expected (as in Figure 4A). The higher correlation between "muddy flavor" and MIB content in the water in Figure 4B ($R^2 = 0.61$) than in Figure 4A ($R^2 = 0.16$) may reflect that the most "odd" samples occurred in production ponds and were thus omitted in Figure 4B.

Table 4. Relationships between Sensory Categories and Total Content of Geosmin in Rainbow Trout

	Robertson et al.[11]	Robin et al.[4]	this study[a]
not tainted	no data	1.12 ± 0.38	<0.25
very slightly tainted	no data	1.36 ± 0.62	
slightly tainted	1.08 ± 0.09	1.32 ± 0.59	
tainted	1.31 ± 0.21	2.27 ± 0.66	0.80
strongly tainted	2.05 ± 0.24	4.18 ± 0.52	
weight of fish	280 g	250—450 g	308 g

[a] Only two sensory categories (not tainted and tainted) were included in this study.

Sensory Evaluation versus TOC Content. With respect to threshold levels, there are only a few well-conducted studies of sensory thresholds for geosmin and MIB in fish flesh. Robertson et al.[11] determined the sensory threshold of geosmin in rainbow trout flesh to be 0.9 μg/kg. Grimm et al.[23] used professional flavor checkers highly sensitive to geosmin and MIB and reported average odor thresholds between 0.25 and 0.5 μg/kg for geosmin and between 0.1 and 0.2 μg/kg for MIB in channel catfish (*Ictalurus punctatus*). In the present study the sensory panel was trained to attribute the anchor point of 1.5 to "little" intensity. Applying a value of 1.5 to Figure 4B for "muddy flavor", the linear correlation indicates a threshold of <0.25 μg/kg for geosmin, whereas the threshold for MIB appears lower, but the variation is too large to indicate a specific threshold value. Thus, for geosmin our threshold value agrees well with the observations by Grimm et al.[23]

Sensory levels for fish are typically categorized relative to degree of tainting. Results from this work and studies of rainbow trout from British and French aquaculture sites show some inconsistency between content of geosmin and sensory category (Table 4). Thus, fish categorized as "not tainted" may contain from <0.25 to 1.12 μg geosmin/kg, and "strongly tainted" fish may contain from 2.05 to 4.18 μg geosmin/kg. These differences may reflect different approaches by the sensory panels. In this study, samples were served warm (50 °C) to the sensory panel, whereas the serving temperature was not indicated in the studies cited above. Also, the inherent fact that different panels consist of different individuals may lead to variable results, for example, due to differences in individual sensitivity and training. In addition, the routine and size of the panel might influence the final sensory evaluation. The panel in this study consisted of eight panelists having from 5 to >10 years of experience in sensory perception of fish. In the studies by Robertson et al. and Robin et al., the panels consisted of 4 and 16 members, respectively.[11,4] In a review by Howgate[10] it is concluded that the threshold for sensory perception of geosmin and MIB in fish is influenced by the actual fish species being examined, its fat content, and possible masking by other flavors. In addition, sensory procedures and the criteria used for defining the threshold may influence the sensory evaluation. In our study, the content of geosmin was found to vary from below to well above the threshold, whereas the content of MIB was below or close to the threshold and made it difficult to establish significant correlations between "muddy flavor" and MIB concentrations in the water.

Effect of Depuration Ponds. In three of the facilities, the geosmin concentration in water of the depuration ponds was equal to or higher than the concentration in the production ponds (Table 3). This was not expected. It is regular practice to

Table 5. Number and Percentage of Fish Having Distinct Muddy Flavor (Muddy (F) > 3) in Ponds with Different Geosmin Concentrations[a]

	geosmin in water				
fish having muddy (F) > 3	<10 ng/L	10−20 ng/L	>20 ng/L	total	significance of relationship
all ponds					
number	9 of 50	26 of 74	24 of 25	149	$p < 0.0001$
%	18	35	96		
depuration ponds					
number	1 of 31	7 of 31	8 of 8	70	$p < 0.0001$
%	3	23	100		

[a] p values from chi-square tests are shown.

replace the water in the depuration ponds and remove biofilm on walls of basins by mechanical or chemical means to reduce TOC levels. Growth of TOC-producing microorganisms in the basins is, however, a continuous process, and it is therefore difficult for the fish farmer to know when water replacement or cleaning is needed. One fish farmer (pond facility 7) has been successful in establishing wall-cleaning routines at weekly intervals, and this has significantly reduced the TOC content in both water and fish.

Influence of Fat Content in Fish. The fat content in the fish varied from 1.9 to 10.6%, with a mean of 5.6 ± 1.6% (data not shown). No correlations were found between fat content and any of the measured parameters (except for a positive correlation with size of the fish). Neither was any influence of fat content found when calibration curves were constructed to be used for quantification of geosmin and MIB in fish flesh. At first, it was assumed that separate calibration curves had to be used depending on the fat content of the fish, but the calibration curves were independent of the fat content, and identical calibrations were used for all fish.

Several authors have shown that fat content in fish influences rate of uptake, level at equilibrium, and rate of depuration of TOCs.[12,24,25] In catfish (*I. punctatus*) it has been shown that content of MIB is related to muscle fat (fish with >2.5% muscle fat accumulated nearly 3 times more MIB than fish with <2% fat),[23] but no relationships between muddy odor and fat content were found in a study of Australian barramundi (*Lates calcarifer*).[7] The lack of effects from fat content on geosmin and MIB content in fish flesh in the present study may be due to the rather similar contents of fat in the fish (average, 5.6%); only three fish had fat content below 2.5%.

Recommendations for Production of Minimum-Tainted Fish. In the aquaculture industry there is a need for tools to monitor levels of TOC compounds to meet expectations by the consumers. From the present relationsships between concentrations of especially geosmin in water and fish, and relationships between content in fish and sensory evaluation, recommendations for production of a minimum-tainted fish can be given. On the basis of the observed variation of our results, it was decided to conduct a chi-square test on categorized data. In this analysis the distribution of a categorical response variable (fish with or without distinct muddy flavor) as conditioned by the values of a categorical factor (high, intermediate, or low concentration of geosmin in water) was examined. From the sensory testing, a level of "muddy flavor" of ≥3 was considered to be distinct, and fish from water with levels of geosmin being <10 ng/L, between 10 and 20 ng/L, and >20 ng/L, were compared. The chi-square tests clearly show that there is a highly significant effect of geosmin in the water on the proportion of fish having distinct muddy flavor (Table 5). This relationship is obvious whether all ponds are analyzed together or production ponds and depuration ponds are analyzed separately (only data from depuration ponds are shown). In fact, a clear recommendation can be given for the highest allowed geosmin content in the water, because at a geosmin level of ≤10 ng/L, practically no fish will have distinct muddy flavor, whereas almost all fish from ponds with geosmin levels >20 ng/L are expected to have distinct muddy flavor.

Today, several companies offer analysis of TOCs in water samples, whereas characterization of TOC content in fish by chemical analysis or sensory evaluation is complicated and performed only by special laboratories. Knowledge on the relationship between a specific geosmin concentration in the water and an expected content in the fish will, however, allow farmers to act on critical geosmin concentrations.

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