

## A comparison of biofilms from macrophytes and rocks for taste and odour producers in the St. Lawrence River

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**Abstract** Given their widespread and prolific annual development in the St. Lawrence River (SLR), macrophytes (i.e. submerged aquatic plants) represent large surface areas for biofilm growth and potentially important sites for associated production of taste and odour (T&O) compounds. We therefore evaluated the importance of submerged macrophytes and their associated biofilms for production of T&O compounds, 2-methylisoborneol (MIB) and geosmin (GM), compared with biofilms from adjacent rocks. We also tested the hypothesis that production of these compounds would differ between macrophyte species, based on the premise that they are not inert substrates but directly influence the communities that colonise their surfaces. Samples collected from transects across the SLR between Kingston and Cornwall, ON were dominated by the flat-bladed *Vallisneria* spp., and the leafed *Myriophyllum spicatum*, *Elodea canadensis*, *Chara* spp., *Potamogeton* spp., and *Ceratophyllum* spp. Overall, MIB and GM levels in biofilms ranged widely between samples. Expressed per g dry weight of biofilm, median levels from macrophyte were 50 (range 1–5,000) ng MIB g<sup>-1</sup> and 10 (< 1 to 580) ng GM g<sup>-1</sup> compared with 50 (range 5–970) ng MIB g<sup>-1</sup> and 160 (1–1,600) ng GM g<sup>-1</sup> from rocks. Based on non-parametric statistical analysis, levels of GM were higher on a g dry weight basis in biofilms from rocks than macrophytes ( $P = 0.02$ ), but MIB levels were similar ( $P = 0.94$ ). However, when normalised for differences in substrate surface area (i.e. ng cm<sup>-2</sup>), levels of both MIB and GM were higher in biofilms from rocks than from macrophytes ( $P < 0.01$ ). There were no discernible differences in MIB and GM concentrations from biofilms of different macrophytes based on either g dry weight sample or surface area ( $P > 0.05$ ). Overlying water (OLW) concentrations ranged between 2–45 ng L<sup>-1</sup> for MIB and 5–30 ng L<sup>-1</sup> for GM and were not correlated with levels in adjacent biofilms. However, OLW concentrations peaked in shallow, low energy embayments consistent with enhanced production and release of MIB and GM in nearshore areas. The results support our previous work showing the importance of biofilms on various surfaces (rocks, macrophytes and zebra mussels) for MIB and GM production in the SLR, but suggest that inert surfaces like rocks are more productive sites per unit surface area than macrophytes.

**Keywords** Geosmin; macrophytes; 2-methylisoborneol; periphyton; St. Lawrence River; taste and odour sources

### Introduction

In the past decade, significant annual outbreaks of musty-earthly taste and odour (T&O) in drinking water drawn from the St. Lawrence River (SLR; Ontario Canada) have affected over 0.5 million consumers along a 200 km international stretch of the river. Our early investigations identified some striking characteristics of these events. They are caused by both of the two sesquiterpenoids most frequently implicated in T&O events, geosmin (GM) and 2-methylisoborneol (MIB). These two major volatile organic compounds attain (and often sustain) concentrations some 5–10 times greater than their odour threshold levels from late summer through late autumn. GM and MIB are generated primarily within the river, rather than in Lake Ontario which discharges into the St. Lawrence, as was clearly shown by the significant increase in their levels in the river

compared with those measured in the lake (Ridal *et al.*, 2000). Surface water monitoring data show significant inter-annual variations ( $\sim 10\text{--}80\text{ ng L}^{-1}$ ) in the relative proportions and levels of GM and MIB depending on location and time of year (Ridal and Watson, unpublished data). Nevertheless, the GM and MIB concentrations measured in the SLR represent impressive production rates ( $7\text{--}55\text{ kg day}^{-1}$ ) given the dilution rates of the river which has an average flow rate of approximately  $8,000\text{ m}^3\text{ s}^{-1}$  at Cornwall, ON.

In an earlier paper, we identified shallow nearshore zones as major T&O production sites, as opposed to the open water or deeper river bottom, based on increasing MIB and GM concentration gradients from mid-river to nearshore and on high levels in biofilms from rocks, zebra mussels and submerged macrophytes (Watson and Ridal, 2004). Some macrophytes are known to produce potent allelopathic or cuticular material which may act against planktonic or epiphytic algae competing with the plants for light and other essential resources (e.g. Goldsborough and Hickman, 1991; Nakai *et al.*, 2000; Gross *et al.*, 2003). We therefore evaluated the relative importance of rocks and macrophytes as substrates for T&O producers. We also examined differences between macrophyte taxa as sources of T&O, testing the hypothesis that, unlike abiotic substrates, their surfaces are not inert, but directly influence T&O production. In this paper we report the results of this investigation, which provides new insight into the biological sources, fates and variability in GM and MIB in the St. Lawrence and other rivers.

## Methods

Our sampling coincided with the height of the seasonal T&O event in the river between mid-September and mid-October 2003. Using divers, we took representative samples of macrophytes and rocks along selected transects in the upper St. Lawrence River between Kingston and Cornwall, Ontario, Canada. For the transects, macrophyte and rock samples were collected at 1 m depth intervals beginning at nearshore to the maximum depth of macrophyte colonisation (approximately 6–9 m depth) using randomised sampling of  $0.1\text{ m}^2$  quadrats. Macrophytes and transportable sized rocks were placed in net collection bags by the divers and returned to the laboratory for further workup. To examine the variation in water concentrations of GM and MIB near potential sources, we collected overlying water samples (OLW) above the substrates at the sampling points. Surface water samples were taken at other locations to identify regional and temporal T&O trends.

Collected macrophytes were measured for total wet mass (excluding roots) then separated into individual species for identification and percent abundance estimates. Subsamples of the three most abundant species were selected for further analysis. Periphyton was removed from rock ( $25\text{ cm}^2$ ,  $n = 20$ ) and macrophyte surfaces (entire surface of subsamples,  $n = 70$ ) by gentle brushing into a small amount of overlying water for analysis of GM and MIB contents, total suspended solids, particulate carbon and nitrogen (GP and SI transects only) and for microscopic examination. Surface area per dry plant weight relationships were estimated for the reported macrophyte species based on measurements of their leaf fine structure for 10 samples with the aid of dissecting microscopes and by applying simple geometric formulas to estimate total surface areas. The weight of each plant sampled for biofilms was recorded and the above relationships used to derive colonisable (i.e. upper leaf) surface area.

GM and MIB samples (measured volumes) were transferred into pre-cleaned 40 mL septum capped VOC vials, allowing a small headspace in the vials, and refrigerated. MIB and GM were measured in all biofilm and overlying water samples within 1 week of collection by solid phase microextraction (SPME) GC-MS headspace analysis (Watson *et al.*, 1999). Loss of MIB and GM in these vials from microbial action was not

controlled for, but assumed to be similar among samples; this therefore represents a potential source of variance in the results which are thus conservative estimates. Surface water samples were extracted with dichloromethane and analysed using GC-MS, following the method of Brownlee *et al.* (2004). Surface water samples were collected from a small outboard boat and portable equipment to measure *in situ* location (GPS, Magellan<sup>®</sup>), light penetration (Secchi disk), water temperature, conductivity (YSI Model 30), dissolved oxygen (YSI Model 55) and water velocity (Type “AA”, Scientific Instruments, Inc.) were used. Subsamples were withdrawn while stirring and filtered through glass fibre filters for total suspended solids and CHN analysis. Reproducibility of the MIB and GM analyses for these samples were similar to that for previous investigations with these techniques (Watson *et al.*, 1999; Watson and Ridal, 2004), and was approximately  $\pm 15\%$  for duplicate analyses of the same samples ( $n = 3$ , this study). Variability of MIB and GM results for periphyton from adjacent surfaces within a site has been found to be approximately  $\pm 50\%$ , whereas differences between sites can vary by an order magnitude (Watson and Ridal, 2004). The data, therefore, exhibit large sample to sample variability and non-normal distributions, and were compared by the non-parametric Mann–Whitney and Kruskal–Wallis tests using Minitab<sup>®</sup> Release 14.20 statistical software.

## Results

Site and water quality characteristics for the detailed sampling transects are provided in Table 1. Water temperatures ranged between 15 and 21 °C with a gradual decrease from mid-September through mid-October. Water transparency (6.0–8.5 m Secchi disk depth), specific conductivity (303–328  $\mu\text{S cm}^{-1}$ ) and total phosphorus (0.006–0.024  $\text{mg L}^{-1}$ ) were similar between most sampling sites, with slight increases for the nearshore area of the JB site, a site with some nearby commercial activities. Concentrations in overlying water ranged from 2–45  $\text{ng MIB L}^{-1}$  and 5–30  $\text{ng geosmin L}^{-1}$ .

Macrophyte beds were highly diverse along all transects, with a few patches dominated by single taxa. The flat-bladed *Vallisneria* spp., and the leafed *Myriophyllum spicatum*, *Elodea canadensis*, *Chara* spp., *Potamogeton* spp and *Ceratophyllum* spp. were the most commonly encountered taxa. Overall, macrophytes covered  $32 \pm 50\%$  and rocks  $18 \pm 29\%$  of the bottom along the four transects sampled, with rocks observed in shallower depths of water (most  $< 4\text{ m}$ ). Particulate C and N levels were lower in biofilms from macrophytes than from rocks ( $P = 0.003$ ) with median values of 17 (range 1–62)  $\text{mg C g}^{-1}$  dry weight for macrophytes and 50 (range 19–89)  $\text{mg C g}^{-1}$  dry weight for rocks, while particulate N values were 1.2 (0.1–4.2) and 5.3 (1.5–7.0)  $\text{mg N g}^{-1}$  dry weight, respectively.

**Table 1** Site details and associated water quality information with sampling transects taken upstream of Cornwall, ON (GP, SI), near Prescott, ON (JB) and downstream of Kingston (K)

Transect	Location (start of transect)	Sampling depths (m)	T (°C)	Secchi disk depth (m)	Total P ( $\text{mg L}^{-1}$ )	MIB ( $\text{ng L}^{-1}$ )	GM ( $\text{ng L}^{-1}$ )
GP	45°01'27 N 74°50'58 E	0.8–9.0	20.9–21.1	6.0	0.006–0.013	4–44	7–28
SI	45°01'20 N 74°50'58 E	1.0–7.6	20.9–21.1	6.5	0.006–0.014	4–6	13–18
JB	44°44'11N 75°28'02E	1.0–9.0	14.7–15.9	8.5	0.011–0.024	1–35	25–37
K	44°14'42N 76°24'31E	0.7–6.0	16.6–19.6	8.5	0.008–0.010	1–7	1–9

MIB and GM contents of the plant and rock biofilms varied widely (Table 2), with median values ranging from 2–130 ng g<sup>-1</sup> dry weight for MIB and from 1–160 ng g<sup>-1</sup> dry weight for GM. Statistical analysis of the data on a dry weight basis indicates that the GM content of rock biofilm was greater than for macrophytes ( $P = 0.02$ ) but MIB values were similar ( $P > 0.05$ ). Similar relative values were observed when concentrations were calculated as function of carbon or nitrogen (i.e. ng mg<sup>-1</sup> C or N).

However, on a surface area (i.e. ng cm<sup>-2</sup>) basis, both MIB and GM in rock biofilms were greater than in macrophyte biofilms ( $P < 0.01$ ), as shown in Figure 1. Median values were 0.07 (range 0.01–7.2) ng MIB cm<sup>-2</sup> and 0.02 (range 0.01–2.0) ng GM cm<sup>-2</sup> for macrophytes while biofilms collected from adjacent rocks had median values of 0.2 (range 0.1–5.3) ng MIB cm<sup>-2</sup> and 0.8 (range 0.1–8.8) ng GM cm<sup>-2</sup>. No differences in MIB and GM concentrations could be discerned for biofilms collected from different macrophyte species ( $P > 0.05$ , Table 2).

#### GM and MIB concentrations in overlying water

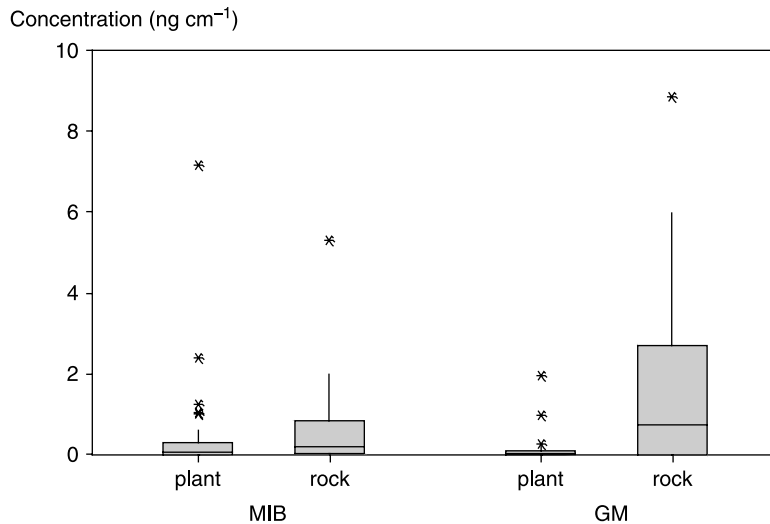
MIB and GM concentrations in overlying water taken above the substrate sampling points had the greatest variations in shallow waters (0.5–4 m depth, Figure 2) but overall did not correlate strongly with MIB and GM levels in the biofilms from the rocks or macrophytes collected at the sites. Factors such as water column mixing, variation in rates of release from biofilms, and loss processes likely vary between sites and contribute to variability in MIB and GM concentrations in the OLW samples. For example, the bathymetry along the transect shown in Figure 1 is characterised by a shallow shelf approximately 50 m in width and depths ranging to 4 m with low current velocities (<0.2 m s<sup>-1</sup>), followed by a gradual slope to 10 m depth where current velocities increased to 0.2–0.4 m/s. Samples from deeper depths, more closely influenced by the river's main flow, were more uniform in their concentrations of MIB and GM and were similar to main channel surface water concentrations (20 ± 5, 5 ± 1,  $n = 7$ ).

#### Discussion

We found biofilms from rocks contained more GM per g dry weight than biofilms from macrophytes. Both GM and MIB were higher in rock biofilms than macrophyte biofilms when expressed on the basis of surface area. C and N contents were also higher per g dry weight on rocks than macrophytes. Rocks are inert surfaces whereas

**Table 2** Median values and ranges for MIB and GM concentrations in biofilms collected from rock and macrophyte surfaces from all transects expressed as ng g<sup>-1</sup> dry weight (dw) biofilm and ng cm<sup>-2</sup> of surface sampled. Also provided is the number of samples analysed (N). Individual macrophyte species are listed in approximate order of increasing leaf complexity

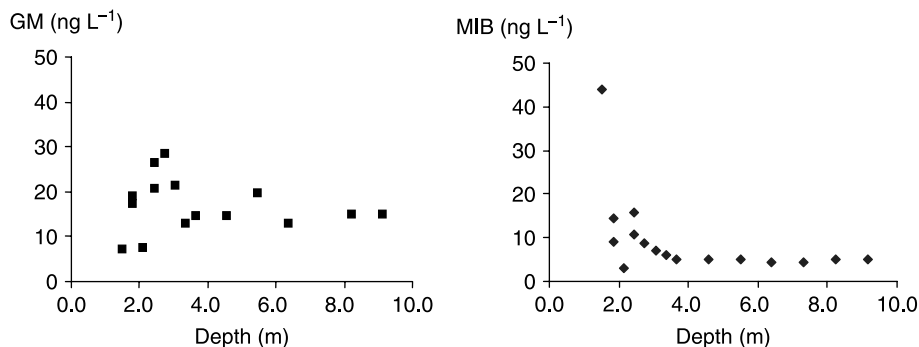
Sample	N	MIB		GM	
		ng g <sup>-1</sup> dw	ng cm <sup>-2</sup>	ng g <sup>-1</sup> dw	ng cm <sup>-2</sup>
Rock	20	50 (5–970)	0.2 (0.03–5.3)	160 (1–1600)	0.8 (<0.01–8.8)
All macrophytes	70	50 (1–5,000)	0.07 (<0.01–7.2)	10 (<0.1–580)	0.02 (<0.01–2.0)
<i>Vallisneria</i> spp.	13	50 (5–750)	0.04 (0.01–1.0)	4 (<0.1–42)	0.01 (<0.01–0.07)
<i>Chara</i> spp.	11	2 (1–480)	0.05 (<0.01–0.58)	1 (<0.1–200)	<0.01 (<0.01–0.25)
<i>Potamogetan</i> spp.	5	130 (60–2,400)	0.07 (0.02–2.4)	87 (2–270)	0.03 (<0.01–0.30)
<i>Elodea</i> spp.	22	40 (1–5,000)	0.09 (<0.01–1.0)	26 (1–580)	0.05 (<0.01–0.18)
<i>Myriophyllum</i> spp.	5	120 (12–220)	0.11 (<0.01–7.2)	15 (1–29)	0.05 (<0.01–1.0)
<i>Ceratophyllum</i> spp.	14	90 (6–400)	0.08 (<0.01–1.2)	40 (1–250)	0.04 (<0.01–2.0)



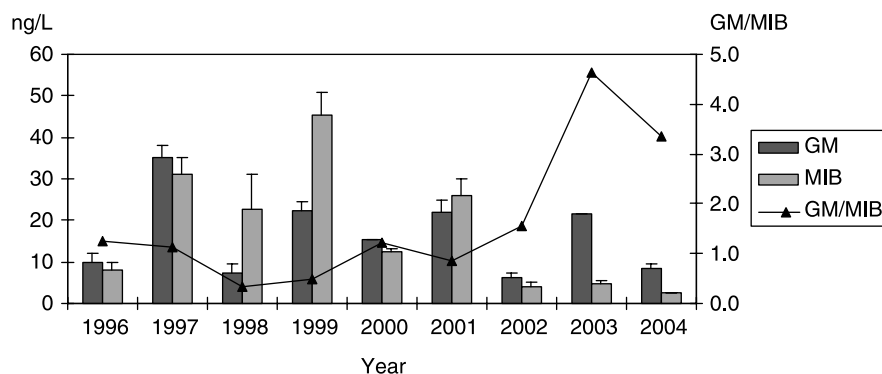
**Figure 1** Boxplots of MIB and GM concentrations expressed on the basis of substrate surface area comparing plant and rock biofilms. Boxes indicate the medians and interquartile ranges; the whiskers are the upper data limits, and asterisks indicate outliers

macrophytes may be able to suppress epiphytic algae competing with the plants for light and other essential resources through the production of allelopathic compounds or cuticular material (Nakai *et al.*, 2000). Complex macrophyte surfaces may have higher self-shading potential and may also increase the amount of silt entrapped on the leaf, thereby reducing biofilm growth.

We did not, however, observe discernable differences in MIB and GM content of biofilms from different macrophytes on either a g dry weight basis or on the basis of estimate surface area (which accounts for differences in plant leaf complexity). In addition, the relative amounts of MIB and GM present in biofilms showed no consistent differences among surface types; the proportion (mass) of MIB recovered from biofilms ranged from 3 to 95% of the total mass of the two terpenoids. Such variations suggest differences in biofilm community structure and/or MIB AND GM production among sites. Factors such as location, local water quality as well as site depth, light intensity (Huggins *et al.*, 2004) and grazing pressure (Lamberti *et al.*, 1987) could obscure differences in biofilm composition between macrophyte species.



**Figure 2** Variations in GM and MIB concentrations in overlying water along a depth transect sampled perpendicular to the shoreline in the St. Lawrence River near Cornwall



**Figure 3** Average surface water concentrations (bars) and standard deviations (error lines) of GM and MIB measured at Cornwall in September. Trend line denotes ratio of GM versus MIB concentrations

#### Long term trends in water concentrations

Data collected over the last 8 years show significant inter-annual variations in the relative proportions and levels of GM and MIB in the surface waters of the SLR (Figure 3). Prior to 2002, the GM/MIB ratio was typically less than or near one. Subsequent years have seen MIB concentrations decline more than four-fold (reaching less than  $15 \text{ ng L}^{-1}$  in 2003 max seasonal value), while GM concentrations have remained relatively constant at  $10\text{--}40 \text{ ng L}^{-1}$  (max seasonal). As a result, the GM/MIB ratio has increased to  $\sim 3\text{--}5$ . These trends suggest a major shift in activity, abundance and/or composition of the biota producing these compounds, likely a response to large scale environmental or ecological drivers.

Despite wide variability in MIB and GM contents amongst samples, biofilms are clearly key sources of these compounds in the St. Lawrence River. While the availability of inert surfaces, such as rocks for colonisation, would be relatively constant from year to year varying mainly with water levels, annual variations in macrophyte abundances depend on biophysical drivers and may be expected to undergo large interannual variabilities. Annual changes in macrophyte abundances would contribute to year to year differences in MIB and GM concentrations by changing the available substrate for biofilm colonization. Hudon *et al.* (2000) investigated the factors affecting macrophyte biomass and depth distribution in the St. Lawrence River, finding that exposure to wind and waves, plant growth forms, water depth and light intensity affected total biomass. Using a hierarchical analysis approach, Hudon *et al.* (2000) identified that the transparent SLR waters favour canopy-forming macrophytes (e.g. *Myriophyllum* sp., *Elodea canadensis*) in shallow ( $< 2.2 \text{ m}$ ), sheltered areas and in deeper exposed areas ( $> 4 \text{ m}$ ). Non-canopy macrophytes (*Vallisneria americana*, *Ceratophyllum demersum*, *Chara* sp.) tend to be dominant in shallow and intermediate depths of exposed sites. Annual water level fluctuations in the SLR will therefore alter the composition and biomass of macrophytes available for biofilm colonisation.

#### Conclusion

In summary, the majority of biofilms from rocks and macrophytes contained T&O producers, although levels vary widely between sample to sample, consistent with our previous work (Watson and Ridal, 2004). Rock biofilms harbour greater amounts of GM and MIB than macrophyte biofilms, which though not demonstrated unequivocally here may suppress biofilm growth. We found no consistent differences between different species of macrophytes although high variability in T&O contents made differences difficult to

detect. Longer term water quality data indicates that MIB and GM water concentrations in the St. Lawrence River have gradually decreased over the past 5 years. With the lack of compelling evidence for planktonic sources (S. Watson, unpublished results) in the St. Lawrence River, T&O producing attached algae are likely the main sources to the overlying water. Factors controlling their abundance, distribution, production and release of T&O compounds are therefore important determinants of short and longer term variations in water concentrations, and are the focus of our continued research.

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