Algal production and trihalomethane formation potential: an experimental assessment and inter-river comparison

Jeffrey Jack, Tim Sellers, and Paul A. Bukaveckas

Abstract: Trihalomethanes (THMs) are byproducts produced during the disinfection of drinking water. We combined survey and experimental approaches to identify factors that influence THM formation potential (THMFP) in the Ohio River drainage basin. Two surveys of the Ohio River and its five principal tributaries were conducted to characterize spatial variation in THMFP in relation to algal abundance and suspended organic matter. We performed three experiments by placing Ohio River water in 2000-L outdoor mesocosms and manipulating algal senescence and bloom development by shading. Increases in THMFP among high- and low-light and dark tanks suggest that algal production, algal senescence, and possibly photolysis increased THMFP by as much as 50% over 3–6 days. Comparable yields of THMs (per unit of chlorophyll) were observed in both survey and experimental settings. Comparison of input waters with outputs indicates that the Ohio River at times acts to attenuate downstream transport of THM precursors. Our findings suggest that both watershed-scale and internal processes regulating THMFP should be considered as utilities develop strategies to meet new drinking water guidelines.

Résumé : Les trihalométhanes (THMs) sont des produits secondaires générés durant la désinfection de l'eau potable. La combinaison d'inventaires et d'expériences nous a permis d'identifier les facteurs qui influencent le potentiel de formation de THM (THMFP) dans le bassin hydrographique de l'Ohio. Deux inventaires de l'Ohio et de ses cinq principaux tributaires ont servi à caractériser la variation spatiale du THMFP en fonction de l'abondance des algues et de la matière organique en suspension. Nos trois expériences consistaient à placer de l'eau de l'Ohio dans des mésocosmes extérieurs de 2000 L et à manipuler la sénescence et la prolifération des algues au moyen de l'ombrage. L'augmentation du THMFP dans les mésocosmes exposés à la lumière forte ou faible, ou gardés à l'obscurité, semble indiquer que la production d'algues, la sénescence des algues et peut-être aussi la photolyse augmentent le THMFP d'une valeur pouvant atteindre 50 % sur une période de 3–6 jours. Des quantités comparables de THM (par unité de chlorophylle) ont été observées dans les inventaires et dans les expériences. La comparaison des eaux à l'arrivée et à la sortie montre que l'Ohio peut quelquefois atténuer le transport vers l'aval des précurseurs des THM. Nos résultats indiquent que, lorsque les services publics mettront au point des stratégies pour se conformer aux nouvelles normes régissant l'eau potable, ils devront tenir compte tant des processus qui agissent à l'échelle du bassin hydrographique que de ceux qui fonctionnent au niveau interne dans la régulation du THMFP.

[Traduit par la Rédaction]

Introduction

Chlorination is a common practice worldwide used to ensure the safety of water for drinking supplies. Although this procedure is effective in ensuring potable water, the process of chlorination may lead to the formation of disinfection byproducts (DBPs). These include trihalomethanes (THMs) such as chloroform and bromoform (Rook 1976) that have been shown to be carcinogenic in laboratory animals (Singer 1999). New United States Environmental Protection Agency (USEPA) regulations require water utilities to take steps to reduce regulated DBPs by 2004 (Cooke and Kennedy 2001). As a result, there is an increased urgency to understand how source waters differ in THM formation potential (THMFP) and what role ecological processes play in regulating the production and fate of THM precursors. THM levels in raw water are usually very low (Arguello et al.1979), and therefore utilities have focused on engineering solutions to reduce THM formation during treatment. Although effective, such proximate solutions do not address the important question of why source waters vary widely in THMFP. If the environmental factors favoring high THMFP can be better understood, this would provide utilities with options for managing source waters to meet the new guidelines. Various site-specific studies have correlated THMFP with environmental factors such as temperature, pH, and concentrations of particulate organic carbon (POC) and dissolved organic carbon (DOC) or their constituent components (e.g., humic acids; Singer 1999; Lin et al. 2000). Although these studies have broadly characterized the environmental conditions favoring THM formation, there has been much less emphasis placed

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on understanding the processes which give rise to THM precursors. Lakes, rivers, and reservoirs are commonly used as source waters and THM precursors may originate from autochthonous or allochthonous inputs of DOC and POC. Potential autochthonous sources include sediments, macrophytes, and algae (Palmstrom et al. 1988). Algae are of particular interest because they attain high numbers in some water supplies and produce extracellular products (ECPs) that may serve as THM precursors (Hoehn et al. 1980). In a combined reservoir and experimental study, Graham et al. (1998) found a positive correlation between THM and chlorophyll concentrations. They also assessed THMFP in algal cultures and found that THM yield was highest in the exponential and death stages of the cultures and was lowest in the stationary phase.

Allochthonous sources of THM precursors include terrestrially derived organic materials that have undergone varying degrees of diagenesis. External sources of THM precursors may be particularly important in rivers and reservoirs because these systems receive higher allochthonous inputs of organic matter than lentic waters. In addition, their higher loads of suspended particulate material reduce light penetration and may limit internal generation of THM precursors associated with phytoplankton production (Alpine and Cloern 1992; Cole et al. 1992). Stepczuk et al. (1998*a*, 1998*b*, 1998*c*) used estimates of organic carbon loading and other parameters to model temporal and spatial variability of THM precursors in a river–reservoir system in New York state (U.S.A.). Landscape level approaches have potential for identifying important sources and sinks at the basin level and for developing whole-catchment strategies for mitigating THM formation.

Although rivers are widely used as sources of drinking water, we know of no prior process-oriented studies on THMFP in rivers. The Ohio River basin drains 10% of the continental United States and includes a population of over 25 million people. More than three million users depend directly on the Ohio River as a water supply. The tributaries contributing to the Ohio River drainage network differ in their degree of hydrologic regulation, watershed land use, and water quality, all of which may influence THMFP. In this study, we combine survey and experimental approaches to examine the relationship between THMFP, algal production, and DOC and POC. We tested the hypothesis that algal growth enhanced THMFP by (*i*) comparing rivers that differed in algal production, and (*ii*) experimentally manipulating algal production in mesocosms containing riverine phytoplankton communities. We predicted that differences in THMFP among river basins could be explained in part by variation in algal abundance (chlorophyll) and that experimental inducement of algal blooms in mesocosms would result in increases of THMFP.

Materials and methods

Comparative studies

Our assessment of spatial covariation in THMFP, chlorophyll (chl *a*), and organic carbon (POC and DOC) focused on the lower Ohio River and its major tributaries (Kentucky, Green, Wabash, Tennessee, and Cumberland rivers; Fig. 1). These tributaries represent all inflows within the study reach that contribute at least 1% of the volume of the Ohio River at its confluence with the Mississippi. Surveys were conducted during the warmwater, low-flow period in August and October of 2000. Sampling followed a semi-Lagrangian design with upstream sites visited 3–7 days prior to downstream sites depending on transit time (estimated from river stage). The mainstem Ohio River was represented by an upstream site located near Vevay, Ind., at Ohio River kilometre (ORK) 854, a midstream site near Evansville, Ind. (ORK 1251) and a downstream site near the confluence with the Mississippi River (ORK 1501). Tributary samples were collected a minimum of 1 km above their confluence with the Ohio River to ensure that the sample was representative of water originating within the sub-basin. Water and ancillary field data (temperature, pH, turbidity) were taken at a midpoint in the river channel and within 1 m of the surface. A single water sample was collected at each site and analyzed for THMFP, chl *a*, POC, and DOC. Flux rates were derived from measured concentrations and 10-day averages of river discharge obtained from United States Geological Survey (USGS) and Tennessee Valley Authority (TVA) gaging stations. Input fluxes from tributaries and the upper Ohio River were compared with outputs near the confluence with the Mississippi to determine whether the Ohio River was a net source or sink for THMFP, chl *a*, and total organic carbon (TOC).

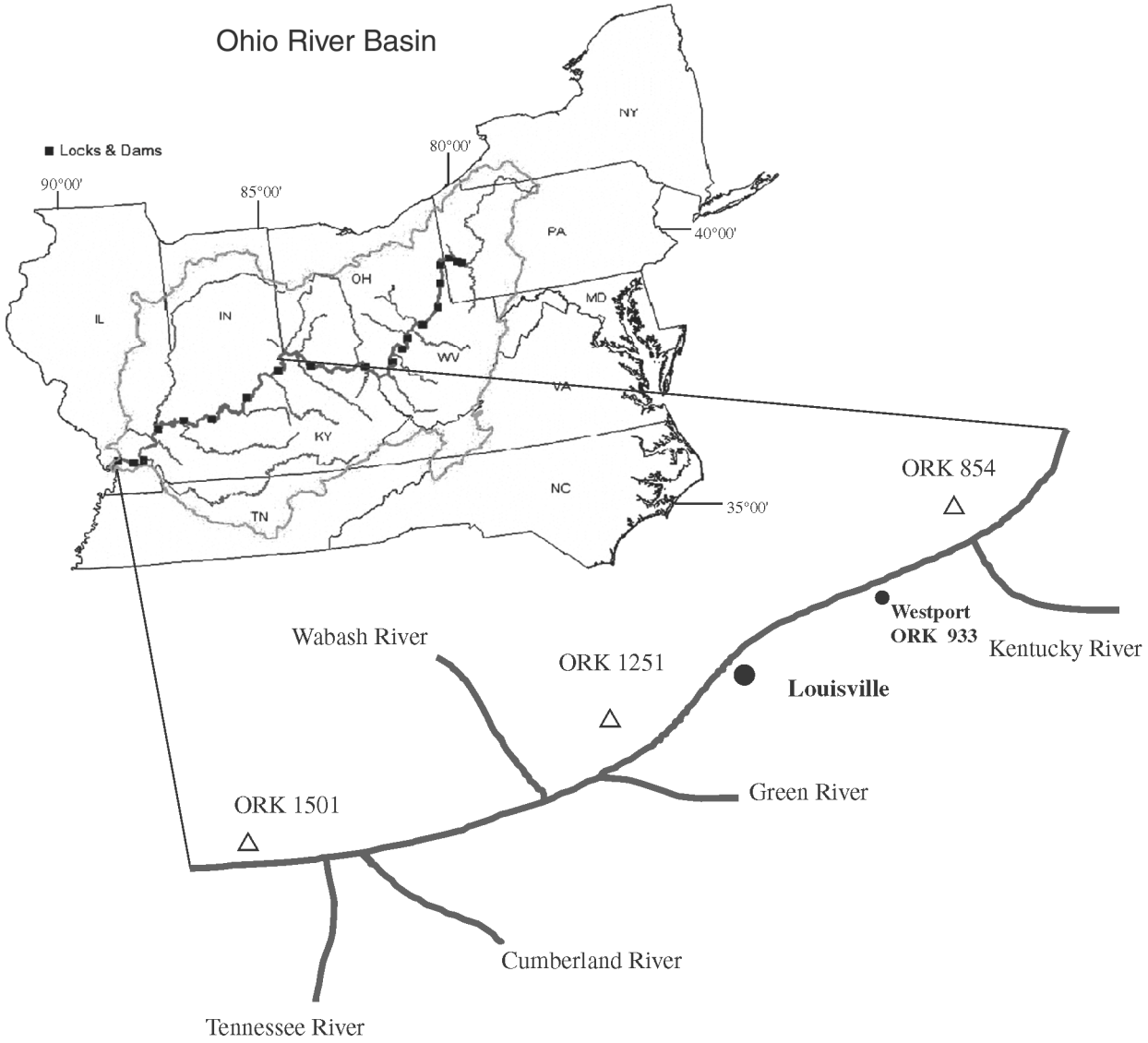
Experimental studies

Experiments designed to assess the effects of algal blooms on THMFP were conducted at the Ohio River Experimental Station located near Westport, Ky. (ORK 933). The facility consists of 24 large (2000-L) outdoor tanks that were filled with water pumped directly from the Ohio River. Three experiments were conducted in July, August, and September of 2000 to capture a range of conditions characteristic of the summer, base-flow period. Submersible pumps maintained particulate matter in suspension during the 6-day experiments. Polyvinyl chloride tank covers with varying aperture dimensions regulated light levels within the tanks, and thereby controlled algal bloom development. "Low" light tanks simulated the light environment occurring in deeper sections of the river (8- to 10-m average cross-sectional depth, chl *a* values $<5\ \mu g \cdot L^{-1}$) where net algal production is near zero; "high" light tanks simulated shallow reaches of the river (3- to 5-m depth, chl *a* values $10–15\ \mu g \cdot L^{-1}$) where higher algal growth rates result in greater bloom development (Sellers 2002). Three replicate high and low-light tanks were sampled on days 0, 3, and 6 during each experiment. In the September experiment, three additional tanks ("dark" treatment; zero aperture) were used to assess the effects of algal senescence on THMFP. Sample analyses for all treatments included determination of THMFP, chl *a*, POC, and DOC. Ancillary data (temperature, pH, turbidity, and dissolved oxygen) were collected to ensure that tank conditions mimicked those observed in the river.

Analytical procedures

An operationally defined procedure has been developed to quantify THMFP in samples incubated under standardized conditions (Method 5710B; APHA 1998). Both tank and river samples were collected in 1-L glass bottles and stored

Fig. 1. Locations of Ohio River sampling sites and five major tributaries, U.S.A. Inset map is modified from United States Army Corps of Engineers (available at http://f7784.lrh.usace.army.mil).



on ice in the dark for no more than 24 h. Samples were buffered to pH 7.0, chlorinated with an excess of free chlorine, and incubated at 25°C. Chlorine demand, dose, and free residual were determined according to Standard Methods (APHA 1998). After the 7-day reaction period, the samples were quenched with ascorbic acid. Concentrations of individual THMs (bromoform, bromodichloromethane, dibromochloromethane, and chloroform) were determined by the purge and trap gas chromatographic – mass spectrometric method (Method 6232C; APHA 1998). We report total THMFP as the sum of the four species.

Samples for chlorophyll analyses were collected on 0.5-μm filters, extracted in acetone (12 h), and analyzed by fluorometry (Turner Designs 10-AU, Sunnyvale, Calif.) with acid correction (Arar and Collins 1997). POC was collected on 0.5-μm filters, dried and analyzed using a Perkin-Elmer 2400 series II CHNS/O analyzer (Shelton, Conn.). Filtrate was analyzed for DOC on an automated total organic carbon analyzer (Shimadzu Model TOC-5050A, Kyoto, Japan) after sparging for 4 min to remove inorganic carbon. Temperature, pH, and dissolved oxygen were measured with a Hydrolab SOND IV (Austin, Tex.) and turbidity was measured with a Hach 2100P turbidimeter (Hach Co., Loveland, Colo.). Experimental data were analyzed by one-way analysis of variance (ANOVA) following tests for normality and homogeneity of variance using SYSTAT v. 7.0. Survey data were analyzed using univariate regressions to identify significant predictors of variation in THMFP across sites.

Results

Comparative studies

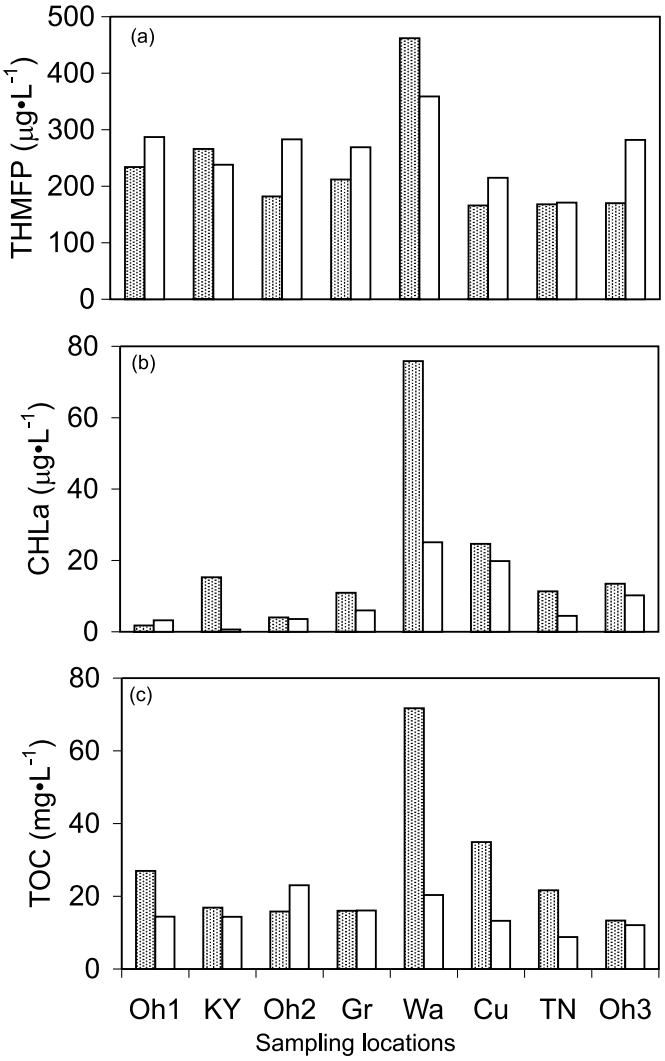
Drainage basin characteristics and C, N, and P concentrations for the Ohio River and its major tributaries are shown in Table 1. THMFP concentrations throughout the Ohio River basin ranged between 150 and 300 $\mu g \cdot L^{-1}$ (Fig. 2), with the exception of the Wabash River, which exhibited the highest

Table 1. Ohio River kilometre (ORK) denotes the location of mainstem sampling sites and for tributaries their confluence with the Ohio River.

	ORK	Basin area (km^2)	Discharge ($km^3 \cdot year^{-1}$)	Total N ($\mu g \cdot L^{-1}$)	Total P ($\mu g \cdot L^{-1}$)	Total OC ($mg \cdot L^{-1}$)
Ohio River (upper)	854	215 410	103.2[a]	1697	88	10.8
Kentucky River	879	18 052	7.6[a]	1030	89	9.1
Ohio River (middle)	1251	251 230	115.8[a]	398	90	11.4
Green River	1254	23 906	10.8[a]	1669	85	13.1
Wabash River	1356	85 729	25.0[a]	2805	219	24.6
Cumberland River	1472	46 413	33.2[a]	538	118	12.1
Tennessee River	1491	105 957	57.4[b]	521	73	8.7
Ohio River (lower)	1501	528 205	246.6[a]	1143	99	9.7

Note: Chemistry data are averages for August and September sampling dates in 1998, 1999, and 2000. OC, organic carbon.
[a]Data from Water Resources Data, Kentucky, Water Year 1997 (USGS KY-97-1) (http://ky.water.usgs.gov/).
[b]Data from Tennessee Valley Authority, Tennessee Water Management Office, Knoxville, Tenn.

Fig. 2. Concentrations of (*a*) trihalomethane formation potential (THMFP), (*b*) chlorophyll *a* (CHLa), and (*c*) total organic carbon (TOC) within the Ohio River and its major tributaries during August (shaded) and October (open) of 2000. Sampling sites were upper (Oh1), middle (Oh2), and lower Ohio (Oh3), Kentucky (KY), Green (Gr), Wabash (Wa), Cumberland (Cu), and Tennessee (TN) rivers.



THMFP concentrations during both the August (462 $\mu g \cdot L^{-1}$) and the October surveys (359 $\mu g \cdot L^{-1}$). The Wabash River also exhibited elevated chl *a* and TOC concentrations (Fig. 2). A univariate regression based on chl *a* accounted for 44% of the variation in THMFP, whereas a similar model using TOC as a predictor of THMFP accounted for 40% of variation. These models predicted comparable yields of THMFP per each unit increase in chl *a* (2.9 ± 0.9 $\mu g \cdot L^{-1}$) or TOC (3.4 ± 1.1 $\mu g \cdot L^{-1}$) over the range of chl *a* (1–25 $\mu g \cdot L^{-1}$) and TOC (10–30 $mg \cdot L^{-1}$) observed in our study. TOC was composed of nearly equal fractions of dissolved (45%) and particulate (55%) forms (average for all samples). Algal carbon (estimated as 30:1 C : chl *a*) was 4% of POC (range 1–7%), suggesting that riverine organic matter was predominantly of allochthonous origin.

Discharge from the Ohio River basin was similar during the two periods with, outputs averaging 3600 cm (August) and 3900 cm (October; Fig. 3). The relative contributions of various sub-basins differed between the two surveys. In August, the upper Ohio (39%) and Tennessee rivers (30%) dominated inputs with the Cumberland (15%), Wabash (10%), and Green–Kentucky rivers (5% combined), accounting for a smaller fraction of inflow. In October, the upper Ohio (36%) and Wabash (32%) rivers were the largest contributors, whereas the Tennessee (21%), Cumberland (10%), and Green–Kentucky rivers (1%) accounted for a smaller proportion of inputs. Material flux rates generally followed patterns in discharge although some differences were noted among sub-basins in their relative contributions of water and dissolved constituents (Fig. 3). In August, the Wabash River was a modest contributor of water (11% of inputs), but accounted for a disproportionately large fraction of THM precursors (22%), DOC (22%), and chl *a* (49%). A similar pattern was observed in October when the Wabash River accounted for 32% of water, 41% of THM precursors, 66% of chl *a*, and 45% of DOC inputs. In contrast, the upper Ohio River contributed similar proportions of water (39%), THMFP (40%), and DOC (35%) inputs, but accounted for only 4% of chl *a* inputs (values for August).

Total inflows to the study reach (from upper Ohio River and tributaries) were equivalent to 85 and 88% of the outflow volume during the August and October index periods, respectively. Observed declines in river stage (USGS data available at http://waterdata.usgs.gov/ky/nwis) during both

Fig. 3. Input and output fluxes of water, trihalomethane formation potential (THMFP), chlorophyll *a* (CHLa), dissolved organic carbon (DOC), and particulate organic carbon (POC) to the Ohio River during (*a*) August and (*b*) October of 2000. Input sources include the Tennessee (horizontal bars), Cumberland (dotted), Wabash (vertical bars), Kentucky + Green (cross-hatched), and Ohio (solid) rivers.

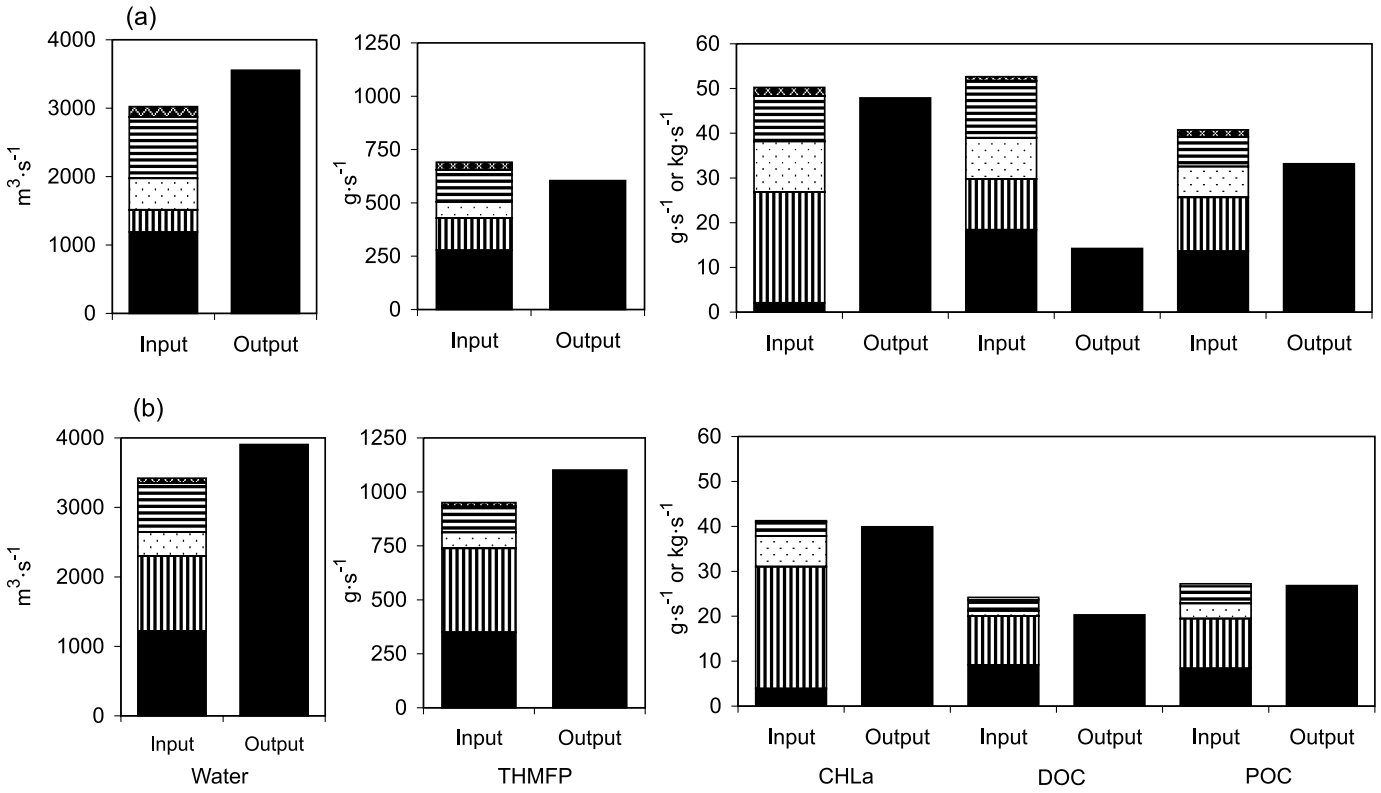


Table 2. Mean and range (in parentheses) of temperature, pH and dissolved oxygen (DO) in the Ohio River and experimental mesocosms during July, August, and September experiments.

Month	Location	Temperature (°C)	pH	DO ($mg \cdot L^{-1}$)
July	River	26.6 (25–27)	7.8 (7.6–8.0)	6.3 (6.2–6.4)
	High light	27.2 (26–29)	8.4 (8.2–8.9)	8.0 (7.9–8.0)
	Low light	27.1 (25–29)	8.3 (8.1–8.5)	7.3 (7.1–7.4)
August	River	27.1 (26–29)	7.8 (7.6–8.2)	6.1 (5.4–7.1)
	High light	26.5 (25–28)	8.5 (7.9–8.8)	7.5 (6.4–8.6)
	Low light	26.4 (25–28)	8.2 (7.9–8.4)	7.2 (6.4–8.0)
September	River	22.5 (20–25)	8.1 (7.8–8.5)	6.5 (5.7–7.3)
	High light	17.9 (15–25)	8.2 (8.2–8.4)	8.2 (7.2–9.2)
	Low light	17.7 (15–25)	8.3 (7.9–8.4)	8.1 (7.3–8.7)
	Dark	19.0 (15–25)	8.2 (8.1–8.2)	8.0 (7.1–8.6)

Note: Mesocosm data are shown for high-light, low-light, and dark (September only) treatments.

index periods suggest that surveys were conducted during a period of decreasing water storage. Therefore, we calculated retention estimates with a correction for the change in storage by assuming an outflow volume equivalent to the inflow volume. In August, input fluxes of THM precursors were 691 $g \cdot s^{-1}$, while outputs (corrected for storage) were 514 $g \cdot s^{-1}$. Retention estimates (as percentage of inputs) were 26% for THM precursors, 19% for chl *a*, 77% for DOC, and 31% for POC. In October, input fluxes of THMFP (951 $g \cdot s^{-1}$) were comparable to output fluxes (964 $g \cdot s^{-1}$), suggesting that the Ohio River was neither a net sink nor net source of THM precursors at this time. Retention estimates for chl *a* (15%), DOC (26%), and POC (14%) were also lower than those observed in August.

Experimental studies

Physical–chemical conditions in the mesocosms were comparable to concurrent river conditions (Table 2). Water temperature within the mesocosms was within 1°C of the river, except during the September experiment when cool nights resulted in more rapid heat loss from the tanks compared with the river. The pH of the mesocosms (8.2–8.4) was slightly higher than that of the river (7.8–8.1), particularly among tanks experiencing large algal blooms (high-

Fig. 4. Mean (± standard error) of chlorophyll concentrations on days 0, 3, and 6 in high-light (open) and low-light (shaded) tanks during (*a*) July, (*b*) August, and (*c*) September experiments. Asterisks denote significant differences between high- and low-light tanks. Concurrent river concentrations are shown for comparison.

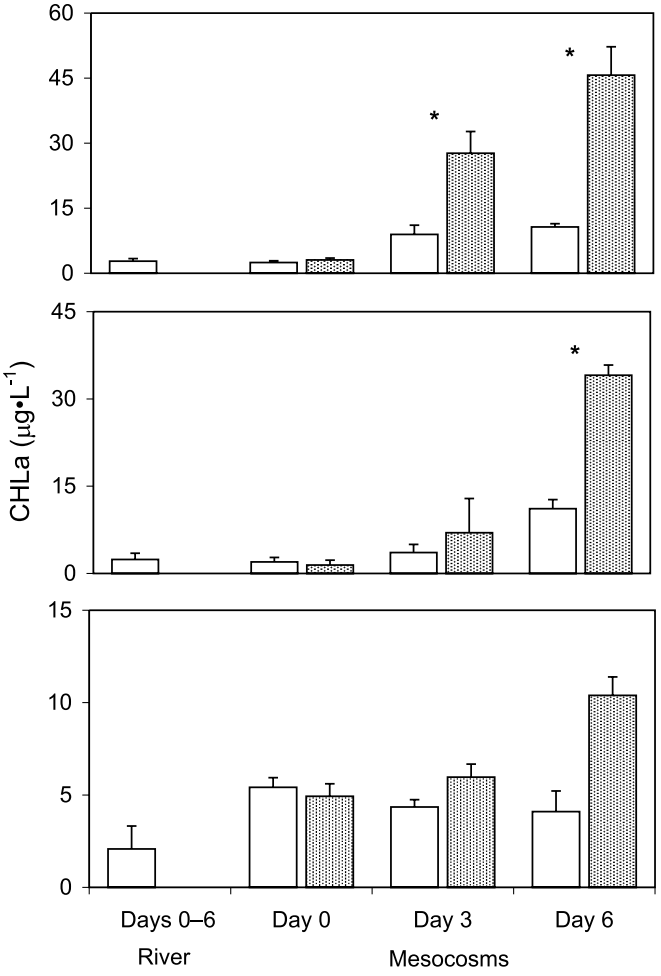
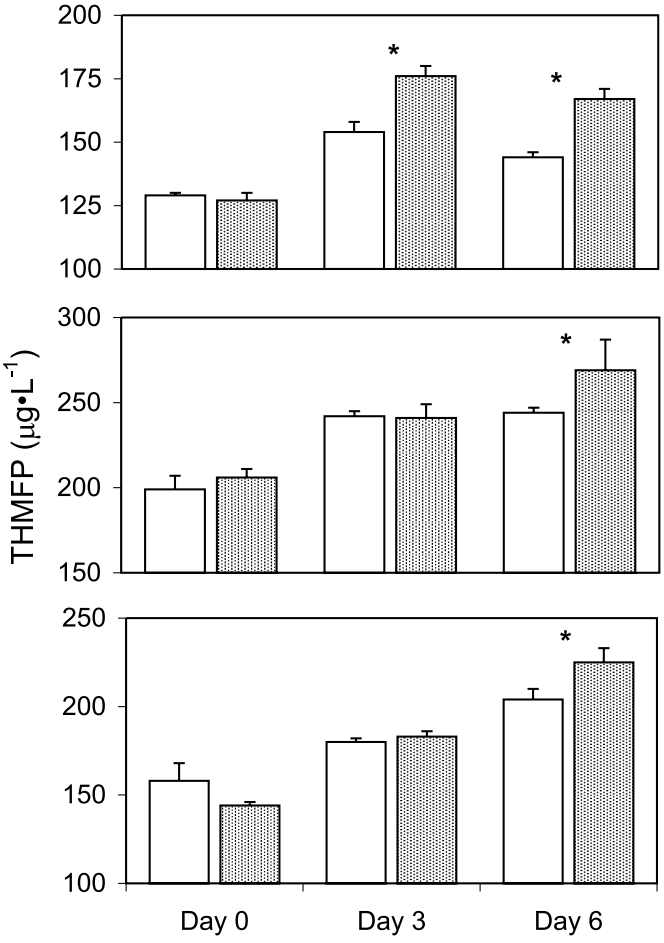


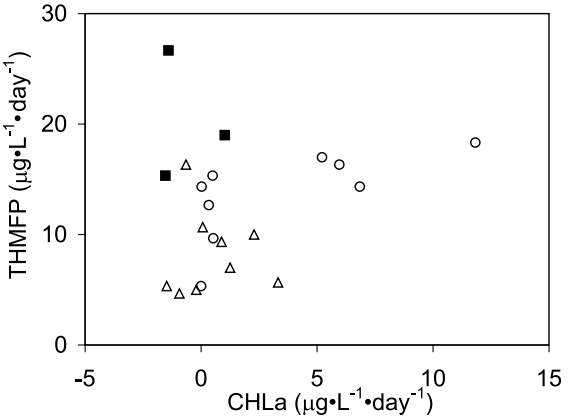
Fig. 5. Mean (± standard error) of trihalomethane formation potential (THMFP) on days 0, 3, and 6 in high-light (open) and low-light (shaded) tanks during (*a*) July, (*b*) August, and (*c*) September experiments. Asterisks denote significant differences between high- and low-light tanks.



light treatment; July and August experiments). Dissolved oxygen differences followed a similar trend with tank concentrations 1–1.5 mg·L^{-1} higher than the river and the largest differences occurring in high-light tanks. Variable shading produced the desired differences in algal bloom formation between high- and low-light tanks (Fig. 4). Differences in chlorophyll were apparent by day 3, and peak concentrations in high-light tanks (day 6) were three- to four-fold higher than those observed in low-light tanks. Low-light tanks exhibited modest gains in chlorophyll with peak concentrations (4–10 µg·L^{-1}) exceeding concurrent levels in the river (1–5 µg·L^{-1}). Although shading levels were held constant for all three experiments, differences in day length, cloud cover, and river turbidity resulted in variable light dosages and algal bloom formation among experiments. Highest light dosages and chlorophyll concentrations (ca. 45 µg·L^{-1}) occurred during the July experiment when water temperature was high (mean 27°C) and turbidity was low (mean 15 nephelometric turbidity units (NTU)). During the August experiment, water temperature was similar (mean 25°C), but turbidity was higher (mean 40 NTU), resulting in somewhat lower peak chlorophyll concentrations (30 mg·L^{-1}). Lowest chlorophyll concentrations (maximum 10 mg·L^{-1}) were observed during the September experiments when water temperature was lowest (mean 19°C). dark tanks (September experiment only) showed expected declines in algal abundance with chlorophyll decreasing from starting concentrations of 5 µg·L^{-1} to less than 1 µg·L^{-1} by day 6 (data not shown).

Initial (day-0) concentrations of THMFP among tanks ranged from 125 µg·L^{-1} (July) to 200 µg·L^{-1} (August) and were comparable to Ohio River concentrations during the August and October surveys (Figs. 2 and 5). Chloroform was always the dominant species, constituting >80% of the total THMFP. By comparing paired whole-water and filtered (0.5 µm) samples on three dates, we found that most of the THMFP (>91%) was associated with the dissolved fraction. THMFP concentrations increased in both high- and low-light tanks during all experiments. Larger increases were observed in high-light tanks (average 39% for three experiments) in comparison with low-light tanks (average 21%). By day 6, differences were statistically significant ($p < 0.05$) in all

Fig. 6. Rates of change in chlorophyll and trihalomethane formation potential (THMFP) between days 0 and 6 among high-light (○), low-light (△), and dark (■) tanks. Symbols denote individual tanks (N = 3) from July, August, and September experiments (dark tanks September only).



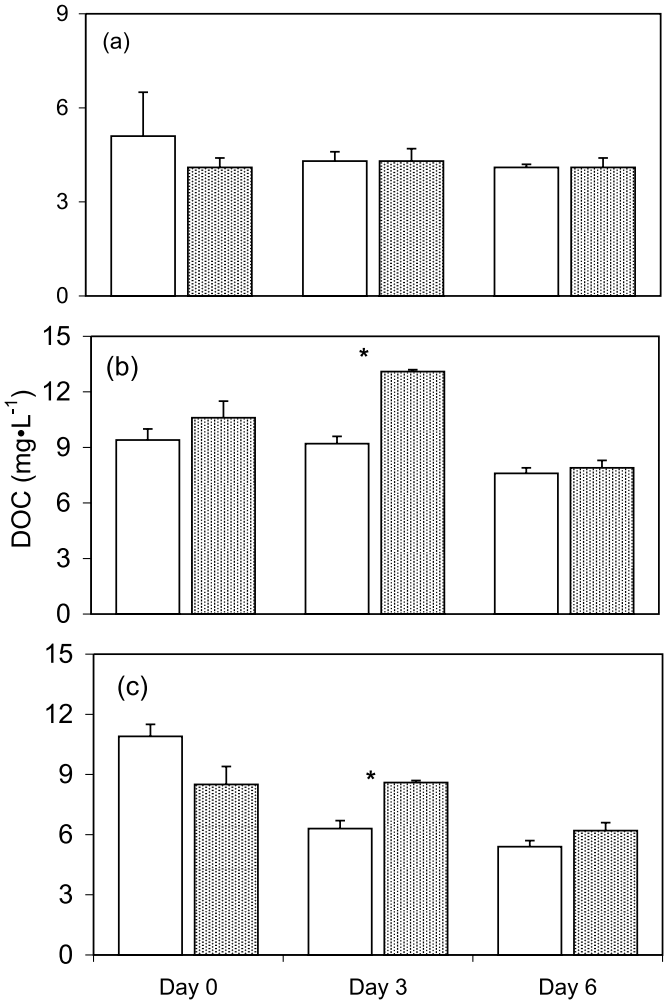
three experiments with high-light tanks exceeding low-light tanks by 20–25 μg THMFP·L^{-1}. Within each experiment, increases in THMFP generally followed increases in chl *a*, but comparisons of data from the three experiments did not reveal a consistent, linear relationship between chl *a* production and generation of THM precursors (Fig. 6). High-light tanks exhibiting large algal blooms (July and August experiments; chl *a* > 5 μg·L^{-1}·day^{-1}) also exhibited large increases in THMFP (14–18 μg·L^{-1}·day^{-1}). Among high- and low-light tanks exhibiting smaller blooms, changes in THMFP were variable (5–15 μg·L^{-1}·day^{-1}) but generally lower (mean 9 μg·L^{-1}·day^{-1}). The largest increases in THMFP (mean ± standard error 47 ± 18 μg·L^{-1}·day^{-1}) were observed among the three dark tanks (September experiment) that exhibited low or negative rates of change in chl *a*.

Variable tank shading and algal bloom development did not result in consistent differences in tank DOC concentrations (Fig. 7). Final (day-6) concentrations were not significantly different between high- and low-light tanks in any of the three experiments.

Transient effects were noted on day 3 of the August and September experiments when DOC concentrations differed by 3 mg·L^{-1} between high- and low-light tanks. DOC concentrations were low (ca. 5 mg·L^{-1}) during the July experiment and higher (ca. 10 mg·L^{-1}) during the August and September experiments. DOC concentrations generally declined during these two experiments from starting concentrations near 10 to 5–8 mg·L^{-1}. Rates of change in DOC among individual tanks were not correlated with changes in THMFP or chlorophyll.

Previous studies have calculated THM yields as a function of DOC or TOC (Graham et al. 1998; Stepczuk et al. 1998*a*; Galapate et al. 1999). Since the majority of our THMFP originated in the dissolved fraction, we derived THM yields based on DOC (μg THM·mg^{-1} DOC). In our river surveys, THM yields ranged from 8 to 65 μg THM·mg^{-1} DOC across all sites. On average, yields were higher among October samples compared to August (40 vs. 25 μg THM·mg^{-1} DOC, respectively; paired t test, p = 0.06). THM yields among mesocosms ranged from 10 to 54 μg THM·mg^{-1} DOC (mean 34 μg THM·mg^{-1} DOC) and were similar during July, August, and September experiments. There was no significant difference in THM yield between high- and low-light treatments (July and August experiments; paired t tests, p > 0.44) or among the low-light, high-light, and dark treatments (September experiment; ANOVA p = 0.48). THM yields increased from day 0 to day 6 because THMFP increased while DOC concentrations remained stable.

Fig. 7. Mean (± standard error) of dissolved organic carbon concentrations on days 0, 3, and 6 in high-light (unshaded) and low-light (shaded) tanks during July, August, and September experiments. Asterisks denote significant differences between high- and low-light tanks.



Discussion

Our survey revealed a two-fold range of variation in THMFP among large rivers composing the Ohio River basin. THM concentrations were comparable to those reported for other rivers and reservoirs (Veenstra and Schnoor 1980; Graham et al. 1998; Stepczuk et al. 1998*a*). Most of the THMFP (>91%) was associated with the dissolved fraction and chloroform was always the dominant species, as has been reported in prior studies (Graham et al. 1998; Stepczuk et al. 1998*a*, 1998*b*). Previous studies have also assessed THMFP

yield from organic carbon by comparing ratios of THMFP/DOC (Galapate et al. 1999; Stepczuk et al. 1998*a*), THMFP/TOC (Graham et al. 1998), or comparing chloroform produced by algal cultures normalized to moles of available halides and organic carbon (Watcher and Andelman 1984). Unfortunately, the latter three studies all used shorter incubation periods (24 h) than is currently recommended (APHA 1998). It would be inappropriate to compare yields among these experiments, since it is well established that THMFP is affected by contact time with chlorine, as well as other factors. Stepczuk et al. (1998*a*) used 7-day incubations (as in our study) and reported an average yield of 119 μg THMFP·mg^{-1} DOC. Our yields of THMFP (<50 μg THM·mg^{-1} DOC) were 50% lower and may reflect watershed-level differences in inputs of allochthonous (terrestrially derived) precursors or internal rates of precursor formation. Previous studies have also reported that THM yields from DOC can vary tremendously, particularly among lotic systems (Veenstra and Schnoor 1980).

Our field and experimental data were generally supportive of the hypothesis that algal production enhances THM formation. The highest THMFP concentrations were found in the Wabash River that also exhibited the highest chlorophyll concentrations. In manipulative experiments, tanks exhibiting large algal blooms showed large increases in THMFP. Yields of THMFP within mesocosms (2.4 ± 0.4 μg THMFP per microgram chl *a*) were comparable to those obtained from survey data (2.9 ± 0.9 microgram THMFP per microgram chl *a*). To our knowledge, this is the first study to establish a correlative linkage between THM formation and algal abundance in both experimental and environmental settings. A positive correlation between chl *a* and THMs has also been reported for reservoirs in England (Graham et al. 1998). In contrast, Stepczuk et al. (1998*a*) found no relationship between THMFP and chlorophyll concentrations in the West Branch of the Delaware River. Variation in the strength of the chl *a* – THM relationship may be attributed in part to differences in the importance of autochthonous production among the systems investigated. Riverine carbon sources are dominated by allochthonous inputs of detrital material, but our survey data suggest that conditions that favor algal bloom development are also associated with high THMFP (as in the Wabash River). Chlorophyll concentrations within the Ohio River and its tributaries were within the range reported for large rivers (Admiraal et al. 1990; Basu and Pick 1995; Spaink et al. 1998), suggesting that autochthonous sources of THM precursors may be important in other rivers. Furthermore, our experimental data show that even modest increases in chl *a* (5–25 μg·L^{-1}) result in 40–50% increases in THMFP. It is interesting to note that although concentrations of chlorophyll and THM precursors were correlated in our survey data set, their flux rates were not. In October, input and output fluxes of THM precursors were higher than those observed in August, despite generally lower chlorophyll fluxes. This pattern arises from seasonal changes in the relative importance of inflows among the major tributaries of the Ohio River. Greater THMFP inputs during October were largely due to elevated fluxes from the Wabash River, while reductions in chl *a* inputs were due to reduced inflows from the Tennessee and Cumberland rivers. These data suggest that the relative importance of autochthonous and allochthonous sources of THM precursors varies among the tributaries and may account for a substantive fraction of the unexplained variation in our chl *a* – THMFP model. Although our limited temporal resolution precludes an adequate assessment of inter-basin differences, these findings suggest that seasonal changes in hydrologic routing within the Ohio River basin may have an appreciable influence on downstream THMFP.

Our experimental data demonstrate a positive association between algal growth and THMFP, but comparisons across experiments show that the generation of THM precursors did not follow a linear relationship with chl *a* production. These findings suggest that multiple mechanisms may act to influence THMFP in our mesocosms including algal growth and senescence as well as photolytic effects on ambient DOC pools. Graham et al. (1998) reported that THM yields from both actively growing and senescent algal cultures were greater than those of cultures in the stationary phase. Others have suggested that ECPs released during algal growth may yield greater quantities of THM precursors than the subsequent decomposition of algal cells (Watcher and Andelman 1984). We derived inferred values of ECP production to assess their potential importance during our mesocosm studies. Chlorophyll concentrations at the start and end of each experiment were converted to C units (based on 30:1 C : chl *a*) and used to estimate net production of algal C during the 6-day experiment. Release of ECPs was estimated as 13% of algal production (Baines and Pace 1991). Among high-light tanks during the July and August experiments, the amount of C released as ECP (127 and 167 μg C·L^{-1}, respectively) exceeded by more than 20-fold the amount of C required to produce the observed increase in THM as chloroform, which was the dominant species of THM in our study rivers (>84%). Since C composes only 10% of the mass of THMs (when chloroform predominates), the formation of THMs represented a very small C demand (4 μgC·L^{-1} and 6 μgC·L^{-1}, respectively) relative to ECP production. Among tanks exhibiting lower rates of algal growth, THM formation corresponded to 5–38% of C released as ECPs. C demand for THM formation exceeded ECP release only among low-light tanks during the September experiment (when net algal production was lowest). These analyses suggest that extracellular release of photosynthate was sufficient to account for the observed changes in THMFP, though current methodology does not enable us to partition ECPs vs. diagenetic sources of THM precursors.

Large increases in THMFP occurred in our dark tanks (September experiment only) suggesting that algal senescence and subsequent release of DOC is a source of THM precursors in low-light environments. This process may be particularly important in large rivers, unstratified reservoirs, and the hypolimnia of lakes and reservoirs where unfavorable light conditions result in decompositional environments. We estimated potential C fluxes arising from algal senescence based on measured declines in chl *a* (30:1 C : chl *a*) and found that these were seven-fold higher than the C required to account for the observed increases in THM. Much of this algal-derived C is labile and therefore is likely to be respired. With correction for respiration losses (assuming 15% bacterial growth efficiency), C release from algal biomass still exceeded C demand for THM formation by two-

fold. These analyses underscore the difficulty of tracking sources of THM precursors since they represent a small proportion of potential fluxes associated with algal growth and senescence and an even smaller fraction (<0.1%) of the background pool of DOC.

For tanks exhibiting modest changes in chlorophyll (<5 $\mu g \cdot L^{-1} \cdot day^{-1}$), increases in THMFP were generally higher among high-light tanks than low-light tanks. Therefore, we cannot exclude the possibility that solar radiation has direct effects on THMFP. Solar radiation in the ultraviolet (UV) range has been shown to cause photolysis of certain types of DOC (Moran and Zepp 1997; Opsahl and Benner 1998) that could result in the production of THM precursors. Li et al. (1996) found that TOC decreased and the yield of chloroform and total THMs from a humic acid sample increased after irradiation at a wavelength of 253.7 nm. Light attenuation in the Ohio River and its tributaries is regulated by suspended particulate materials and varies seasonally with discharge. Penetration of UV light into the water column is limited compared with other systems like low DOC lakes (Williamson et al. 1999; Bukaveckas and Robbins-Forbes 2000). However, during the period of summer base flow (typically July–October), turbidity is at its lowest point of the year (<7 NTU) and conditions may be favorable for photolysis, particularly in shallower reaches. Further study is needed to resolve the possible role of UV effects on the production of THM precursors.

Increases in THMFP among high- and low-light and dark tanks suggest that algal production, algal senescence, and photolysis may all play a role in the internal generation of THM precursors in large rivers. These processes were of sufficient magnitude to increase THMFP by as much as 50% over 3–6 days within our experimental mesocosms. Their importance in situ will depend on rates of biotic and photolytic activity as well as water residence time in the rivers, which varies (e.g., from <1 to >20 days for the McAlpine Pool) depending on discharge. The net effect of riverine processes on precursor production and loss can be inferred from comparisons of inputs and outputs. In August, tributary and upstream inputs of THMFP as well as chl *a*, DOC, and POC exceeded outputs from the Ohio River. Differences between input and output fluxes were larger (e.g., 26% for THMFP) than the error typically ascribed to measurements of discharge and solute concentration (10–15%). Our estimation of retention was based on the assumption that the observed imbalances in the water budget (<20%) were attributable to decreases in channel storage coinciding with falling river stage. We cannot discount the possibility that underestimation of total inputs due to small tributaries and other ungauged sources may contribute to this imbalance. We estimated additional inputs from these sources assuming an inflow volume equivalent to that required for balancing the water budget and concentrations derived from volume-weighted averages for the measured inflows. Retention derived by this method yielded a similar estimate (e.g., 27% for THMFP) to that calculated based on a correction for changing storage. We conclude that at this time, the river was acting as a net sink for THM precursors, dissolved organic matter, and algal C. These patterns are indicative of a predominance of bacterial respiration over photosynthesis that is generally characteristic of river and reservoir environments (Benner et al. 1995; Howarth et al. 1996; Bukaveckas et al. 2002). By October, water temperatures were lower and the associated decline in algal and bacterial activity may account for the river being in equilibrium with respect to inputs and outputs of THMFP, chl *a*, and DOC. The Wabash River accounted for a large fraction of THMFP and chl *a* inputs during October, and since its confluence with the Ohio River is near the downstream end of our study reach, short residence time may have also contributed to low retention. Overall, our findings suggest riverine processes may attenuate THMFP and thereby benefit water quality downstream, but that these effects are seasonally variable. More detailed studies are needed to quantify the importance of these processes on an annualized basis, since our data are based on low flow conditions.

Our results indicate that water utilities may face considerable challenges as they prepare strategies to meet the new THM guidelines from the USEPA. First, it is clear that there are watershed-level differences in THMFP among source waters within the Ohio River basin that may make it difficult for utilities in the region to adopt a uniform strategy for reducing THMs. For example, water companies whose intakes draw from the Wabash River face greater challenges in meeting THM guidelines than plants located along the Kentucky or Green rivers. Similarly, the instream processes that make the Ohio River a sink for THMFP could benefit utilities located downstream, while those further upstream may have to be more aggressive in their approach to the management of THM. Secondly, the importance of photosynthetically derived THM precursors highlights the need for continued efforts to mitigate algal blooms in water bodies receiving anthropogenic nutrient inputs. Traditional approaches have relied on in-plant solutions such as removal of algae through coagulation and flocculation, but these may not reduce concentrations of algal ECPs (El-Dib and Ali 1994; Graham et al. 1998). Prechlorination of intake waters may increase levels of THM precursors as the contents of the lysed algal cells become available to form disinfection by-products. As regulations for THM levels in finished water become more stringent, such narrowly focused solutions may no longer be economically attractive and greater attention needs to be directed to the ecological processes in rivers and other source waters that determine THMFP. Improved understanding of both watershed-level and insteam processes that influence THMFP would help utilities to develop more innovative and cost-effective ways of meeting their obligation to regulatory agencies and customers.

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