Utility of a multi-tracer approach as a component of adaptive monitoring for municipal wastewater impacts

Rajiv N. Tanna, Michael C. Moncur, S. Jean Birks, John J. Gibson, Carol J. Ptacek, Bernhard Mayer, Michael E. Wieser, Fred J. Wrona and Kelly R. Munkittrick

ABSTRACT

Distinguishing municipal wastewater effluent (MWWE) from other industrial effluents or through an urbanized watershed can be challenging. In complex receiving environments, linking environmental responses to specific compounds or effluents is not always straightforward. In order to characterize the inherent complexity of tracing MWWE in aquatic systems influenced by multiple stressors, a proposed multi-tracer suite is intended to highlight areas of potential biological concern. Characterization and quantification of effluent exposure to aquatic biota in this manner is essential to shape policies intended to encourage wastewater infrastructure development (i.e. treatment plant upgrade) and broader environmental management. This paper describes the use of a comprehensive suite of tracers that includes isotopes in support of a core surveillance program, demonstrating its effectiveness both empirically and with respect to diagnostic value contributed to monitoring programs.

Key words | emerging contaminants, environmental tracers, monitoring design, wastewater

HIGHLIGHTS

- Characterizing presence of trace organic compounds.
- Developing predictive capacity of effects using water chemistry.
- Linking effects-driven water chemistry analysis (science) to decision making (policy).

INTRODUCTION

In Canada, there are 3,700 municipal wastewater treatment plants that release 6 trillion liters of treated municipal wastewater effluent (MWWE) annually, presenting a major threat to aquatic environments (Chambers et al. 1997); over 20% of this release occurs into Alberta waters (Government of Canada 2010). Growing populations are continually
introducing emerging contaminants comprising mainly of organic pollutants into the waste streams through broad consumption and disposal. This phenomenon has increased the need to trace the fate of effluent in the environment. It is therefore the intent of this paper to identify the value in using multiple tracers to guide where and when intervention should occur in waters receiving MWWE and when upgrades to infrastructure may be required. Ideal tracers for MWWE support characterization of exposure in the receiving environment and thus should be wastewater-specific and not ubiquitous in the environment (Gasser et al. 2014). Additional properties of good tracers as presented by Lalor & Pitt (1999) are:

1. Significant difference in pollutant concentrations between possible source waters;
2. Small variations in pollutant concentrations within each likely source water;
3. Conservative behavior (i.e. concentrations do not change due to physical, chemical or biological processes); and
4. Ease of measurement with adequate detection limits, good sensitivity and repeatability.

As these properties are specific to quantification and resolution, capability of tracers to produce meaningful data can be evaluated by considering desirable properties of indicators (Cairns et al. 1993):

- Cost-effective
- Historical data
- Lack of redundancy
- Timeliness
- Biologically relevant
- Socially relevant
- Interpretable
- Anticipatory
- Integrative
- Diagnostic

This subset of applicable properties of indicators (those overlapping with tracer properties and not relevant to water quality were omitted) presented in the seminal work by Cairns et al. (1993) ensures that compounds being quantified in the environment are both relevant and meaningful when combined with tracer objectives. Current MWWE discharge regulations are limited and place wastewater effluent quality limits on biological oxygen demand, suspended solids, residual chlorine and unionized ammonia (Government of Canada 2012). These analytes, unfortunately, do not provide insight into fate and potential responses of receiving environments due to MWWE exposure and specifically to the over 40,000 trace organic compounds currently manufactured, consumed and potentially present in MWWE (Diamond et al. 2011). Biological organisms exposed to MWWE accumulate compounds which may lead to impacts (Wang et al. 2011). It is possible to characterize exposure and identify areas where potential impacts would be most likely using a multi-tracer method.

A multi-tracer approach for MWWE leverages the utility of multiple analytes to enable the modeling of fate, degradation, potential synergistic or additive effects and food-web dynamics. This study proposes the use of trace organic compounds in concert with isotopes and metals to maximize diagnostic potential of receiving environment water quality monitoring for MWWE. The suite of analytes needs to incorporate a variety of chemicals that have differing characteristics, the potential to ‘pass-through’ wastewater treatment systems, and are relatively easy to measure. The utility of anti-epileptics such as carbamazepine as well as nonsteroidal anti-inflammatory drugs such as naproxen and ibuprofen in tracing wastewater exposure in receiving environments has been well documented due to the tendency of these chemicals to be recalcitrant through treatment (Metcalfe et al. 2003; Conkle et al. 2008). Illicit drugs (Metcalfe et al. 2010), artificial sweeteners (Liu et al. 2014) and caffeine (Thomas & Foster 2005) have also been shown to have utility as tracers for MWWE. Metals, although more ubiquitous in the environment are introduced in significant quantities through MWWE and in dissolved form can elicit toxic responses (Gagnon & Saulnier 2003; AEP 2018). Isotopes are incorporated into the multi-tracer analytical suite to enable confirmation of exposure of resident aquatic organisms to wastewater and to trace exposure through food-webs (Loomer et al. 2015; Robinson et al. 2016). In addition to determining the spatial extent of biotic exposure, isotopes can also enable discrimination between multiple complex effluents introduced to rivers in more developed watersheds (Dubé et al. 2005).

To test the potential utility of the approach in a system with a relatively high level of dilution, samples of river
water were collected in the Bow River in Calgary, Alberta and analyzed for several potential tracers to track wastewater from a major municipal wastewater treatment plant (MWWTP). MMWE is a significant point-source stressor on the Bow River, with the Bonnybrook MWWTP releasing the largest volume of treated effluent of the three MWWTPs within the City of Calgary. The Bonnybrook MWWTP is a tertiary treatment plant with an average discharge per day in 2011 and 2012 of 300,000 m³/day released on the left bank of the river (V Moore, pers. comm. 2019). Treatment technologies include ammonia, nitrogen, phosphorous control and ultra-violet disinfection (Alberta Environment 2009). The objective of the study was to assess the persistence of several target analytes relative to conventional wastewater performance parameters to determine their effectiveness as tracers and to define their diagnostic value.

**MATERIALS AND METHODS**

**Water sampling**

Samples of river water were collected at 10 locations (Figure 1) over a distance of 14.8 km along the Bow River through the city of Calgary (southern Alberta, Canada) in October 2011 and May 2012. Samples were obtained at half-depth from a flat-bottomed boat along the channel thalweg, except at the sewage outfall location BR-2 (this sample collected from the middle of the plume). Winter samples could not be obtained due to safety and access limitations. The locations are labeled BR-1 through BR-10. BR-1 is upstream of the Bonnybrook WWTP. Average discharge of the Bow River was measured by combining flow along the main stem, with the Elbow River and Nose Creek flows (sum of Water Survey Canada stations 05BH004, 05BJ001 and 05BH901, respectively, with diverted flow at 05BM015 subtracted) and was 94.6 m³/s for sampling in October 2011 and 93.6 m³/s for sampling in May 2012. These conditions are representative of 'low flow' conditions in the Bow River within Calgary, where the Bonnybrook MWWTP effluent makes up to 4.5% of the flow in the river (Supplementary Material, Table S1).

Water samples were collected from the middle of the wastewater plume discharging from the Bonnybrook WWTP. The wastewater plume was previously delineated in detail by Vandenberg et al. (2005). In addition, the plume is clearly visible from publicly available aerial photographs. Measurements of pH, Eh, temperature, electrical conductivity (EC) and dissolved oxygen (DO) were made in the field using a Hydrolab. All probes were calibrated prior to sampling; Eh electrode was calibrated in Zobell’s (Nordstrom, 1977) and Light’s solutions (Light, 1972); the pH electrode was calibrated with standard buffer solutions at pH 4, 7 and 10. All river samples were collected in a consistent manner utilizing a peristaltic pump and a 6.4 mm (¼-inch) diameter polyethylene tubing attached to a stainless steel weight and lowered into the upper 0.5 m of the water column. At each location, water samples were collected into pre-cleaned Nalgene bottles for major anions and cations, dissolved metals, dissolved organic carbon (DOC), nutrients (NH₃-N, NO₂-N, NO₃-N, PO₄-P), H₂S, Fe(II) and stable isotopes δ¹⁸O, δ³H, δ¹³C(DIC), δ¹³C(DOC), δ³⁴S + δ¹⁸O(SO₄), δ¹⁵N + δ¹⁸O(NO₃), ⁸⁷/⁸⁶Sr, and δ⁵²Cl. Dissolved metals and major cations were acidified in the field with 12 N trace-metal grade HNO₃ to a pH of <1 and anions, organic acids, and DOC were unacidified. Samples for trace organic compounds including pharmaceuticals and artificial sweetener were collected in pre-cleaned 1 L amber glass bottle and preserved in the field with trace-grade H₂SO₄. All water samples were field filtered with 0.45 μm high-volume inline filters with the exception of δ¹³C(DIC). All water samples were immediately refrigerated at 4°C until analysis.

**Water analysis**

Water samples were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) for major cations, inductively coupled plasma mass spectrometry (ICP-MS) for dissolved metals and ion chromatography (IC) for anions. Combustion and infrared detection was used for DOC analysis. Dissolved H₂S, Fe(II) and NH₃-N were determined in-field using a Hach DR 2800 portable spectrometer following the procedure outlined by SMEWW (2005).

The oxygen isotopic composition of water samples was determined by the H₂O–CO₂ equilibration method. Hydrogen (deuterium) isotopic composition in water was
Figure 1 | Map of the Bow River within the City of Calgary, Alberta, Canada. 'Bonnybrook WWTP' and the adjacent filled star indicates the effluent outfall of the Bonnybrook municipal wastewater treatment plant. Dotted line within inset map identifies study area as presented. Remaining open stars denote sampling locations visited in the fall of 2011 and spring of 2012 with numbering corresponding to successive locations downstream and data (Figures 2–6).
measured on an isotope ratio mass spectrometer (MS) determined by reduction of water to H₂ gas using chromium metal as an active reducing agent. All isotopic ratios are reported as ‰o against an internationally accepted standard. The results of oxygen and deuterium isotope measurements are expressed in delta notation (δ¹⁸O and δ²H) relative to Vienna Standard Mean Ocean Water (V-SMOW) standard. The δ¹⁸O and δ²H composition of groundwater was measured to identify the degree of evaporative enrichment. The method of Assayag et al. (2006) was used to determine δ¹³C in dissolved inorganic carbon (DIC), reported relative to Vienna Pee Dee Belemnite (V-PDB) standard. The δ¹³C of DIC was used to identify processes such as carbonate dissolution and bacterial reduction. Values of δ¹³C-DOC were determined using continuous flow isotope mass spectrometry after preparation to remove the inorganic carbon using phosphoric acid and saturated potassium persulfate. The δ¹³C-DOC results were reported relative to V-PDB with an uncertainty of ±0.3‰. Sulfur-isotope ratios of dissolved sulfate were determined by adding BaCl₂ to precipitate BaSO₄, which was then converted into SO₂ in an elemental analyzer coupled to a MS in continuous flow mode (Giesemann et al. 1994). Sulfur-isotope measurements are reported in delta notation (δ³⁴S) relative to the Vienna Canyon Diablo Troilite (V-CDT) standard. Oxygen-isotope analysis on BaSO₄ samples was carried out by pyrolysis in a high temperature conversion/elemental analyzer interfaced to an isotope-ratio MS. Boron isotope abundance measurements were made on water samples that were purified by microsublimation (Gröbe et al. 2000) and analyzed on a thermal ionization MS (Williams et al. 2001). Strontium was extracted from the waters using ion exchange resin columns, eluted with deionized water, evaporated and then analyzed using positive ion thermal ionization mass spectrometry with a Thermo Electron Triton (PTIMS) instrument using the method described by Burton et al. (2002) using reference material SRM987. Measurement uncertainty in the ⁸⁷Sr/⁸⁶Sr ranged was ±0.0001. Chlorine isotope analysis was measured by continuous flow isotope ratio mass spectrometry using the method described by Shouakar-Stash et al. (2005). The method involved the precipitation of dissolved chloride to silver chloride (AgCl), followed by the conversion of silver chloride to methyl chloride (CH₃Cl). Methyl chloride was then isolated on a GC column and the isotope ratio was measured using an IRMS. Results are reported relative to Standard Mean Ocean Chloride (SMOC) with an uncertainty of ±0.2‰.

ACE-K and the eight pharmaceutical compounds were analyzed using solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) followed by tandem mass spectrometry using previously published methods (Vanderford & Snyder 2006; Stafiej et al. 2007; Scheurer et al. 2009) with slight modifications such as inclusion of isotope dilution techniques for each target compound and optimization of instrument operating conditions. Native compounds were supplied by Sigma-Aldrich (Oakville, Canada) with the exception of ACE-K obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Isotope labeled standards, including ACE-K-d₄ and SMX-d₄ (Toronto Research Chemicals Inc., Toronto, Canada), MDA-d₅ and MDMA-d₅ (Cerilliant Inc., Texas, USA), and CBZ-d₁₀, CAF-d₃, IBU-d₃, GEM-d₆, and [¹³C]-NAP (Cambridge Isotope Laboratory Inc., Cambridge, USA) were obtained as dry powders. Standard stock solutions of 1,000 mg L⁻¹ were prepared by dissolving each compound in methanol. Working standard solutions containing all analytes were prepared by serial dilution in methanol: water 50:50 vol/vol. HPLC-grade ammonium acetate and formic acid were obtained from Sigma-Aldrich (Oakville, Canada). Ultrapure (Type 1) water was generated using a Milli-Q A10 water system (18.2 MU cm @ 25 °C).

Prior to analysis, 600 mL aliquots of aqueous samples were spiked with a consistent amount of internal standard mixture. These samples were passed through SPE cartridges (Oasis HLB 6 mL glass cartridges; Waters Corp., Mississauga, Canada) pre-conditioned with 3 × 2 mL methanol and then equilibrated with 3 × 2 mL ultrapure water. After loading the 600 mL samples, the cartridges were washed using 3 × 2 mL 5 vol% methanol then eluted with 3 × 2 mL methanol. The eluate was collected in an amber glass vial and stored at 4 °C until analysis.

The extracts were analyzed using an Agilent 1100 HPLC (Agilent Technologies, Mississauga, Canada) followed by electrospray tandem mass spectrometry (MS/MS; 4000 Q TRAP, Applied Biosystems, Foster City, USA). Caffeine, SMX, CBZ, MDA and MDMA were analyzed in electrospray ionization positive (ESI+) mode, while IBU, GEM, NAP and CE-K were analyzed in ESI negative (ESI-) mode.
mode. The gradient and mobile phases were changed from previous methods based on the HPLC analytical columns and MS requirements. The compound and source-dependent parameters of the MS were modified to obtain an optimum signal response. The mobile phases for analysis in ESI+ mode consisted of 0.1% formic acid and 5 mM ammonium acetate in water (phase A) and 99.9% MeOH with 0.1% formic acid (phase B). A gradient elution started at 10% B for 5 min, increased to 90% B in 10 min and then held at 90% B min for 10 min. The flow rate was 1,000 mL min⁻¹ and the injection volume was 15 mL. The mobile phases for analysis in ESI/- mode consisted of 6.9 mM acetic acid in 300 mL acetonitrile and 700 mL water (phase A) and 100% acetonitrile (phase B). A gradient elution started at 12% B, then increased linearly to 40% in 10 min and held at 40% for 10 min. The injection volume was 10 mL and the flow rate was 1,000 mL min⁻¹. For ACE-K analyzed in ESI- mode, the mobile phases consisted of 20 mM ammonium acetate in water (phase A) and 20 mM ammonium acetate in methanol (phase B). The gradient elution started at 2% B, then increased linearly to 75% in 8 min and held at 75% for 8 min. The injection volume was 10 mL and the flow rate was 1,000 mL min⁻¹.

Statistics

All data were subjected to quality assurance and quality control measures outlined in Surface Water Quality Programs in Alberta (Alberta Environment 2006), with values below detection limit set to 0. Principal component analysis (PCA) was used to determine the reduced number of latent variables (principal components) explaining the most variance in the dataset. Samples were normalized using the STANDARDIZE function in Microsoft Excel (version 1908) to a mean of 0 and a variance of 1 prior to analysis. This undirected method served to visually represent patterns of a large number of variables which are spatially autocorrelated. Variables following a correlation with traditional wastewater indicators such as chloride are expected to coalesce with larger component loadings across component 1. The PCA was completed with IBM SPSS Statistics 26 using Varimax Rotation to explain the influence of various tracers. Analytes which were significantly correlated to chloride, a conventional tracer of wastewater, were explored graphically using normalized data.

Correlational analyses were conducted using IBM SPSS Statistics 26 using a Pearson Correlation test with a two-tailed test of significance (α) at 0.01 with raw nonnormalized data.

RESULTS AND DISCUSSION

Conventional parameters, major ions and nutrients

Flow conditions in both sampling campaigns were similar (94.6 and 93.6 m³/s for 2011 and 2012, respectively) and thus supported comparisons in both summer (May) and fall (October) conditions. DO levels consistently averaged ∼10 mg/L in both years (Figure 2), with the lowest concentrations in the river immediately downstream of the effluent plume due to increased oxygen demand. Similarly, pH values were consistent between years with average pH at ∼8, with the lowest values (∼7–7.3) observed at locations immediately downstream of the discharge point as is characteristic of MWWE. Reduction potential showed minimum variation across sites within years but showed a higher reduction potential in fall of 2011, which is believed to be the driver in differences in ammonia concentrations (Figure 3) between years. Temperature differentials between effluent and receiving environment were observed as warmer conditions (up to 7 °C) caused by effluent release. All major ions and nutrients (Figure 3) were considerably elevated and attenuated across downstream sampling locations, corresponding to increased conductivity as expected. With exposure profile for traditional (albeit ubiquitous) ions such as chloride established, these compounds provided the basis to conduct statistical comparisons to correlate and assess exposure against. Conventional parameters commonly have guidelines associated with them due to the increased potential for acute toxicity; however, the other classes of compounds in this study are more likely to result in chronic toxicity.

Metals

Dissolved metals showed consistent patterns of more than doubling at the discharge point relative to upstream concentrations (Figure 4) with the exception of dissolved barium which was diluted by wastewater effluent. Dissolved iron was substantially lower downstream of the discharge point.
Figure 2  | Field parameters and conventional wastewater performance analyte concentrations in samples obtained from the Bow River across a MWWE gradient.

Figure 3  | Major ion and nutrient parameters including nitrogen sulfate and phosphate expressed as concentration in samples obtained from the Bow River across a MWWE gradient.
in fall of 2011, which may have been a result of higher oxidation-reduction potential across sites in the fall despite similar flows. Increases in dissolved metals at the outfall may have been further exacerbated by low pH which can act to bring metals which are present into solution. These spikes in metal concentrations were sustained and took up to 15 km to attenuate to background levels. Metals are well documented as presenting a potential for toxicity to invertebrates and chronic toxicity to fish via direct toxicity (e.g. gill damage) or at critical life stages (i.e. embryo), resulting in environmental protection guidelines or ‘limits’ (AEP 2018). These guidelines do not necessarily account for site specific sensitivities and are continuously being re-evaluated based on toxicity mechanisms (AEP 2018). This knowledge combined with the quantities of metals released via MWWE makes metals relevant and nonredundant in the tracer suite from both a concentration and a total loading perspective.

Trace organic compounds

Trace organic compounds including pharmaceuticals quantified in this study are compounds which are known to be recalcitrant through treatment processes and are commonly used to characterize effluent exposure. Anti-epileptic drugs like carbamazepine and artificial sweeteners like acesulfame are largely resistant to biodegradation (Metcalfe et al. 2003; Liu et al. 2014) making them good candidate tracers for MWWE. Despite high dilution capacity afforded by the receiving environment, artificial sweeteners, anti-epileptic,
anti-inflammatory and lipid controlling compounds all showed varying effluent-driven increases downstream of the discharge point (Figure 5). The reducing environment observed through summer conditions and oxidizing in the fall appear to impact concentration and fate of pharmaceuticals in a manner similar to iron and ammonia.

**Stable isotope ratios**

In analysis of the stable isotope compositions of water: sulfate, nitrate, DIC, DOC, strontium and chloride showed patterns, which corresponded in varying degrees to effluent discharge (Figure 6) $\delta^{2}$H and $\delta^{18}$O increases in receiving water at the effluent outfall can be attributed to evaporation and ‘off-gassing’ of $O_2$ and $H_2$ through the treatment process (Wayland & Hobson 2001; Bedard-Haughn et al. 2003). Conversely, decreases in $\delta^{15}$C of DIC and to a lesser extent $\delta^{15}$C of DOC in the outfall relative to upstream and downstream conditions are a product of the large quantities of terrestrially derived organic material entering treatment plants, as well as the microbial treatment process (Wayland & Hobson, 2001; Bridgeman et al. 2014). $\delta^{34}$S and $\delta^{18}$O values decreased from +10 and +2‰ above the effluent to +1 and −2‰, respectively downstream of the effluent, due to the low $\delta^{34}$S and $\delta^{18}$O values of effluent sulfate (Kruk et al. 2020). Downstream of the effluent $\delta^{34}$S and $\delta^{18}$O values of sulfate increased to +8 and +1‰ due to mixing with riverine sulfate. $\delta^{15}$N values of nitrate increased from +7 ‰ above the effluent to +10 ‰ downstream of the effluent input, while $\delta^{18}$O value of nitrate decreased markedly from +2‰ to −9‰ due to the influx of effluent nitrate with elevated $\delta^{15}$N and very low $\delta^{18}$O values (Kruk et al. 2020). As sampling was conducted during characteristic low flow periods where a higher level of groundwater influence is observed, Sr minimal differences in isotope ratios are presumed to be driven by groundwater recharge (Armstrong et al. 1998). No evidence of effluent impact was apparent in Cl isotope ratios.

Isotopes are important to verifying that changes appearing in potential biological treatment can be correctly attributed to wastewater. Bi-plots between isotope ratios for specific compounds can show distinct signatures or ‘fingerprints’ that can provide greater utility in verification of exposure in biological receptors and food web dynamics to better inform cumulative effects assessment at the watershed scale. Similar patterns in isotope ratios as seen in a number of compounds have been observed previously in wastewater studies and specifically with carbon isotopes; however, these

![Figure 5](http://iwaponline.com/wqrj/article-pdf/55/3/327/730094/wqrjc0550327.pdf)  
**Figure 5** | Dissolved concentrations of acesulfame (ACE), select pharmaceuticals (CBZ – Carbamazepine; GEM – Gemfibrozil; IBU – Ibuprofen; NAP – Naproxen; SMX – Sulfamethoxazole), caffeine (CAF) and illicit drug Ecstasy (MDMA) in samples obtained from the Bow River across a MWWE gradient.
patterns and directionality of relative differences are also reliant upon the nature of the receiving environment (Kuuppo et al. 2006; Leavitt et al. 2006; Loomer et al. 2015).

**Statistical analysis**

Results from a PCA resulted in 56.9% component loadings on Principal Component 1 and 8.2% on Principal Component 2 due to the high correlation between analytes in response to wastewater release. In comparing these compounds through PCA, compounds which had strong correlations with traditional wastewater indicators such as chloride showed high positive loadings on Component 1 (Figure 7). Conversely where values for tracers followed a negative correlation against wastewater as observed with pH, these were represented as high negative loadings on Component 1. Isotope ratios such as δ18O-NO3 and δ15N-NO3 did not correspond to such patterns and instead showed greater loadings on Component 2 with modifying factors such as oxidation-reduction potential, alkalinity and distance from effluent release.

Pearson product moment correlation coefficients were calculated for tracers against chloride to identify correlations with two-tailed significance at $\alpha = 0.01$. Prior to evaluating data for correlations against chloride, correlation against field EC was first confirmed ($r = 0.991$, $p = 5.1 \times 10^{-10}$). Insignificant correlations were limited to those between chloride and: turbidity, ORP, alkalinity, selenium, thallium, thorium, uranium, sulfamethoxazole, δ13CDOC, δ15NNO3, δ18ONO3 and δ37Cl. Figures representing significant correlations ($p \leq 0.01$, $r > 0.8$ or $r < -0.8$) graphically for conventional parameters, major ions and nutrients, metals, trace organics and isotopes are included as Supplementary material, Figures S1 through S5.

**Utility of a multi-tracer approach**

The appeal in utilizing multiple tracers in monitoring wastewater effluent is (A) the added specificity in detecting the wastewater signal in the receiving environment and (B) the
development of a chemical baseline to interpret potential effects-based (biological) changes. Rivers that flow through urban centers are often influenced by a number of sources of environmental substances of concern. These sources can include but are not limited to agriculture, urban runoff, hospital effluents, septic tanks, subsurface storage of household and industrial waste, landfill leachate and others. When faced with the need to distinguish the signal of wastewater and characterize its potential influence in a system to manage wastewater infrastructure: conventional parameters, metals, isotopes and pharmaceuticals and personal care products collectively meet the criteria of good tracers and desirable properties of indicators to support these needs.

Establishing a multi-tracer suite of compounds for MWWE that incorporates traditional compounds (i.e. metals), wastewater performance indicators (i.e. chloride and ammonia), pharmaceuticals and isotopes in concert enables a dynamic baseline to be established. Benefits of this approach in the context of desirable properties of the indicators and tracers presented in this study are summarized in Table 1. These select compounds can be utilized to characterize local receiving environments and enable comparisons within and across watersheds impacted by wastewater effluent release. In the study completed by Liu et al. (2014) on the Grand River, similar concentrations of pharmaceuticals and other analytes were observed across sampling locations despite higher and lower dilution ratios relative to the current study (Supplementary Material Table S1). It is important to note that through the period of time in which the survey on the Grand River was completed, that parallel research programs indicated biochemical, organ and community level responses in fish in response to effluent release (Tetreault et al. 2011, 2013; Tanna et al. 2013). Although the pharmaceutical tracers utilized in both the present study and by Liu et al. (2014) are not directly implicated in biological responses, they serve as general tracers of organic compounds to establish site conditions where a risk to aquatic wildlife might exist. In studies where impacts to wildlife are observed, isotopic analysis has been critical in confirming that biological effects are elicited by wastewater exposure (Dubé et al. 2005).

Characterization of receiving environments using a multi-tracer approach with simultaneous biological monitoring can help to establish adverse outcome pathways (Ankley
Table 1 | Summary of tracer categories and associated tracers for use as a comprehensive suite in routine monitoring of wastewater influenced systems, with biological relevance and relevant criteria for selection identified. (Tracer criteria 1 through 4 presented in Introduction)

<table>
<thead>
<tr>
<th>Tracer category</th>
<th>Proposed tracers</th>
<th>Fate</th>
<th>Diagnostic capacity</th>
<th>Biological relevance</th>
<th>Tracer criteria met</th>
<th>Indicator criteria met</th>
</tr>
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<tbody>
<tr>
<td>Conventional wastewater parameters</td>
<td>pH, temperature, conductivity, turbidity, DO, dissolved organic carbon</td>
<td>N/A – physicochemical in nature</td>
<td>Low – confounded by other point and nonpoint sources</td>
<td>Indicative of potential acute toxicity</td>
<td>1, 2, 4</td>
<td>Cost-effective</td>
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<td>Historical</td>
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<td>Biologically relevant</td>
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<td>Interpretable</td>
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<td>Major ions and nutrients</td>
<td>Chloride, nitrate, nitrite, phosphate</td>
<td>Remained in solution and are processed or consumed</td>
<td>Low – confounded by runoff and other point and nonpoint sources</td>
<td>Drive primary productivity and acute toxicity</td>
<td>1, 2, 4</td>
<td>Biologically relevant</td>
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<td>Broadly applicable</td>
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<td>Metals</td>
<td>Aluminum, arsenic, barium, boron, copper, chromium, iron, manganese, molybdenum, nickel, lead, lithium, selenium, strontium, zinc*</td>
<td>Largely sequestered in treatment plant sludge, dissolved metals remain in solution, influenced by pH</td>
<td>Medium – Known driver of toxicity impacting development and respiration</td>
<td>Known driver of toxicity impacting development and respiration</td>
<td>1, 2, 3, 4</td>
<td>Biologically relevant</td>
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<td>Diagnostic</td>
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<td>Trace organic contaminants</td>
<td>Carbamazepine, gemfibrozil, fluoxetine, sulfamethoxazole, naproxen, ibuprofen</td>
<td>Sequestered in sludge, in solution, adsorbed to sediment based on $K_{ow}$</td>
<td>High – Specific to wastewater inputs (including septic systems) with no overlap with other point-sources/non-point sources</td>
<td>Known driver of toxicity via endocrine disruption</td>
<td>1, 2, 3, 4</td>
<td>Biologically relevant</td>
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<td>Lack of redundancy</td>
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<td>Diagnostic</td>
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<td>Isotopes</td>
<td>C-dic/doc, N-nitrate, o-nitrate, s-sulfate, o-sulfate, N, C, O18, H2</td>
<td>N/A – Signatures influenced by mixture of effluent influenced by plant and animal metabolism and evaporation</td>
<td>Neutral – Provides baseline signature that includes an understanding of effluent mixture and contributions of other source waters</td>
<td>Confirms direct and indirect exposure to invertebrates and higher order organisms</td>
<td>1, 2, 4</td>
<td>Biologically relevant</td>
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<td>Interpretive</td>
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</tbody>
</table>

*Metals are analyzed as a suite of ‘regulated metals’ by laboratories corresponding to freshwater aquatic life guidelines (AEP 2018).
et al. 2010) that are MWWE-specific and support targeted investigation of specific chemical compounds should impacts be observed. This approach enables the ability to establish conditions where MWWE poses a risk to receptors and supports development of predictive capacity related to effluent quantity and quality. Upon establishing baseline conditions, where there is an absence of degradation in biological data or trends in chemistry, sampling intensity in time and space can be adapted (Arciszewski et al. 2017) to reflect these conditions.

Of pollutants that are released through MWWE, trace organic compounds can be of the highest potency but are the most varied and can elicit a response below analytical detection limits. For this reason, effects are not easily generalized across sites in the context of specific or groups of compounds within effluent as complex as MWWE. The proposed multi-tracer suite used in conjunction with established biological criteria for MWWE (Kilgour et al. 2005) can serve to support stronger comparisons and better identify scenarios that merit further investigation of trace organic compounds and potential management.

Consistent collection of data using a multi-tracer suite enables important questions to be asked of the data. Specifically, we can adapt the questions identified by Diamond et al. (2010) for screening of high-risk CECs and adapt them to tracers for surveillance monitoring by asking:

- Where tracers (some or all) are elevated, do we find biological effects?
- Are tracers elevated where we find effects?
- Where tracers are found to be elevated, what type of conditions (i.e. physical or chemical) are present that create risk?

Further validation of tracers is necessary through synoptic study across multiple MWWE releases in effluent dominated systems. Specifically, planned next steps include sampling across two additional downstream outfalls, two tributaries and a series of stormwater outfalls within the watershed, leveraging historical data collected by the City of Calgary. Identifying areas of exposure and potential concern in a multi-tracer approach enables the characterization of relevant chronic effects where they may exist.

This approach is thus seen as adaptive due to forward compatibility considering ever-changing production and consumption of high risk (i.e. organic) compounds entering MWWTPs. Ultimately, receiving environments which are not monitored for change via surveillance monitoring which considers the fate of stressors leaves wastewater infrastructure operators and environmental managers unable to plan and adapt appropriately. Implementation of a multi-tracer approach enables exploration of current conditions in the context of historical conditions and to consider the relevance of US and European chemistry frameworks that consider pollutants introduced by wastewater for provincial and federal regulation revisions. Due to the absence of effects-directed monitoring in existing federal wastewater regulations, local efforts to ensure ecosystem health and in-turn continued protection of ecosystem services, in particular to meet the objectives of Alberta’s renewed Water for Life strategy (Government of Alberta 2008).

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SUPPLEMENTARY MATERIAL

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