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Trace organic contaminant (TOrC) mixtures in Minnesota littoral zones: Effects of on-site wastewater treatment system (OWTS) proximity and biological impact

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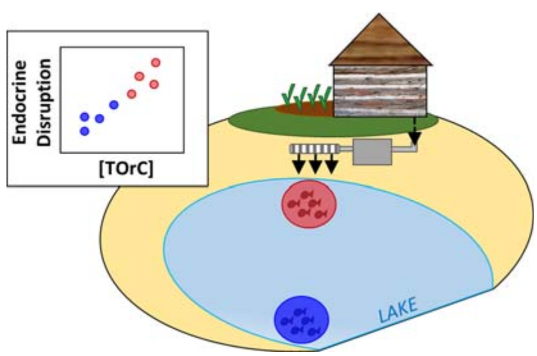
HIGHLIGHTS

- OWTSs are a potential diffuse source of TOrCs in Minnesota Lakes.
- TOrC concentrations increase with residential proximity.
- Increased endpoints of endocrine disruption in fish near residences
- Targeted analysis may limit understanding of mixture bioactivity.

GRAPHICAL ABSTRACT



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ABSTRACT

On-site wastewater treatment systems (OWTSs) are an international wastewater management strategy for rural and semi-rural communities without access to centralized sewage treatment. These systems are a suspected source of trace organic contaminants (TOrCs) that may be responsible for endocrine disrupting effects to resident fish species in Minnesota Lakes. This study assessed localized porewater concentrations of TOrCs in near-shore environments across five Minnesota Lakes. Sampling sites were designated as either likely (HOME) or unlikely (REF) to receive OWTS discharges based on their proximity to shoreline households. Sampling sites also served as sunfish spawning habitats concurrently studied for biological impacts to resident adult males. Two-group hypothesis tests demonstrated significantly ($p = .02$) higher total TOrC concentrations in HOME (Mean = 841 ng/L) versus REF (Mean = 222 ng/L) sites. HOME sites also contained a wider suite of TOrC detections relative to REF sites. The distance to the nearest household (most proximal distance; MPD) negatively correlated ($r = -0.62$) with total TOrC concentrations. However, 2,4-D and DEET were major contributors to these total concentrations, suggesting that anthropogenic influence from households may not be exclusively attributed to OWTS discharges. Further, TOrC presence and elevated nitrogen concentrations in REF site porewater suggest additional, non-household TOrC discharges to these lakes. Significantly higher

Abbreviations: HOME, site impacted by domestic wastewater; REF, reference site; MPD, most proximal distance; TOrC, trace organic contaminant; OWTS, on-site wastewater treatment system.

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blood concentrations of vitellogenin ($p = .03$) and 11-ketotestosterone ($p = .01$) were observed in adult male sunfish captured from HOME versus REF sites. Comparisons between chemical and biological data indicate enhanced bioactive effects of co-contaminants. The findings from this study demonstrate multiple diffuse transport pathways contribute to the presence of biologically active TOrC mixtures in Minnesota Lakes, and mitigation efforts should consider minimizing residential inputs of chemicals associated with both outdoor and OWTS activity.

1. Introduction

Trace organic contaminants (TOrCs) represent many emerging contaminants prioritized in current environmental monitoring efforts. TOrCs encompass pharmaceuticals, herbicides, pesticides, hormones, steroids, personal care products, cleaning agents, and food preservatives detected at low (ng/L) concentrations throughout the environment (Fatta-Kassinos et al., 2011; Kolpin et al., 2002; Lapworth et al., 2012). Many of these chemicals are not currently regulated despite the association of several TOrCs with adverse biological impacts, particularly endocrine disrupting effects (Ortiz de García et al., 2014). Endocrine disrupting chemicals mimic or inhibit normal androgen or estrogen receptor function, resulting in abnormal masculinization or feminization of affected species, respectively (Diamanti-Kandarakis et al., 2009; Söffker et al., 2015). The endocrine disruption capabilities of individual TOrCs are defined using laboratory exposure experiments through biochemical, histological, and behavioral endpoints (Blair et al., 2000; Elliott et al., 2014; Han et al., 2010; Oropesa et al., 2016). Still, the endocrine activity of environmental TOrC mixtures are poorly understood, particularly in light of the likely co-occurrence of unknown TOrCs (with unknown biological activities) and the potential for co-contaminants to enhance biological impacts (McCarty and Borgert, 2006).

Minnesota littoral zones are a prime field environment for studying diffuse sources of TOrCs and subsequent effects to aquatic life. Previous assessments of nutrient loadings in the United States (US) have led to the consensus that diffuse sources, such as agricultural runoff, groundwater infiltration, and atmospheric deposition, are responsible for most water quality degradation (U.S. Environmental Protection Agency, 1996). The lack of discrete inputs within proximity of surveyed Minnesota water resources indicates diffuse sources are also responsible for widespread TOrC occurrence in these waters (Erickson et al., 2014; Ferrey et al., 2015; Writer et al., 2010). In addition, many lakes in the state (90% of those surveyed by Writer et al.) also contain adult male fish with elevated vitellogenin concentrations, a biomarker of fish feminization (Writer et al., 2010). The spatial and seasonal heterogeneity of TOrC presence, both across lakes and within the same lake, impedes alignment of current chemical and biological observations (Baker et al., 2014). More strategic sampling methods, specifically sampling porewater in littoral zones (near-shore environments with depth < 5 m) during the spring and summer months should enable better characterization of biologically active TOrC mixtures that affect Minnesota fish species. Littoral zones serve as spawning habitats for fish species, such as the commonly studied bluegill sunfish *Lepomis macrochirus*. Spawning season is a critical time of TOrC exposure for larvae and the adult male sunfish that guard them (Becker, 1983). On-site wastewater treatment systems (OWTSs) are one of the proposed diffuse sources affecting the health of these fish (Baker et al., 2014; Writer et al., 2010). Analysis of sediment porewater in these locations advantageously provides insight into the TOrC concentrations of inflowing, potentially OWTS-impacted shallow groundwater and relevant exposure concentrations to fish interacting with lake sediments while spawning.

OWTSs are a documented diffuse source of wastewater-derived contaminants in groundwater, drinking water wells, and surface waters around the world (Gago-Ferrero et al., 2017; Godfrey et al., 2007; Phillips et al., 2015; Schaider et al., 2014; Subedi et al., 2015). This method of treatment typically serves rural and semi-rural populations without access to centralized sewage, around 25% of the population in the United States and 20% of Minnesotans (U.S. Environmental Protection Agency, 2014; West, 2008). Removal of nutrients, suspended solids, and pathogens is achieved by percolating pre-treated wastewater through unsaturated native soils (Crites and Tchobanoglous, 1998; Stanford et al., 2010). TOrCs readily sorbed or biotransformed in these subsurface conditions are also effectively attenuated, even though their removal is not considered in OWTS design (Conn et al., 2006, 2010; Teerlink et al., 2012b). Nevertheless, certain TOrCs remain recalcitrant to modern wastewater treatment technologies (Du et al., 2014; Wode et al., 2015). For this reason, several TOrCs, such as carbamazepine, are now designated as environmental domestic wastewater indicators (Kahle et al., 2009). Furthermore, the ability of OWTSs to effectively treat heterogeneous inputs of TOrCs at their small sewershed scale is highly variable (Teerlink et al., 2012a). Out-of-compliance systems, attributed to either improper installation or maintenance, allow insufficiently treated wastewater to reach the water table and enter shallow groundwater flow paths (Bremer and Harter, 2012; Yates, 1985). In addition, cesspool and leach pit OWTS designs have a decreased ability to remove TOrCs compared to a conventional two-stage system (Schaider et al., 2017). Transport through the subsurface poses an environmental health risk, and can lead to diarrhea outbreaks in children consuming water from OWTS-impacted drinking wells (Borchardt et al., 2003). An estimated 21% of OWTSs in Minnesota are operated out of compliance (Robinson and Schultz, 2015), but subsurface clay moraines with low hydraulic conductivity may also compromise soil driven treatment in the region (Engelking and Kovacevic, 2016). While advanced treatment options, such as aerated biofilters, are available, current OWTS regulation does not require their implementation (Jantrania and Gross, 2006). In light of the potential for OWTSs to serve as sources of TOrCs to Minnesota waters, it seems prudent to evaluate their occurrence in relation to OWTSs and their potential biological impacts before additional steps are taken to reduce these potential impacts.

The objectives of this study were to characterize targeted TOrC mixtures in littoral zones affected by discharges from OWTSs and evaluate potential associations between these TOrCs and biological impacts to adult fish. We hypothesized that locations more proximal to shoreline households would have more TOrC detections at higher concentrations and these locations would contain sunfish with elevated biomarkers of endocrine disruption. The following research questions were addressed: (1) what TOrC mixtures are present at near-shore environments in Minnesota Lakes, (2) are there significant compositional differences between sites likely impacted by OWTSs versus those which likely are not, (3) how are localized environmental TOrC mixtures related to biological responses in fish species? To address these questions, targeted aqueous analysis of porewater grab samples from spawning habitats in five Minnesota Lakes were compared to endpoints of biological impact in captured adult male sunfish.

2. Materials and methods

2.1. Site selection

Five lakes were selected for this study: Cedar Lake (Wright County, MN), Franklin Lake (Otter Tail County, MN), Lake Mary (Wright County, MN), Pearl Lake (Stearns County, MN), and Sullivan Lake (Wright

County, MN) (Fig. 1). Lakes were chosen based on the following criteria: influence of groundwater, presence of suitable bluegill nesting habitats, shoreline development >30%, and the use of OWTSs for wastewater treatment of domestic wastewater (regardless of OWTS functionality). The lakes are from the North Central Hardwood Forest Ecoregion from the EPA's EcoRegion III classifications (U.S. Environmental Protection Agency, 2013). Lakes were surrounded by residences, agricultural croplands, and several municipal buildings including churches and a summer camp. Each lake is also associated with recreational activities, including a public access boat ramp and regular stocking for recreational fishing (Minnesota Department of Natural Resources, 2017). Likelihood of groundwater influx was examined using historical water tables, groundwater flow, and stable isotope data collected from U.S. Geological Survey (USGS) Hydrologic Atlases and Minnesota Department of Natural Resources County Geologic Atlases (Adams, 2016; Lujan Jr. and Peck, 1992). A preliminary survey in the summer of 2015 verified groundwater influx at the candidate lakes. Areas with persistent temperature and specific conductance differences between sediment porewater and overlying surface water were deemed as “gaining” due to groundwater input and selected as sites for the study. Sediment porewater was pumped to the surface with a mini piezometer to monitor basic water quality measurements, such as temperature and specific conductance for verification of the influence of groundwater. The use of bed sediment temperatures as an indicator of groundwater inputs is relatively accurate in determining the influence of groundwater on surface water in riverine systems (Conant, 2004) and lakes (Constantz et al., 2007; Jones, 2006).

Four active sunfish spawning habitats per lake were chosen according to their proximity to shoreline households: two sites which likely received discharges from household OWTSs (HOME) and two reference sites (REF) likely unaffected by OWTS inputs. This study design assumes that the littoral zone in each lake sampled was not well mixed and discrete inputs would result in localized chemical and biological signatures of impact from OWTSs. Explicitly, REF sites are used as a control group in this study rather than reference lakes. As undeveloped lakes are anomalous with respect to land use characteristics in the Upper Midwest where lakeshore properties are highly desirable, the use of reference lakes would not have strengthened the experimental design of the study. HOME and REF were distinguished in the field as sites with or without a household in eyesight of the spawning habitat, respectively.

Site distinctions for two group analysis were re-examined after sampling using aerial imagery analysis. OWTS locations are extremely difficult to obtain; even with access to their public records, designs are usually detailed by hand-drawn representations with minimal geographic information. Therefore, household locations were used as a proxy. Household locations were recorded based on Google Maps imagery (Maps, 2017). Latitude and longitude direct decimal (DD) coordinates were recorded as the center of visible households or the center of household plots covered by trees after verifying addresses with Google Map streetview. Sampling location coordinates were obtained in the field using a global positioning system (GPS). The distance between each sampling location and all shoreline households within 100 m of lakeshore were determined using the Euclidean distance technique (Gower, 1982). The nearest household at each sampling location was determined by calculating the minimum of the set of distances attributed to each sampling location. The minimum distance at each site is herein referred to as the most proximal distance (MPD).

2.2. Sample collection

2.2.1. Aqueous samples

Porewater grab samples were collected between the months of May and July 2016. Samples were collected by pumping porewater through piezometers to the surface and accumulating water in appropriate sampling vessels. Piezometers were driven into the sediment until the screened terminal end of the probe reached saturated conditions determined to contain inflowing groundwater (~0.5–1 m depth), as verified by the methods described in the site selection section. Once placed, a peristaltic pump (Geotech Environmental Supply, Denver, CO, U.S.)

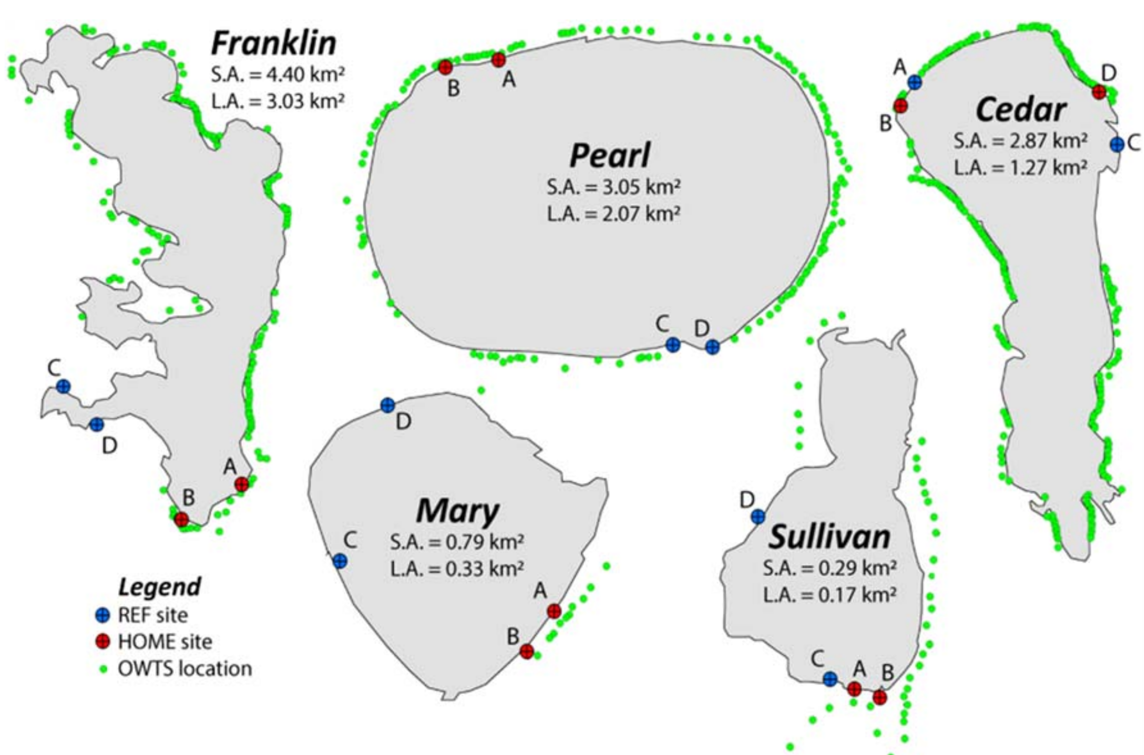

Fig. 1. Summary of sampling locations within each of the five lakes. Lake characteristics are provided under each lake's name, where S.A. = surface area, L.A. = littoral area, and bars represent 0.25 or 0.5 km distances.

was connected to the piezometer and porewater was pumped (~35 mL/min) for collection at the surface. This pumping rate was chosen so that the rate of groundwater replenishment at the sampling point was not exceeded. Pumping equipment was rinsed thoroughly with filtered water (Omni Water; Thermo Fisher Scientific, Walthman, MA, U.S.) before collection at each site to prevent cross contamination. Samples intended for TOrC analysis were collected in 1 L amber glass bottles pre-cleaned by scrubbing with liquinox, rinsing with DI water, and triple rinsing with reagent grade methanol. Samples for inorganic analysis were collected in 50 mL polypropylene tubes. All samples were stored and shipped at 4 °C to the Colorado School of Mines (CSM; Golden, CO, U.S.) for further analysis.

2.2.2. Biological samples

Resident sunfish samples were collected concurrently with the aqueous grab samples. Male fish were collected directly off spawning beds by rod and reel (permitted by Minnesota Department of Natural Resources). During the spawning season, male sunfish will continuously defend their small nest site (approximately 0.5 m in diameter). Consequently, the fish are unable to forage for food and will readily accept baited hooks. This behavior ensures that only nest defending males, who have likely been site-bound for days or weeks, were captured as non-nest holding sneaker males are able to feed between forays into the spawning grounds. A total of 124 male fish were collected from OWTS-influenced sites and 116 from reference sites. On a lake basis, 98 sunfish were collected from Sullivan Lake, 83 from Lake Mary, 35 from Cedar Lake, and 24 from Lake Franklin. Fish were not collected at Pearl Lake because early ice-off in the spring of 2016 disrupted spawning activity (i.e. $n_{bio} = 8$ rather than 10). Males captured were immediately euthanized using a buffered MS-222 solution approved by the St. Cloud State University Institutional Animal Care and Use Committee (IACUC # 8-77). A whole blood sample from the caudal vein was taken using a 22-gauge needle, stored on ice, and transferred to St. Cloud State University (SCSU; St. Cloud, MN, U.S.) for centrifugation. Fish carcasses were placed on ice and transferred to SCSU for dissection and further analysis.

2.3. Sample analysis

2.3.1. Chemical analysis

Basic porewater characteristics at each site, including temperature, pH, specific conductance, and dissolved oxygen (DO) concentrations were collected in the field using a Yellow Spring Instrument (YSI; Yellow Springs, OH, U.S.) probe. Surface water at each site was also measured for DO concentrations, specific conductance, and temperature to confirm adequate environmental conditions to support fish spawning.

Samples received at CSM intended for TOrC analysis were filtered using Whatman GF/F filters and spiked with surrogate standards (20 ng each of 48 unique stable isotope standards; Sigma Aldrich, St. Louis, MO, U.S.) within 48 h of sampling. Samples were then enriched using solid phase extraction (SPE) within two weeks of spiking with surrogate. SPE was executed using an AutoTrace 280 as follows: 6cm^3 500 mg Oasis HLB cartridges were pre-conditioned with 5 mL methyl tert-butyl ether (MTBE), followed by 5 mL methanol, and then 5 mL HPLC water. The 1 L sample was then loaded onto the cartridge. The cartridge was washed with 10 mL HPLC water, and then dried for 60 min using nitrogen gas. Cartridges were eluted with 5 mL methanol followed by 5 mL of 90/10 Methanol/MTBE (% v/v). The extract was blown down to 500 μL using an N-Evap system, then reconstituted in 2 mL of methanol. Methanol extracts were diluted 10:1 in ultra-pure water (Thermo Fisher Scientific, Walthman MA, U.S) and analyzed using a AB Sciex 3200 QTRAP liquid chromatograph tandem mass spectrometer (LC–MS/MS). Sample data was acquired with two 1 mL injections: one positive electrospray ionization method (ESI +) and one negative electrospray ionization (ESI -) method. Targeted analytes were selected to represent common indicators of domestic wastewater and TOrCs with known endocrine disrupting capabilities (Table S1). For ESI + runs, a Phenomenex Luna C18 column (Phenomenex, Torrance, CA, U.S) was used with water and methanol eluents buffered with 10 mM ammonium formate and 1 mL formic acid (Thermo Fisher Scientific, Walthman MA, U.S). The ESI – method used a Phenomenex Gemini C18 column (Phenomenex, Torrance, CA, U.S) with water and methanol eluents buffered with 5 mM ammonium fluoride (Thermo Fisher Scientific, Walthman, MA, U.S).

Inorganic samples were prepped and preserved at CSM in accordance with their intended instrument analysis. All inorganic samples were filtered using 0.4 μm syringe filter within 48 h of sampling. Major nutrients were analyzed using ion chromatography (Dionex Thermo Fisher ICS-900) using unacidifed aliquots of the filtered sample. Total nitrogen and total organic carbon were assessed with sample acidified using hydrochloric acid on a Schimadzu TOC system (Shimadzu TOCV-TNM-LCSH). Trace metals were quantified using inductively coupled plasma atomic emission electroscopy (ICP-AES) with filtered aliquots acidified with nitric acid.

2.3.2. Biological analysis

Glucose concentrations in whole blood samples were measured using a TRUEbalance Blood Glucose Monitor (Moore Medical, Farmington, CT, U.S.). Blood samples were then centrifuged (8000 ×g) for 12 min at 4 °C, plasma was pipetted into separate vials and frozen at −80 °C until analysis. Plasma vitellogenin concentration was determined through an enzyme linked immunosorbent assay (ELISA) using purified sunfish vitellogenin and sunfish validated vitellogenin antibodies. The protocol followed parameters as used in Schultz et al. (2013). 11-ketotestosterone concentrations were determined using an ELISA (Cayman Chemical Company, Kit #582751) following the manufacturer's guidelines. Wet weight of fish carcasses was determined upon return to SCSU (within 6 h of fish capture), and liver and gonad were excised and weight. From these values, body condition factor (weight / (total length)^3 × 100,000), hepatosomatic index (liver weight / mass fish × 100), and gonadal somatic index (gonad weight / mass fish × 100) were calculated (Bolger and Connolly, 1989; Fulton, 1904).

2.3.3. Quality assurance and quality control

Reported aqueous chemistry data were subject to various field and laboratory quality control measures. All grab samples were collected in triplicate along with field blanks and equipment blanks collected at each lake. Field blanks were collected at each lake by passing purified water (OmniWater, Thermo Fisher Scientific, Walthman, MA, U.S.) through the piezometer pumping set up. Laboratory blanks and instrument blanks were also included to ensure contamination introduced during sample handling could not skew reported results. Raw data from targeted LC–MS/MS data acquisition was initially processed using Sciex's MultiQuant software. Quantitation limits for each analyte were set as the concentration of the lowest calibration standard included in a valid calibration curve. Valid calibration curves needed to include at least four points, exclude points with possible instrument or laboratory contamination (defined as containing analyte peak area greater than three times analyte peak area in the blank run before the calibration standards), and have a (1/x)- or (1/x^2)-fit trendline with a Pearson's r value >0.99. Analyte signals influenced by field contamination were redacted by setting reporting limits above the quantitation limit. Reporting limits were set as the value of the average field and equipment blank concentrations plus three times the standard deviation. For analytes with only one field blank or equipment blank with quantifiable contamination, this value was set as three times the field blank concentration (Table S2). Average concentrations were reported if at least two out of three replicates had quantified results. Sites with detections in zero or only one of the three replicates were reported as below the quantitation limit. Below reporting limit and below quantitation limit values were handled as censored values during statistical analysis according to Helsel (2012). Censored data analysis methods are explained in the Statistical analysis section. Instrument performance was verified with regular calibration standards and calibration verification standards. TOrC results were also constrained by matrix spike and surrogate recoveries (Table S1). Furthermore, analytes were redacted if their matrix spike recoveries were outside the 70–130% range or if surrogate recoveries were <10%. Thirty-seven of the TOrCs analyzed met all QA/QC requirements and were used for statistical comparisons between HOME and REF sites.

Biological data were also subject to quality assurance and quality control procedures. For plasma vitellogenin measurements, all samples were analyzed at three dilutions (1:50, 1:250, and 1:1000). An eight-point standard curve was then used to reference absorbance readings of samples. Four replicate samples were added to each plate and replicate samples were added across plates. All samples were randomized across plates.

2.3.4. Statistical analysis

Non-parametric statistical comparisons of biological and chemical data were executed as two-group sample hypothesis tests between HOME ($n_{bio} = 8$, $n_{chem} = 10$) and REF ($n_{bio} = 8$, $n_{chem} = 10$) sites. Rejection of the null hypothesis was considered valid for p-values < .05. Tests on aqueous chemistry data were conducted using the “NADA” package in RStudio to ensure proper handling of the numerous left-censored data in both organic and inorganic targeted datasets (Lee, 2017; Rstudio Team, 2015). Average concentrations of all inorganic and organic analytes, as well as the sum of the average targeted TOrC concentrations, referred to hereafter as total TOrC concentration, in HOME and REF groups were compared using the cendiff( ) function, a Mann-Whitney-Wilcoxon test of the empirical cumulative distribution functions within each group. This non-parametric statistical test does not assume a normal distribution of values within each group, which is appropriate for comparing concentrations across lakes from different geographical regions, unique OWTS owners, and different resultant baseline TOrCs in the respective lake systems. The larger number of biological samples collected allowed for HOME and REF two group tests to be executed at both inter- and intra-lake levels. Intra-lake comparisons were assumed to have a normal distribution in biological endpoints. Mean comparisons from biological data were conducted with Tukey's honest significant difference (HSD) test and two-sided t-tests.

A bivariate analysis of the transformed variables ΔMPD and ΔTOrC provide a parametric assessment of OWTS proximity on TOrC concentrations. The variables are defined as:

$$\Delta MPD = MPD_{site} - MPD_{median} \tag{1a}$$

$$\Delta TOrC = TOrC_{site} - TOrC_{median} \tag{1b}$$

where MPD_{site} is the MPD value specific to a sampling location and MPD_{median} is the calculated median MPD value attributed to the four sampling locations at each lake. Similarly, $TOrC_{site}$ is the total TOrC concentration specific to a sampling location and $TOrC_{median}$ is the calculated median total TOrC concentration attributed to the four sampling locations at each lake. These transformed variables were used to allow better comparison across lakes. Explicitly, MPD values were modified to ΔMPD to better compare HOME/REF site selection across lakes with varied surface areas, and TOrC values were modified to $\Delta TOrC$ to better compare across lakes with varied background concentrations.

3. Results and discussion

3.1. Site distinction analysis

MPDs were significantly (p = .005) lower at HOME sites relative to REF sites (Fig. S1). This corroborates the two-group distinctions used for non-parametric hypothesis testing of the chemical and biological results. Importantly, the MPD metric of proximity does not consider pronounced chemical and biological effects in regions that could be impacted by OWTS leachate from more than one system. Assessing impact from density, such as the approach used in Bremer and Harter, was also considered (Bremer and Harter, 2012); however, obtaining the exact location and compliance status of all relevant OWTSs would require a level of cooperation from homeowners unattainable at this time. Analysis of OWTS density in this study was, therefore, determined to be unreliably speculative.

3.2. TOrC detections and non-parametric two group comparisons

Fifteen of the reported TOrCs were detected in at least one of the sites sampled in this study (Table S3). Pharmaceuticals, such as carbamazepine, are a preferred indicator of wastewater presence in an environmental matrix (Subedi et al., 2015). While detections of particular pharmaceuticals were not widespread enough to generate meaningful statistics comparing HOME and REF sites, it is noted that the pharmaceuticals carbamazepine, dilantin, and ibuprofen were only detected at HOME sites (Tables S3 and S4). Interestingly, the synthetic estrogen 17α-ethinylestradiol (EE2) was only detected in two REF sites. EE2 is a known endocrine disruptor that was shown to collapse a fish population during a lake dosing study in Canada (Kidd et al., 2007). EE2 is used as both a synthetic birth control hormone and a livestock hormone to improve productivity and treat livestock diseases (Gadd et al., 2010). The REF sites with EE2 detections, SUL_D at 25 ng/L and CED_A at 5 ng/L are suspected to receive shallow groundwater carrying agricultural runoff from fields that use hormone-fed livestock manure as fertilizer (Zaharin Aris et al., 2014). These concentrations match the upper end of previously observed EE2 concentrations in surface waters (Zaharin Aris et al., 2014). In addition, bed sediments usually have higher EE2 concentrations attributed to the contaminants hydrophobic and persistent properties (Zaharin Aris et al., 2014). Therefore, the observed concentrations of EE2 in porewater sampled during this study are consistent with those expected in bed sediments (Zaharin Aris et al., 2014). Other known or suspected endocrine active compounds that were commonly detected include the cosmetic preservative methylparaben and estrone, which were detected in 40% and 20% of the sites sampled, respectively (Bergman et al., 2012). The steroidal hormones androstenedione and testosterone, as well as 4-tert-octylphenol (used in the manufacturing of anionic surfactants, such as detergents or, less commonly, as an emulsifier in personal care products such as insect repellents) were also detected, but less frequently (detection frequency ≤ 10%). Some TOrCs typically detected in environments down-gradient of OWTSs, such as sulfamethoxazole, were not detected in this study. These non-detections are attributed to the heterogeneity of inputs to wastewater treatment systems at such a small sewershed scale (Teerlink et al., 2012a), as well as the variability of subsurface conditions that affect TOrC removal in soil treatment units. Explicitly, these TOrCs may simply not have been used at the households within proximity to sampling locations, or soil regions were anaerobic and suitable for sulfamethoxazole attenuation (Massmann et al., 2008).

The most frequently detected analyte (detection frequency = 85%) was *N*,*N*-diethyltoluamide, more commonly referred to as DEET. DEET is neither a persistent nor bioaccumulative organic pollutant, with a half-life in the order of days to weeks as well as acute and chronic effect concentrations orders of magnitude above observed environmental concentration (Weeks et al., 2012). This insect repellent ingredient has been previously detected in lakes across the state of Minnesota where lake recreation and mosquitoes are very common in the summer months (Ferrey et al., 2015; Writer et al., 2010). This study is the first to note significantly higher concentrations of DEET in Minnesota lake sites more proximal to households across all lakes sampled (Fig. 2a). DEET could enter household wastewater streams through bathing or clothes washing which could then enter OWTS discharges. Gago-Fererro et al. also noted seasonally high concentrations of DEET in surface waters adjacent to OWTSs at concentrations an order of magnitude lower than

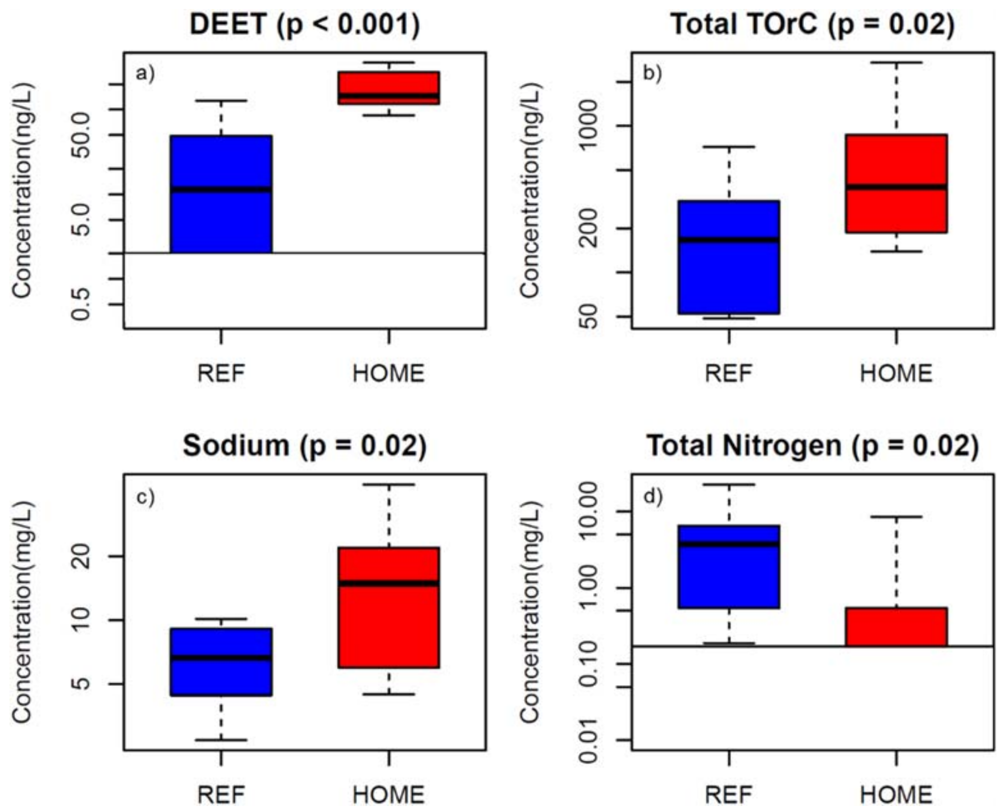

Fig. 2. Two-group comparison of average site concentrations across all five lakes. The analytes a) DEET, b) total targeted TOrCs, c) sodium, and d) total nitrogen had significantly different concentrations in HOME and REF sample groups. Each boxplot displays minimum, first quartile, median, third quartile, and maximum values specific to each group. The detection limits of DEET and Total Nitrogen are displayed using horizontal lines at 2 ng/L and 0.17 mg/L, respectively. First quartiles estimated below these values through censored statistical analysis are omitted from display.

those observed in our study (average summer sampling concentration of 13 ng/L) (Gago-Ferrero et al., 2017). However, designating it as a wastewater indicator in these lake systems is inappropriate (Tran et al., 2014). The associated outdoor usage of DEET suggests that this contaminant may also be entering lake systems from general anthropogenic activity in and around lakes.

The total TOrC concentrations at HOME sites were significantly ($p = .02$) higher compared to those at REF sites (Fig. 2b). A more detailed presentation of the measured analyte concentrations at each lake site is provided in Fig. 3. HOME sites contained a wider suite of targeted analytes (14 out of the 37 reported) as compared to REF sites (7 out of the 37 reported). Fig. 3 also shows the consistently higher concentrations of specific TOrCs detected at the HOME versus REF sites. Lake Mary's HOME sites, MAR_A and MAR_B, had the highest measured total TOrC concentration across all sites assessed in this study. This is mainly attributed to the measured concentrations of the herbicide 2,4-D at both of the lake's HOME sites (mean of 2200 ng/L). 2,4-D is commonly used for outdoor home gardening applications suggesting that, similar to DEET, household activity other than OWTSs are impacting littoral porewater of these lakes (Mnif et al., 2011). 2,4-D is also one of the few measured TOrCs monitored by the EPA, with a maximum contaminant level (MCL) in drinking water of 50 μg/L as a result of its association with blood, kidney, and liver toxicity (EPA, 1998).

Non-household diffuse sources are expected to contribute to the "background" presence of TOrCs, such as DEET and oxybenzone, observed at many of the REF sites (Fig. 3). The only sites DEET was not detected at were the REF sites PRL_C, FRK_C, and FRK_D. As suggested before, agricultural operations could contribute to TOrC occurrence, particularly herbicides, pesticides, and feedlot hormones, in the REF sites sampled. Agricultural fields surround all of the lakes, and the boundaries of these operations are in closer proximity to lake shoreline unoccupied by household lots. Recreational activities, such as boating, may also act as a non-point source of TOrCs in lake locations distant from households. Each lake sampled is a stocked fishery with household and public boat ramp access points (Minnesota Department of Natural Resources, 2017). Most lakeshore households have their own docks and boats (verified with aerial imagery; Google Maps accessed March 2017) (Maps, 2017). Oxybenzone, common in sunscreens, and DEET, common in insect repellents, are both TOrCs integrated into personal care products associated with these lake recreational activities. The transport processes resulting in their presence in groundwater-impacted lake porewaters for these contaminants is not immediately clear. Ferrey et al. proposed atmospheric deposition as a diffuse TOrC transport mechanism to Minnesota Lakes (Ferrey et al., 2015). DEET has been reported to be widely present in atmospheric samples (Balducci et al., 2012; Cheng and Lehmann, 1985). However, the significantly higher concentrations of DEET in sediment porewater near households in this study suggest long range aerial transport is unlikely. TOrCs introduced at the lake surface may enter into shallow groundwater after application near the water's surface. Contaminants may then settle with suspended solids in the lake and accumulate in the sediment where they may then partition into sediment porewater and reenter littoral zones with the influx of shallow groundwater (Winter et al., 1999).

3.3. Patterns in inorganic non-point source indicators

Basic water quality assessments of the sampled porewater confirm inflowing groundwater had "young" or shallow flowpaths (Table S5). The consistently low (<5 mg/L) dissolved oxygen (DO) measurements of the porewater are typical for groundwater (Peterson and Risberg, 2009). There were no significant ($p > .05$) differences between porewater DO concentrations at HOME versus REF sites, but the

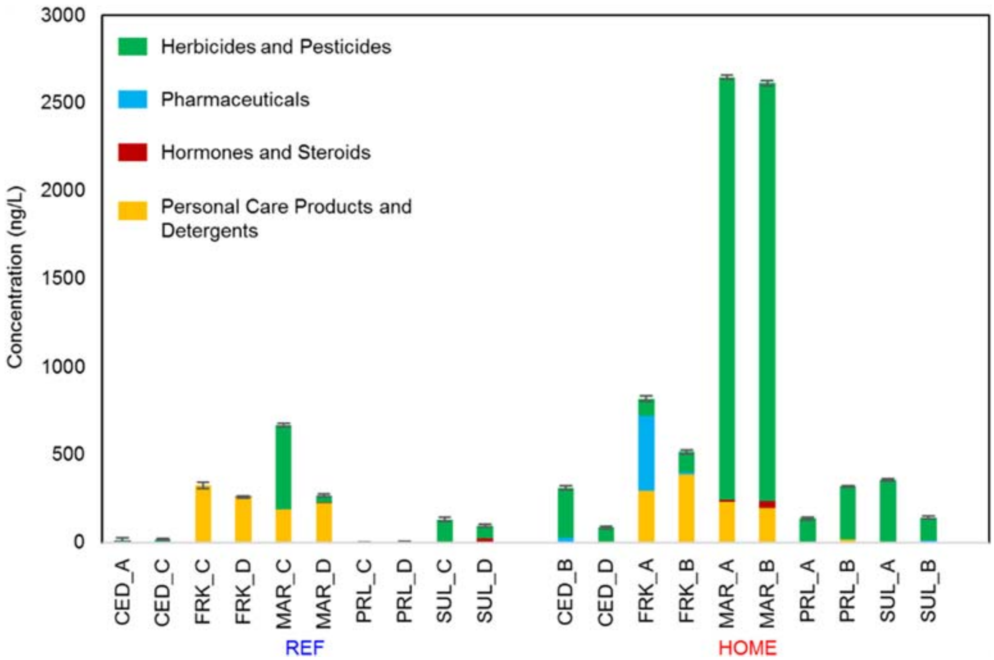

Fig. 3. Average total TOrC concentrations measured at each site. Detected analytes are color coded by compound use. Average analyte-specific concentrations measured at each site are displayed in Table S5. Error bars represent ± average standard deviation across all TOrCs measured at each site.

maximum observed values were present in the REF sites. In addition, conductivity values across all sites' porewater samplers were at the lower end of the typical groundwater values (typical range 50–50,000 μS/cm, (Sanders, 1998)), suggesting influence from shorter groundwater flowpaths (Erickson et al., 2014). Lake Mary notably had the lowest porewater DO concentrations. Previous studies have noted that anoxic regions lead to longer range transport of TOrCs, as these pollutants are generally more effectively attenuated through aerobic degradation (Carrara et al., 2008; Phillips et al., 2015). We speculate anoxic regions are present in the subsurface surrounding Lake Mary and contribute to the higher detected TOrC concentrations in Lake Mary littoral sediment porewaters; however, a more thorough characterization of groundwater flow paths and redox conditions at this lake are required to test this hypothesis.

Nutrient and trace metal data were also compared to assess chemical differences between HOME and REF sites (Tables S6 and S7). Previous studies have shown total nitrogen concentrations to positively correlate with TOrC occurrence, particularly TOrCs derived from OWTSs (Del Rosario et al., 2014; Phillips et al., 2015; Schaider et al., 2017). Surprisingly, there were significantly ($p = .02$) higher total nitrogen concentrations at the REF sites, further indicating the presence of an additional non-point source in the region (Fig. 2d). This was not reflected by significant differences ($p > .05$) in the measured concentrations of nitrite or nitrate. Unmeasured ammonia or organic nitrogen are suspected to be the dominant nitrogen species at these locations, with the exception of Pearl Lake's REF site C which had nitrate as the dominant nitrogen species. Pearl Lake's REF site C also had the highest total nitrogen concentration measured across all sampling locations. Each of the lakes sampled have agricultural fields surrounding them, particularly in non-residential parts of the shoreline, that could contribute to observed elevated nitrogen concentrations at REF versus HOME sites. There was no significant difference in total organic carbon between HOME and REF sites ($p > .05$). Many of the other nutrients analyzed were below detection limits. Chloride and bromide ratios can be used to assess sources of groundwater (Katz et al., 2011), but consistent censoring of bromide concentrations hindered this calculation. Trace metal analysis only showed significantly higher ($p = .019$) sodium concentrations in the HOME sites (Fig. 2a). Sodium salts are common in detergents and other common household products as well as softening systems, which could explain this significant difference in HOME and REF concentrations. The wastewater tracer boron, also common in household products (Woods, 1994), showed no significant ($p > .05$) difference in concentrations between HOME and REF sites.

3.4. MPD effect on total TOrC concentration

Results from the parametric analysis are displayed in Fig. 4. We anticipated HOME sites would have more positive ΔTOrC and negative ΔMPD values, in agreement with the hypothesis that sites more proximal to household OWTSs would have higher total TOrC concentrations. ΔMPD negatively ($r = -0.62$, $p < .001$) correlated with ΔTOrC, supporting this hypothesis. Certain lakes showed more pronounced differences between HOME and REF sites than others. Specifically, Lake Mary's HOME and REF total TOrC concentrations showed the greatest difference, attributed to the high concentrations (~2200 ng/L) of 2,4-D at the lake's HOME sites. Sullivan, Cedar and Pearl lakes clustered around the origin of the plot, demonstrating poorer distinction between HOME and REF sites at these lakes. As expected, these sites with similar MPDs had less differentiation in total measured TOrC concentrations.

The high concentrations measured at Lake Mary may be attributed to the lake's small surface area, along with many households at the south and eastern shorelines where the HOME site samples were collected. This spatial arrangement of households and OWTSs creates the potential for multiple wastewater streams to impact the adjacent littoral environments with minimal effects from dilution and attenuation. In addition, the DO readings for this porewater were very low (~1 mg/L), which has been associated with longer range transport of untransformed TOrC species in OWTS plumes (Carrara et al., 2008).

3.5. Biological data two group hypothesis tests

Field measurements of the lake surface water affirmed that the littoral environments could support healthy spawning at the sites sampled. DO levels were still hospitable for aquatic life (>6 mg/L). Further, all observed aqueous temperatures were suitable for spawning with the exception of Pearl Lake (average = 12 °C), which experienced an early

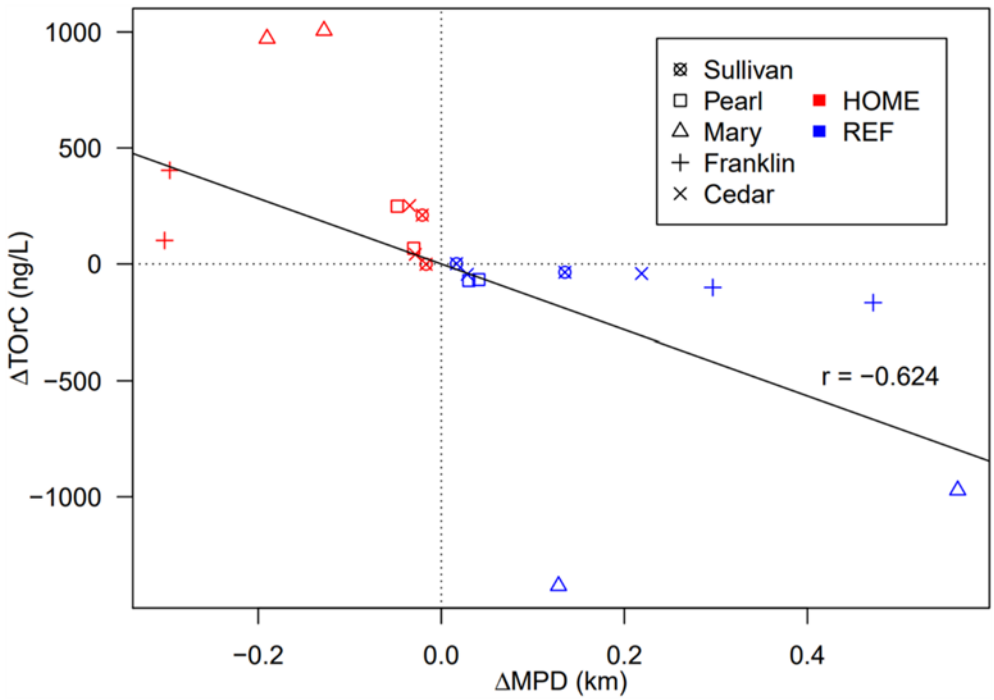

Fig. 4. Bivariate analysis of residential proximity's effect on total TOrC concentration in littoral site porewater. Variables ΔMPD and ΔTOrC are defined in Eqs. (1a) and (1b), respectively. Trendline: $y = a * x$, where $a = -1413.0 \pm 427.8$ ($p = .00374$); $r^2 = 0.3313$.

ice off. No sunfish were captured or analyzed from Pearl Lake; therefore, only chemical data are reported from this lake.

Two group comparison of biological data demonstrated more pronounced biological impacts in HOME site spawning male sunfish with respect to vitellogenin and 11-KT concentrations (Fig. 5). Increased concentrations of vitellogenin in male fish is a Tier 1 indicator of estrogen agonism and elevated concentrations of 11-ketotestosterone in male fish is a Tier 3 indicator of steroidogenesis according to the EPA's Endocrine Disruptor Screening Program (Borgert et al., 2014). All other biological endpoints measured, i.e. gonadal somatic index, glucose concentrations, and body condition factor showed no significant ($p > .05$) difference for captured fish at HOME and REF. Vitellogenin concentrations were significantly ($p = .0108$) higher in HOME versus REF sites, driven by the results from Lake Mary ($p \leq .0001$). Higher vitellogenin concentrations in HOME versus REF fish, particularly those inhabiting Lake Mary, suggest these males are being exposed to mixtures of TOrCs with estrogenic activity. This finding is particularly interesting when considering Lake Mary's HOME sites had the most detections with the highest concentrations. We speculate the notably high concentrations of 2,4-D may be enhancing estrogen agonist effects of known endocrine active co-contaminants measured at these sites. Even though 2,4-D is not itself consider an endocrine disruptor by the EPA's EDSP for the 21st century (EDSP21) dashboard (U.S. Environmental Protection Agency, 2017), a study by Kim et al. demonstrated 2,4-D and its transformation product 2,4-dichlorophenol (DCP) could enhance the androgenic effects of 5-dihydroxytestosterone (Kim et al., 2005). Steroidogenesis effects were also more pronounced at HOME sites as demonstrated by significantly higher concentrations of 11-KT in blood

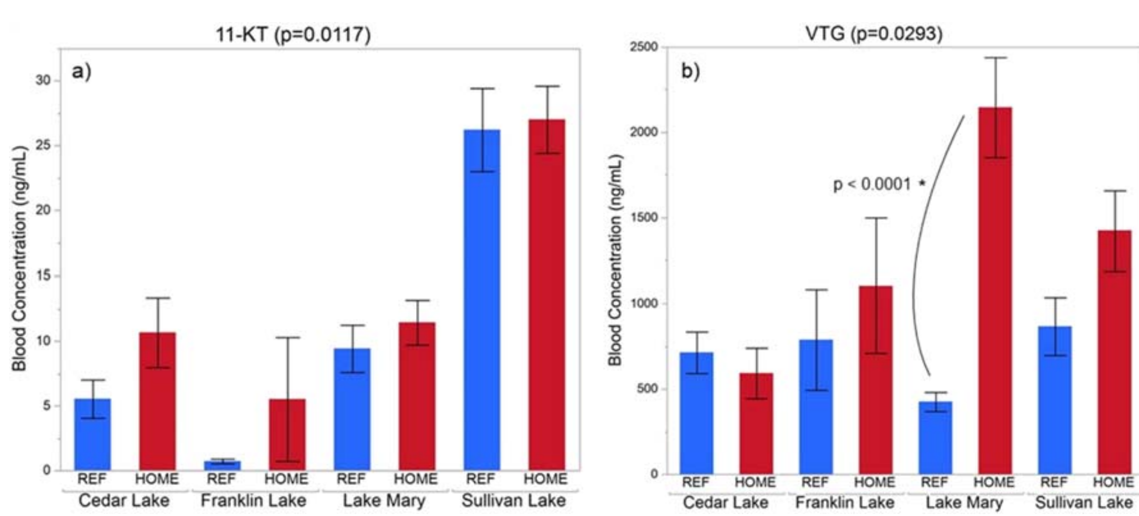

Fig. 5. Average blood concentrations of a) 11-Ketotestostrone and b) vitellogenin in fish captured at HOME and REF within each lake. Error bars represent the standard deviation of values from the average concentration at each lake. p-Values above the plots are comparisons of concentrations in fish captured from HOME and REF sites across all lakes. In panel b, the in-plot p-value is the two group comparison of HOME and REF captured fish from Lake Mary only (color, 2 panel).

samples ($p = .0117$) when compared across all lakes. Cedar and Franklin Lake showed the most pronounced intralake site differences in 11-KT concentrations (Fig. 5a). Interestingly, all fish sampled from Sullivan Lake, from both HOME and REF sites, had significantly higher ($p = .003$, two way ANOVA) 11-KT concentrations than fish sampled from other lakes. Previously reported 11-KT blood concentrations for adult male sunfish averaged at 13.8 ng/mL (Knapp and Neff, 2007), suggesting Sullivan Lake's fish were anomalously high and Franklin Lake's REF site was anomalously low. Chemical analyses did not indicate the presence of potent endocrine disruptors at Sullivan Lake, suggesting that the targeted analysis in this study may not sufficiently describe the localized TOrC mixtures present at the Sullivan Lake sites that may be impacting observed 11-KT concentrations.

4. Implications

The findings of this study suggest that TOrC occurrence in sunfish spawning habitats of Minnesota littoral environments are affected by groundwater inflows. Lakeshore households increase concentrations and detection frequency at adjacent lake locations. Minimizing TOrC loadings from households requires consideration of heterogeneous outdoor activity and domestic wastewater chemical compositions at shoreline residential locations. Hydrologic processes, such as stormwater infiltration, are suspected to increase the mobility of TOrCs in the subsurface and encourage transport from residences to littoral zones. Inputs from agricultural operations and recreational activity separate from residential locations must also be considered during mitigation efforts, particularly as this study suggests they may be sources of potent endocrine disruptors. Resultant endocrine disrupting effects are only partially justified by the TOrCs detected, suggesting total concentrations reported are merely a proxy for all components of biologically active mixtures in these environments. Non-targeted analysis with high resolution mass spectrometry could better resolve components of environmental TOrC mixtures that contribute to pronounced biologic activity (Schymanski et al., 2015).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.123.

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References

Adams, R., 2016. Water-Table Elevation and Depth to Water Table Minnesota Hydrogeology Atlas Series Atlas HG-03 Minnesota. Department of Natural Resources Ecological and Water Resources Division County Geologic Atlas Program.

Baker, B.H., Martinovic-Weigelt, D., Ferrey, M., Barber, L.B., Writer, J.H., Rosenberry, D.O., Kiesling, R.L., Lundy, J.R., Schoenfuss, H.L., 2014. Identifying non-point sources of endocrine active compounds and their biological impacts in freshwater lakes. Arch. Environ. Contam. Toxicol. 67:374–388. https://doi.org/10.1007/s00244-014-0052-4.

Balducci, C., Perilli, M., Romagnoli, P., Cecinato, A., 2012. New Developments on Emerging Organic Pollutants in the Atmosphere. https://doi.org/10.1007/s11356-012-0815-2.

Becker, G.C., 1983. The Fishes of Wisconsin. Univ. Wisconsin Press, Madison.

Bergman, Å., Heindel, J., Jobling, S., Kidd, K., Zoeller, R.T., 2012. State of the science of endocrine disrupting chemicals—2012. Toxicol. Lett. https://doi.org/10.1016/j.toxlet.2012.03.020.

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R., Sheehan, D.M., 2000. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. Toxicol. Sci. 54: 138–153. https://doi.org/10.1093/toxsci/54.1.138.

Bolger, T., Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish Biol. 34:171–182. https://doi.org/10.1111/j.1095-8649.1989.tb03300.x.

Borchardt, M.A., Chyou, P.H., DeVries, E.O., Belongia, E.A., 2003. Septic system density and infectious diarrhea in a defined population of children. Environ. Health Perspect. 111: 742–748. https://doi.org/10.1289/ehp.5914.

Borgert, C.J., Stuchal, L.D., Mihaich, E.M., Becker, R.A., Bentley, K.S., Brausch, J.M., Coady, K., Geter, D.R., Gordon, E., Guiney, P.D., Hess, F., Holmes, C.M., LeBaron, M.J., Levine, S., Marty, S., Mukhi, S., Neal, B.H., Ortego, L.S., Saltmiras, D.A., Snajdr, S., Staveley, J., Tobia, A., 2014. Relevance weighting of tier 1 endocrine screening endpoints by rank order. Birth Defects Res. B Dev. Reprod. Toxicol. 101:90–113. https://doi.org/10.1002/bdrb.21096.

Bremer, J.E., Harter, T., 2012. Domestic wells have high probability of pumping septic tank leachate. Hydrol. Earth Syst. Sci. 16:2453–2467. https://doi.org/10.5194/hess-16-2453-2012.

Carrara, C., Robertson, W.D., Blowes, D.W., 2008. Fate of Pharmaceutical and Trace Organic Compounds in Three Septic System Plumes, Ontario, Canada. 42 pp. 2805–2811.

Cheng, H.H., Lehmann, R.G., 1985. Characterization of herbicide degradation under field conditions. Weed Sci. 33:7–10. https://doi.org/10.1017/s0043174500083740.

Conant Jr., B., 2004. Delineating and quantifying ground water discharge zones using streambed temperatures. Ground Water 42, 243–257.

Conn, K.E., Barber, L.B., Brown, G.K., Siegrist, R.L., 2006. Occurrence and fate of organic contaminants during onsite wastewater treatment. Environ. Sci. Technol. 40: 7358–7366. https://doi.org/10.1021/ES0605117.

Conn, K.E., Siegrist, R.L., Barber, L.B., Meyer, M.T., 2010. Fate of trace organic compounds during vadose zone soil treatment in an onsite wastewater system. Environ. Toxicol. Chem. 29:285–293. https://doi.org/10.1002/etc.40.

Constantz, J.E., Niswonger, R.G., Stewart, A.E., 2007. Analysis of Temperature Gradients to Determine Stream Exchanges With Ground Water Field Techniques for Estimating Water Fluxes Between Surface Water and Ground Water. Reston, VA.

Crites, R., Tchobanoglous, 1998. Small and Decentralized Wastewater Treatment Systems.

Del Rosario, K.L., Mitra, S., Humphrey, C.P., O'Driscoll, M.A., 2014. Detection of pharmaceuticals and other personal care products in groundwater beneath and adjacent to onsite wastewater treatment systems in a coastal plain shallow aquifer. Sci. Total Environ. 487:216–223. https://doi.org/10.1016/j.scitotenv.2014.03.135.

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr. Rev. 30:293–342. https://doi.org/10.1210/er.2009-0002.

Du, B., Price, A.E., Scott, W.C., Kristofco, L.A., Ramirez, A.J., Chambliss, C.K., Yelderman, J.C., Brooks, B.W., 2014. Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent. Sci. Total Environ. 466:976–984. https://doi.org/10.1016/j.scitotenv.2013.07.126.

Elliott, S.M., Kiesling, R.L., Jorgenson, Z.G., Rearick, D.C., Schoenfuss, H.L., Fredricks, K.T., Gaikowski, M.P., 2014. Fathead minnow and bluegill sunfish life-stage responses to 17β-estradiol exposure in outdoor mesocosms. J. Am. Water Resour. Assoc. 50: 376–387. https://doi.org/10.1111/jawr.12169.

Engelking, P., Kovacevic, A., 2016. 2016 Pollution Report to the Legislature.

EPA, E.P.A., 1998. Ambient water quality value for protection of sources of potable water - beta-hexachlorocyclohexane. J. Chem. Inf. Model. 53:1689–1699. https://doi.org/10.1017/CBO9781107415324.004.

Erickson, M.L., Langer, S.K., Roth, J.L., Kroening, S.E., 2014. Scientific Investigations Report 2014–5096 Contaminants of Emerging Concern in Ambient Groundwater in Urbanized Areas of Minnesota.

Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal. Bioanal. Chem. 399:251–275. https://doi.org/10.1007/s00216-010-4300-9.

Ferrey, M.L., Heiskary, S., Grace, R., Hamilton, M.C., Lueck, A., 2015. Pharmaceuticals and other anthropogenic tracers in surface water: a randomized survey of 50 Minnesota lakes. Environ. Toxicol. Chem. 34:2475–2488. https://doi.org/10.1002/etc.3125.

Fulton, T.W., 1904. The rate of growth of fishes. 22nd Ann. Rep. Fish. Board Scotland 3, pp. 141–241.

Gadd, J.B., Tremblay, L.A., Northcott, G.L., 2010. Steroid Estrogens, Conjugated Estrogens and Estrogenic Activity in Farm Dairy Shed Effluents. https://doi.org/10.1016/j.envpol.2009.10.015.

Gago-Ferrero, P., Gros, M., Ahrens, L., Wiberg, K., 2017. Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters. Sci. Total Environ. 601–602:1289–1297. https://doi.org/10.1016/j.scitotenv.2017.05.258.

Godfrey, E., Woessner, W.W., Benotti, M.J., 2007. Pharmaceuticals in On-site Sewage Effluent and Ground Water, Western Montana. 45:pp. 263–271. https://doi.org/10.1111/j.1745-6584.2006.00288.x.

Gower, J.C., 1982. Euclidean distance geometry. Math. Sci. 7, 1–14.

Han, S., Choi, K., Kim, J., Ji, K., Kim, S., Ahn, B., Yun, J., Choi, K., Khim, J.S., Zhang, X., Giesy, J. P., 2010. Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*. Aquat. Toxicol. 98:256–264. https://doi.org/10.1016/j.aquatox.2010.02.013.

Helsel, D.R., 2012. Statistics for Censored Environmental Data Using Minitab and R. John Wiley & Sons, Inc., Hoboken, NJ.

Jantrania, A.R., Gross, M.A., 2006. Advanced Onsite Wastewater Systems Technologies. CRC Press.
Jones, P.M., 2006. Ground-Water/Surface-Water Interaction in Nearshore Areas of Three Lakes on the Grand Portage Reservation, Northeastern Minnesota, 2003–04 Scientific Investigations Report 2006–5034. Reston, VA.
Kahle, M., Buerge, I.J., Müller, M.D., Poiger, T., 2009. Hydrophilic anthropogenic markers for quantification of wastewater contamination in ground- and surface waters. Environ. Toxicol. Chem. 28:2528. https://doi.org/10.1897/08-606.1.
Katz, B.G., Eberts, S.M., Kauffman, L.J., 2011. Using Cl/Br Ratios and Other Indicators to Assess Potential Impacts on Groundwater Quality From Septic Systems: A Review and Examples From Principal Aquifers in the United States. 397:pp. 151–166. https://doi.org/10.1016/j.jhydrol.2010.11.017.
Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick, R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. U. S. A. 104:8897–8901. https://doi.org/10.1073/pnas.0609568104.
Kim, H.-J., Park, Y.I., Dong, M.-S., 2005. Effects of 2,4-D and DCP on the DHT-induced androgenic action in human prostate cancer cells. Toxicol. Sci. 88:52–59. https://doi.org/10.1093/toxsci/kfi287.
Knapp, R., Neff, B.D., 2007. Steroid hormones in bluegill, a species with male alternative reproductive tactics including female mimicry. Biol. Lett. 3:628–631. https://doi.org/10.1098/rsbl.2007.0379.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H. T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: a National Reconnaissance. Environ. Sci. Technol. 36, 1202–1211.
Lapworth, D.J., Baran, N., Stuart, M.E., Ward, R.S., 2012. Emerging organic contaminants in groundwater: a review of sources, fate and occurrence. Environ. Pollut. 163:287–303. https://doi.org/10.1016/j.envpol.2011.12.034.
Lee, L., 2017. NADA: Nondetects and Data Analysis for Environmental Data.
Lujan Jr., M., Peck, D.L., 1992. Ground Water Atlas of the United States: Hydrologic Investigations Atlas 730-J. Reston, VA.
Maps, G., 2017. Google Maps [WWW Document]. Retrieved from. https://www.google.com/maps/place/Minnesota/@46.3540035,-97.848883,6z/data=!3m1!4b1!4m5!3m4!1s0x4d585b9a60780b9b:0x2a2c99b10fea20f!8m2!3d46.729553!4d-94.6858998, Accessed date: 11 April 2017.
Massmann, G., Dünnbier, U., Heberer, T., Taute, T., 2008. Behaviour and redox sensitivity of pharmaceutical residues during bank filtration - investigation of residues of phenazone-type analgesics. Chemosphere 71:1476–1485. https://doi.org/10.1016/j.chemosphere.2007.12.017.
McCarty, L.S., Borgert, C.J., 2006. Review of the toxicity of chemical mixtures: theory, policy, and regulatory practice. Regul. Toxicol. Pharmacol. 45:119–143. https://doi.org/10.1016/j.yrtph.2006.03.004.
Minnesota Department of Natural Resources, 2017. LakeFinder: Minnesota DNR [WWW Document]. URL. http://www.dnr.state.mn.us/lakefind/index.html, Accessed date: 3 August 2017.
Mnif, W., Hassine, A.I.H., Bouaziz, A., Bartegi, A., Thomas, O., Roig, B., 2011. Effect of endocrine disruptor pesticides: a review. Int. J. Environ. Res. Public Health 8:2265–2303. https://doi.org/10.3390/ijerph8062265.
Oropesa, A.L., Floro, A.M., Palma, P., 2016. Assessment of the effects of the carbamazepine on the endogenous endocrine system of *Daphnia magna*. Environ. Sci. Pollut. Res. 23: 17311–17321. https://doi.org/10.1007/s11356-016-6907-7.
Ortiz de García, S.A., Pinto, G.P., García-Encina, P.A., Irusta-Mata, R., 2014. Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. Ecotoxicity 23:1517–1533. https://doi.org/10.1007/s10646-014-1293-8.
Peterson, F., Risberg, J., 2009. Low Dissolved Oxygen in Water - Causes, Impact on Aquatic Life - An Overview.
Phillips, P.J., Schubert, C., Argue, D., Fisher, I., Furlong, E.T., Foreman, W., Gray, J., Chalmers, A., 2015. Science of the Total Environment Concentrations of Hormones, Pharmaceuticals and other Micropollutants in groundwater Affected by Septic Systems in New England and New York. 513 pp. 43–54.
Robinson, C., Schultz, P., 2015. 2015 SSTS Annual Report Subsurface Sewage Treatment Systems in Minnesota. Minnesota Pollution Control Agency Photo Credit.
Rstudio Team, 2015. RStudio: Integrated Development for R.
Sanders, L.L., 1998. A Manual of Field Hydrology. Prentice-Hall, NJ.
Schaider, L.A., Rudel, R.A., Ackerman, J.M., Dunagan, S.C., Brody, J.G., 2014. Pharmaceuticals, perfluorosurfactants, and other organic wastewater compounds in public drinking water wells in a shallow sand and gravel aquifer. Sci. Total Environ. 468: 384–393. https://doi.org/10.1016/j.scitotenv.2013.08.067.
Schaider, L.A., Rodgers, K.M., Rudel, R.A., 2017. Review of organic wastewater compound concentrations and removal in onsite wastewater treatment systems. Environ. Sci. Technol. 51:7304–7317. https://doi.org/10.1021/acs.est.6b04778.
Schultz, M.M., Minarik, T.A., Martinovic-Weigelt, D., Curran, E.M., Bartell, S.E., Schoenfuss, H.L., 2013. Environmental estrogens in an urban aquatic ecosystem: II. Biological effects. Environ. Int. 61:138–149. https://doi.org/10.1016/j.envint.2013.08.006.
Schymanski, E.L., Singer, H.P., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., Thomaidis, N.S., Bletsou, A., Zwiener, C., Ibáñez, M., Portolés, T., de Boer, R., Reid, M.J., Onghena, M., Kunkel, U., Schulz, W., Guillon, A., Noyon, N., Leroy, G., Bados, P., Bogialli, S., Stipaničev, D., Rostkowski, P., Hollender, J., 2015. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. Anal. Bioanal. Chem. 407: 6237–6255. https://doi.org/10.1007/s00216-015-8681-7.
Söffker, M., Tyler, C.R., Söffker, M., Tyler, C.R., 2015. Endocrine Disrupting Chemicals and Sexual Behaviors in Fish – A Critical Review on Effects and Possible Consequences Fish. :p. 8444 https://doi.org/10.3109/10408444.2012.692114.
Stanford, B.D., Amoozegar, A., Weinberg, H.S., 2010. The impact of co-contaminants and septic system effluent quality on the transport of estrogens and nonylphenols through soil. Water Res. 44, 1598–1606.
Subedi, B., Codru, N., Dziewulski, D.M., Wilson, L.R., Xue, J., Yun, S., Braun-Howland, E., Minihane, C., Kannan, K., 2015. A pilot study on the assessment of trace organic contaminants including pharmaceuticals and personal care products from on-site wastewater treatment systems along Skaneateles Lake in New York State, USA. Water Res. 72:28–39. https://doi.org/10.1016/j.watres.2014.10.049.
Teerlink, J., Hering, A.S., Higgins, C.P., Drewes, J.E., 2012a. Variability of trace organic chemical concentrations in raw wastewater at three distinct sewershed scales. Water Res. 46:3261–3271. https://doi.org/10.1016/j.watres.2012.03.018.
Teerlink, J., Martínez-Herná Ndez, V., Higgins, C.P., Drewes, J.E., 2012b. Removal of trace organic chemicals in onsite wastewater soil treatment units: a laboratory experiment. WR 46:5174–5184. https://doi.org/10.1016/j.watres.2012.06.024.
Tran, N.H., Li, J., Hu, J., Ong, S.L., 2014. Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater. Environ. Sci. Pollut. Res. 21:4727–4740. https://doi.org/10.1007/s11356-013-2428-9.
U.S. Environmental Protection Agency, 1996. Nonpoint Source Pollution: The Nation's Largest Water Quality Problem. Washington, DC.
U.S. Environmental Protection Agency, 2013. Level III Ecoregions of the Continental United States. Corvallis, Oregon.
U.S. Environmental Protection Agency, 2014. Annual Report 2013: Decentralized Wastewater Management Program Highlights.
U.S. Environmental Protection Agency, 2017. Endocrine Disruptor Screening Program for the 21st Century. [WWW Document]. URL. https://actor.epa.gov/edsp21/, Accessed date: 1 November 2018.
Weeks, J., Guiney, P., Nikiforov, A., 2012. Assessment of the environmental fate and ecotoxicity of *N*,*N*-diethyl-m-toluamide (DEET). Integr. Environ. Assess. Manag. 8: 120–134. https://doi.org/10.1002/ieam.1246.
West, M., 2008. Soil-Based Sewage Treatment Systems. St. Paul, MN.
Winter, T.C., Harvey, J.W., Franke, O.L., Alley, W.M., 1999. Ground water and surface water: a single resource. U.S. Geol. Surv. Circ. 1139.
Wode, F., Van Baar, P., Dü Nnbier, U., Hecht, F., Taute, T., Jekel, M., Reemtsma, T., 2015. Search for over 2000 current and legacy micropollutants on a wastewater infiltration site with a UPLC-high resolution MS target screening method. Water Res. 69: 274–283. https://doi.org/10.1016/j.watres.2014.11.034.
Woods, W.G., 1994. An introduction to boron: history, sources, uses, and chemistry. Environ. Health Perspect. 102 (Suppl. 7), 5–11.
Writer, J.H., Barber, L.B., Brown, G.K., Taylor, H.E., Kiesling, R.L., Ferrey, M.L., Jahns, N.D., Bartell, S.E., Schoenfuss, H.L., 2010. Science of the Total Environment Anthropogenic tracers, endocrine disrupting chemicals, and endocrine disruption in Minnesota lakes. Sci. Total Environ. 409:100–111. https://doi.org/10.1016/j.scitotenv.2010.07.018.
Yates, M.V., 1985. Septic tank density and ground-water contamination. Ground Water 23:586–591. https://doi.org/10.1111/j.1745-6584.1985.tb01506.x.
Zaharin Aris, A., Soraya Shamsuddin, A., Mangala Praveena, S., 2014. Occurrence of 17α-ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. Environ. Int. 69:104–119. https://doi.org/10.1016/j.envint.2014.04.011.