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Interactive effects of temperature and water exchange of depuration tanks on geosmin excretion by Atlantic salmon (*Salmo salar)*

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ABSTRACT

Fish farmers utilize the reversibility of the bioconcentration process to depurate geosmin and other off- flavour causing chemicals from their fish by placing them in clean water just before harvest. To better understand and improve this process, we investigated effects of temperature and water exchange of depuration tanks on geosmin elimination from Atlantic salmon (*Salmo salar*). Fish loaded with geosmin were depurated for 144 h during which they were subjected to combinations of water exchange (stagnant water or a water exchange rate of ~1200 L/kg fish/d) and temperature (~11.5 or ~14.5 °C) treatments. Model predictions indicated enhanced depuration by water exchange, elevated temperature and interactive effects of these two factors, plus geosmin accumulation in the depuration tank water. The latter was predicted but not observed in the experiment. Furthermore the elevated temperature did not enhance geosmin elimination from Atlantic salmon, possibly because in the tanks with water exchange, temperature treatments did not cause differences in oxygen levels and thus gill ventilation rates. The water exchange significantly increased geosmin elimination from Atlantic salmon, indicating that removal of excreted geosmin from the direct environment of this fish is needed to obtain maximal geosmin elimination from the fish.

1. Introduction

According to the general fish aquatic bioaccumulation model (Arnot and Gobas, 2006; OECD, 2012) the uptake and excretion of moderately lipophilic chemicals by fish is a process of passive diffusion driven by differences in concentrations and affinities of chemicals for water and lipids. This model has been used to describe the bioaccumulation in fish of moderately lipophilic chemicals like for example the pesticide atrazine (El-Amrani et al., 2012) and geosmin (Howgate, 2004). According to this model, exchange of moderately lipophilic chemicals predominantly takes place over the water/blood barrier in the fish gill. This bioconcentration process is dynamic and reversible.

Geosmin is one of the chemicals responsible for earthy/musty off-flavours in fish, which is considered a quality defect by human consumers. In land-based aquaculture systems off-flavour chemicals are often produced by microbiota in biofilters (reviewed by Azaria and van Rijn, 2018). Following release to the water, these chemicals are quickly bioconcentrated in fish, rendering it off-flavoured (reviewed by Howgate, 2004). Fish farmers use the reversibility of the bioconcentration process to remove off-flavour causing chemicals from their fish. Before harvest, fish are placed in clean water where they are usually not fed until off-flavour chemical levels have declined below their human sensory detection thresholds. This depuration process however is not always effective and predictable. Also, it adds significantly to the production costs due to biomass losses (Schram et al., 2008; Burr et al., 2012) and additional operational and investments costs associated to depuration systems. Further optimization of the depuration process is needed to prevent market entrance of off-flavoured fish.

According to the general fish aquatic bioaccumulation model, the geosmin concentration in the fish declines exponentially when the fish is depurated in geosmin free water, depending only on the geosmin concentration in the fish at the start of the depuration process and the excretion rate constant (Howgate, 2004). For a given initial geosmin concentration in the fish the time required to depurate geosmin from fish can then be reduced by increasing the excretion rate by e.g. increasing the water temperature (Howgate, 2004).

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In industrial off-flavour depuration systems with high fish densities, excreted geosmin accumulates in the water (Podduturi et al., 2021) where it is available for re-uptake in the fish. Under such conditions, net excretion will also depend on the uptake rate constant, the fish density and the geosmin removal rate from the depuration tank. The time required to depurate geosmin from the fish can then also be reduced by increasing the removal rate from the direct environment of the fish by e.g. increasing the water exchange rate of the depuration tank. We hypothesize that these two basic mechanisms for reduction of the depuration time interact: a maximum net effect of an increased excretion rate on depuration time requires removal of excreted geosmin from the direct environment of the fish.

The effect of increased water exchange rate of depuration tanks on geosmin excretion was previously studied for European eel (Schram et al., 2017) and Atlantic salmon (Davidson et al., 2020). Geosmin depuration from eel did not increase with water exchange, which was explained by possible biotransformation of geosmin by the eel. In the recent study by Davidson et al. (2020) with Atlantic salmon, water exchange rate of depuration tanks did enhance net geosmin excretion. They observed the lowest off-flavour levels in Atlantic salmon depurated at the highest water exchange rates. The above mentioned interaction between geosmin excretion by the fish and removal from the depuration tank by water exchange has however not yet been studied.

Therefore, this study aims to unveil main and interactive effects of increased geosmin excretion and geosmin removal on the net elimination of geosmin by fish. Elevated water temperature was used to enhance geosmin excretion rates (Howgate, 2004). Depuration tanks with and without water exchange were used to create different geosmin removal rates from the tanks. Experiments were conducted with Atlantic salmon (*Salmo salar*) and the results compared to model predictions (Schram et al., 2017).

2. Materials and methods

2.1. Depuration model and prediction of experimental results

The derivation of the equations for the geosmin concentrations in the water ($C_{W(t)}$) and the fish ($C_{F(t)}$) over time as a function of uptake, excretion, fish density and water flow rate over the depuration tank were described in detail in Schram et al. (2017). In brief: Howgate (2004) obtained a simplified model for geosmin bioconcentration in fish by excluding biotransformation, faecal egestion and growth dilution from the general fish bioconcentration model for moderately lipophilic chemicals (reviewed Arnot and Gobas (2006):

$$\frac{dC_F}{dt} = k_1 C_W - k_2 C_F \quad (1)$$

where dC_F/dt is the change of the chemical concentration in the fish C_F (g/kg) over time, C_W the constant chemical concentration in the water (g/l), k_1 the rate constant (1/d) for the uptake from the water and k_2 the rate constant (1/d) for excretion to the water.

This model assumes a constant chemical concentration (C_W) in the water. However, at high fish densities we predict accumulation of excreted geosmin in the water. The model was therefore extended by allowing C_W to vary over time as a function of chemical uptake and excretion by the fish and chemical outflow via the tank effluent, which is described by:

$$\frac{dC_W}{\mathrm{dt}} = zk_2 C_F - zk_1 C_W - QC_W \quad (2)$$

where the first term describes the increase in concentration as a result of elimination from fish, the second term is the change rate of the concentration in the water as a result of uptake by fish, and the third term is the loss rate from the inflow of clean water (at rate Q). The tank volume is assumed constant and hence there is also an implicit outflow of water with concentration C_W The parameter z is the ratio of fish to water volume (BM/V, Table 1), which is used to account for the different volumes of water and masses of fish present. Fish and water were assumed to have an identical density (one). Other assumptions to Eq. (2) are that the water inflow does not contain any chemical.

The system formed by Eqs. (1) and (2) was solved mathematically using Mathematica 9.0 (Wolfram Research, Champaign, Illinois, USA) to yield equations for $C_F(t)$ and $C_W(t)$ (Schram et al., 2017). We used these equations for $C_F(t)$ and $C_W(t)$ to predict the results of the depuration experiment with Atlantic salmon. Therefore model input (Table 1) closely resembled the actual experimental conditions for Atlantic salmon (Table 2). As geosmin uptake (k_1) and excretion (k_2) rate constants are unknown for Atlantic salmon, we used the rate constants calculated by Howgate (2004) for rainbow trout with a lipid content of 10% (*w*/w). We obtained rate constants specific for the fish weight and temperatures in the current experiment by interpolation of Howgate's (2004) rate constants for different body weights and temperatures. As the excretion rate constant depends on lipid volume (Gobas and MacKay, 1987), the k_2 values were recalculated to the lipid content of 3.5% (w/w) of the Atlantic salmon in the current experiment. We assumed equal gill uptake efficiency for chemicals and the gill ventilation rate for rainbow trout and Atlantic salmon.

2.2. Depuration experiment

2.2.1. Ethics statement and origin of the experimental animals

The treatment of the fish was in accordance with Dutch law concerning animal welfare, as approved by the ethical committee for animal experimentation of Wageningen UR Livestock Research (protocol 2013146.a). Atlantic salmon fry obtained from Meridian salmon Ltd., UK was raised to smolts of ca. 200 g and transferred to seawater in the research facilities of Wageningen Marine Research, The Netherlands.

2.2.2. Experimental design and procedures

The experiment was set up as a 2x2x3 factorial block design with water exchange (Q), water temperature (T) and depuration time (Ti) as factors and two sessions in time as blocks to obtain replication of the experimental treatments.

Prior to each session a batch of Atlantic salmon ($n = 80$ per batch) with mean (SD) individual weights of 199.1(42.0) (batch 1) and 202.(35.8)g (batch 2) and a mean (SD) lipid content of 3.5 (0.6)% (*w*/w) was loaded with geosmin by exposure to waterborne geosmin for 5 days. To this end a batch of fish was stocked in a polyethylene tank filled with 830 l seawater with a salinity of 33 g/l. On day 1 of the geosmin loading of the fish, tank water was spiked with geosmin to a nominal concentration of 0.6 μg/l by adding 1 l of a 0.5 μg/ml geosmin stock solution in

Table 1
Model input.

Parameter	Symbol	Value or range	Unit
Geosmin concentration inflowing water	$C_{WD(0)}$	0	(ng/kg)
Uptake rate constant geosmin at high temperature	k_1	229	(1/d)
Excretion rate constant geosmin at high temperature	k_2	0.57	(1/d)
Uptake rate constant geosmin at low temperature	k_1	172	(1/d)
Excretion rate constant geosmin at low temperature	k_2	0.42	(1/d)
Initial geosmin concentration in the fish	$C_{B(0)}$	8500	(ng/kg)
Water flow rate – tanks with water exchange	Q	1200	(l/kg fish/d)
Water flow rate – tanks with stagnant water	Q	0	(l/kg fish/d)
Tank volume	V	170	(l)
Individual fish weight	W	200	(g)
Total fish biomass per tank	BM	1.0	(kg)

Table 2
Experimental conditions. Mean (SD) values for water flow rate, water temperature and dissolved oxygen concentration.

Session	Water exchange treatment (Q)	Temperature treatment (T)	Water flow rate		Water temperature	$[O_2]$
			(l/kg fish/d)	l/d	(°C)	(mg/l)
1	Water exchange	High	1203 (52)	1218 (36)	14.6 (0.2)	10.3 (3.0)
	Water exchange	Low	1267 (162)	1319 (114)	11.6 (0.2)	11.4 (2.8)
	Stagnant	High	0	0	14.5 (0.1)	10.8 (4.2)
	Stagnant	Low	0	0	11.4 (0.4)	14.5 (4.6)
2	Water exchange	High	1150 (149)	1119 (106)	14.7 (0.1)	8.3 (0.9)
	Water exchange	Low	1271 (117)	1224 (64)	11.8 (0.2)	9.8 (2.2)
	Stagnant	High	0	0	14.6 (0.1)	11.7 (4.0)
	Stagnant	Low	0	0	11.6 (0.2)	12.9 (5.4)

water (Sigma Aldrich). On day 2 to 4, 80% of the tank water was replaced daily with new seawater and then spiked again with geosmin. Water temperature was kept at 14.0 °C. Tank water was aerated by an air stone to supply oxygen to the fish. Fish were not fed the day before the start and during the 5 days of geosmin exposure.

The two sessions were identical and during each session fish were depurated at two different water exchange rates Q (water flow rates were ~ 1200 and 0 l/kg fish/d, Table 2) and two different water temperatures T (~ 11.5 and ~ 14.5 °C, Table 2) for three different depuration times Ti (24, 72 and 144 h), with one tank for each of the twelve different combinations of Q, T and Ti. At the start of each session ($t = 0$), fish ($n = 80$) loaded with geosmin were randomly split into 16 groups of five fish and weighed per group. Four randomly assigned groups served to determine the initial geosmin content of the salmon. Each of the 12 remaining groups was randomly assigned to one of 12, 180 l polyester tanks.

Water exchange was installed by pumping water to the fish tanks by peristaltic pumps (Watson Marlow 505, Rotterdam, The Netherlands). The water pumped to the fish tanks was extracted from one of two temperature controlled water reservoirs of 400 l. The temperature controlled water reservoirs were continuously supplied with new, geosmin free seawater. The effluent of the experimental tanks was discharged. No water was supplied to the tanks assigned to the stagnant water treatments for the entire duration the experiment. The tanks with stagnant water were placed *au bain marie* in a larger tank which was flown through with water originating from one of the two temperature controlled water reservoirs to install the temperature treatments.

Oxygen was supplied to each tank via a minimal flow of pure oxygen to prevent volatizing of excreted, waterborne geosmin. Each tank was covered by a 6 mm glass sheet. The glass cover sheets were equipped with a circular hatch (diameter 150 mm) to allow introduction of fish and collection of water samples. The hatches were covered by glass sheets (200 × 200 mm) during the experiment, leaving a minimal opening for passage of aeration tubing. Water flow rate, temperature and dissolved oxygen concentration (Hach Lange Multimeter) were monitored in each individual tank up to the moment the fish were sampled and the tank removed from the experiment. Water quality was measured at Ti = 0, 24, 48, 72, 96, 120 and 144 h. Oxygen supply, temperature and water flow rate were adjusted when necessary.

A preliminary stability study in seawater at 14.0 °C assessed geosmin loss from the tanks via other routes than the outflowing water. The stability study was limited to the higher temperature used in the experiment as chemical losses due to volatilization would be the highest at the highest temperature. Three tanks identical to those used in the experiment but with stagnant water and without fish were spiked with 300 ng/l geosmin. Monitoring geosmin concentrations over time established that 95% to 100% of the initial geosmin concentration remained in the exposure tanks after 144 h in seawater at 14 °C.

2.2.3. Sampling

Four samples of five fish were collected at the start of each the two sessions. During each session fish were sampled at Ti = 24, 72 and 144 h. At each sampling time Ti, four tanks (one tank for the each of the four treatment combinations of QxT) were taken out of the experiment and all fish in these tanks were sampled. For sampling, fish were rapidly netted and anaesthetised in 0.1% (*v*/v) 2-phenoxyethanol (Sigma, St. Louis, USA). Fish were then filleted. Entire fillets were de-skinned, pooled per tank ($n = 5$), homogenized and stored at −20 °C until analysis. Individual fish weight was measured at stocking and upon fish sampling (Mettler PM40). Water samples of 250 ml were collected from each tank just before fish stocking and upon fish sampling at the various sampling times. Water samples were stored in entirely filled glass bottles, closed with lids with a Teflon inlay and stored at 4 °C until further analysis.

2.2.4. Geosmin and lipid analysis

Fish fillet samples were thawed overnight at 4 °C. From each fish fillet a subsample of approximately 1 g was taken and 100 µl of internal standard solution (D5-geosmine in water, 1 µg/ml, Sigma Aldrich) was added. Samples were extracted by accelerated solvent extraction (ASE, Dionex, Amsterdam, the Netherlands) at 40 °C using a 15:85 (v/v) penthane-dichlorinemethane mixture. After extraction, 1 ml of hexane was added to the extract. Extracts were concentrated to 1 ml by gently evaporating the penthane-dichlorinemethane mixture (Rotavap, Heidolph) and stored in 2 ml amber coloured glass vials at −20 °C until geosmin measurement. To each water sample (250 ml) 100 µl of internal standard solution (D5-geosmine in water, 1 µg/ml) was added. Water samples were led over extraction cartridges (Sep-Pak® Vac 6 cc (1 g) Certified tC18) that were subsequently eluted with 5 ml diethylether. Water was removed from the collected diethylether by addition of sodium sulphate. Diethylether samples were concentrated to about 1 ml under a gently nitrogen gas flow and stored in an amber coloured glass vial at −20 °C until geosmin measurement. The method for geosmin measurement is described in detail in Schram et al. (2018). We validated the method for geosmin extraction, concentration and measurement in low fat (6% *w*/w) fish samples according to NEN 7777 (Anonymous, 2011) and established a limit of detection of 6.1 ng/g, a recovery of 93.5 to 99.2% and an extended uncertainty of 27.8%. Lipid content of fish samples was determined using the gravimetric method according to Bligh and Dyer (1959) modified by De Boer (1988).

2.2.5. Calculations and statistics

Geosmin concentrations in fish fillets (ng/g) were normalized for lipid content (%, w/w). The decline of the geosmin concentration in the fish over time was predicted by fitting a log linear straight line regression model with a common constant and eight separate straight lines and slopes for the four duplicated treatment combinations of Q and T to the measured geosmin concentrations in the fish:

$$ln(\mu) = \beta_0 + \beta_{1j} * \text{time} \quad (3)$$

with different slopes β_{1j}, j = 1, 2, 3, 4 for the four different Q x T treatments. Here μ denotes the mean geosmin concentration as predicted by the model. Possible lack-of-fit of the straight lines model was assessed from residual plots and by adding quadratic and cubic time effects to the straight lines model. Quadratic nor cubic terms were needed in the

model as they were not significant (F-tests, $P > 0.10$). The same constant could be used for the two sessions (F-tests, $P > 0.10$). The measured geosmin concentrations (Y) were considered as pseudo Poisson data with variance proportional to Poisson variance, i.e.,

$$\text{variance}(Y) = \varphi\mu \quad (4)$$

Here φ denotes the dispersion parameter. Estimates for the model parameters and F-tests for the terms in the model were obtained using the general linear model procedure in GenStat. An estimate for φ was calculated from Pearson's chi-square.

Pairwise differences of slopes between the four Q x T treatments were tested by *t*-tests. Main and interactive effects of Q and T were assessed by accumulated analysis of deviance by fitting the measured geosmin concentrations in the fish to:

$$ln(\mu) = \beta_0 + (\beta_1 + \beta_{1Q}{}^*X_Q + \beta_{1T}{}^*X_T + \beta_{1QT}{}^*X_Q X_T)^*\text{time} \quad (5)$$

Here X_Q, X_T are dummy variables with the values −1 for the low levels and + 1 for the high levels of Q and T. The importance of main effects and interaction were assessed by F-tests using the general linear model procedure in GenStat.

3. Results

3.1. Prediction of experimental results

Model predictions revealed a marked effect of water exchange on geosmin accumulation in the water of depuration tanks (Fig. 1A) and geosmin depuration from the fish (Fig, 1B). In stagnant water predicted geosmin levels in water and fish reach an equilibrium after approximately 48 h (Fig. 1A and B). When water is exchanged, the geosmin level in water initially increases, peaks (in this specific case after approximately 8 h of depuration) at a much lower level than in stagnant water and then declines over time (Fig. 1A). The predicted effect of water temperature on geosmin accumulation in depuration tanks and geosmin depuration from fish is less pronounced than the predicted effect of water exchange. The model predictions reveal an interaction between the effects of water temperature and water exchange on geosmin depuration from fish: in stagnant water there is hardly an effect of increased water temperature, while when water is exchanged, the higher temperature is predicted to result in a faster decline of the geosmin level of the fish (Fig. 1B).

3.2. Depuration experiment

Averaged over all treatments, a strong reduction of the geosmin concentration in fillets of Atlantic salmon over time ($P_{Time} \leq 0.001$) was observed. In addition to this main effect of depuration time, each of the four QxT treatment combinations also showed a significant reduction of the geosmin concentration over time (Fig. 2, Table 3). The main effect of exchanging the water of a depuration tank (Q) affected the decline of the geosmin concentration in salmon with time ($P_{TimeQ} = 0.009$), while the main effect of temperature had no impact on the time-related decline ($P_{TimeT} = 0.39$). There was a tendency for an interactive effect of water exchange and temperature on the decline of the geosmin concentration with time ($P_{TimeQxT} = 0.09$). In the water exchange treatments no geosmin was detected in the water, both at high and low temperature, except for a very small amount at Ti = 24 h in one replicate of the water exchange – high temperature treatment. In the stagnant water treatments geosmin was detected in the water of four depuration tanks, two times at Ti = 24 and two times at 72 h. Geosmin concentrations ranged from 21 to 3.6 ng/l. Geosmin did not accumulate in the tanks with stagnant water: at the last sampling point at Ti = 144 h, no geosmin was detected.

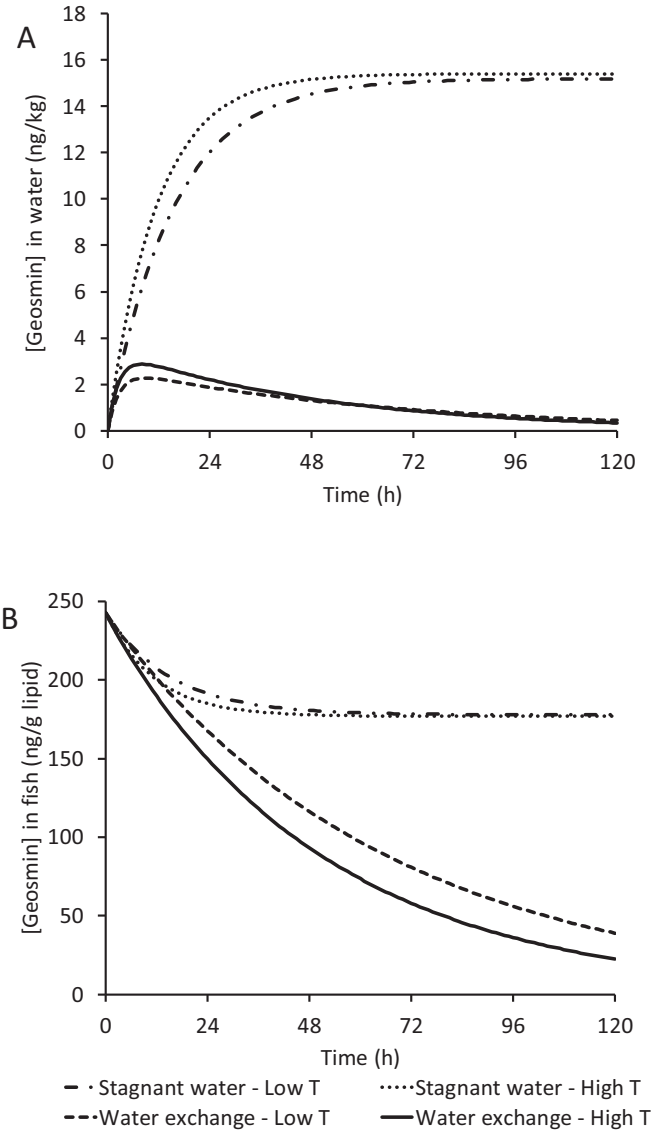

Fig. 1. Model predictions of the effect of water exchange (Q) and temperature treatments (T) on the accumulation of geosmin in the water (A) and on the decline of the geosmin concentration in fish (B) during geosmin depuration from Atlantic salmon with an initial geosmin content of 243 ng/g lipid (8.5 ng/g, lipid content 3.5% w/w).

4. Discussion

We studied two basic mechanisms which enhance the depuration rate of geosmin from fish: the effect of increased temperature on the excretion rate from the fish body to the water and the effect of water exchange removing geosmin from the direct environment of the fish. A model approach was used to predict the effects of these two mechanisms on the development of geosmin in water and fish over time. A depuration experiment with Atlantic salmon aimed to validate the predicted effects.

The model indeed predicted a strong positive effect of water exchange on the net excretion of geosmin by the fish, a slighter positive effect of increased temperature, and revealed an interaction effect. To illustrate these effects, we calculated the depuration times required to reach an arbitrary target concentration in the fish of 1 ng/g for each of the four modelled combinations of water exchange rate and temperature. In stagnant water this target concentration is not reached as the geosmin concentration in the fish reaches an equilibrium just below 8

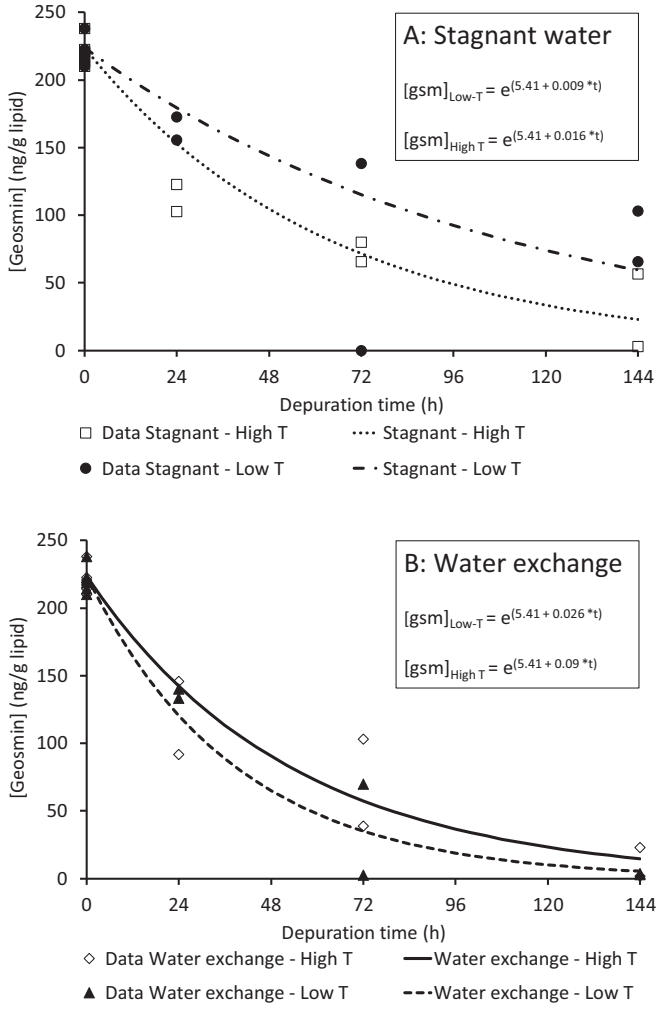

Fig. 2. Observed decline of the geosmin concentration in Atlantic salmon over time in depuration tanks with stagnant water (A) and water exchange (B) at low (11.6 °C) and high (14.6 °C) water temperature.

Table 3
Estimated slopes for the decline of the geosmin concentration in Atlantic salmon.

Treatment	Slope ß	SE(b)	*P*-value slope ß
Water exchange - High T	−0.0189	0.0041	< 0.001
Water exchange – Low T	−0.0257	0.0056	< 0.001
Stagnant – High T	−0.0158	0.0036	< 0.001
Stagnant - Low T	−0.0092	0.0025	< 0.001

ng/g (Fig. 1B), irrespective of the temperature. With water exchange the required depuration time is 151 h at the low temperature and 117 h at the high temperature. These theoretical depuration times are specific for the model input (Table 1). However, they do illustrate the generic observation that increasing the temperature, and thereby the chemical excretion rate, only has a relevant effect on depuration time in case the excreted chemical is removed from the fish's direct environment.

In accordance with model predictions and previous research by Davidson et al. (2020), the depuration experiment showed a significant main effect of water exchange of the depuration tank on geosmin elimination by salmon. This may be explained by prevention of re-uptake of excreted geosmin by salmon as a result of its removal from the depuration tank by the outflowing water. In other words, in agreement with the consensus that lipophilic compounds freely move in and out of fish, this experiment indirectly shows that re-uptake of excreted geosmin indeed may occur. Except for a minute amount at $t = 24$ h in one replicate, no geosmin was detected in any of the depuration tanks with water exchange, which is consistent with the notion that water exchange removes excreted geosmin from the depuration tank. The difference between stagnant water and water exchange treatments was not as pronounced as predicted by the model (Fig. 1 vs. Fig. 2). In stagnant water, the geosmin concentrations in water and salmon are predicted to quickly stabilize after an initial decrease in the fish and increase in the water. Neither were observed in the experiment. Geosmin was detected at t = 24 h and $t = 72$ h in some of the tanks, but in all cases no geosmin was present in the water at $t = 144$ h, the end of the depuration experiment. Under the assumption that all geosmin excreted by the fish appears in the water, the observed geosmin decline in the fish should have resulted in geosmin accumulation to levels ranging from 23 to 45 ng/l in the water of the stagnant tanks at t = 144 h. Geosmin initially appeared in the water but the predicted geosmin accumulation in the water over time did certainly not occur. The geosmin concentrations in the Atlantic salmon in the stagnant tanks continuously declined during the depuration experiment, suggesting that geosmin removal by water exchange was not essential for geosmin elimination from the fish. Apparently geosmin sinks are present in the stagnant depuration tanks. System losses from the water phase, e.g. evaporation and adsorption to the tank walls or to any of the auxiliary pieces of equipment in the depuration tanks are unlikely geosmin sinks because the preliminary geosmin stability test without fish showed no significant reduction of geosmin from the water phase after 144 h. Whether this also excludes microbial degradation of geosmin (Azaria and van Rijn, 2018) and binding to particles (Durrer et al., 1999) is not clear. Both geosmin degrading microbiota and particles may have been introduced in the experimental tanks with the fish, but this is unknown. Another possible geosmin sink is biotransformation in the fish, either in the fish liver or in the intestinal microbiome. Although geosmin biotransformation is generally assumed to be absent in fish, the required biotransformation pathways for lipophilic compounds have been established in fish (Kleinow et al., 1987). Our previous work indeed indicated a possible role of biotransformation in geosmin elimination from European eel (Schram et al., 2017) and rainbow trout (Schram et al., 2018). Clearly, continuous exchange of the water in depuration tanks enhances geosmin elimination from Atlantic salmon. At the same time, without this water exchange elimination also takes place; water exchange seems not essential for continuous elimination. Whether this is because geosmin is removed from the water in other ways or that the fish metabolizes geosmin is not clear. When a fish would excrete a geosmin metabolite instead of the parent compound, this would not be detected with the current chemical analysis. Our observations may be thus explained by geosmin biotransformation by the Atlantic salmon but they do not provide direct evidence for biotransformation. With more understanding about mechanisms behind the observed disappearance of geosmin, this phenomenon could perhaps be manipulated to enhance depuration without increasing water exchange.

No significant main effect of temperature on the decline of the geosmin concentration in fillets of salmon over time was observed. However, there is some evidence for interaction between water exchange and temperature treatments. Judging from Fig. 2, the effect of temperature seems more pronounced within the stagnant tanks than within the tanks with water exchange. It cannot be excluded that a higher temperature may enhance microbial degradation of geosmin, either in the tank or in the fish. The effect of temperature on geosmin excretion rate has been postulated to act through the increase in gill ventilation rate to compensate for the decrease in oxygen solubility in water and increase in physiological oxygen demand concurring with temperature increase (Howgate, 2004; Neely, 1979). In the stagnant depuration tanks the mean dissolved oxygen concentration was indeed higher at the low temperature (14.5 versus 10.8 mg/l). In the depuration tanks with water exchange, the contrast in dissolved oxygen concentration between the low and the high temperature is much smaller (11.4 versus 10.3 mg/l),

probably caused by the water exchange. It then seems likely that in the tanks with water exchange, temperature had no effect on geosmin excretion because it caused no difference in oxygen levels and thus gill ventilation rates. Although we have no gill ventilation rate data to corroborate this, we attribute the absence of a main temperature effect on geosmin excretion to a lack of contrast in gill ventilation rates between the temperature treatments in the tanks with water exchange.

The predicted interaction effect between temperature and water exchange implies that the temperature effect on the net geosmin excretion depends on the water exchange rate. The model prediction shows a very small temperature effect in tanks with stagnant water and a larger temperature effect on geosmin elimination in depuration tanks with water exchange. The experiment however suggests the opposite, i.e., no temperature effect when water is exchanged and possibly an effect in stagnant water. This contrast in predicted and observed interaction may be explained by the experimental conditions. The predicted interaction effect requires that 1. the temperature effect is present in all treatments and 2. in the stagnant depuration tanks the net effect of temperature is reduced because geosmin is not removed. Since both criteria were not met in the experiment, it is not surprising that treatments did not interact as predicted. Although we cannot draw a clear conclusion regarding our interaction hypothesis. The observed main effect of water exchange indicates that removal of excreted geosmin from the direct environment of this fish is needed to obtain maximal geosmin elimination from the fish. We therefore can reject nor accept our interaction hypothesis. It is clear that more data are needed to falsify our hypothesis. Also in the depuration tanks without water exchange geosmin was ultimately removed from the direct environment of this fish. It is very important to unveil the mechanisms underlying this removal because they may offer opportunities to enhance geosmin removal without the need for increased water exchange.

Besides removal via the outflowing water other geosmin sinks seem to have played a role in the current experiment. As they were not accounted for in the depuration model, we could not obtain estimates for the uptake and excretion rates constants k_1 and k_2 by fitting the experimental data to the depuration model. Since no geosmin could be detected in the water of the depuration tanks with water exchange, the estimated slopes for the exponential decay of the geosmin concentration in salmon represent the total elimination rate constant. This yields total elimination rate constants of 0.46 and 0.62 1/d (estimates for the slopes (1/h), Table 2, multiplied by 24) for the high and low temperatures in the experiment. These slopes are not significantly different, i.e., there is no main effect of temperature. The excretion rate constants for rainbow trout of the same size and lipid content derived from Howgate (2004) as described above are 0.54 1/d and 0.47 1/d for the higher and lower temperature, so within the range of the rate constants obtained from the depuration experiment. Possibly the excretion rate constants presented by Howgate (2004) are reasonable estimates for Atlantic salmon.

In conclusion, exchanging the water of depuration tanks significantly affects geosmin elimination by Atlantic salmon but temperature effects on geosmin excretion were not detected. Based on this experiment we cannot draw a clear conclusion regarding our interaction hypothesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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