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20-00637

Environmental Toxicology

C. Doose et al.

Thorium and fatty acid transfer from biofilms to grazers

Thorium Exposure Drives Fatty Acid and Metal Transfer From Biofilms to the Grazer *Lymnaea* sp.

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(Submitted 17 August 2020; Returned for Revision 05 October 2020; Accepted 06 April 2021)

Abstract: Aquatic ecotoxicological risks associated with tetravalent metallic elements such as thorium (Th) are still poorly understood. Periphytic biofilm represents an important food source in aquatic environments, thus such risks could severely affect nutrient and energy cycling in these ecosystems. The present study investigated the potential for Th to change fatty acid compositions of biofilm communities. Thorium bioaccumulation and fatty acids (FAs) were measured after 4 weeks to two exposure conditions: a control (C0) and Th (C10). Some major FAs such as C16:1n-7 and the docosahexaenoic acid C22:6n-3 differed significantly between control and C10 conditions. To determine if the Th can be trophically transferred and to investigate the impacts of nutritional quality changes on primary consumers, common pond snails

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.5067.

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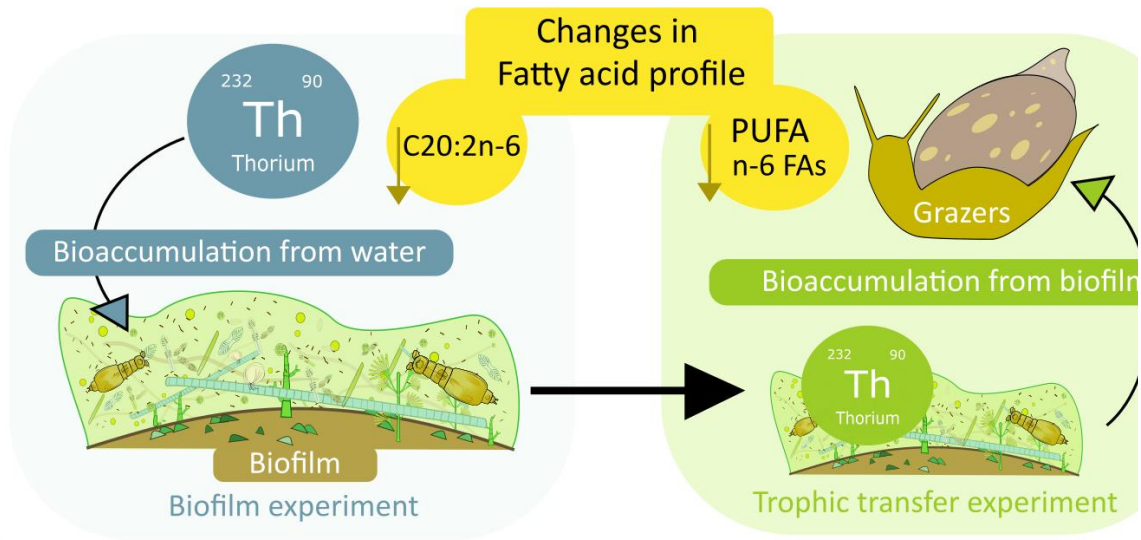
(Lymnaea sp.) were fed for 4 weeks with control and Th-exposed biofilm. Thorium appeared to be trophically transferable to the grazers although we cannot exclude that part of the Th accumulated by the snails may have been taken from the water through release from the biofilms. The composition of major FAs observed in the grazers was also significantly affected, notably by a decrease of total polyunsaturated FAs. These results indicate that very low Th concentrations can decrease the nutritional quality of organisms at the base of the food chain.

Graphical Abstract



Graphical Abstract. Thorium (Th) waterborne exposure induced bioaccumulation in biofilms and a reduction in fatty acids (FAs) such as C20:2n-6. The Th-exposed biofilm diet caused a decrease in polyunsaturated FAs (PUFA) and n-6 FAs in grazers, as well as Th bioaccumulation in soft bodies.

Keywords: food chain, freshwater toxicology, metal toxicity, trace metals, trophic transfer, periphytic biofilm, grazers, thorium

This article includes online-only Supplemental Data.

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Published online XXXX 2021 in Wiley Online Library (www.wileyonlinelibrary.com).

DOI: 10.1002/etc.xxxx

1. INTRODUCTION

Aquatic benthic biofilms (periphyton) are composed of various microorganisms belonging to bacteria, fungi, algae and micromeiofauna taxa, and are embedded in a matrix of extracellular polymeric substances (EPS) and host a diverse ecosystem that provides different ecological services. These biofilms are often very productive and can recycle nutrients, stabilize sediments, and thus play an important role in benthic organism recruitment (Decho, 2000). Their perturbation can lead to important changes in aquatic environments (Romaní et al., 2016).

Benthic diatoms, unicellular algae within biofilms, are known to be particularly rich in polyunsaturated fatty acids (PUFA) which provide a high-quality food source to periphyton grazers, such as cladoceran or insects (Ahlgren et al., 1990; Leland and Carter, 1984). These PUFA can be divided into 3 groups depending on the first unsaturation location (n-3, n-6 and n-9). They are major constituents of cellular membranes, important energy sources and are required in various biochemical pathways. They are exclusively synthesized by primary producers and are the most-transferred molecules across food webs (Gonçalves et al., 2016). Animals are capable of modifying PUFA through elongation and desaturation reactions but cannot synthetize them *de novo* and thus need to obtain them from their diet (Brett and Muller-Navarra, 1997). Other biofilm-produced fatty acids (FA), such as saturated and monounsaturated fatty acids (SFA and MUFA) represent an important energy source for their consumers (Brett and Muller-Navarra, 1997; Masclaux et al., 2012; Monroig

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and Kabeya, 2018). Consequently, a perturbation within periphytic communities can lead to changes in their nutritional quality and can perturb energy transfer in freshwater ecosystems (Boëchat et al., 2011). Therefore, periphyton represents an important source of these FA for animals in aquatic ecosystems (Masclaux et al., 2012; Monroig and Kabeya, 2018).

The presence of contaminants, such as metals, could impact aquatic food webs by affecting FA synthesis (Drerup and Vis, 2018; Fadhlaoui et al., 2020). Metallic contaminants can generate oxidative stress, and the PUFA composing the cell membrane are especially prone to lipid peroxidation (Rocchetta et al., 2006). For example, copper was shown to affect lipid composition of the diatoms *Tabellaria flocculosa* and *Thalassiosira weissflogii* by decreasing the amount of unsaturated FA (UFA) (Filimonova et al., 2016; Gauthier et al., 2020; Gonçalves et al., 2018). It also affects lipid composition of the green alga *Chlorella* sp. by altering glycerophospholipid biosynthesis pathways (Zhang et al., 2015). In addition, it is well established that benthic microorganisms can internalize metallic elements such as selenium (Conley et al., 2013), cadmium (Xie et al., 2010), zinc (Kim et al., 2012), copper (Meylan et al., 2004) and nickel (Fadhlaoui et al., 2020), and can retain them up to several days after exposure (Holding et al., 2003). As diet can be a predominant source of metal exposure (Luoma and Rainbow, 2005), the capacity for periphytic biofilm to bioaccumulate metals can affect its consumers (Cain et al., 2011; Conley et al., 2011; Xie et al., 2010). Hence, the consumption of metal contaminated biofilm may impact grazers by reducing their food quality concomitantly with direct effects of the metal (Bonnineau et al., 2020). A decrease in food quality can also stimulate the grazers to increase their consumption of contaminated food to compensate for the loss of nutritive values, further increasing contaminant ingestion (Neury-Ormanni et al., 2020).

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Thorium (Th) is a tetravalent metallic element often found in uranium mining waste. Aeronautic, metallurgy and petrochemistry industries use it for its capacity to resist high temperatures (Mernagh and Miezitis, 2008). Because of its affinity for phosphate, it is also found in phosphate fertilizers (Registry Agency for Toxic Substances and Disease, 1990). Moreover, Th is three times more abundant than uranium and will potentially be used as a future nuclear power plant combustible (Loiseaux et al., 2002). While these activities lead to Th release within the environment, aquatic ecotoxicological risks associated with thorium are still poorly understood. Thorium concentrations between 0.003 and 700 $\mu g \cdot L^{-1}$ (0.01 nM and 3.0 µM) have been observed in natural freshwater and up to 1400 $\mu g \cdot L^{-1}$ (6.0 µM) in the drainage water of uranium and iron mines in the south of Brazil (Godoy and Godoy, 2006; Ramli et al., 2005; Veado et al., 2006). In the present study Th accumulation and fatty acid composition in periphytic biofilms were determined after 4 weeks of exposure at 10 nM to explore whether this metal can affect the nutritional quality of the biofilm. The grazer, *Lymnaea* sp. was then exposed to this biofilm to investigate the impact of Th exposure upon food and nutritional characteristic changes. These results will contribute to a better comprehension of the potential for Th trophic transfer from biofilm to grazers and its subsequent impacts by altering the nutritional quality of trophic chains within freshwater ecosystems.

2. MATERIALS AND METHODS

2.1. Experimental setup

Biofilm exposure

To grow periphyton, ceramic tiles of 23 cm^2 were submerged for one month in a large colonization channel of 60 L(made of acrylic with dimensions of 210 x 40 x 7 cm) in

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which growth medium was recirculated (1.5 ± 0.1 L·min^{-1}). This colonization channel was inoculated with a periphyton suspension sampled from the Cap-Rouge River (Quebec, Canada, geographical coordinates: 46°45'48.8"N 71°21'24.0"W). One week before Th exposure, independent experimental channels of 6 L (also made of acrylic with dimensions of 100 x 20 x 10 cm) were filled with the different exposure media (C0 and C10) to pre-equilibrate the adsorption sites of walls, tubes and reservoirs with the metal in solution (C10). Then, the biofilm was exposed to Th in recirculating exposure channels (1.5 ± 0.1 L·min^{-1}) for 4 weeks. To proceed to this exposure, the obtained biofilm covered tiles were randomly distributed from the large colonization channel to 8 experimental channels (4 channels per condition) containing a synthetic culture medium (Dauta) (Dauta, 1982) with targeted Th concentrations of 0 (C0) and 10 nM (C10) (measured weekly). Following 4 weeks of exposure, 8 tiles per channel (32 per exposure concentration) were randomly sampled, pooled, and homogenized in 200 mL final volume of Dauta medium to make one composite sample per condition. Biofilm dry weight was quantified after filtration of a 20 mL aliquot (on pre-weighted and dried GF/F filters) and lyophilisation according to the NF EN 872 standard method (AFNOR, 2005). Aliquots of 1.5 mL were sampled from the 200 mL of homogenized biofilm suspension and kept frozen at -80°C to be later given as a food source to grazers and to conduct fatty acid analyses.

Trophic transfer experiment

A total of 48 snails acclimated for 1 week were distributed in 3 control and 3 exposed-food aquaria of 2 L (17 x 24 x 12 cm) filled with dechlorinated tap water, maintained at 21.8 ± 0.2 °C. Snails in control aquaria were fed with C0 biofilm and snails in the exposed-food aquaria were fed with the C10 biofilm for 4 weeks (Figure 1). Each aquarium contained 8 snails which were acclimated for 1 week in an aquarium. One

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aliquot of 1.5 mL of control or Th-exposed biofilm (corresponding to 6.3 ± 1.6 mg dw) per aquarium was given per day as a food source to the grazers. Half of the media volume was renewed daily. An aliquot of water was sampled at the beginning of the experiment and at the end of each week before the daily renewal to determine the concentration of Th in the media of each aquaria. At the end of the 4 weeks of food-exposure, the live snails were sampled and placed in a Petri dish on ice, then soft bodies were separated from the shell and weighted before being frozen in liquid nitrogen and stored at -80°C before further analysis. To realize the FA and Th analyses, 6 snails for each analysis were randomly taken in the total of 24 snails per exposure concentration. During the experiment, 3 snails died, 2 were in different aquaria of the control condition and 1 was in a C10-fed aquarium.

2.2. Fatty acid analysis

To obtain technical replicates, 6 aliquots of each of the pooled control and Th-exposed biofilm samples were used. Total lipids were extracted from the whole biofilm in those aliquots according to Folch et al. (1957). The detailed procedure for lipid extraction was described in Fadhlaoui et al. (2020) (Fadhlaoui et al., 2020). Total lipids were extracted in a chloroform/methanol mix (1V/2V). The obtained fraction was then esterified in boron trifluoride (BF3, 4 % methanol) to obtain fatty acid methyl esters (FAME). These FAME were analyzed by gas chromatography-flame ionization detector (GC-FID) and the relative FAME content was determined by comparing chromatograms to reference standards (mixtures of 37 fatty acids, NHI-F, a fatty acid methyl ester mix, PUFA NO. 2, an animal source and fatty acid methyl esters kit; Sigma-Aldrich, Canada).

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2.3. Thorium quantification

Thorium content was quantified using inductively coupled plasma-atomic emission spectrometry (ICP-MS). The ICP-MS calibration curve was validated with certified control solution 406 (SCP Science, Baie-d'Urfé, Canada). Thorium in the biofilm and in the snails was determined after samples were lyophilized and digested according to Fadhlaoui et al. (2020). Aqueous Th concentrations in the biofilm exposure media as well as in the snail water were monitored weekly during the experiment. Thorium concentrations in control C0 and C10 exposure solutions of biofilms were 0.004 ± 0.002 nM and 8.7 ± 3.4 nM Th respectively (n = 8). The aqueous Th concentrations in the snail exposure media were 0.0014 ± 0.0009 and 0.20 ± 0.01 nM for the control and Th-exposed biofilm fed conditions, respectively.

2.4. Data treatment and analysis

To assess the presence of differences among treatments, one-way ANOVA statistical tests were performed with the R software (vegan package) as well as Kruskal–Wallis non-parametric test. A principal components analysis (PCA) was also constructed on biofilm and snails FA analysis data with R (FactoMineR package). The PCA's function in this package automatically normalizes the data during the calculation. Ellipses were built around centroids to represent 95 % of data from biofilm and grazer's conditions.

In order to estimate fatty acid desaturase and elongase activities in the biofilms, the product/precursor ratios were used as described in Fadhlaoui et al. (2016). The ratios 16:1n-7/16:0 and 18:1n-9/18:0 were used to estimate the Δ9-desaturase (D9D; stearoyl- CoA- desaturase), the ratio 18:2n-6/18:1n-9 for the Δ12- desaturase (D12D), and 18:3n-3/18:2n-6 for the Δ15- desaturase activity (D15D). The ratio

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18:0/16:0 was used to calculate the elongase activity (ELOVL, long chain fatty acids elongation).

3. RESULTS AND DISCUSSION

3.1. Fatty acid profiles of control biofilms and grazers

The biofilm FA profile can give qualitative information about bacteria, green algae, and diatom composition. These biofilm FA profiles were obtained for the control (sampled after 4 weeks of exposure, Table 1). The main SFA were palmitic acid C16:0 and stearic acid C18:0 representing respectively 25.2 ± 0.8 and 14.1 ± 3.3 % of the FA measured in the control condition biofilm. The MUFA were dominated by palmitoleic acid C16:1n-7 and oleic acid C18:1n-9, PUFA by C18:3n-3, arachidonic acid C20:4n-6 and eicosapentaenoic acid C20:5n-3. The dominance of C16:0, C16:1n-7, C18:3n-3 in the control biofilm FA profile suggested a dominance of green algae and cyanobacteria (Kelly and Scheibling, 2012). These results are in line with the taxonomic observations made in the same biofilm samples (Doose, PhD thesis, INRS ETE, Quebec, Qc, Canada), especially concerning the presence of the cyanobacteria genus *Pseudanabaena*. The n-3, n-6 and n-9 were present in the same proportion, each representing around 10 to 11 % of the total FA in the control biofilm.

The FA compositions were also determined in the grazers *Lymnaea* sp. (Table 1). The SFA were dominated by C16:0 and C18:0 representing 10.5 ± 0.4 % and 14.4 ± 0.7 %, respectively. The predominant MUFA were C18:1n-9 and C20:1n-9 representing 9.8 ± 0.4 % and 5.0 ± 0.6 %, respectively. The predominant PUFA were C18:2n-6, C20:2n-6, C20:4n-6, C20:5n-3 and C22:5n-3 representing 5.1 ± 0.3, 7.2 ± 1.1, 21.6 ± 2.0, 4.4 ± 0.4 and 6.2 ± 2.8 %, respectively. This composition is in accordance with of the FA profiles of other snails found in the literature (Çelik et al., 2019; Ekin and

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Şeşen, 2017; Panayotova et al., 2019; Silva et al., 2017), except for the presence of C20:5n-3 in this study (see supporting information section). This PUFA was found to be characteristic of fatty acid profiles for marine invertebrates (mostly molluscs) and was also a major component of the marine gastropods *Rapana venosa* and *Turbo cornutus* (Isay and Busarova, 1984; Saito and Aono, 2014). The FA content in animal tissues observed in Kelly et al. (2012) is similar to the levels we observed (Figure 2). In addition, the SFA, MUFA and PUFA amounts in the diet have been reported to directly affect the FA composition of the consumers (Ikauniece et al., 2014; Milinsk et al., 2003), and C18:1n-9, C18:2n-6 and C20:5n-3 in animal tissues are known to originate from dietary sources. For example, C20:5n-3 is a typical biomarker for diatoms and their presence in the snail's tissue highlights the presence of diatoms in the grazed biofilm (Napolitano, 1999). Burns et al. (2011) observed that C18:2n-6 abundance in the grazers Daphnia and Ceriodaphnia fed with Chlorophyceae and Cyanobacteria were significantly correlated with its abundance in their diet. Moreover, some molluscs, such as pulmonated snails, are expected to be able to synthesize PUFA *de novo* (Kabeya et al., 2018; Weinert et al., 1993), but we didn't find any information about such metabolic pathways occurring in the genera *Lymnaea*. Thus, these results are in line with the observation of Kelly et al. (2012) and show that the grazers are able to assimilate and retain these PUFA from a biofilm diet (Kelly and Scheibling, 2012). The C20:4n-6 is known to be accumulated at a relatively high level in several molluscs, which Kharlamenko et al. (2001) attributed to the consumption of fungi (Kharlamenko et al., 2001). However, it has been suggested that the accumulation of C20:4n-6 might be due to its biosynthesis from its precursor C18:2n-6, since this has been observed in other molluscs like the scallop *Pecten maximus* (Kelly and Scheibling, 2012; Soudant et al., 1996). These

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interpretations are consistent with our results regarding the high C18:2n-6 accumulation in the grazers' tissues.

3.2. Thorium concentrations in biofilm and snails

The Th concentrations measured in the biofilm after the 4 weeks of exposure are presented in Table 2. The control biofilm contained a baseline Th content of 0.009 ± 0.004 ng mg^{-1} dw despite that Th was not added into the exposure medium. Thorium, like all other metallic elements, is naturally present in environmental freshwater and tap water (Correa et al., 2009; Godoy and Godoy, 2006). Therefore, it is normal to find a background signal in the biofilm. The Th content determined in the C10 condition was significantly higher than the control conditions with 11.6 ± 1.4 ng mg^{-1} dw ($p = 4 \times 10^{-12}$). These results show the ability of the periphytic biofilm to accumulate Th. Thorium accumulation in the biofilm can result from internalization and adsorption onto the cells surface (Bonnineau et al., 2020).

The Th content measured in the grazer's (*Lymnaea* sp.) soft bodies fed with the control and Th-exposed biofilm are presented in Table 2. Thorium bioaccumulation by the snails fed with the C10 biofilm was significantly higher than by snails fed with the control biofilm, with 12 ± 1 and 22 ± 2 ng·mg^{-1} dw, respectively ($p = 0.02$). Also, no significant difference was observed between the biomass of the control and C10 fed snails (16.9 ± 1.8 and 16.2 ± 1.0 mg dw respectively), suggesting that the Th presence did not affect the weight and the food intake of the grazers. These results suggest that the Th bioaccumulated by the biofilm could be trophically transferred to grazers with a transfer trophic factor (TTF) of 1.9 ± 0.2. The Th concentrations in the snail's tissues were almost two time higher than in the biofilm. Moreover, the Th concentration measured in the snail's water in the C10-fed exposure (0.20 ± 0.01 nM) could be due to a partial release of the metal bound to the biofilm or to a

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remobilisation of the biofilm Th through the snail's digestion and excretion. It is thus possible that a part of the Th accumulated by the snails could be attributed to this aqueous exposure.

3.3. Effect of Th exposure on biofilm fatty acid profile

The FA composition in the biofilm samples (Figure 3A) was not significantly different between the control and the C10 condition after 4 weeks of exposure. Whereas the total SFA, MUFA and PUFA in the biofilm were not significantly affected by the Th exposure, the amounts of some minor FA belonging to these groups significantly differed from the control (Table 1). The SFA C15:0 and C17:0 values were significantly higher by a factor of 2.4 ($p = 0.01$) and 1.5 ($p = 0.02$) in the control than in the C10 biofilm, respectively. The MUFA C22:1n-9, C16:2n-4 and C16:3n-4 were not detected in the control biofilms but represented 0.06 ± 0.06, 1.3 ± 0.8 and 0.09 ± 0.05 % of the FA measured in the C10 biofilms, respectively. In addition, the PUFA C18:4n-3 and C22:6n-3, which were not detected in the control biofilm, were present in the C10 biofilm. The C22:6n-3 represented 5.6 ± 2.2 % of the total FA and was thus one of the main FA of the C10 biofilm. This showed that Th at low concentration can induce significant FA metabolism perturbations.

In contrast to the situation for C22:6n-3, the biofilm content for PUFAs C20:2n-6and C20:4n-6 were significantly higher in the control than in the C10 condition ($p = 0.02$ and $p = 0.05$, respectively). The C20:4n-6 is known to play an important role in mollusc cellular signalling (Ye et al., 2017), the nervous system (Piomelli, 1991) and reproduction (Clare et al., 1986; Deridovich and Reunova, 1993). Its decrease in snail tissues could lead to important physiological damages in the grazers and also in their consumers since this PUFA cannot be synthesized by most animals.

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The ratios of enzymatic activities presented in Table 3 provide insights into the effects of Th exposure on elongation and desaturation reactions. The values calculated for D9D, D12D and ELOVL were lower than those previously found in control biofilm observed by Fadhlaoui et al. (2020), but the D15D ratios were similar (around 1.3). These dissimilarities could be explained by differences in the biofilm's taxonomic composition. Despite the significant changes in some FA amounts observed between the control and Th-exposed biofilm, no significant differences were found between ratios of enzyme activities. However, these ratios are calculated from corresponding FA amounts which also depend on previous or subsequent enzymatic reactions of the FA biosynthesis pathways. Thus, the ratios calculation might not allow for observation of Th effects on the enzymatic activities. For example, the significant increase of C18:4n-3 in the C10 biofilm, while C20:2n-6 and C20:4n-6 being more present in the control biofilm, could be partly explained by an increase of the desaturase D15 activity in the C10 exposed biofilm. In the control biofilm, the activities of the elongase (EVOVL) and the desaturase D8 enzymes could be dominant (Figure 2). Since metals are known to induce the presence of reactive oxygen species (ROS) in biofilm organisms which can lead to lipid peroxidation (LPO), the changes induced by the Th exposure could be attributed to LPO rather than to the reduction of the enzymes' activities. The decrease of PUFA measured in biofilm could be also due to the formation of oxylipins in the microorganism's cells. These bioactive lipid metabolites are synthetized from PUFA under the catalytic action of cycloxygenase, lipoxygenase, cytochrome P45 enzyme, and initiated by the abundant presence of ROS in the cells (Mosblech et al., 2009; Ritter et al., 2008).

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3.4. Effect of Th-exposed biofilm on grazers

The proportions of the FA categories measured in the snail soft bodies after 4 weeks of feeding on Th-exposed biofilm were different from the controls (Figure 3A). The proportions of PUFA were significantly higher in the control grazers than in snails fed with the C10 biofilm with 52 ± 2 and 43 ± 5 %, respectively ($p = 0.0005$). On the contrary, SFA tended to be higher in the C10 fed snails as compared to the control biofilm fed snails. Significant differences in the MUFA group were observed (Table 1) for the eicosenoic (C20:1n-9) and erucic (C22:1n-9) acids between the two diet exposure conditions. The C20:1n-9 was higher in the control condition with 5.0 ± 0.6 % than in the C10 fed condition (3.0 ± 0.7 %, $p = 0.01$). On the contrary, C22:1n-9 was found in higher amounts in the C10 fed condition than in control snails ($p = 0.01$). Because the ELOVL enzyme is involved in the elongation of eicosenoic acid to erucic acid, Th exposure could have led to an elongation rate increase but this hypothesis is not supported by the calculated ELOVL activity, which was not significantly different between the control and exposed conditions (Table 3). A change in food intake could also affect the FA profile of the snails but the dry weight of control and C10-fed grazers were not different showing no evidence about a feeding decrease. The n-6 FA were significantly lower in the grazers fed with the C10 biofilm than in those fed with the control biofilm with 35.8 ± 1.9 and 20.4 ± 3.5 %, respectively ($p = 0.003$). Moreover, PUFA C20:2n-6, C20:4n-6 and C22:5n-3 were significantly different and 3 times more abundant in the control as compared to the C10 fed condition ($p = 0.008$, $p = 0.02$ and $p = 0.003$, respectively). Numerous metallic elements are known to produce reactive oxygen species (ROS) which can initiate lipid peroxidation (LPO) when the antioxidant system of the cells is overwhelmed (Valavanidis et al., 2006). Th waterborne exposure from 25.3 ± 3.2 to 609 ± 61 µg L^{-1} are known to induce

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oxidative stress in the bile, gills, liver, muscle, brain, skin, kidney and blood of the silver catfish (*Rhamdia quelen*) (Correa et al., 2009). Because PUFA are relatively easy to breakdown during oxidative reactions, the Th bioaccumulated in the biofilm could have generated ROS in the snail tissues during and after the food uptake (Géret et al., 2002; Livingstone et al., 1990). This could explain the lower amount of PUFA in the C10 fed snails despite the lack of significant differences in the amount of PUFA between control and C10 biofilms. While nonsignificant differences were observed for the C18:2n-6, the snails fed with the control biofilm accumulated around 1.6 times more of this FA than those fed with the Th-exposed biofilm. Significant decrease of accumulation was also observed for the C20:2n-6 and the C20:4n-6 (ANOVA, $p < 0.05$). Because n-3 and n-6 PUFA such as C22:5n-3 and C20:4n-6 are essential for the consumer's physiology and metabolism, their decrease in the C10-fed grazer tissues could affect the snail's fitness (Brett and Muller-Navarra, 1997). Moreover, these PUFA can only be synthesized de novo in plants and algae and can only be provided to the consumers through diet. Therefore, their decrease in the grazers could affect the rate of PUFA flow in the upper trophic levels (Müller-Navarra et al., 2000; Torres-Ruiz et al., 2007; Torres-Ruiz and Wehr, 2010).

The principal component analysis (PCA; Figure 4) shows the distribution of biofilm and snail samples as a function of their FA profiles. Biofilms and snails appeared well separated along Dim1. Biofilm samples were characterized by high SFA and MUFA content, while snails were more characterized by PUFA, notably by the n-6. This shows the ability of the grazer to accumulate the n-6 PUFA from the biofilm, as reported previously (Kelly et al., 2012).

Biofilm FA profiles in control and Th-exposed conditions were not separated by the PCA and thus seemed non-affected by the exposure. It has been shown that metal

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contamination in acid mine drainage waters leads to FA profile changes in biofilm (Drerup and Vis, 2016), but the present study's Th exposure may have been too low to induce such changes. However, the centroids of PCA points for control and Th-exposed snails appeared to be spatially discriminated along Dim1. The control snails were characterized by higher n-6 PUFA compare to those fed with Th-exposed biofilm. In the grazers, Th diet exposure appeared to affect the amount of n-6. This could be explained by the tendency of those PUFA to decrease in the Th-exposed biofilm and/or by a lipid peroxidation occurring during feeding, as suggested previously. Moreover, the Th diet-exposed snail samples were more dispersed in the PCA biplot than the control ones. This shows that dietary exposure to Th-exposed biofilm leads to changes in the FA profile of the grazers. The two patterns of changes induced by Th diet exposure could be a consequence of differences with respect to variables such as the rate of egg production and laying which involve PUFA in hormone synthesis and nutritive stock for the eggs and could lead to a change of fatty acid profile (Clare et al., 1986). Moreover, laying could be a Th excretion pathway for the snails if the Th bioaccumulates inside the eggs like Cu and Zn in squid (Craig and Overnell, 2003). To conclude, the Th-exposed biofilm diet affected the snail's FA composition, notably by reducing PUFA and n-6 abundance andthe grazers, when fed with the Th-exposed biofilm, presented more heterogeneous FA profiles than the control grazers, probably due to individual physiologic and metabolic variations.

4. CONCLUSION

In this study, Th was shown to be accumulated by a periphytic biofilm and to affect its fatty acid composition. When this biofilm was consumed by the grazer *Lymnaea* sp., Th bioaccumulated and affected the biofilm nutritional quality, notably by a significant decrease of C20:2n-6, which plays essential roles in metazoan

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metabolisms. Snail exposure to Th through the diet together with changes in the fatty acid composition of the biofilm, led to changes in the FA composition of the grazer. Indeed, the grazers fed with Th-laden biofilms had less PUFA and n-6 FA compared to the snails fed with the control biofilm. These results show that a very low concentration of Th can induce a change in the nutritional quality of the organisms studied. Thus, Th can represent a hazard for the entire freshwater ecosystem by being accumulated and affecting the energy transfer along the trophic chain. This study shows the pertinence of examining sublethal endpoints, such as FA profiles, and multiple trophic levels in order to improve our knowledge of metal impacts on the aquatic food chain. As these effects were observed at very low Th concentrations, more work should be initiated on the potential impacts of this data-poor element on aquatic ecosystems.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Acknowledgment— Financial support from the Fonds de recherche du Québec sur la nature et les technologies (FRQNT; grant number 2015-MI-190537) is acknowledged. Caroline Doose acknowledges the language assistance received from Scott Hepditch. Claude Fortin is supported by the Canada Research Chair program (grant number 950-231107).

Disclaimer— The authors declare no conflict of interest.

Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (caroline.doose@ete.inrs.ca).

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FIGURE CAPTIONS

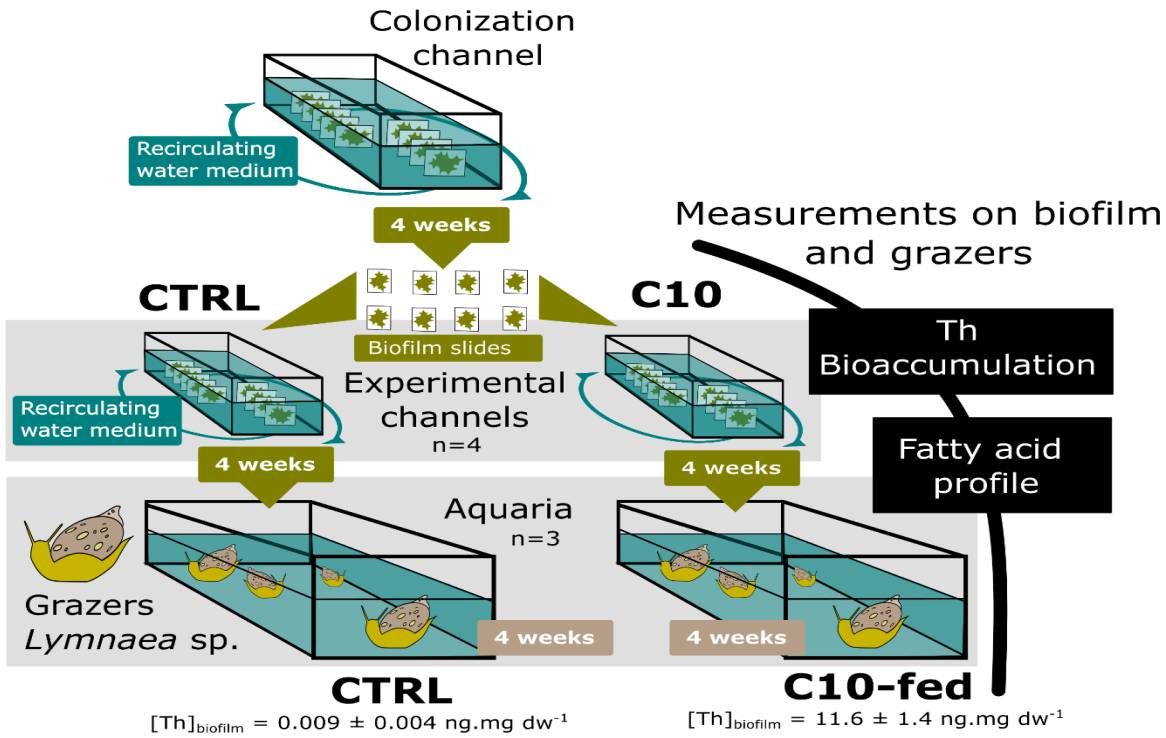

Figure 1. Overview of the experimental setup of biofilm Th exposure and the subsequent trophic transfer experiment.

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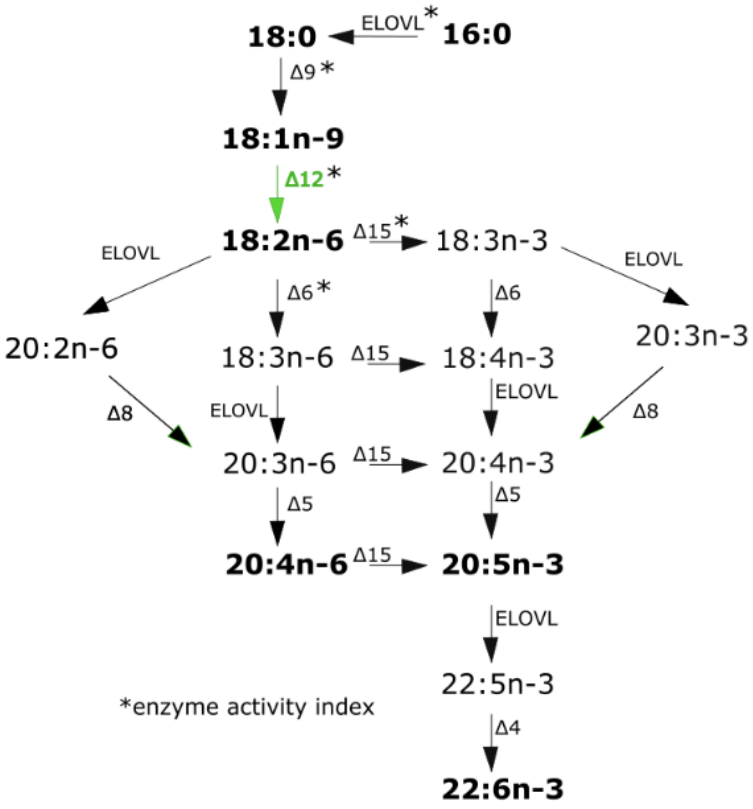

Figure 2. Biosynthetic pathways of polyunsaturated fatty acids (FA) in algae and invertebrates by desaturation and elongation (modified from Fadhlaoui et al. 2020, Monroig et al. 2018 and Guschina and Harwood 2006) (Fadhlaoui et al., 2020; Guschina and Harwood, 2006; Monroig and Kabeya, 2018). C18:2n-6 = linoleic acid; C20:4n-6 = arachidonic acid; C22:6n-3 = docosahexaenoic acid; C20:5n-3 = eicosapentaenoic acid; C18:3n-3 = linolenic acid, FA shown in bold are known to accumulate in animal tissues with high concentration. The D12D: Δ12-desaturase (in green) occurs only in algae, bacteria and protists of biofilm.

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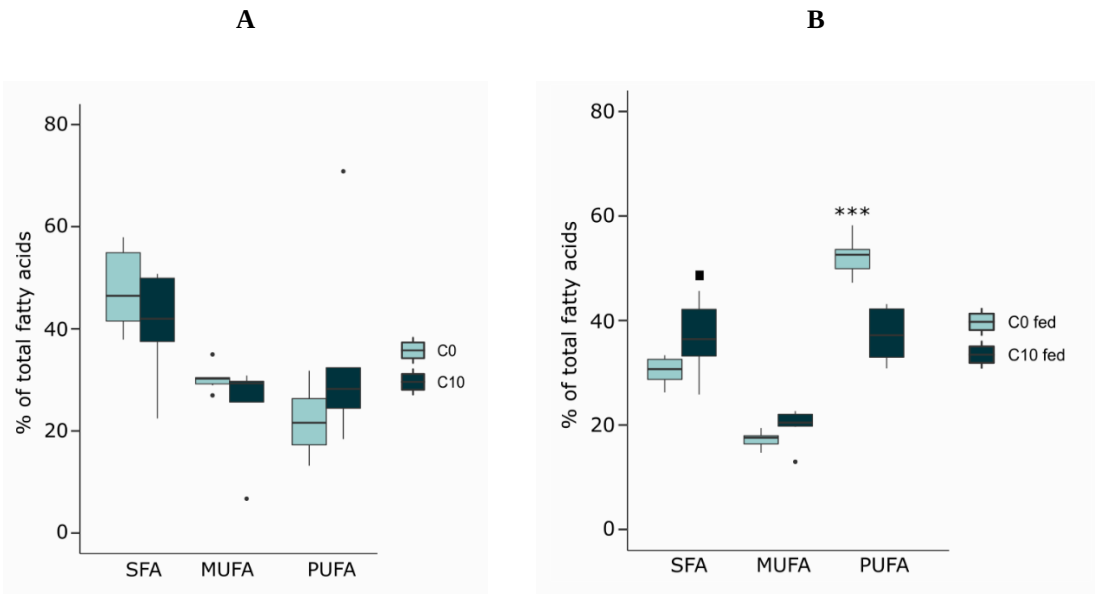

Figure 3. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids measured in: A. biofilm samples after 4 weeks of exposure in control C0 (0.004 ± 0.002 nM Th) or C10 (8.7 ± 3.4 nM Th) (n = 6). B. in the snail *Lymnaea* sp. following 4 weeks of feeding with C0 (9.4 ± 4.4 ng mg^{-1} dw) or C10 (11.6 ± 1.4 $x10^3$ ng mg^{-1} dw) biofilm. Significant differences between the two biofilm or grazer's conditions for each fatty acid are shown by *** ($p < 0.001$), **($p < 0.01$), *($p < 0.05$) and ▪ ($p < 0.1$), one-way ANOVA (n = 6, except Th-exposed biofilm group n = 5).

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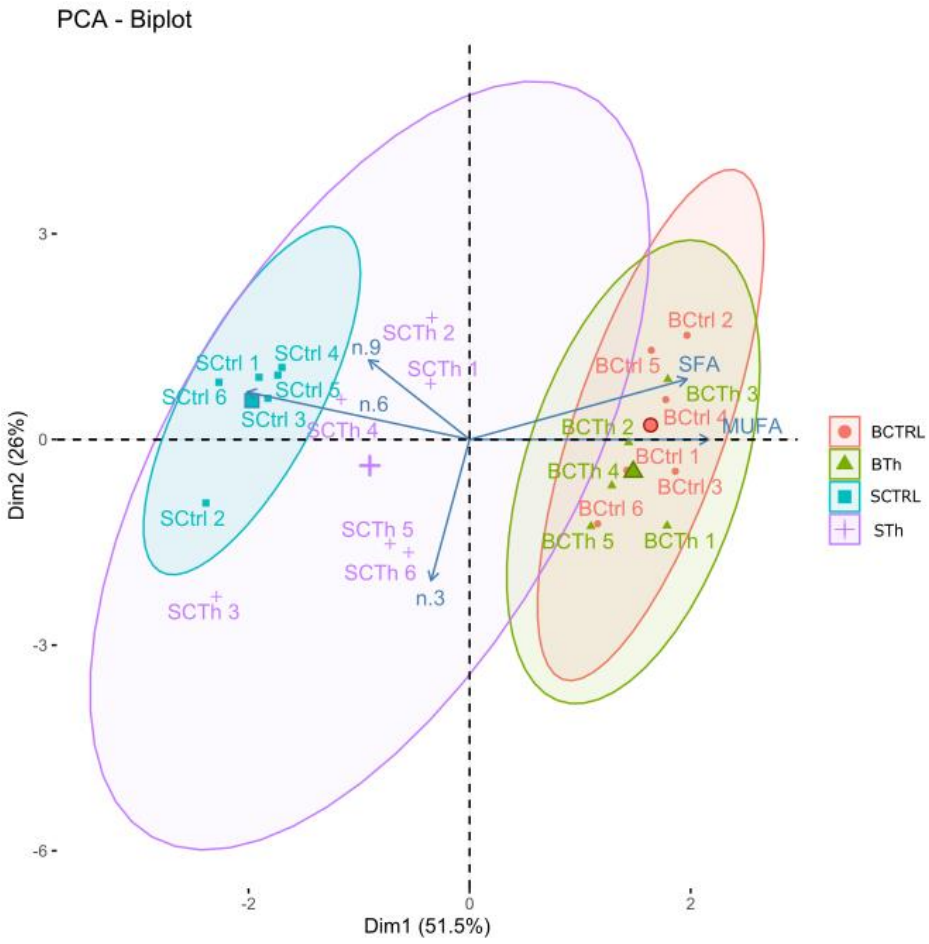

Figure 4. Principal component analysis (PCA) realized on FA data of samples of biofilms and snails, under control and Th-exposure conditions. n = 6, except Th-exposed biofilm group for which n = 5. Abbreviations: BCtrl (control biofilm), BCTh (exposed biofilm), SCtrl (control snails), SCTh (exposed Snails).

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Table 1. Fatty acid composition of control biofilm, Th exposed biofilm and grazers (*Lymnaea* sp., control group and group feeding on contaminated biofilm). Values are shown as % mean ± SEM of total measured FA for each biofilm or grazer's conditions (n = 6, for control biofilm and grazer, n = 5 for exposed biofilm). The significant differences between biofilm or grazer's conditions correspond to * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ (one-way ANOVA, n = 6). U/S = unsaturated to saturated fatty acid ratio, Σn-3 = n-3 unsaturated fatty acid sum, Σn-6 = n-6 unsaturated fatty acid sum and Σn-9 = n-9 unsaturated fatty acid sum. Dashes represent values below 10^{-3} %.

	Control biofilm	Contaminated biofilm	Control grazers	Grazers feeding contaminated biofilm
C14:0	3.9 ± 0.3	3.4 ± 0.5	1.5 ± 0.3	2.7 ± 0.5
C15:0	1.2 ± 0.2	0.5 ± 0.1 *	0.5 ± 0.1	0.8 ± 0.2
C16:0	25.2 ± 0.8	27.1 ± 1.7	10.5 ± 0.4	12.3 ± 1.7
C17:0	1.1 ± 0.1	0.7 ± 0.1 *	1.3 ± 0.1	1.5 ± 0.2
C18:0	14.1 ± 3.3	12.8 ± 1.3	14.4 ± 0.7	15.3 ± 1.8
C20:0	0.7 ± 0.2	0.2 ± 0.1	1.3 ± 0.5	0.7 ± 0.2
C:21	-	0.5 ± 0.4	0.7 ± 0.2	2.7 ± 1.2
C22:0	0.1 ± 0.1	0.3 ± 0.2	0.04 ± 0.04	0.06 ± 0.04
C24:0	1.5 ± 0.6	0.5 ± 0.2	0.2 ± 0.1	0.7 ± 0.1
C14:1n-5	0.3 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
C15:1	0.4 ± 0.2	0.4 ± 0.3	-	0.04 ± 0.04
C16:1n-7	16.9 ± 2.9	15.1 ± 0.7 *	1.6 ± 0.3	1.8 ± 0.4
C17:1	1.7 ± 0.2	1.6 ± 0.4	0.4 ± 0.1	0.6 ± 0.1
C18:1n-9	10.5 ± 2.2	10.4 ± 1.12	9.8 ± 0.4	10.9 ± 0.8
C20:1n-9	0.4 ± 0.2	0.3 ± 0.1	5.0 ± 0.6	3.0 ± 0.7 *
C22:1n-9	-	0.1 ± 0.1 *	0.1 ± 0.1	0.2 ± 0.1 **

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C24:1n-9	-	0.2 ± 0.1	0.2 ± 0.2	3.2 ± 1.6
C16:2n-4	-	1.3 ± 0.8	-	0.3 ± 0.3
C16:3n-4	-	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.04
C18:2n-6	3.0 ± 0.5	3.4 ± 0.9	5.1 ± 0.3	4.8 ± 0.7
C18:3n-6	1.0 ± 0.2	1.3 ± 0.4	-	1.0 ± 0.1 ***
C18:3n-4	-	-	0.1 ± 0.1	0.2 ± 0.1
C18:3n-3	3.9 ± 0.6	3.7 ± 1.2	1.2 ± 0.2	1.2 ± 0.1
C18:4n-3	-	0.3 ± 0.2 *	0.5 ± 0.2	0.3 ± 0.2
C20:2n-6	1.9 ± 0.4	0.4 ± 0.3 *	7.2 ± 1.1	2.2 ± 1.0 **
C20:3n-6	0.2 ± 0.1	0.4 ± 0.2	1.3 ± 0.1	2.9 ± 0.7
C20:4n-6	3.7 ± 0.7	1.5 ± 0.7 *	21.6 ± 2.0	10.2 ± 3.4 *
C20:3n-3	-	1.2 ± 0.7	0.2 ± 0.1	7.5 ± 4.7
C20:4n-3	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.2 ± 0.1
C20:5n-3	5.4 ± 0.2	5.0 ± 1.0	4.4 ± 0.4	6.0 ± 0.9
C22:2n-6	1.6 ± 0.2	0.3 ± 0.2 **	0.6 ± 0.3	0.2 ± 0.1
C22:5n-3	1.2 ± 1.2	0.6 ± 0.2	6.2 ± 2.8	0.1 ± 0.1 **
C22:6n-3	-	5.6 ± 2.2 **	3.7 ± 2.1	6.2 ± 4.4
Σn-3	10.5 ± 1.4	16.1 ± 2.4	10.1 ± 2.4	21.5 ± 5.1
Σn-6	11.5 ± 1.6	7.4 ± 1.0	35.8 ± 1.9	20.4 ± 3.5 **
Σn-9	10.9 ± 2.4	10.9 ± 1.3	15.0 ± 0.6	17.3 ± 1.4
U/S	1.2 ± 0.2	1.2 ± 0.1	2.3 ± 0.1	1.6 ± 0.1

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Table 2. Thorium content in control (C0) and Th-exposed (C10) biofilms after 4 weeks of exposure and in the grazer *Lymnaea* sp. fed for 4 weeks with C0 and C10 biofilms, the significant differences between biofilm or grazer Th content is indicated by * (ANOVA for the biofilm data and Kruskal–Wallis non-parametric test for the snail's data, $p < 0.05$, $n = 6$).

		C0	**C10**
Biofilm	**Th (ng mg^{-1} dw)**	0.009 ± 0.004	11.6 ± 1.4 *
Grazers	**Th (ng mg^{-1} dw)**	12 ± 1	22 ± 2 *

Table 3. Estimated fatty acid desaturase and elongase activities in biofilms and grazers from different exposure conditions. Values are shown as % mean ± SEM for each condition (n = 6, except for exposed biofilm n = 5). D9D: Δ9-desaturase (stearoyl-CoA-desaturase, [1]16:1n-7/16:0 and [2]18:1n-9/18:0); D12D: Δ12-desaturase (18:2n-6/18:1n-9); D15D: Δ15-desaturase (18:3n-3/18:2n-6), Δ6-desaturase (18:3n-6/18:2n-6 and 18:4n-3/18:3n-3) and elongase: ELOVL (18:0/16:0).

	Biofilm		Grazers	
	Control biofilm	Contaminated biofilm	Control grazers	Grazers feeding on contaminated biofilm
D9D[1]	0.69 ± 0.13	0.57 ± 0.05	0.15 ± 0.03	0.16 ± 0.03
D9D2	0.78 ± 0.06	0.81 ± 0.06	0.68 ± 0.03	0.74 ± 0.06
D12D	0.41 ± 0.13	0.33 ± 0.09	n.c.	n.c.
D15D	1.31 ± 0.03	2.2 ± 1.5	n.c.	n.c.
D6D	0.32 ± 0.04	0.38 ± 0.18	0.13 ± 0.09	0.12 ± 0.08
ELOVL	0.55 ± 0.12	0.48 ± 0.05	1.37 ± 0.04	1.26 ± 0.04

n.c. = not calculated (because of the absence of either the precursor or the product).

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