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The effects of urbanization on trophic relationships in constructed wetlands

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Abstract: Constructed stormwater wetlands are one strategy for mitigating the negative effects of urbanization on aquatic ecosystems. However, the biotic community in these wetlands generally is dominated by organisms able to tolerate poor water quality. Reduced macroinvertebrate diversity and abundance in comparison to natural wetlands, and prevalence of invasive species, such as Gambusia, can influence the flow of energy through food webs. We used stable-isotope analysis (δ¹³C and δ¹⁵N) of food webs to assess whether the amount of catchment urbanization (total imperviousness [imperviousness] = % catchment covered in impervious surfaces) influenced basal resources and trophic relationships in constructed wetlands in Melbourne, Australia. As imperviousness increased, the abundance and diversity of macroinvertebrates decreased significantly and the values of δ¹³C and δ¹⁵N recorded for fishes and macroinvertebrates increased significantly. An increase in imperviousness was associated with a decrease in the mean trophic position of fishes and an increase in the mean trophic position of macroinvertebrates. Our results suggest that sources of C differed between sites of low and high imperviousness and that N sources increased with increasing imperviousness. Our study provides an understanding of the likely consequences of disturbance associated with urbanization on the foodweb structure of constructed wetlands.

Key words: constructed wetlands, total imperviousness, macroinvertebrates, fish, food web, stable isotope analysis

Urban development causes considerable degradation in aquatic ecosystems worldwide, and managing the water cycle in urban landscapes is a significant research frontier (Smucker and Detenbeck 2014). Sixty-six percent of the world’s population is expected to inhabit urban areas by 2050 (United Nations 2014). The correlated increase in extent of urban areas will alter patterns of hydrology, local climate, and biochemical processes and will affect natural ecosystems globally (Grimm et al. 2008, Smucker and Detenbeck 2014, United Nations 2014).

Constructing wetlands to intercept stormwater is one management strategy to mitigate the negative effects of urbanization on aquatic ecosystems (Hughes et al. 2014, Smucker and Detenbeck 2014). Constructed wetlands are engineered solutions designed to reduce the flashiness of storm flows and reduce the passage of nutrients, sediment, and pollutants to downstream ecosystems (Zhang et al. 2012, Smucker and Detenbeck 2014). Pollutants are removed by various physical, chemical, and biological processes in constructed wetlands (Brauman et al. 2007, Zhang et al. 2012). In addition, constructed wetlands provide habitat for native wildlife, including aquatic invertebrates, fish, and water birds because they are designed to mimic the processes that operate in natural wetlands (Ehrenfeld 2000, Sundaravadiel and Vigneswaran 2001, Grimm et al. 2008).

The amount of stormwater entering aquatic systems is related to the percentage of the area of a catchment covered by impervious surfaces, such as paved areas and roofs (total imperviousness; hereafter referred to as imperviousness) (Morse et al. 2003, Walsh et al. 2005a, Cuffney et al. 2010). In stream systems, an increase in imperviousness causes a decrease in the richness and abundance of fishes and macroinvertebrates (Walsh et al. 2001, Grimm et al. 2008, Eitzmann and Paukert 2010). The mechanisms that underlie these effects are a combination of altered hydrology, resulting in flashier flows, and increased pollutant loads (Walsh et al. 2005b). Imperviousness is an important variable to consider when designing constructed wetlands because it can indicate pollutant-loading rate and predict the hydraulic effectiveness of the wetland (Melbourne Water 2015). Imperviousness is a surrogate measure that represents a simplification of underlying processes, but it can be used as an index to indicate the loading rate of contaminants entering a wetland because these contaminants are
sourced primarily from impervious surfaces (Schiff and Benoit 2007).

Constructed wetlands provide a habitat for native macroinvertebrates and fish, but these systems are usually dominated by organisms that can tolerate poor water quality. The organisms include macroinvertebrates, such as chironomid midges and oligochaete worms, and nonnative fishes, such as Common Carp, *Cyprinus carpio* and Mosquito Fish, *Gambusia holbrooki* (Batty et al. 2005, Pyke 2008, O’Connor et al. 2012, Brown and Morgan 2015). In aquatic systems, low macroinvertebrate richness and abundance and the prevalence of invasive species, such as *G. holbrooki*, can alter aquatic food webs by modifying the flow of energy (Pankratz et al. 2007, Eitzmann and Paukert 2010, Ho et al. 2011). For example, *Gambusia* can cause a shift in numerical dominance from larger to smaller zooplankton species via selective feeding on larger species (Pen and Potter 1991, Pyke 2008). Anthropogenic activities can affect aquatic systems in a multitude of ways. For example, nutrient inputs can increase primary productivity, potentially increasing energy supply to higher trophic levels (Freedman et al. 2012). Urbanization and its associated decrease in water quality can cause food chains to lengthen as a consequence of higher energy supply or to shorten as a result of the loss of large-bodied and more sensitive predatory taxa (Cabana and Rasmussen 1994, Schriever and Williams 2013). Furthermore, changes in food webs can be used to predict the consequences of disturbance on ecosystems (Carscallen et al. 2012).

Authors of several studies have described the effects of urbanization on food webs in aquatic systems (Eitzmann and Paukert 2010, Freedman et al. 2012, Hirama et al. 2014). However, few investigators have studied foodweb structure in constructed wetlands (Jackson et al. 2013). This type of research is important because stormwater entering constructed wetlands can contain nutrients, contaminants, and pollutants, all of which can alter food webs. A large number of wetlands have been constructed in Melbourne, a major city in southeastern Australia, for the purpose of reducing N, P, suspended solids, and heavy metals discharging into downstream waters and entering a large marine embayment, Port Phillip Bay (Melbourne Water 2005). Information exists on the abundance and diversity of taxa in these wetlands (Mackintosh et al. 2015), but less is known about the drivers of foodweb structure in these systems. Stable isotope analysis (SIA) is a useful tool for quantifying food webs and assessing the effects of urbanization on watersheds (Steffy and Kilham 2004). Isotopic values (δ13C and δ15N) generally reflect the isotopic value of organic matter assimilated by organisms, and SIA can be used to trace the flow of organic matter through food webs (Hamilton et al. 1992).

The objective of our study was to assess whether the amount of catchment urbanization (as imperviousness) influences trophic relationships in the aquatic food webs of constructed wetlands in Melbourne, Australia. We addressed the following questions: 1) Does increasing imperviousness alter the richness, evenness, and abundance of macroinvertebrates and fish? 2) Does increasing imperviousness alter the basal sources (as estimated by δ13C signatures) available to macroinvertebrates and fish in constructed wetlands? 3) Does increasing imperviousness alter the trophic position of invertebrates and fish (as estimated by δ15N signatures) in constructed wetlands?

We predicted that an increase in imperviousness would increase the volume of stormwater and associated pollutants entering constructed urban wetlands, thereby reducing the richness and abundance of aquatic invertebrates and fish. We also predicted that moderate levels of imperviousness would lengthen food chains because of an increase in productivity (Steffy and Kilham 2004), but that high levels would shorten food chains as a consequence of the loss of large-bodied species.

**METHODS**

**Site selection and assessment of imperviousness**

We conducted this study in the Melbourne metropolitan area, an area of 9800 km2 in southeastern Australia with a population of 3.81 million people (73% of the inhabitants of the state of Victoria). Land use includes residential, industrial, and commercial areas (ABS 2013). The underlying lithology of the region comprises 2 distinct geological regions, basalt in the west and sedimentary material in the east and south, which has been shown to influence aquatic communities (Pettigrove and Hoffman 2003).

We examined 9 constructed wetlands situated across the western basalt region. The constructed wetlands were built between 1997 and 2004 and are managed by a single water utility authority (Melbourne Water). The design objective for the construction of the wetlands was largely to manage N (Melbourne Water 2005). Wetlands design is consistent, with water flowing into an inlet zone, then through a gross pollutant trap, a ponding area, a vegetated zone, and an outlet pond before returning to the stream (Fig. 1). Constructed wetlands vary in size because they are designed to a size specification that depends on the volume and concentration of incoming water in a catchment. However, they are often retrofits or constructed in ‘leftover’ space, which can also determine size. The constructed wetlands in our study have been designed to be either ‘online’ or ‘offline’ (see Mackintosh et al. 2015). Online sites were built on an existing watercourse, and all stormwater entering the system is treated by the wetland. At offline sites, stormwater is diverted from an existing waterway into the wetland during a rain event. Once a maximum level is reached and a risk exists for flooding beyond the wetland margins, water is diverted to a high-flow bypass and returned to the river channel (Fig. 1) (Melbourne Water 2005, Mackintosh et al. 2015).
The 9 wetlands were situated in predominantly urban catchments. Sites were chosen based on previously published studies and had heavy-metal-contaminated sediment (Pettigrove and Hoffmann 2003, Carew et al. 2007). We chose wetlands along a gradient of subcatchment imperviousness calculated by applying the method of Walsh and Kunapo (2009) to data supplied by Melbourne Water. We used ArcGIS (version 10; Environmental Systems Research Institute, Redlands, California) software to calculate the percentage of the catchment upstream of wetlands covered by sealed surfaces, including roads, paths, and buildings. Imperviousness values ranged from 4 to 47% for the 9 wetlands (see Mackintosh et al. 2015). We sampled sites once between November 2011 and January 2012. These dates were selected to encompass the period of maximum abundance of common macroinvertebrate taxa.

Environmental variables
Turbidity, pH, conductivity, dissolved O2 (DO), and temperature were recorded in situ at each site with a Horiba U50 Water Quality Checker (Horiba, Kyoto, Japan) once in the outlet zone of each wetland between November 2011 and January 2012. These spot water measurements were made between 1000 and 1600 h. We used Google™ Earth Pro software (version 7.1.2.2014; Google, Mountain View, California; Map data: Google SKM 2013).

Collection of basal resources and aquatic consumers
We collected macroinvertebrates with a long-handled sweep net (210- × 240-mm, 250-µm mesh). We took 3- × 10-m sweeps from the littoral zone, the region considered to contain the maximum number of macroinvertebrate species and individuals. Upon collection, we stored samples in Ziploc® bags on ice for transport to the laboratory where we froze them for later identification with the aid of compound and dissecting microscopes. Most invertebrates in each sample were identified to genus (~95% of taxa) or to family (when genus-level information were not available) with reference to Davis and Christidis (1997) and Gooderham and Tsyrilin (2002).

We set 2 unbaited single-winged fyke nets (4-mm mesh, 5-m wing, 60-cm drop) and five 3-mm-mesh standard bait traps (45 × 25 × 25 cm) overnight (12–14 h soak time) in each wetland to sample fish communities. The native species captured were Anguilla australis, Philypnodon grandiceps, and Galaxias maculatus. Nonnative species captured were Cyprinus carpio, Gambusia holbrooki, Perca fluviatilis, Carassius auratus, Tinca, and Misgurnus anguillicaudatus. We also captured freshwater crayfish, Cherax destructor, and eastern long-necked turtles, Chelodina longicollis. The latter were removed from nets and released immediately. All other individuals were removed from the nets and euthanized immediately with a lethal concentration of clove oil in accordance with approved animal ethics procedures (BSCI/2011/29). We stored all specimens in plastic bags and froze them for later processing.

We collected the dominant sources of organic matter at each wetland as follows. Fringing vegetation and emergent and submerged macrophytes were collected by hand. Periphyton were washed from submersed plant parts by gentle agitation and brushing until biofilm was no longer evident on the surfaces. Coarse particulate organic matter (CPOM) was collected with a long-handled net as part of the macroinvertebrate sampling process. Phytoplankton was not sampled because concentrations in these benthic-dominated systems are extremely low.
Sample processing

We rinsed periphyton, fringing vegetation, and submerged and emergent macrophyte samples with distilled water in the laboratory to remove dirt and debris. We used a 500-μm sieve to separate the CPOM fraction from other benthos. All organic material was oven dried at 60°C for ≥ 72 h. We ground plant samples (leaves only) in a coffee grinder or mill grinder for ~3 min or until the sample had been reduced to a fine powder. A subsample of this powder was used for the stable-isotope analysis. We thoroughly cleaned and rinsed all equipment with distilled water between processing of each type of plant species.

We defrosted, rinsed, and dried all aquatic macroinvertebrates before grinding them with a mortar and pestle. With the exception of predators, individuals were ground whole. We removed the heads, cerci, and gut contents of predators before processing them. We removed guts from only predators because Jardine et al. (2005) suggested that clearance or removal of gut contents in primary consumers is not necessary because the volume of gut contents is minimal relative to the whole animal and the gut material has low C and N content. We pooled ground individuals collected from the same location and sampling date to ensure that sufficient mass of each genus or family was available for stable-isotope analysis. The exoskeletons of all aquatic crustaceans were removed (before drying) to ensure that accumulated calcium carbonate did not influence C isotope values. C incorporated into carbonate must be removed before analysis because it is of a different origin than C in tissue and differs in δ13C (Jacob et al. 2005).

We defrosted fish and decapods, measured them to the nearest 0.1 mm (standard length for fishes and total length for decapods), and weighed them to the nearest gram. We sampled dorsal muscle of fish and abdominal muscle of decapods. We pooled up to 3 similar-sized individuals of *G. holbrooki* per sample to ensure adequate sampling mass. We removed muscle tissue by dissection as outlined by Zeug and Winemiller (2008) and placed muscle samples in acid-washed 50-mL plastic sample containers for freeze drying at −60 to −70°C for 5 d in a Dynavac FD12 freeze dryer (Dynavac, Hingham, Massachusetts). After drying, we ground fish tissue to a fine powder with a coffee or mill grinder depending on the size of the sample.

We weighed ground samples to the nearest 0.001 mg and encapsulated them in preweighed 8- × 5-mm Sn capsules for measurement of δ13C, δ15N, and C and N concentration. The samples were analyzed at the Water Studies Centre (Monash University) on an ANCA GSL2 elemental analyzer interfaced to a Hydra 20-22 continuous-flow isotope ratio mass-spectrometer (Sercon, Crewe, UK). The precision of the elemental analysis was 0.5 μg for both C and N (n = 5). The precision of the stable-isotope analysis was ±0.1%o for 13C and ±0.2%o for 15N (SD for n = 5). Precision values are based on bream and gelatin, and referenced against IAEA7, USGS40, and USGS41.

Stable-isotope data are expressed in the δ notation (δ13C and δ15N), relative to the stable isotopic ratio of Vienna Pee Dee Belemnite standard (RVPDB = 0.0111797) for C and atmospheric N (RAir = 0.0036765). Stable-isotope results are expressed as per mil (%o) deviations from the standards, as defined by the equation:

\[ \delta^{13}C \text{ or } \delta^{15}N = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \]  

where \( R = ^{13}C/^{12}C \) for C and \( ^{15}N/^{14}N \) for N.

The isotopic value of δ13C undergoes little change and can be used to identify the sources of organic material supporting consumers. In contrast, δ15N becomes more enriched in food webs, so values can be used to estimate trophic position (Post 2002).

Data analysis

We constructed and visually interpreted foodweb biplots based on isotopic signatures across pools. Trophic position estimates were based on fractionation of δ15N between consumers and basal production sources, and trophic position was calculated as:

\[ \text{trophic position} = \lambda + (\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{base}})/\Delta_n \]  

where \( \lambda = \) trophic position of the organism used to estimate \( \delta^{15}N_{\text{base}} \) (e.g., \( \lambda = 1 \) for primary producers) and \( \Delta_n \) is the enrichment per trophic level (Post 2002). The trophic fractionation of N \( \Delta_n \) is generally assumed to be between 3 and 4‰, so we used an enrichment value of 3.4‰ based on empirical review by Post (2002). This fractionation value has been used in other studies to estimate trophic position of consumers (e.g., Vander Zanden et al. 1999, Anderson and Cabana 2007). \( \delta^{15}N_{\text{base}} \) values were calculated separately for each habitat to ensure that estimates of trophic position were comparable among the 9 study wetlands (Zeug and Winemiller 2008).

We did not apply mixing models because of the absence of a clear difference among the signatures of basal resources (error bars overlapped) and because we did not collect some basal food resources (e.g., zooplankton). Mixing models are based on the assumption that all food sources are included. They yield multiple indistinguishable solutions when basal resources have strongly overlapping signatures (Phillips 2012).

We used PRIMER (version 6) software (PRIMER-E, Plymouth, UK) to calculate evenness based on Pielou’s evenness index.

RESULTS

Physicochemical conditions

Physicochemical conditions varied across wetlands. Values for pH ranged from 6.62 to 8.33, conductivities ranged from...
from 162 to 1757 μS/cm, and turbidities ranged from 5.3 to 364.5 NTUs. Imperviousness ranged from 4 to 47% (see Mackintosh et al. 2015). Imperviousness did not affect these 3 water-quality variables.

Agricultural communities

The basal resources commonly recorded at each site included the common reed (Phragmites australis), broadleaf cumbungi (Typha orientalis), and river club rush (Schoenoplectus validus). Water-ribbon (Triglochin procerum) occurred at only 3 wetlands, and its distribution appeared to be unrelated to imperviousness. Slender knotweed (Persicaria decipiens) was the most common fringing species. Terrestrial vegetation was sparse at all sites, and the river red gum (Eucalyptus camaldulensis) was the most common species.

Thirty families of aquatic invertebrates were recorded from the constructed wetlands (Table 1). Chironomidae were found at all sites. Other common taxa included Corixidae, Coenagrionidae, Micronectidae, Notonectidae, Glos-siphonidae, and Physidae. Ordination of macroinvertebrate communities did not show distinct clustering by wetland.

Nine fish species were collected from the constructed wetlands (Table 2). Nonnative fish species were found at all wetlands. Of these non-natives, Gambusia holbrooki was found at the greatest number of sites. With the exception of Merlynton (MS), the site with the highest imperviousness, ≥1 native fish species was found at each wetland. Anguilla australis was the native fish species present at the greatest number of sites.

Stable-isotope analysis

Most consumers had isotopic signatures that were intermediate between the δ13C signatures of periphyton and fringing vegetation (Fig. 2A–I). This result suggests that energy entered the food web primarily via these 2 sources. Most consumer δ15N signatures were higher than ≥1 basal source, but some exceptions did exist. At Shankland (SH; Fig. 2H), Naucoris spp. (Hemiptera) had a more depleted δ15N signature than any producer. At Merlynton (MS; Fig. 2I), Physella (Gastropoda), Micronecta (Hemiptera), and Chironomidae (Diptera) had more depleted δ15N signatures than producers, a result indicating that ≥1 primary producer that had been assimilated was not sampled. Agaptoctoridae spp. (Hemiptera) was more enriched than the fish species at Leamington (LW; Fig. 2E) and Saint Albans (SA; Fig. 2G) wetlands. This unexpected result might indicate that the hemipterans were scavenging on dead fish or killed and ate small fish or other vertebrates.

Predatory and nonpredatory macroinvertebrates had similar δ15N signatures at most wetlands (Fig. 2A–I). However, nonpredatory invertebrate consumer δ13C signatures appeared to occupy intermediate positions between predatory invertebrate consumers at some wetlands sites (PM, CL, SA, and SH) (Fig. 2A, F, G, H). The leech, Placobdelloides, was more 15N-enriched than any other invertebrate taxon at Leamington (LW; Fig. 2E), Cherry Lake (CL; Fig. 2F), Saint Albans (SA; Fig. 2G), and Merlynton (MS; Fig. 2I).

Hemiptera was the dominant predatory taxon (Noto-nectidae, Naucoridae, Belostomatidae, and Corixidae) at most sites. δ13C values ranged from −33.8 to −22.0‰ (Fig. 2A–I). The δ15C signatures of coleopteran predators (Dytiscidae, Hydrophilidae, and Hydraenidae), dipteran predators (Cera-topogonidae and Tabanidae), and odonatan predators (Coenagrionidae, Corduliidae, and Aeshnidae) ranged from −35.4 to −29.3‰. The δ15N signature of Hemipteran predators ranged from 1.1 to 19.8‰, whereas the δ15N signatures of coleopteran, dipteran, and odonatan predators ranged from 0.7 to 12.4‰ (Fig. 2A–I).

Among nonpredatory consumers, δ13C values of Chironomidae ranged from −34.2 to 25.4‰ and of Hemiptera (Sigara sp., juvenile Corixidae, and Micronectidae) ranged from −35.9 to 25.5‰ (Fig. 2A–I). These taxa were predominant at all wetlands. δ15N values of Chironomidae (which included the predatory Tanytarsinae) had the widest range (0.8–15.2‰), whereas values of Hemiptera ranged from 4.3 to 10.9‰.

The native piscivore species (A. australis) was more 13C enriched than the nonnative species (P. fluviatilis) with δ13C values ranging from −23.1 to −26.8‰ and −30.3 to −27.1‰, respectively. The native omnivore species (G. maculatus, P. grandiceps) were more 13C depleted than the nonnative omnivore species (C. auratus, C. carpio, T. tinca) with δ13C values ranging from −33.2 to −28.7‰ and −27.4 to −27.9‰, respectively (Fig. 2A–I).

Consumer δ15N for the native omnivore species (G. maculatus and P. grandiceps) had the greatest range of δ15N of all the fish species at 6.5 to 18.2‰ (Fig. 2A–I). The native piscivore species (A. australis) had a wider range of δ15N values than the nonnative species (P. fluviatilis) at 11.2 to 18.3‰ and 12.2 to 14.0‰, respectively.

Effects of imperviousness

Imperviousness vs density and diversity

As imperviousness increased, both density and number of invertebrate families decreased and evenness (Pielou’s evenness index) increased significantly (density: $R^2 = 0.25, p = 0.008$; number of families: $R^2 = 0.22, p = 0.01$; evenness: $R^2 = 0.56, p < 0.001$, respectively) (Fig. 3A–C). Imperviousness was not significantly related to abundance, number of fish species, or evenness (abundance: $R^2 = 0.11, p = 0.37$; number of species: $R^2 = 0.14, p = 0.31$; evenness: $R^2 = 0.13, p = 0.33$, respectively).

Imperviousness vs δ1C

Imperviousness was significantly positively related to mean δ13C signatures of fish and macro-invertebrates and aquatic basal resources (fish: $R^2 = 0.52, p = 0.02$; macroinvertebrates: $R^2 = 0.36, p = 0.05$; basal re-
sources: $R^2 = 0.53, p = 0.02$; respectively; Fig. 4A–C). The wetland (Pipemaker [PM]) with the lowest imperviousness also contained the most $^{13}$C-depleted fish ($-31.26\%$), whereas macroinvertebrates were most $^{13}$C-depleted at Edge-water (EW) ($-32.84\%$).

**Imperviousness vs $\delta^N$** Imperviousness and mean $\delta^{15}$N signature of fish, macroinvertebrates, and aquatic basal resources were significantly positively related (fish: $R^2 = 0.17, p < 0.001$; macroinvertebrates: $R^2 = 0.37, p < 0.001$; basal resources: $R^2 = 0.30, p < 0.001$) (Fig. 4A–C). The wetland with the lowest imperviousness (PM) contained the most $^{15}$N-depleted fish, macroinvertebrates, and aquatic basal resources (fish: 10.23%; macroinvertebrates: 2.66%, basal resources: 1.96%).

**Imperviousness vs trophic position** The mean trophic position of fish consumers ranged from 2.89 to 4.19. Imperviousness and fish trophic position were significantly negatively related ($R^2 = 0.52, p < 0.001$) (Fig. 5). The mean trophic position of macroinvertebrate consumers ranged from 0.96 to 1.88. Imperviousness and macroinvertebrate trophic position were weakly positively related ($R^2 = 0.10, p < 0.001$).

### Table 1. Summary of macroinvertebrate taxa, number of genera, and number of individuals recorded at constructed wetlands western Melbourne. Predators (PR), gathering collectors (GC), filtering collectors (FC), scrapers (SC), shredders (SH), macrophyte piercers (MP). Classifications after Hamilton et al. (1992) and Goodeham and Tsyrin (2002). Data pooled from 3 samples. Numbers in brackets indicate % catchment covered by impervious surface.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Number of genera</th>
<th>Functional feeding group</th>
<th>PM (4)</th>
<th>JC (15)</th>
<th>TA (20)</th>
<th>EW (20)</th>
<th>LW (23)</th>
<th>CL (38)</th>
<th>SA (40)</th>
<th>SH (45)</th>
<th>MS (47)</th>
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DISCUSSION

All constructed stormwater wetlands in our study were dominated by species of macroinvertebrates and fish that are considered to be tolerant of poor water quality. An increase in the imperviousness of the catchments of these wetlands was associated with a significant decrease in the densities and number of invertebrate families present. Increasing imperviousness was associated with an increase in the $\delta^{13}$C and $\delta^{15}$N values of fish, macroinvertebrates, and aquatic basal resources but not terrestrially derived basal resources (leaf matter). The mean trophic position of macroinvertebrates increased significantly, whereas the mean trophic position of fish decreased significantly with imperviousness.

We found little evidence that urbanization affected the basal energy sources of the food webs. A reduction in habitat heterogeneity, often associated with increasing urbanization, can lead to a narrower range of basal resources, which can cause organisms to feed on similar items because the resource base is less diverse (Eitzmann and Paukert 2010, Calizza et al. 2012). Consumers at some wetlands had $\delta^{13}$C values higher than those of the basal resources. This finding suggests that additional but unknown sources of C were also important food sources, a finding similar to that of Bunn and Boon (1993) who concluded that consumers were being supported by an unknown primary producer in a study of wetlands in New South Wales, Australia. Epiphytic algae can be an important energy source in wetland food webs, but it is often challenging to sample this component effectively to measure stable-isotope signatures (Hamilton et al. 1992, Bunn and Boon 1993, Benetti et al. 2014). Epiphytic algae may have contributed to the unknown $\delta^{13}$C values recorded at the wetlands in our study. Our sampling method collected the entire biofilm associated with aquatic plants, not just epilithic algae, and the biomass of the latter may have been high at other times of the year. Phytoplankton also can be an important energy source in wetlands and might be a potential component of the basal energy sources not accounted for in our study. However, evidence of algal blooms (i.e., large phytoplankton biomass or productivity) is scant in these predominantly turbid wetlands.

The significant decrease in macroinvertebrate richness and abundance and increase in evenness as imperviousness increased was expected. As in urban rivers, the biota of constructed wetlands is generally limited to organisms able to tolerate poor water quality, such as Chironomidae and Corixidae (Pankratz et al. 2007, Awal and Svozil 2010, Mackintosh et al. 2015). Chironomidae, Corixidae, and Micronectidae were major components of the food webs in our study, consistent with degradation of the systems by poor water quality and high inputs of organic matter from human and animal waste (Pettigrove and Hoffmann 2005, Gresens et al. 2007). Larval chironomids provided a food source for predatory aquatic invertebrates (including the Tanytarsineae), and groups such as the damselflies (Xanthagrion spp.) appeared to be transferring energy to some wetland fishes. The exotic and invasive G. holbrooki was the species with a $\delta^{13}$C value consistently most similar to that of the Chironomidae at all wetlands.

The absence of an association between increasing imperviousness and fish species richness and abundance also was expected because all wetlands were dominated by exotic species (mainly G. holbrooki, but the exotic Redfin Perch [Perca fluviatilis] was present in the 2 wetlands with the highest imperviousness). These species are able to survive in degraded conditions (Gkenas et al. 2012). The native Short-Finned Eel Anguilla australis was found at most sites and had higher values for both $\delta^{13}$C and $\delta^{15}$N than the other fish species present. This suggests that A. australis fed on a different C source from that consumed by other fish species. Their higher value for $\delta^{15}$N indicates the pred-


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They are opportunistic, mainly carnivorous feeders, and their prey items include fish, insects, freshwater crayfish, shrimps, mollusks, and frogs.

**Imperviousness vs δ¹³C values**

Our finding that an increase in imperviousness corresponded with a significant increase in the δ¹³C values of fish, macroinvertebrates, and aquatic basal resources, is similar to results of other studies. Eitzmann and Paukert (2010) and Calizza et al. (2012) also found that δ¹³C values were higher in urbanized than in nonurban streams (Eitzmann and Paukert 2010, Calizza et al. 2012). Calizza et al. (2012) attributed this result to an increase in the proportion of aquatic primary producers in the food web. Our findings could be caused by the loss of riparian vegetation associated with urban catchments, resulting in a lower contribution of terrestrial C to wetland food webs. Imberger et al. (2014) found no correlation between increasing urbanization of streams (measured as connectivity to stormwater drains) and detrital and benthic CPOM or other forms of organic material (including algae, macrophytes, and sediments). Furthermore, Olsen et al. (2010) showed that sites with a high degree of urbanization had significantly lower δ¹³C values across all trophic levels. Authors of both studies suggested that differences in δ¹³C

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values may be a result of changes in the relative input of C rather than directly by urbanization. Inputs of allochthonous organic matter may overwhelm an increase in algae or macrophytes, resulting in lower $\delta^{13}$C values across all trophic levels (Olsen et al. 2010, Imberger et al. 2014). The contrast between these results and our findings suggests that aquatic basal resources, rather than terrestrial inputs, are the dominant C source in the wetlands with highest imperviousness.

### Imperviousness vs $\delta^{15}$N values

We found a significant positive relationship between imperviousness and the mean $\delta^{15}$N value of fish, invertebrates, and aquatic basal resources, but not of terrestrially derived basal resources. The absence of a relationship for terrestrially derived basal resources may have been caused by our inability to include terrestrially derived detritus other than leaves. Our results support those of several studies in which a positive association was found between increasing urbanization and N loads and corresponding increases in the $\delta^{15}$N values of aquatic producers and consumers (Steffy and Kilham 2004, Olsen et al. 2010, Calizza et al. 2012). Elevated $\delta^{15}$N values in basal organic matter and primary consumers can be attributed to inputs of stormwater with an enriched $\delta^{15}$N signal from human activity (Steffy and Kilham 2004, Kohzu et al. 2009).

Inputs of human sewage or pet waste could explain the increase in $\delta^{15}$N with imperviousness in our study. As urban populations grow, so does the input of N from anthropogenic sources, and a shift to higher $\delta^{15}$N value can arise from inputs of human sewage, e.g., from leaky septic tanks (Cabana and Rasmussen 1996, Steffy and Kilham 2004, Kohzu et al. 2009). Pet waste also can be a significant source of N in urban aquatic systems (Carpenter et al. 1998, Baker et al. 2001, Wollheim et al. 2005).

### Imperviousness vs trophic position

Our finding that the trophic levels of wetland fish, as estimated by $\delta^{15}$N, appeared to decrease as imperviousness increased supports findings by Eitzmann and Paukert (2010) who found the mean trophic position of fish was higher in less-urbanized stream environments. Their suggestion that urbanization reduced habitat heterogeneity and led to an associated compression of the stream food web applies well to our findings for constructed stormwater wetlands (Eitzmann and Paukert 2010).

Macroinvertebrate trophic position increased with imperviousness. The dynamic stability hypothesis states that less predictable environments should have shorter food chains because longer food chains are less resilient to perturbation (Vander Zanden et al. 1999). An increase in urbanization can cause a decrease in invertivorous fish that can lead to alterations in stream food webs (Eitzmann and Paukert 2010). Systems that are hydrologically disconnected from the river channel often have a higher abundance of piscivorous fish and, as a consequence, the numbers of herbivorous and invertivorous fish present are reduced by heavy predation (Jackson et al. 2013). Cabana and Rasmussen (1994) found that food chains were shortest when pelagic forage fish were absent because higher consumers were then forced to feed on plankton and benthic organisms. Fish can exert a strong top-down effect in wetland habitats, and the exclusion of invertivorous predators can lead to increased invertebrate abundance and species richness (Gilinsky 1984, Batzer et al. 2000).

Our finding of a decrease in the mean trophic level of fish with increasing imperviousness could indicate a change in feeding strategy or quality of prey items. Perturbations can lead to shorter food chains (Cabana and Rasmussen 1994, Schriever and Williams 2013), but nutrient enrichment in urban settings can lead to longer food chains (Freedman et al. 2012), as has been documented in systems that are supported by phytoplankton rather than by periphyton and detritus (Jackson et al. 2013). Increased algal productivity can
increase foodweb complexity through an increase in the abundance and diversity of primary consumers, but it can reduce complexity if the DO demand reaches levels that have a negative effect on the aquatic fauna (Freedman et al. 2012). The potentially nonlinear nature of some of these relationships emphasizes the value of the type of gradient study we conducted.

Temporal limitations

Stable-isotope analysis is an important tool in characterizing food webs, but the seasonal variability in isotopic signatures at the base of the food web must be considered when interpreting results (Hart and Lovvorn 2002, Jardine et al. 2014). For example, isotopic signatures of short-lived producers and consumers with short life spans are likely to respond quickly to a high turnover of body N and its associated C (Jardine et al. 2014). $\delta^{15}$N can display a much larger range of temporal variability in urban environments, where the main sources of N are anthropogenically derived, than in nonurbanized systems (Pastor et al. 2014). Strategies for dealing with temporal variability include sampling short-lived primary producers and consumers over large spatial scales and several times before collection of larger and longer-lived consumers (Jardine et al. 2014). We found a significant positive relationship between impervious and $\delta^{13}$C and $\delta^{15}$N values, but our study should be repeated.

Figure 4. Total % catchment covered by impervious surface (imperviousness) vs mean (±SE) unadjusted $\delta^{13}$C and $\delta^{15}$N for fish (A), macroinvertebrates (B), and aquatic basal resources (C) in constructed wetlands.
with sampling at regular intervals over a longer period to gain insight into temporal variation in the patterns we observed.

Conclusion

The effects of urbanization, measured as imperviousness, on constructed stormwater wetlands, were associated with decreased abundance and diversity of macroinvertebrates, decreased trophic position of fishes, and increased trophic position of macroinvertebrates. Sources of C differed between sites of low and high imperviousness, and sources of N increased with imperviousness. Tolerant macroinvertebrates and fish dominated at these wetlands. Our findings suggest that imperviousness, and by implication, urbanization, affects food webs in constructed wetlands. Further studies are required to determine the potential consequences of the changes in energy transfer to higher trophic levels, such as predatory fish and water birds.

Acknowledgements

Author contributions: TJM developed the idea of the study and undertook all field and laboratory work, including analyzing data. She was the main author of the manuscript. RMT and JAD provided input, feedback, and comments on the study and manuscript.

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