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δ^{15}N tracks changes in the assimilation of sewage-derived nutrients into a riverine food web before and after major process alterations at two municipal wastewater treatment plants

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ABSTRACT

Stable isotopes (δ^{15}N and δ^{13}C) were used to assess the changes in exposure and assimilation of sewage-derived nutrients in an aquatic food web following changes in effluent quality over an 8 year period at two municipal wastewater treatment plants (WWTPs) that discharge to the Grand River, in southern Ontario. Upgrades at the Kitchener WWTP started in late 2012 to enhance nitrification, while the Waterloo WWTP had a series of construction issues at the plant that resulted in a deterioration of its effluent quality over the study period (2007–2014). Fish (rainbow darter, *Etheostoma caeruleum*) and primary consumers (benthic invertebrates) were sampled in the receiving waters associated with each outfall. Upgrades at the Kitchener WWTP resulted in improved effluent quality with total annual ammonia output dropping by nearly sixfold (583–100 t), while the Waterloo WWTP increased its total annual ammonia output by nearly fourfold (135–500 t) over the duration of the study. Downstream of the Kitchener WWTP, the reduction in total ammonia output negatively correlated with changes in δ^{15}N of rainbow darter from being depleted (prior to the upgrade) to reflecting signatures similar to those at the upstream reference site. The biota downstream of the Waterloo WWTP showed the opposite trend, going from slightly enriched, to being depleted relative to the upstream reference sites. δ^{13}C was consistently higher downstream of both WWTPs regardless of changing effluent quality, and annual variability in δ^{13}C was associated with annual river discharge. In a laboratory based dietary switch study conducted with rainbow darter, the isotope half-life in muscle (29 days for δ^{15}N and 33 days for δ^{13}C) were determined and these rapid changes were consistent with responses in muscle of wild fish. This is a unique study that was able to contrast two WWTPs in the same watershed as they underwent major changes in treatment processes. Stable isotopes were very effective as a tool to trace the changes in aquatic biota due to changes in wastewater effluent quality, both improvements and deterioration over time.

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1. Introduction

Municipal wastewater treatment plants (WWTPs) discharge among the highest volumes of effluent compared to other industries in Canada (Chambers et al., 1997). WWTP effluents contain a mixture of chemicals including total suspended solids (TSS), nutrients (phosphorous and nitrogen products), metals, and pharmaceuticals and personal care products (Chambers et al., 1997; Daughton and Ternes, 1999; Metcalfe et al., 2003; Lishman et al., 2006). Environmental impacts associated with municipal WWTP effluents released into aquatic environments have been associated with eutrophication and oxygen depletion (Gücker et al., 2006; Carey and Migliaccio, 2009; Kiedrzyńska et al., 2014), endocrine disruption (Jobling et al., 1998; Tetreault et al., 2011), impacts on fish assemblages (Tetreault et al., 2013), and alterations of food webs (deBruyn et al., 2003).

Stable isotope ratios of carbon (δ^{13}C) and more commonly nitrogen (δ^{15}N) have successfully been used to track the exposure and assimilation of sewage-derived nutrients into aquatic food webs (deBruyn and Rasmussen, 2002; Morrissey et al., 2013; Loomer

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et al., 2015). Wastewater constituents enter the aquatic food web through ingestion of particulate organic matter by consumers or through the uptake of sewage-derived inorganic nutrients by primary producers (Tucker et al., 1999). $\delta^{15}N$ measured in organisms exposed to WWTP effluent will depend on the treatment processes utilized at the plant, final effluent quality, and the characteristics of the receiving environment. Organisms exposed to secondary or greater treated effluent typically results in enriched $\delta^{15}N$ values (Gaston et al., 2004; Morrissey et al., 2013; Robinson et al., 2016). This is because nitrification and denitrification processes associated with secondary treatment tend to result in the accumulation of the heavier nitrogen isotope, ^{15}N (Heaton, 1986). A lack of nitrification and denitrification processes (e.g. in raw sewage or primary treated effluent) usually result in an accumulating pool of ammonia depleted in ^{15}N and when released into the receiving environment, and primary producers will preferentially take up $^{14}NH_4$ over $^{15}NH_4$ (Birgand et al., 2007), resulting in organisms depleted in ^{15}N (deBruyn and Rasmussen, 2002; Gaston and Suthers, 2004; Daskin et al., 2008). The carbon discharged from municipal WWTP effluent is primarily terrestrial in origin which has a relatively constant $\delta^{13}C$ value of about −28‰, hence it is possibly discriminated from aquatically derived (autotrophic) sources which can range between −40 and −20‰ (France, 1995).

The Grand River watershed is the largest drainage basin in southern Ontario, Canada, which flows into the northeastern part of Lake Erie. This watershed assimilates effluent from 30 municipal WWTPs serving almost one million people. The largest WWTPs, Kitchener and Waterloo, (collectively serving >370,000 people in 2014), both use secondary conventional activated sludge processes, and discharge into the central reaches of the Grand River. The effluents from these WWTPs have been historically associated with poor water quality in the receiving environment including hypoxic river conditions (Venkiteswaran et al., 2015), unionized ammonia concentrations above the provincial water quality objective (>0.0165 mg/L) (Loomer and Cooke, 2011), and the presence of elevated levels of selected pharmaceuticals (Arlos et al., 2015). Impacts on fish downstream of these WWTPs include the feminization (Tetreault et al., 2011; Tanna et al., 2013; Bahamonde et al., 2015) and reduced reproductive success (Fuzzen et al., 2015) of male rainbow darter (*Etheostoma caeruleum*). A study conducted in 2007 by Loomer et al. (2015), documented changes in $\delta^{13}C$ and $\delta^{15}N$, in rainbow darter and primary consumers exposed to these WWTP effluents in the Grand River. Exposure to the poorly treated (non-nitrifying) Kitchener effluent resulted in a decrease in $\delta^{15}N$, while exposure to the effluent at the Waterloo WWTP (higher quality effluent with partial nitrification at the time) resulted in little to no change (Loomer et al., 2015). Major planned upgrades at both the Kitchener and Waterloo WWTPs created a unique opportunity to examine how changes in effluent quality impacted the stable isotope ratios of fish (rainbow darter) and primary consumers (benthic invertebrates).

The major planned upgrades at the Waterloo and Kitchener WWTPs were to convert them from carbonaceous activated sludge treatment (primarily for BOD removal) to fully nitrifying activated sludge. In August 2012, the Kitchener WWTP had initiated its upgrades for nitrification, and by January 2013 it achieved full nitrification. Nitrification was achieved by retrofitting the current WWTP with return activated sludge (RAS) reaeration and replacing the old aeration system with more efficient fine bubblers (Table 1) (Bicudo et al., 2016). At the same time, the Waterloo WWTP initiated upgrades, but a number of changes and construction issues led to a decrease in effluent quality (e.g. increasing total ammonia) over several years. Similar to the Kitchener WWTP, the Waterloo WWTP was retrofit with RAS reaeration in 2014; however, fine bubblers had not been installed to achieve full nitrification (Table 1) (Region of Waterloo, 2016).

The primary objective of the present study was to assess how the changing effluent quality at two WWTPs altered the stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) throughout an aquatic food web, using two trophic levels, primary consumers (benthic invertebrates) and a secondary consumer (rainbow darter). The rainbow darter was selected for this study since it had been used as a sentinel species in a variety of recent biomonitoring studies in the Grand River (Tetreault et al., 2011; Tanna et al., 2013; Bahamonde et al., 2015). Using new collections, archived samples, and previously published data, the patterns of stable isotopes in rainbow darter and selected primary consumers collected adjacent to the Waterloo and Kitchener WWTPs were assessed before and after the process changes (2007–2014). There were two specific research questions addressed in this study. The first question was to test whether a difference could be detected in $\delta^{15}N$ and $\delta^{13}C$ in fish and primary consumers before and after the Kitchener WWTP upgrade and in the years the Waterloo WWTP had deteriorating effluent quality. The second question was to test whether any changes in $\delta^{15}N$ and $\delta^{13}C$ could be linked to changing effluent quality. To help with the interpretation of the isotope data, a laboratory-based diet switch experiment was conducted with rainbow darter to estimate the relative isotopic turnover rate in muscle and liver tissues. The contrasting changes in effluent quality at the Kitchener and Waterloo WWTPs, with either improvements or deteriorations over time, provided a unique opportunity to follow these changes, and how they may alter the flow of nutrients in a riverine food web.

2. Materials and methods

2.1. Sampling sites

Sampling sites selected for this study were based on previously published or unpublished studies related to the impacts of municipal WWTPs on rainbow darter in the Grand River, Ontario, Canada (Tetreault et al., 2011; Bahamonde et al., 2015; Fuzzen et al., 2015; Loomer et al., 2015). These sites were selected due to their proximity to the Kitchener and Waterloo WWTP outfalls and to represent similar riffle/run habitats (Fig. 1). This study comprised a total of nine sites all located on the Grand River and spanning a distance of 60 km from the furthest upstream to the furthest downstream site (Fig. 1). These sites were sampled between 2007 and 2014 in spring and/or fall seasons, however fish and primary consumer samples from archived collections (2007 to spring 2013) were not always available for all sites in every year/seasons due different study objectives (Table S1).

Two of the nine sites (REF 1 and REF 2) in this study were in non-urban environments, outside of the Kitchener and Waterloo city limits. These sites were included in this study to characterize any change related to urbanization and also to characterize spatial heterogeneity typical of a river system (Vannote et al., 1980). The first urban reference site (REF 3) is located 5 km above the Waterloo WWTP outfall. The first near-field exposure site (DSW 1) is located 1 km downstream from the Waterloo WWTP outfall. There are two sites located further downstream from the Waterloo WWTP but upstream of the Kitchener WWTP. INT 1, which is 12 km downstream from the Waterloo WWTP outfall and INT 2 which is located 19 km downstream from the Waterloo WWTP outfall and 1 km upstream from the Kitchener WWTP outfall. There are three additional exposure sites each located at 0.5 km (DSK 1), 1.5 km (DSK 2), and 5 km (DSK 3) downstream of the Kitchener WWTP outfall.

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