# Determining the Benthic Macroinvertebrate Community Composition of Freshwater Streams from Fish-Gut Analysis

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**Abstract** - The monitoring of changes in benthic macroinvertebrate communities over time facilitates the evaluation of any changes that occur in the function and structure of aquatic ecosystems. We hypothesized that it would be possible to determine, through running simulations, which trophic group of fishes' gut content can and should be used to best determine benthic macroinvertebrate community composition. Researchers could use this knowledge to estimate historic benthic macroinvertebrate communities of aquatic systems from fishes catalogued in museums. These historical data could then be compared to current data to see how macroinvertebrate communities have changed over time. In this study, we identified the fishes whose gut content most accurately reflected the benthic macroinvertebrate community of Marshalls Creek in East Stroudsburg, PA. We collected fish species and benthic macroinvertebrate samples at various sites and at different times of year to reflect seasonal variation. *Enneacanthus gloriosus* (Bluespotted Sunfish), *Lepomis auritus* (Redbreast Sunfish), and *Catostomus commersonii* (White Sucker) were the species that best represented the benthic macroinvertebrate community from their gut content. We determined that these species predicted 81% of all taxa that occur in summer. To estimate sampling distribution, we ran 100 simulations in R 3.0.2 on each combination of 3 fish species to determine the average quantity of taxa consumed (to the family level) along with sampling variation. Data obtained from the dissection of museum specimens could then be compared to data obtained from more recently collected specimens and a comparison made to determine changes in the macroinvertebrate community over time.

## Introduction

Generally, when ichthyologists sample a body of water, they preserve the majority of fishes caught and place them into 1 or more natural history museums. Many studies conducted by aquatic entomologists, however, focus on specific taxa of interest, and, therefore a complete representative sample of the benthic macroinvertebrate fauna may not be collected. Thus, it is difficult to determine the entire benthic macroinvertebrate community of a particular freshwater stream from historical collections catalogued into entomology museums alone.

The composition of the benthic macroinvertebrate community in freshwater streams reflects overall stream health, with certain taxa present only if pristine conditions exist (Cairns and Pratt 1993). Conversely, other taxa survive when stream-water quality is poor (Chapman et al. 1982, Zimmerman 1993). Different

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Manuscript Editor: Joseph Rachlin

families of benthic macroinvertebrates have specific functions within the stream habitat based on diverse feeding habits; therefore, their presence or absence can result in changes within aquatic food chains. In general, benthic macroinvertebrates are sedentary, so any type of disturbance at a site is reflected in the presence or absence of specific taxa (Chessman 1995). Aquatic biologists rely on the structure and function of aquatic macroinvertebrate communities to assess stream health (Stauffer and Hocutt 1980, Warkentine and Rachlin 2015).

In order to evaluate changes in the structure and function of aquatic ecosystems, it is useful to track changes in benthic macroinvertebrate communities over time. We undertook this study because we hypothesized that it was possible to determine, through running simulations, which trophic group of fishes' gut content can and should be used to best determine benthic macroinvertebrate community composition. Rachlin and Warkentine (1987) first proposed using stomach contents to reconstruct invertebrate fauna. Researchers can use this knowledge to estimate the benthic macroinvertebrate community for streams from which we have museum specimens of fish. Historic and current data can be compared to see how a particular stream's water-quality may have changed over time.

## Field-site Description

Marshalls Creek originates from Otter Lake in East Stroudsburg, Monroe County, PA (Fig. 1). It flows for 16.8 km into Lower Brodhead Creek, which then drains into the Delaware River. According to the Pennsylvania Department of Environmental Protection, Pennsylvania Code Title, Chapter 93, unnamed tributaries of Brodhead Creek are designated as high-quality, coldwater fisheries (PADEP 2013). A 1998 survey conducted by Tom Shervinskie (Pennsylvania Fish and Boat

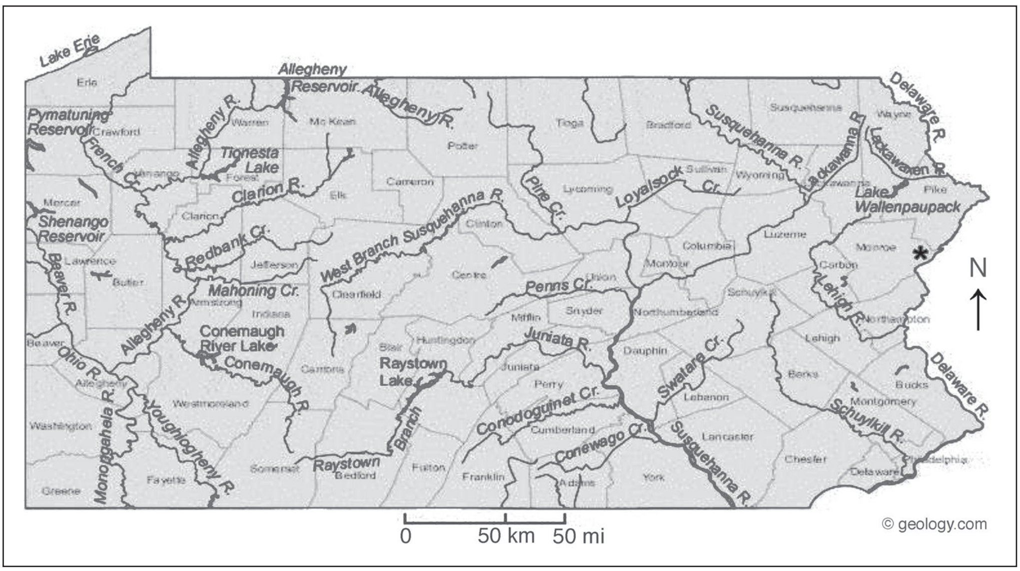


Figure 1. State of Pennsylvania with study-site area designated with bold asterisk in the eastern part of the state, Monroe County (downloaded from Google maps).

Commission, Harrisburg, PA) recorded 29 fish species in the Marshalls Creek drainage (Leckvarcik 2001). The survey conducted by the Stauffer Laboratory at The Pennsylvania State University in 2010, upon which this research is based, yielded 20 fish species.

## Methods

According to Bunn et al. (1986:85), "major temporal changes were observed in the community structure of the invertebrate fauna" when the macroinvertebrate population was sampled every 6 weeks for a 1-y period in their study conducted in Australia. To mitigate temporal variation, we sampled for both benthic macroinvertebrates and fishes in March, August, and December 2010. Fish species collected were: *Lethenteron appendix* (DeKay) (American Brook Lamprey), *Anguilla rostrata* (Lesueur) (American Eel), *Catostomus commersonii* (Lacepède) (White Sucker), *Erimyzon oblongus* (Mitchill) (Eastern Creek Chubsucker), *Rhinichthys cataractae* (Valenciennes) (Longnose Dace), *Rhinichthys atratulus* (Hermann) (Blacknose Dace), *Exoglossum maxillingua* (Lesueur) (Cutlips Minnow), *Luxilus cornutus* (Mitchill) (Common Shiner), *Notropis bifrenatus* (Cope) (Bridle Shiner), *Notropis chalybaeus* (Cope) (Ironcolor Shiner), *Semotilus corporalis* (Mitchill) (Fallfish), *Noturus insignis* (Richardson) (Margined Madtom), *Ameiurus nebulosus* (Lesueur) (Brown Bullhead), *Esox niger* (Lesueur) (Chain Pickerel), *Salmo trutta* (L.) (Brown Trout), *Micropterus salmoides* (Lacepède) (Largemouth Black Bass), *Enneacanthus gloriosus* (Holbrook) (Bluespotted Sunfish), *Lepomis auritus* (L.) (Redbreast Sunfish), *Percina peltata* (Stauffer) (Shield Darter), and *Etheostoma olmstedi* (Storer) (Tessellated Darter). We sampled 6 different sites and various habitat types within the Marshalls Creek drainage either for benthic macroinvertebrates, fishes, or both. We employed a Smith-Root LR-24 Electrofisher (Smith-Root, Vancouver, WA) to conduct single-pass backpack electrofishing for 100-m stretches at each site. We completed our sampling during the day; therefore, nocturnal fish such as ictalurids were caught in limited numbers. The battery-powered electrofisher was set at pulsed 300 volts of direct current.

### Fish collection

We euthanized fishes in a buffered solution of MS-222 at a concentration of 250 mg/L. All fish speciments were left in the solution for at least 10 min after all opercula movement stopped (PSARP 2010), fixed in formalin for a 2-week period, rinsed, stored in 80% ethanol, and catalogued into the Pennsylvania State University Fish Museum.

We measured total length for each fish. We removed and opened the entire foregut, stomach, and hindgut and placed the contents into 70% ethanol for identification. For this research, we did not dissect cyprinid species because they masticate prey with their pharyngeal teeth, which makes prey items difficult to identify (Litvak and Hansell 1988). We also did not dissect the 7 larval American Brook Lamprey because they are filter feeders; thus, the majority of their diet consists of diatoms (Moore and Mallatt 1980).

**Benthic-macroinvertebrate collection**

We used a standard D-frame kick-net with a 30-cm opening and a 1200-µm mesh size in order to obtain a representative benthic macroinvertebrate sample. Benthic macroinvertebrate samples taken with a kick-net have less variation among replicates than those collected with a Surber sampler (Hornig and Pollard 1978), and kick-net samples collect a larger number of taxa than does a Surber sampler (Mackey et al. 1984). We made 10 collections from different habitat types at each site to ensure a representative sample of the entire benthic macroinvertebrate community.

**Benthic-macroinvertebrate identification**

We used taxonomic keys to identify gut contents to the lowest taxonomic designation possible (Merritt et al. 2008, Peckarsky et al. 1990, Wiggins 1996). The data used for statistical analysis were at the family level, due to the degree of difficulty in identifying gut contents to genus.

In the case of Ephemeroptera, Plecoptera, and Trichoptera, sometimes only mandibles remained; if 2 mandibles were found, we determined 1 individual had been consumed. Amphipoda specimens were sometimes torn into pieces; thus, we counted the number of heads, and for psephenids, if individuals were not whole, we found pieces that could form a whole and counted accordingly. In the case of chironomids, we counted the number of head capsules. When counting Ostracoda, Copepoda, and Chironomidae in White Suckers, we placed the entire gut contents in a 50-mm-diameter petri dish, placed graph paper with each square numbered under the dish, and randomly counted gut contents in 20% of the squares. We multiplied by 5 the totals of each (Ostracoda, Copepoda, and Chironomidae) to get estimates of total number of individuals consumed.

We conducted all analyses in R 3.0.2 (R Core Team 2013). We employed simulations to obtain sampling distribution estimates, by sampling without replacement using the original dataset obtained from our fish and macroinvertebrate collections (Hallgren 2013). We eliminated White suckers <80 mm in length from simulations because they eat primarily microorganisms until they are ~2 years of age (Stewart 1926). Minus the cyprinid species, we collected 13 species of fish. We discarded 7 of these from simulations because of the small sample size (<7 total specimens). After these adjustments, we ran simulations with the remaining 6 fish species. We ran our simulations at the family level; thus, when we refer to taxa, we mean families.

To determine which species were most important to estimate a stream's benthic macroinvertebrate population, we ran 100 simulations each ($n = 5$) of all 20 possible combinations of 3 of the remaining 6 fish species. After completing 100 simulations on each combination of 3 species, we found the sums (total number of benthic macroinvertebrate families consumed) of the 100 simulations, averaged them and determined sampling variability by examining the standard deviation of the sample mean (Table 1).

## Results

We collected a total of 360 fish representing 18 taxa during the summer of 2010. Gut contents included 10,895 individuals representing 60 benthic macroinvertebrate taxa and 8 terrestrial arthropod taxa, including 13 unique taxa (or 22% of the total) in the gut-content samples. These unique taxa included Coleoptera (Dytiscidae, Haliplidae, Hydrophilidae), Diptera (Ceratopogonidae, Ephydridae, Simuliidae), Ephemeroptera (Leptophlebiidae), Hemiptera (Belostomatidae), Lepidoptera (Crambidae, Noctuidae), Odonata (Libellulidae), Plecoptera (Chloroperlidae), and Trichoptera (Limnephilidae). The kick-net samples collected during this summer yielded 2495 individuals representing 47 benthic macroinvertebrate taxa, including 8 unique taxa (or 13% of the total) in the kick-net samples (Table 2). These unique taxa included Diptera (Athericidae), Ephemeroptera (Ameletidae, Caenidae, Leptohyphidae), Gastropoda (Physidae, Viviparidae), Hemiptera (Gerridae), and Odonata (Calopterygidae). The taxa whose gut content best represented the macroinvertebrate community were Bluespotted Sunfish with 30 taxa, White Sucker with 28 taxa, and Redbreast Sunfish with 36 taxa (Table 3).

We recorded several macroinvertebrate taxa in kick-net samples but not in the gut contents of any fish specimens over the 3 collection seasons. These include Ephemeroptera (Caenidae), Odonata (Calopterygidae), Plecoptera (Chloroperlidae), Hemiptera (Gerridae, Notonectidae, Pleidae), Trichoptera (Apataniidae, Uenoidae), Ephemeroptera (Leptohyphidae), Diptera (Athericidae), Gastropoda (Physidae, Viviparidae), and Unionoida (Unionidae). We

Table 1. Possible combinations of 3 of 6 total fish species with the average and standard deviation obtained from 100 simulations run in R. White = White Sucker, Red = Redbreast Sunfish, Blue = Bluespotted Sunfish, Eel = American Eel, Tess = Tessellated Darter, and Shield = Shield Darter.

| Fish combinations | Average | SD |
|---|---|---|
| WhiteRedBlue | 31.68 | 2.40 |
| RedBlueEel | 29.46 | 2.24 |
| WhiteRedEel | 29.45 | 2.56 |
| WhiteBlueEel | 29.04 | 2.37 |
| RedBlueTess | 28.68 | 2.75 |
| WhiteRedTess | 28.31 | 2.40 |
| WhiteBlueTess | 27.56 | 2.32 |
| WhiteRedShield | 27.53 | 2.75 |
| RedBlueShield | 26.95 | 2.44 |
| WhiteBlueShield | 26.11 | 2.56 |
| RedEelTess | 23.72 | 2.32 |
| WhiteEelTess | 23.05 | 2.37 |
| RedEelShield | 22.56 | 2.41 |
| RedTessShield | 22.33 | 3.11 |
| BlueEelTess | 22.30 | 2.37 |
| WhiteEelShield | 22.24 | 2.16 |
| WhiteTessShield | 21.13 | 2.39 |
| BlueEelShield | 20.45 | 2.14 |
| BlueTessShield | 18.80 | 2.25 |
| EelTessShield | 11.51 | 2.00 |

identified other taxa in gut-content samples, but not in any of the kick-net samples over the 3 seasons. These include Hemiptera (Belostomatidae), Lepidoptera (Crambidae), Lepidoptera (Noctuidae), Coleoptera (Dytiscidae), and Diptera (Empididae, Ephydridae, Muscidae).

During the winter season, we collected 50 fishes, and 38% of them had 1 or 0 taxa in their gut. In winter, there were 11 gut taxa and 44 kick-net taxa (Table 4). Thus, the taxa collected from the gut samples representeed only 24.4% of the total

Table 2. Summer presence/absence kick-net taxa collected 4 August 2010 from Marshalls Creek, East Stroudsburg, PA. X = presence of taxon. *denotes taxa found in summer kick-net samples that were not present in gut contents.

| Order | Family | Presence | Order | Family | Presence |
|---|---|---|---|---|---|
| Amphipoda | Gammaridae | X | Isopoda | Asellidae | X |
| | Talitridae | X | Lepidoptera | Crambidae | |
| Bivalvia | Sphaeriidae | X | | Noctuidae | |
| | Unionidae | | | Pyralidae | X |
| Coleoptera | Dytiscidae | | Megaloptera | Corydalidae | X |
| | Elmidae | X | | Sialidae | X |
| | Gyrinidae | X | Odonata | Aeshnidae | X |
| | Haliplidae | | | Calopterygidae* | X |
| | Hydrophilidae | | | Coenagrionidae | X |
| | Psephenidae | X | | Gomphidae | X |
| Decapoda | Cambaridae | X | | Libellulidae | |
| Diptera | Athericidae* | X | Plecoptera | Chloroperlidae | |
| | Ceratopogonidae | | | Leuctridae | X |
| | Chironomidae | X | | Nemouridae | |
| | Empididae | | | Perlidae | X |
| | Ephydridae | | | Pteronarcyidae | X |
| | Muscidae | | | Taeniopterygidae | |
| | Simuliidae | | Trichoptera | Apataniidae | |
| | Tipulidae | X | | Brachycentridae | X |
| Ephemeroptera | Ameletidae* | X | | Helicopsychidae | X |
| | Baetidae | X | | Hydropsychidae | X |
| | Caenidae* | X | | Hydroptilidae | X |
| | Ephemerellidae | X | | Lepidostomatidae | X |
| | Heptageniidae | X | | Leptoceridae | X |
| | Isonychiidae | X | | Limnephilidae | |
| | Leptohyphidae* | X | | Odontoceridae | X |
| | Leptophlebiidae | | | Philopotamidae | X |
| | Siphlonuridae | X | | Polycentropodidae | X |
| Gastropoda | Physidae* | X | | Psychomyiidae | X |
| | Planorbidae | X | | Rhyacophilidae | X |
| | Valvatidae | X | | Uenoidae | |
| | Viviparidae* | X | | | |
| Hemiptera | Belostomatidae | | | | |
| | Corixidae | X | | | |
| | Gerridae* | X | | | |
| | Notonectidae | | | | |
| | Pleidae | | | | |
| | Veliidae | X | | | |

Table 3. Gut-content data for White Sucker, Bluespotted Sunfish, and Redbreast Sunfish collected 4 August 2010 from Marshalls Creek, in East Stroudsburg, PA. x= presence of taxon and (*n*) = # of specimens containing that taxon in gut content. [Table continued on the following page.]

| Order | Family | White Sucker | Bluespotted Sunfish | Redbreast Sunfish |
|---|---|---|---|---|
| Amphipoda | Gammaridae | x (3) | | |
| | Talitridae | | x (18) | |
| Bivalvia | Sphaeriidae | x (7) | x (1) | |
| | Unionidae | | | |
| Coleoptera | Dytiscidae | | | x (1) |
| | Elmidae | x (8) | | x (9) |
| | Gyrinidae | | | |
| | Haliplidae | x (1) | x (2) | |
| | Hydrophilidae | | x (1) | |
| | Psephenidae | x (6) | x (1) | x (9) |
| Decapoda | Cambaridae | | | x (3) |
| Diptera | Athericidae | | | |
| | Ceratopogonidae | x (1) | x (6) | x (1) |
| | Chironomidae | x (17) | x (19) | x (16) |
| | Empididae | | | |
| | Ephydridae | | x (1) | x (1) |
| | Muscidae | | | |
| | Simuliidae | x (1) | | x (1) |
| | Tipulidae | x (11) | x (2) | x (5) |
| Ephemeroptera | Ameletidae | | | |
| | Baetidae | x (1) | x (1) | |
| | Caenidae | | | |
| | Ephemerellidae | | | x (3) |
| | Heptageniidae | x (2) | x (3) | x (2) |
| | Isonychiidae | x (1) | | x (3) |
| | Leptohyphidae | | | |
| | Leptophlebiidae | | x (2) | |
| | Siphlonuridae | | x (1) | |
| Gastropoda | Physidae | | | |
| | Planorbidae | x (1) | x (1) | |
| | Valvatidae | | x (3) | x (3) |
| | Viviparidae | | | |
| Hemiptera | Belostomatidae | | | x (2) |
| | Corixidae | x (1) | x (10) | x (10) |
| | Gerridae | | | |
| | Notonectidae | | | |
| | Pleidae | | | |
| | Veliidae | | | x (1) |
| Isopoda | Asellidae | | x (11) | x (3) |
| Lepidoptera | Crambidae | | | x (1) |
| | Noctuidae | | | x (1) |
| | Pyralidae | x (4) | x (1) | x (2) |

benthic macroinvertebrate community composition found (both kick-net and gut). We also found 44 taxa in the kick-net samples in the spring season (Table 5), though not all the same taxa as found in the winter.

When we included all data from all 6 fish species used in the simulation ($n$ = 10), we were able to predict 82% of the summer gut taxa (SGT), 72% of the summer kick-net taxa (SKT), and 72% of the summer total taxa (STT). Simulations using samples of 13 White Suckers, 21 Bluespotted Sunfish, and 24 Redbreast Sunfish (the total number caught of each individual fish species in the collection) produced an average of 92% SGT, 76% SKT, and 81% STT. Since spring and winter gut content captured a smaller percentage of the total macroinvertebrate community found, we only used the summer date for the simulations.

Redbreast Sunfish made the largest contribution to the overall total taxa. When we ran 100 simulations ($n$ = 5 fish) with this taxon alone, an average of 27 of the benthic macroinvertebrate families was captured, compared to an average of 19 for White Sucker, and 16 for Bluespotted Sunfish. When we combined presence/absence tables for the 3 species, 47 benthic macroinvertebrate families were consumed, with only 51 SGT found among all the fish species sampled.

Table 3, continued.

| Order | Family | White Sucker | Bluespotted Sunfish | Redbreast Sunfish |
|---|---|---|---|---|
| Megaloptera | Corydalidae | | | |
| | Sialidae | | x (2) | x (2) |
| Odonata | Aeshnidae | | x (1) | |
| | Calopterygidae | | | |
| | Coenagrionidae | x (1) | x (5) | x (1) |
| | Gomphidae | x (5) | | x (9) |
| | Libellulidae | x (1) | x (1) | x (2) |
| Plecoptera | Chloroperlidae | | | |
| | Leuctridae | x (1) | | |
| | Nemouridae | | | |
| | Perlidae | | | x (4) |
| | Pteronarcyidae | | | x (2) |
| | Taeniopterygidae | | | |
| Trichoptera | Apataniidae | | | |
| | Brachycentridae | x (11) | | x (9) |
| | Helicopsychidae | x (1) | | |
| | Hydropsychidae | x (3) | x (1) | x (9) |
| | Hydroptilidae | x (6) | x (12) | x (8) |
| | Lepidostomatidae | | | x (1) |
| | Leptoceridae | x (13) | x (7) | x (16) |
| | Limnephilidae | x (2) | x (2) | x (4) |
| | Odontoceridae | | x (2) | x (2) |
| | Philopotamidae | x (1) | x (1) | x (2) |
| | Polycentropodidae | x (4) | x (18) | x (11) |
| | Psychomyiidae | x (6) | | |
| | Rhyacophilidae | | x (1) | x (2) |
| | Uenoidae | | | |

We also used our experimental data to run 100 simulations ($n$ = 10) of each of those same 3 species and determined that, if there were 10 museum specimens of the selected species available (in this case 10 White Suckers, 10 Bluespotted Sunfish, and 10 Redbreast Sunfish), one need only dissect a total of 30 fish to identify 78% of the taxa that might have been identified within the guts of all fish sampled in a freshwater stream and 73% of the taxa that would have been found in a kick-net sample, had one been collected at the time the fish were captured. This same sample

Table 4. Spring presence/absence kick-net taxa collected 27 March 2010, from Marshalls Creek, East Stroudsburg, PA. X =å presence of taxon. *denotes taxa found in spring kick-net samples that were not present in gut contents.

| Order | Family | Presence | Order | Family | Presence |
|---|---|---|---|---|---|
| Amphipoda | Gammaridae | X | Isopoda | Asellidae | X |
| | Talitridae | X | Lepidoptera | Crambidae | |
| Bivalvia | Sphaeriidae* | X | | Noctuidae | |
| | Unionidae* | X | | Pyralidae* | X |
| Coleoptera | Dytiscidae | | Megaloptera | Corydalidae | X |
| | Elmidae* | X | | Sialidae | X |
| | Gyrinidae | | Odonata | Aeshnidae* | X |
| | Haliplidae* | X | | Calopterygidae | |
| | Hydrophilidae | | | Coenagrionidae* | X |
| | Psephenidae* | X | | Gomphidae | X |
| Decapoda | Cambaridae | X | | Libellulidae | |
| Diptera | Athericidae* | X | Plecoptera | Chloroperlidae* | X |
| | Ceratopogonidae | | | Leuctridae* | X |
| | Chironomidae | X | | Nemouridae* | X |
| | Empididae | | | Perlidae* | X |
| | Ephydridae | | | Pteronarcyidae | |
| | Muscidae | | | Taeniopterygidae* | X |
| | Simuliidae | X | Trichoptera | Apataniidae* | X |
| | Tipulidae | X | | Brachycentridae* | X |
| Ephemeroptera | Ameletidae | X | | Helicopsychidae* | X |
| | Baetidae | X | | Hydropsychidae | X |
| | Caenidae | | | Hydroptilidae | |
| | Ephemerellidae | X | | Lepidostomatidae | X |
| | Heptageniidae | X | | Leptoceridae* | X |
| | Isonychiidae | X | | Limnephilidae* | X |
| | Leptohyphidae | | | Odontoceridae | |
| | Leptophlebiidae | X | | Philopotamidae | X |
| | Siphlonuridae | | | Polycentropodidae* | X |
| Gastropoda | Physidae* | X | | Psychomyiidae | |
| | Planorbidae* | X | | Rhyacophilidae* | X |
| | Valvatidae* | X | | Uenoidae* | X |
| | Viviparidae | | | | |
| Hemiptera | Belostomatidae | | | | |
| | Corixidae | | | | |
| | Gerridae | | | | |
| | Notonectidae | | | | |
| | Pleidae | | | | |
| | Veliidae | | | | |

would yield 68% of the total macroinvertebrate taxa expected to be found in the freshwater stream where the specimens originated.

## Discussion

Based on data collected from the Marshalls Creek drainage, the 3 best representatives to determine benthic-macroinvertebrate population composition from their gut content alone were Redbreast Sunfish, Bluespotted Sunfish, and White

Table 5. Winter presence/absence kick-net taxa collected 17 December 2010, from Marshalls Creek, East Stroudsburg, PA. X = presence of taxon. *denotes taxa found in winter kick-net samples that were not present in gut contents.

| Order | Family | Presence | Order | Family | Presence |
|---|---|---|---|---|---|
| Amphipoda | Gammaridae | X | Isopoda | Asellidae | X |
| | Talitridae | X | Lepidoptera | Crambidae | |
| Bivalvia | Sphaeriidae* | X | | Noctuidae | |
| | Unionidae | | | Pyralidae* | X |
| Coleoptera | Dytiscidae | | Megaloptera | Corydalidae* | X |
| | Elmidae* | X | | Sialidae* | X |
| | Gyrinidae | | Odonata | Aeshnidae* | X |
| | Haliplidae | | | Calopterygidae | |
| | Hydrophilidae | X | | Coenagrionidae | X |
| | Psephenidae* | X | | Gomphidae | |
| Decapoda | Cambaridae | | | Libellulidae* | X |
| Diptera | Athericidae | | Plecoptera | Chloroperlidae | |
| | Ceratopogonidae | X | | Leuctridae | X |
| | Chironomidae | X | | Nemouridae | X |
| | Empididae | | | Perlidae* | X |
| | Ephydridae | | | Pteronarcyidae | |
| | Muscidae | | | Taeniopterygidae | X |
| | Simuliidae | X | Trichoptera | Apataniidae* | X |
| | Tipulidae | | | Brachycentridae* | X |
| Ephemeroptera | Ameletidae* | X | | Helicopsychidae* | X |
| | Baetidae | X | | Hydropsychidae* | X |
| | Caenidae* | X | | Hydroptilidae | |
| | Ephemerellidae | X | | Lepidostomatidae* | X |
| | Heptageniidae* | X | | Leptoceridae* | X |
| | Isonychiidae* | X | | Limnephilidae* | X |
| | Leptohyphidae* | X | | Odontoceridae | |
| | Leptophlebiidae | X | | Philopotamidae | X |
| | Siphlonuridae | | | Polycentropodidae* | X |
| Gastropoda | Physidae* | X | | Psychomyiidae | |
| | Planorbidae* | X | | Rhyacophilidae | |
| | Valvatidae* | X | | Uenoidae | |
| | Viviparidae* | X | | | |
| Hemiptera | Belostomatidae | | | | |
| | Corixidae* | X | | | |
| | Gerridae | | | | |
| | Notonectidae* | X | | | |
| | Pleidae* | X | | | |
| | Veliidae | | | | |

Sucker. These 3 species are native to drainages along the entire Atlantic seaboard, and Redbreast Sunfish and White Sucker are widely distributed. When sampling in an area with little or no abundance of Bluespotted Sunfish, another member of the Centrarchid family could be substituted for this species.

Given the life history of these 3 fish species, it is not surprising that together they best captured a representative benthic macroinvertebrate community. Redbreast Sunfish have the most varied diet of any of the centrarchids and readily feed from the water's surface (Warren 2009). Although an opportunistic feeder, the Bluespotted Sunfish inhabits densely vegetated areas only; therefore, it collects benthic macroinvertebrates in its gut that the Redbreast Sunfish does not encounter, much less consume (Murdy and Musick 2013). The White Sucker forages along the bottom of the water column, filtering detritus, and eating benthic macroinvertebrates buried beneath the substrate (Stewart 1926). Together, these 3 species consume macroinvertebrates from the entire river—the top, the bottom, the sides (among vegetation), and within the water column.

Our simulations of each of Redbreast Sunfish, Bluespotted Sunfish, and White Sucker indicated that one need only dissect a total of 30 fish to identify most (78%) of the taxa present within the guts of all fish of those species in a freshwater stream as well as most (73%) of the taxa detectable using kick-net samples at the time the fish were captured and the majority (68%) of all the macroinvertebrate taxa present in the freshwater stream where the specimens originated. In a study conducted on the diet of demersal fishes off the western coast of Scotland, Gibson and Ezzi (1987) similarly found that 20–30 fish stomachs were required for the cumulative curve to reach its asymptote when the cumulative number of diet categories was plotted against the number of fish guts examined in order to check if the sample size was sufficient.

The benthic macroinvertebrate data obtained from the dissection of museum specimens could then be compared to data obtained from specimens collected more recently (10 White Suckers, 10 Bluespotted Sunfish, and 10 Redbreast Sunfish) to determine changes in the macroinvertebrate community over time. From these data, one can determine if the community has remained stable over time, improved, or deteriorated.

Some of the taxa found in kick-net samples likely will never be found in the guts of fish because of defensive mechanisms that some benthic macroinvertebrates possess. For example, members of the Hemiptera (Gerridae) are rarely predated on by fish due to scent-gland secretions that repel predators (Stonedahl and Lattin 1982). The scent glands are located in the sternum and discharge through a single middle opening; the fluid released is both foul-smelling and distasteful (Anderson and Polhemus 1976). Some benthic macroinvertebrates, such as Plecoptera (Chloroperlidae), inhabit the hyporheic zone (Kondratieff 2008); thus, they may be undetected by 1 or more sampling techniques because some genera in this family are found at considerable depths below the surface of the substrate or within a stream bank (Stanford and Ward 1988).

Although some benthic macroinvertebrates most likely will not be present in fish-gut contents, we found many of the benthic macroinvertebrates that indicate good stream health within the gut content of fishes we examined. Anthropogenic disturbances affect all parts of our ecosystem. If we can monitor their effects on fresh water by monitoring benthic-macroinvertebrate community composition, we can document changes over time.

## Acknowledgments

We thank the following individuals for assisting with field work: Shan Li, Rich Taylor, Bill Hanson, Brent Smith, Bryan Matje, Josh Lynn, Titus Phiri, Sara Mueller, Cindy Nau, and Bethany Thomas. We have special appreciation for Rich Taylor, who assisted with R coding. Dr. Dave Miller provided suggestions in the writing of the thesis on which this manuscript is based. Greg Hoover offered his expertise on benthic macroinvertebrate identification.

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