Characteristics of Macroinvertebrate and Fish Communities From 30 Least Disturbed Small Streams in Connecticut

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Abstract - Water quality programs in Connecticut and nationally have focused on restoring impaired waters, while modest attention has been allocated to healthy watersheds in the least disturbed condition. The objective of our study was to document the geographic location of least disturbed streams in Connecticut, describe the aquatic biota from these systems, and describe important environmental variables that may help explain the distribution of these biota. We used geographic information systems to select drainage basins by their natural attributes and by eliminating anthropogenic stressor variables in order to best approximate a least disturbed watershed condition in Connecticut. We then sampled the fish and macroinvertebrate communities, water chemistry, and associated GIS-derived watershed attributes to determine the variables that best described the sampled biota. We identified 30 least disturbed streams that had drainage areas <29 km^2, whose stream order ranged from 1–4, and that contained <4% total impervious cover in the upstream watershed. Least disturbed streams were generally located in three geographic areas of the state—northwest Connecticut, northeast Connecticut, and the central Connecticut valley—and were absent from the southern coast of Connecticut and southwestern Connecticut. Cluster analysis and nonmetric multidimensional scaling of macroinvertebrate taxa in the Orders Ephemeroptera, Plecoptera, and Trichoptera showed 3 macroinvertebrate stream classes, with 12 significant indicator species ($P < 0.05$). Drainage area, water temperature, alkalinity, hardness, chloride, ammonia, total nitrogen (TN), and total phosphorus (TP) may explain some of the differences in taxa between macroinvertebrate stream classes. Cluster analysis and nonmetric multidimensional scaling of fish species also showed three fish stream classes, with 9 significant indicator species ($P < 0.05$). Drainage area, stratified drift, dam density, water temperature, total suspended solids, alkalinity, hardness, ammonia, TN, and TP may explain some of the differences in species between fish stream classes. Ninety percent of the least disturbed streams sampled contained *Salvelinus fontinalis* (Brook Trout), which can be considered a sentinel fish species for small, least disturbed streams in Connecticut.

Introduction

The history of water quality management in Connecticut dates back to the Connecticut Water Pollution Control Act (CWPCA) of 1967. Public concern over poor water quality led to the CWPCA, which gave the state authority to require more stringent wastewater treatment for municipal sewerage facilities and industrial discharges to the states waters, and is now incorporated into the General

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Statutes of Connecticut (Chapter 446k, Sections 22a-416 to 22a-599). Nationally, amendments to the Federal Water Pollution Control Act in 1972 and 1977 (FWPCA) resulted in the first comprehensive water pollution law for the nation. This legislation and subsequent amendments still serve as the foundation of surface water quality regulations in the United States. As a result of public concern over poor water quality and the promulgation of these state and federal laws, monitoring the chemical and biological quality of the state's water resources became a priority issue to track progress of clean water regulations.

Biological monitoring has been the foundation for assessing water quality in Connecticut's rivers and streams since the early 1980s. The concept behind biological monitoring is to use organisms living in streams (e.g., macroinvertebrates, fish) to measure the health of the waters. Karr (1981) first introduced an index of biological integrity (IBI), a composite measure of ecological characteristics, as an index of fish population health. In 1989, the United States Environmental Protection Agency (EPA) introduced guidance that included assessment protocols for fish and macroinvertebrates that expanded the development of multimetric indices to assess stream health (Plafkin et al. 1989). Following EPA's guidance, the Connecticut Department of Environmental Protection (CTDEP) implemented bioassessment protocols focusing on macroinvertebrates as the foundation of stream health assessment to evaluate the goals of CWPCA and the FWPCA

The goal of the FWPCA is to "restore and maintain the chemical, physical, and biological integrity of the nation's waters." However, much of the national and state effort to monitor and assess its waters from the 1980s to late 1990s focused on the restoration of "impaired" streams that fell on the "high" portion of the stressor gradient rather than the maintenance or preservation of streams that fell on the "low" portion of stressor gradient (Fig.1). Davies and Jackson (2006) introduced the biological condition gradient (BCG) conceptual model of ecological community change in flowing waters with increased anthropogenic stressors. The BCG describes the ecological community change as a continuum, with one end representing communities exposed to low stress and natural biological condition and the other end representing high stress and degraded biological condition. Since much of the historic monitoring of stream biological communities in Connecticut has focused on impaired waters (i.e., mid–high stress on the BCG), biological communities from natural streams under low stress on the BCG continuum are not well documented.

This paper identifies the location of 30 streams in the natural/low-stress portion of the BCG continuum, or least disturbed condition in Connecticut. Given Connecticut's long history of land-use disturbance (Bell 1985), we follow the definition of Stoddard et al. (2006) that the least disturbed condition is the "best available physical, chemical, and biological habitat conditions given today's state of the landscape." We used geographic information system software (GIS, ESRI Arc Map Version 9.2) to select drainage basins by their natural attributes and by eliminating known or suspected anthropogenic stressor variables in order to best approximate a least disturbed watershed condition. Our goal was to describe important fish species and macroinvertebrate taxa, and to use watershed attributes derived from GIS and water chemistry samples to highlight variables that best described these sampled biota. The results of this study can aid our understanding of fish and macroinvertebrate communities along the low-stress/natural portion of the BCG gradient (Fig 1) in Connecticut and lead to a better understanding of how these streams compare to streams with higher anthropogenic stress.

Methods

Selection of least disturbed streams

We used GIS to select least disturbed streams in Connecticut by evaluating land-use characteristics, water quantity stress (diversions), habitat fragmentation (dams and reservoirs), and salmonid fry stocking records. We used a hierarchical approach to select study streams first by screening at the subregional drainage-basin scale using GIS, followed by catchment-level screening using GIS, and we then followed GIS screening with field checks to determine habitat suitability (i.e., wadeable, good mix of riffle habitat and pool habitat) and validate dam

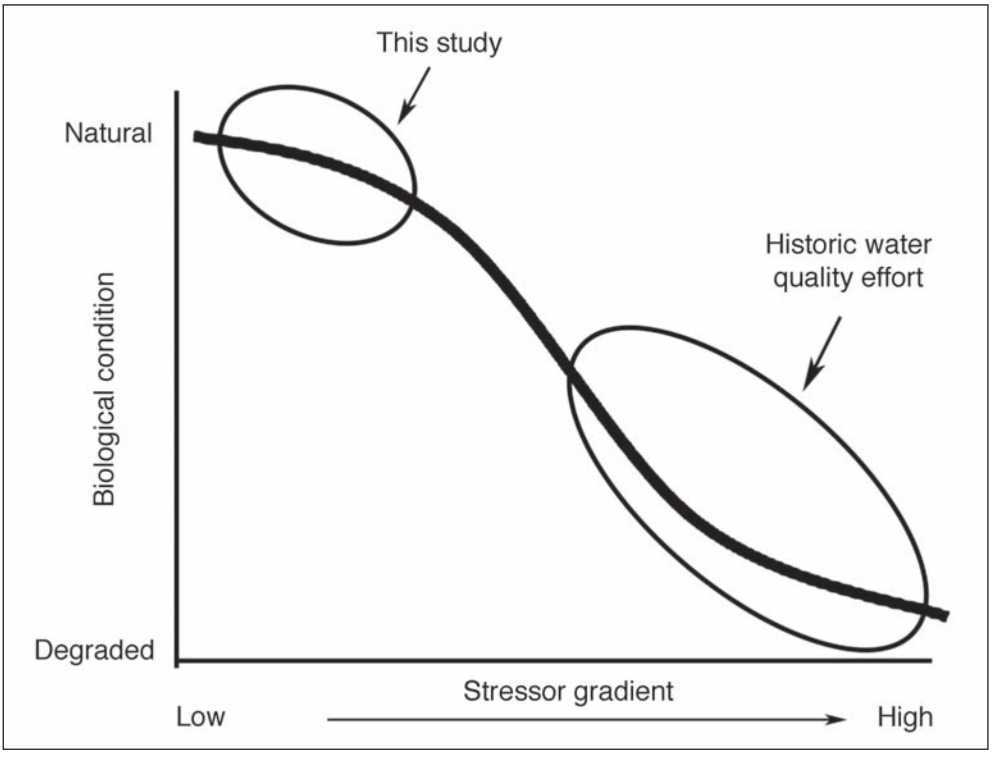


Figure 1. A schematic of the biological condition gradient based on Davies and Jackson (2006), showing focus of water quality efforts on moderately to highly stressed waters since the adoption of Connecticut Clean Water Act of 1967 and Federal Clean Water Act in 1972.

locations shown on our GIS. We only considered wadeable perennial streams with watersheds 2–2000 km^2 for our study.

We first selected subregional drainage basins, as defined in Nosal (1997), with greater than 80% natural land cover. Percent natural land cover was calculated from 2002 land-cover data produced by the University of Connecticut Center for Land-use Education and Research program and derived from 2002 LandSat satellite imagery. Percent natural land cover was an aggregate percentage of deciduous forest, coniferous forest, open water, and wetland land-cover categories. We calculated the percent natural land cover for each of the 334 subregional basins in Connecticut. Subregional basins in Connecticut range in size from 0.21–457.81 km^2, although 95% are less than 101.01 km^2 (median = 27.07 km^2). For those subregional basins that met the >80% natural land-cover criterion, we applied additional criteria for total percent impervious cover (IC)—water diversions, dams and reservoirs, and salmonid fry stocking in catchments within those subregional basins—to obtain a list of least disturbed streams.

Impervious cover has been shown to act as a surrogate measure of negative impacts to aquatic life in streams (Bellucci 2007, Morse et al. 2003, Roy et al. 2005, Stranko et al. 2008, Wang et al. 2001) and therefore is an appropriate screening tool at a broad spatial scale. Subregional basins containing <4% IC were selected for potential study. Subregional basins >4.1% IC were excluded from further analysis. IC was calculated using the Impervious Surface Analysis Tool, an ESRI Arc Map version 9.2 extension, using 2002 Connecticut Land Cover data following the guidelines in Prisloe et al. (2002).

The reduction in stream flow from water diversions can reduce the available aquatic habitat and therefore negatively impact the abundance and diversity of aquatic life in streams (Bain et al. 1988, Freeman and Marcinek 2006, Konrad et al. 2008, Poff et al. 1997). The location of water diversions was evaluated using best available data from the CTDEP Inland Water Resources Division. The diversion database contained the locations of approximately 2236 diversions, and we used GIS to select catchments that did not contain diversions. All catchments that contained diversions were excluded.

Dams are ubiquitous in Connecticut's landscape, and can contribute to stream habitat fragmentation and change the natural dynamics of stream ecosystems (Braatne et al. 2008, Graf 1999, Ligon et al. 1995, Poff and Hart 2002, Stanford and Ward 1989). Because dams are so widespread and common, we could not completely eliminate their presence or we would risk having no streams left in our study population. Therefore, we attempted to eliminate large dams from our analysis and included an acceptable threshold distance downstream from smaller dams. To infer the presence of large dams, we used a combination of a CTDEP database containing Hazard Class C dams and a Connecticut Department of Public Health (CTDPH) database containing information on reservoir size. Hazard Class C dams are defined as dams that impound large volumes of water and could be hazardous if the dam were breached. Waterbodies listed as reservoirs in the CTDPH database are typically used for public water supply storage and are usually not run of river. First, we screened stream segments using GIS and excluded those with Hazard Class C dams or reservoirs in upstream segments. Second, we used the CTDEP dam location database to eliminate stream reaches that were within 1.6 km of a dam and selected free-flowing sections of stream that were located greater than 1.6 km from a dam. We thought that 1.6 km was a reasonable distance to filter immediate ecological impacts from small dams for our study, while still retaining some sections of stream for our study.

Fish stocking can have negative impacts on natural fish populations (Faush 1988, Kreuger and May 1991) and was therefore a consideration to identifying least disturbed streams in Connecticut. *Salmo trutta* L. (Brown Trout) fry and *Salmo salar* L. (Atlantic Salmon) fry stocking records were obtained from the CTDEP Fisheries Division, and streams stocked with these species were eliminated because it is not possible to discriminate naturally reproduced Brown Trout fry from stocked fry; most occurrences of juvenile Atlantic Salmon in Connecticut are stocked fish. We then used GIS to select stream segments that were not influenced by fry stocking of these species. We did not exclude streams that were stocked with adult salmonids because our selection criteria dictated small, remote streams which are typically not stocked with adult salmonids. In addition, we hypothesized that there would be few, if any, adult stocked streams in the potential stream choices given our other selection criteria, and that if captured, adult stocked salmonids would be easily identified in the field.

Field checks were used to evaluate site accessibility, standardize sampling habitat (e.g., reaches with no riffle habitat or too deep to wade were eliminated), and verify dam locations. For watersheds that met all the above GIS screening criteria and field checks, the latitude and longitude of the sampling sites were recorded with a Garmin Model 76 GPS. We then used those coordinates and the Arc Hydro extension of GIS to delineate the watershed upstream of the sampling point. Our GIS selection criteria, followed by site visits, resulted in 30 small least disturbed streams as our study population.

Biological communities and water quality

Benthic macroinvertebrate samples were collected September–October 2007 using an 800-*u*m-mesh kick net. A total of 2 m^2 of riffle habitat (12 kicks composited from multiple riffles of a stream reach) was sampled at each location. Samples were preserved in 70% ethyl alcohol and brought back to the laboratory for subsampling. A 200-organism subsample was taken using a random grid design (Plafkin et al. 1989) from each sampling location. Organisms were identified to the lowest practical taxon, generally species.

A macroinvertebrate multimetric index (MMI) score for each site was calculated using a 200-organism subsample at the genus level (Gerritsen and Jessup 2007). The MMI is composed of 7 metrics: Ephemeroptera (E) taxa, Plecoptera (P) taxa, Trichoptera (T) taxa, percent sensitive EPT, scraper taxa, BCG taxa biotic index, and percent dominant genus (Table 1). The MMI score is the average score of all seven metrics and ranges from 0–100, with low values representing high stress and high values representing least stressed sites. For this paper, we followed the convention of CTDEP to aid in interpretation of the MMI scores as follows: MMI < 44 fails aquatic life goals, MMI range of 45–55 is an inconclusive assessment, and MMI > 56 passes aquatic life goals. These MMI values are typically used by CTDEP as part of the decision criteria for assessing aquatic life for Clean Water Act 305 (b) reporting and Section 303 (d) impaired water listing. We evaluated the MMI values from our study streams along the human-disturbance gradient using a scatter plot of MMI and IC. We included locations in Connecticut that were sampled outside of this study to allow comparison of MMI values from this study to MMI values from streams with higher levels of human disturbance. To accomplish this, 125 sites from wadeable streams in Connecticut with macroinvertebrate samples (Bellucci 2007) collected using the same sampling protocols as in this study were included in the scatter plot.

Fish sampling was conducted from June–September 2007 during periods of low streamflow to maximize sampling efficiency. Typically, 150 m of stream were electrofished using either a backpack unit or a single tow barge electrofishing unit (Hagstrom et al. 1995). A single pass was completed at each location, and all species were measured to the nearest centimeter (total length), counted, and immediately released into the stream.

A surface-water grab sample was collected from mid-channel at least once during spring, summer, and fall 2007 at each site and analyzed for total nitrogen, ammonia, total phosphorus, pH, alkalinity, hardness, and chloride. Water temperature was measured concurrent with site visits from May–September 2007 using a calibrated thermometer.

Statistical analysis

We calculated the percent occurrence of fish taxa from 30 least disturbed study streams and macroinvertebrate taxa from 24 least disturbed study streams. We

Table 1. Description of the seven metrics used to calculate the macroinvertebrate multi-metric index (MMI). The MMI is calculated as the average of the seven metrics. For more details on metrics that compose the MMI, see Gerritsen and Jessup (2007). Trend = trend in response to increasing stress.

Metric	Description	Trend
E taxa	Number of genra in the Order Ephemeroptera (E). This metric is adjusted for watershed size.	Decrease
P taxa	Number of genera in the Order Plecoptera (P).	Decrease
T taxa	Number of genera in the Order Trichoptera (T).	Decrease
% EPT	Number of organisms in the Orders EPT excluding the families Hydropsychidae and Baetidae divided by the total number of organisms in the samples times 100. This metric is adjusted for watershed size.	Decrease
Scraper taxa	Number of genera in the scraper functional feeding group	Decrease
% dominant genus	Number of organisms in the genus with the most individuals divided by total number of organisms multiplied times 100.	Increase
BCG taxa	Average of BCG attributes for each genera.	Increase

also compared the percent occurrence of macroinvertebrates and fish taxa from this study to other streams in Connecticut that were subjected to greater human disturbance. To accomplish this, we established 3 bins using IC as a measure of human disturbance. Bin 1 consisted of the streams for this study with IC < 4%, bin 2 included mid-level stress sites with IC = 4.1–11.9%, and bin 3 contained high-level stress sites with IC > 12%. IC was calculated as described above. We then queried the CTDEP ambient monitoring database for wadeable stream sites where fish and macroinvertebrate taxa were collected using the same methodology used in this study, and we calculated the percent occurrence of taxa for each bin. We only report taxa that were found in this study since our goal was to compare the taxa from least disturbed smaller streams in Connecticut (i.e., taxa that occurred exclusively in bins 2 and 3 were not included in this analysis).

Cluster analysis (CA) was used to explore taxa similarities between least disturbed streams separately for fish species and macroinvertebrate taxa. For macroinvertebrate stream classes, we evaluated taxa from the orders Ephemeroptera or E taxa (mayflies), Plecoptera or P taxa (stoneflies) and Trichoptera or T taxa (caddisflies). EPT were selected because these orders are known to be a dominate component of community richness in least disturbed conditions and as such would provide the most instructive information. For fish, we initially evaluated all species to determine stream fish classes.

For both EPT taxa and fish species, taxa proportional abundances were arcsine square-root transformed to improve normality. The Sorensen distance measure with the flexible beta linkage method (beta = -0.25) was used in all CA. Species that occurred in less than 5% of the samples (McCune and Grace 2002) were removed from the analysis for both the EPT and fish analysis. For fish, in addition to eliminating rare species, stocked salmonids and Cyprinidae <3 cm were also eliminated from the data matrix. The 44 EPT taxa by 24 site matrix for EPT and 17 fish species by 30 site matrix were used to produce dendrograms using PC ORD Version 5 (MjM Software Design, Gleneden Beach, OR).

Indicator species analysis (Dufrene and Legendre 1997) was used as an objective criterion to prune the dendrograms. The *P*-values from the Monte Carlo tests (1000 permutations) were averaged for all species after pruning the cluster dendrogram into 2, 3, 4, 5, 6, and 7 clusters, and the lowest average *P*-values determined the appropriate number of clusters (McCune and Grace 2002). We also used Wishart's (1969) objective function and percent-information-remaining statistic to interpret the site dissimilarity. The percent-information-remaining statistic indicates the relative distance between sites as defined by the location of the dendrogram branches. Sites that span a short distance of percent information remaining have more homogeneous taxa than sites that span a greater distance. Cluster analysis results were displayed as a dendrogram that graphically displays the relationship of sites to each other based on the proportions of taxa present at each site. Sites that span a short distance of the dendrogram (i.e., percent-information-remaining statistic) have more homogeneous taxa than sites that span a greater distance.

Ordination plots using nonmetric multidimensional scaling (NMS) were used as another graphical interpretation of taxa similarities between small, least disturbed streams. We followed recommendations in McCune and Grace (2002) to seek solutions with low stress and select the appropriate number of dimensions. We used the Sorensen distance measure and ran 250 iterations with real data, and then performed a Monte Carlo simulation with random data over 250 iterations to compare the solutions with real data to solutions that might be obtained by chance. We used these results, combined with a scree plot, to determine the solution with lowest stress in relation to dimensionality, then reran the NMS to obtain the final ordination plots for macroinvertebrate stream classes and fish stream classes.

After determining the macroinvertebrate and fish site classes using CA and NMS, indicator species analysis (Dufrene and Legendre 1997) was used to highlight taxa that were indicative of each of the macroinvertebrate and fish stream classes. Indicator species analysis combines a measure of taxa relative abundance and relative frequency of taxa into an indicator value score ranging from 0% (no indication) to 100% (perfect indication). A taxon with perfect indication of 100% would mean that it occurs at all sites in a group and is exclusive to that group (i.e., does not occur in other groups). We noted species that had indicator values greater than expected by chance using a 1000 permutation Monte Carlo test ($P < 0.05$).

We used watershed attributes and water chemistry parameters collected during the study to describe variables that may influence the fish and macroinvertebrate stream classes as determined by the CA. For each catchment, we calculated MMI, drainage area (km^2), percent stratified drift, road density (number per km^2), and dam density (number per km^2) using GIS. For each variable, differences in the data distribution among fish sites class and macroinvertebrate sites class were determined using the Kruskal Wallace test ($P < 0.05$).

Results

Description of 30 least disturbed streams in Connecticut

The 30 least disturbed streams had drainage areas <29 km^2 and Strahler stream order that ranged from 1–4; all contained <3.5% IC in the upstream watershed, and contained a high percentage of forested land use (Table 2). In general, the 30 least disturbed streams were located in three geographic groups: northwest Connecticut, northeast Connecticut, and the central Connecticut River valley (Fig. 2). Pendleton Hill Brook (SID 1748) was the only least disturbed stream that was located outside of these three groups. Four least disturbed streams were located in the town of East Haddam. Ashford, Canaan, and Lyme each contained three least disturbed streams and Barkhamsted, East Hampton, and Torrington each contained two least disturbed streams. Eleven towns contained one least disturbed stream. Least disturbed streams were absent from southwestern Connecticut and along the southern coast because the combination of urbanization, dams, diversions, and stocking practices excluded these streams.

Table 2. Location, drainage area, stream order, percent impervious cover, percent coniferous forest, and percent deciduous forest of thirty least disturbed streams in Connecticut, listed by station identification number (SID). SID's correspond with Figure 2.

SID	Stream	Town	Latitude	Longitude	Drainage area (km^2)	Stream order	% impervious cover	% coniferous forest	% deciduous forest
766	Stickney Hill Brook	Union	41.9833	-72.2179	6	3	2.06	58.95	27.65
1236	Beaver Brook	Lyme	41.4100	-72.3289	21	4	2.43	1.46	78.85
1239	Burhams Brook	East Haddam	41.4603	-72.3343	3	1	2.19	7.90	81.09
1435	Cedar Pond Brook	Lyme	41.4119	-72.3128	21	3	2.66	1.50	78.17
1748	Pendleton Hill Brook	Stonington	41.4748	-71.8342	10	2	2.51	2.70	76.72
1941	Bebbinton Brook	Ashford	41.8447	-72.1593	6	3	3.24	2.17	55.46
1981	Carse Brook	Sharon	41.8552	-73.3755	14	3	2.43	0.66	84.99
2291	Branch Brook	Eastford	41.9108	-72.1245	13	3	1.97	65.78	18.88
2293	Knowlton Brook	Ashford	41.8492	-72.1783	18	4	2.89	1.24	73.13
2294	Gardner Brook	Ashford	41.8643	-72.1598	4	2	3.37	1.42	67.21
2295	Mott Hill Brook	Glastonbury	41.6615	-72.5365	7	2	2.17	1.97	83.75
2296	Beaver Meadow Brook	Haddam	41.4553	-72.5288	4	2	2.97	27.42	60.03
2297	Hemlock Valley Brook	East Haddam	41.4283	-72.4226	7	3	3.00	5.53	65.14
2298	Hungerford Brook	Lyme	41.4255	-72.4094	7	3	3.41	1.35	69.16
2299	Rugg Brook	Winchester	41.9328	-73.1214	5	2	1.93	59.95	23.08
2301	Kettle Brook	Barkhamsted	41.9324	-72.9442	4	3	1.77	63.53	31.37
2302	Roaring Brook	Barkhamsted	41.9454	-72.9475	4	2	1.53	77.61	15.20
2303	Powder Brook	Harwinton	41.7541	-73.0170	3	2	2.23	1.00	62.97
2304	Day Pond Brook	Colchester	41.5623	-72.4338	3	2	3.17	6.57	72.88
2305	Elbow Brook	East Hampton	41.5211	-72.4869	2	2	2.67	0.00	87.66
2306	Flat Brook Central	East Hampton	41.5544	-72.4523	6	2	3.09	1.10	81.88
2307	Early Brook	East Haddam	41.4978	-72.3435	6	2	3.17	0.69	81.49
2308	Muddy Brook	East Haddam	41.4756	-72.3420	3	2	2.91	7.26	79.04
2309	Flat Brook North	Canaan	41.9459	-73.3200	7	2	2.45	15.24	68.84
2310	Whiting Brook	Canaan	41.9730	-73.3178	2	2	1.21	48.89	47.37
2311	Hall Meadow Brook	Torrington	41.8861	-73.1689	27	3	2.13	35.67	50.13
2312	Jakes Brook	Torrington	41.8646	-73.1679	4	3	2.08	23.09	63.94
2331	Stonehouse Brook	Chaplin	41.7812	-72.1509	14	4	2.66	0.29	77.98
2334	Chatfield Hollow Brook	Madison	41.3314	-72.5950	29	4	3.20	0.52	75.86
2342	Brown Brook	Canaan	41.9267	-73.2799	14	3	1.22	50.63	39.66

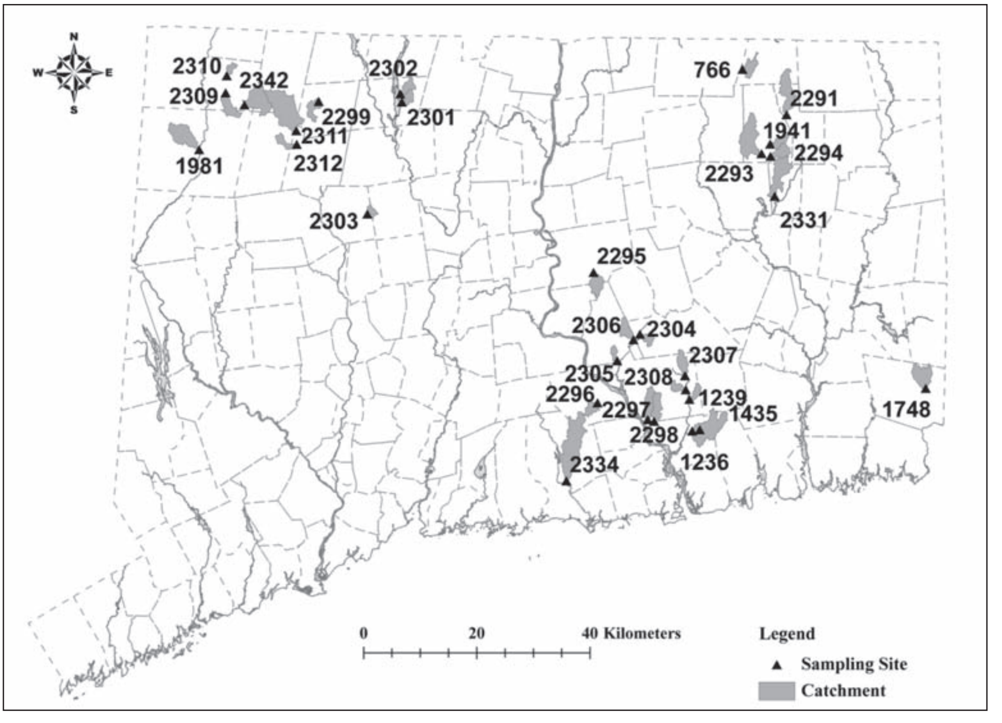


Figure 2. Location of the 30 least disturbed streams in Connecticut. Station identification number (SID) correspond to sites listed in Table 1.

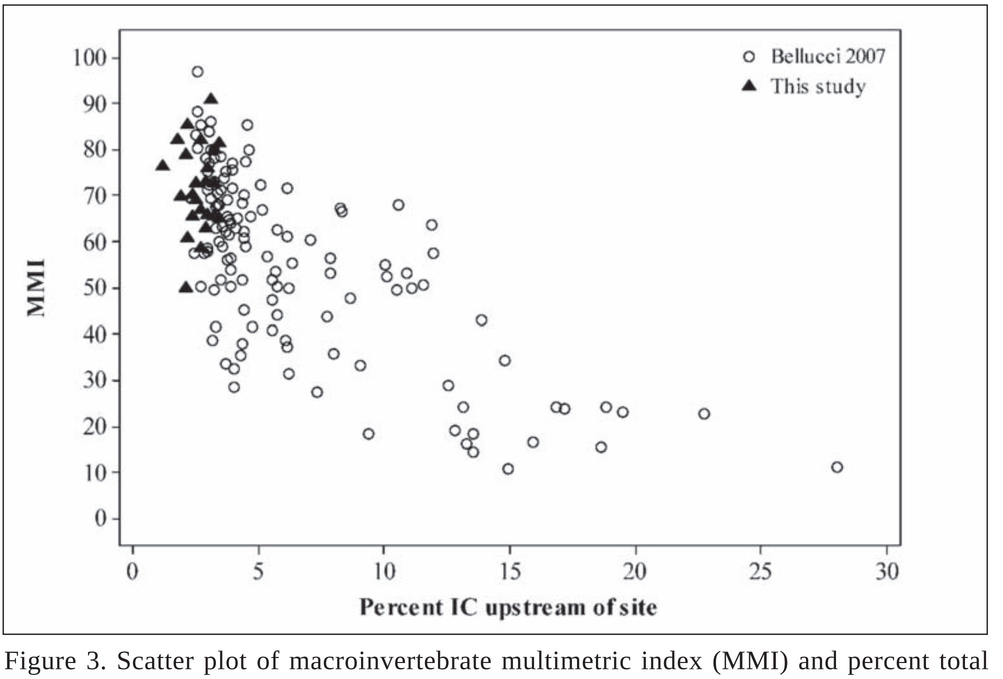


Figure 3. Scatter plot of macroinvertebrate multimetric index (MMI) and percent total impervious cover (IC) upstream of the sampling site. Solid triangles are the 24 least disturbed study streams, and the open circles are other site locations in Connecticut with samples collected in the same manner as used in this study (Bellucci 2007).

Biological communities from least disturbed streams

Macroinvertebrate communities were sampled from 24 of the 30 least disturbed streams. Six streams—Stickney Hill Brook (SID 766), Bebbington Brook (SID 1941), Branch Brook (SID 2291), Roaring Brook (SID 2302), Powder Brook (SID 2303, and Whiting Brook (2310)—were not sampled due to inadequate stream flow during the fall benthic sampling index period (September 15–November 30). Macroinvertebrate MMI scores ranged from 50–91 (average = 72, s.d. = 9.50), indicating the majority of the least disturbed streams passed aquatic life goals (Table 3). The one exception was an MMI value of 50 for Hall Meadow Brook (SID 2311), which was an inconclusive assessment. When compared to other streams in Connecticut along the human-disturbance gradient, the MMI scores from this study were consistent with our understanding of the BCG conceptual model (Fig. 3). That is, the majority of least disturbed streams had MMI values that scored towards the natural (least stressed) portion of the MMI scale and, therefore, the BCG scale as well.

A total of one hundred forty six macroinvertebrate taxa were identified from the 24 least disturbed streams (Appendix 1). Several macroinvertebrate taxa

Table 3. Macroinvertebrate multimetric index (MMI) and metrics: Ephemeroptera (E) taxa, Plecoptera (P) taxa, Trichoptera taxa, percent sensitive EPT (scoring adjusted for watershed size), scraper taxa, biological condition gradient (BCG) taxa biotic index, and percent dominant genus for 24 least disturbed streams by station identification number (SID).

SID	Sample date	MMI	E taxa	P taxa	T taxa	% sensitive EPT	Scraper taxa	BCG taxa biotic index	% dominant genus
1236	9/24/2007	70	100	33	38	100	64	68	87
1239	9/25/2007	85	100	83	62	100	64	89	98
1435	9/25/2007	67	38	17	85	68	82	83	96
1748	9/25/2007	73	42	50	85	82	55	100	95
1981	9/19/2007	66	53	50	38	73	64	100	80
2293	9/28/2007	63	74	17	31	88	73	93	66
2294	9/28/2007	81	100	50	54	100	73	94	100
2295	9/19/2007	61	57	33	62	51	45	83	92
2296	9/19/2007	66	71	33	54	83	45	81	92
2297	9/18/2007	76	90	50	77	74	64	81	97
2298	9/18/2007	65	47	33	69	57	64	96	90
2299	9/21/2007	70	100	33	54	70	82	75	76
2301	9/21/2007	82	100	67	77	100	55	88	89
2304	9/19/2007	80	100	50	77	100	64	70	100
2305	9/19/2007	82	100	33	85	100	64	100	92
2306	9/19/2007	69	49	33	62	84	73	84	97
2307	9/25/2007	73	90	17	77	74	64	87	100
2308	9/25/2007	73	100	33	69	100	55	66	89
2309	9/21/2007	91	89	100	77	96	91	98	84
2311	9/24/2007	50	34	17	46	35	73	57	87
2312	9/24/2007	79	76	67	69	84	73	85	97
2331	9/21/2007	58	39	33	38	70	55	84	89
2334	10/2/2007	65	57	33	69	90	45	65	97
2342	10/9/2007	76	76	67	54	83	73	87	96

documented from the 24 study streams did not occur in other mid-level (4.1–11% IC) or high-level (>12% IC) streams in the CTDEP database. For example, *Adicrophleps hitchcocki* Flint occurred at 16.67% of the 24 least disturbed streams sampled for macroinvertebrates, but did not occur in streams with higher levels of human disturbance.

The percent occurrence of several taxa decreased with increasing human disturbance. For example, *Promoresia tardella* Fall, *Stenelmis, Psephenus herricki* DeKay, *Hexatoma, Tiplua, Maccaffertium* Bednarik, *Nigronia serricornis* Say, *Acroneuria abnormis* Newman, *Diplectrona*, and *Dolophilodes* all occurred in at least 75% of the of the 24 least disturbed streams sampled for macroinvertebrates, but the percent occurrence declined in streams with higher levels of human disturbance. Other taxa such as *Tallaperla*, *Psilotreta*, *Ceratopsyche ventura* (Ross), *Rhyacophila minora* Banks, *Nanocladius*, and *Leuctra* occurred in fewer than 75% of least disturbed streams, but also declined with higher levels of human disturbance.

Some macroinvertebrate taxa showed a positive response to higher levels of human disturbance. For example, *Antocha* occurred at 4.17% of the least disturbed streams, but the percent occurrence increased to 37% and 43% as IC increased in watersheds in Connecticut. Some taxa appear to be neutral to human disturbance in that the percent occurrence is minimally affected by human disturbance. For example, the Elmid beetle *Macronychus glabratus* Say occurred at approximately 8% of sites across the gradient of IC.

The 146 macroinvertebrate taxa contained 68 EPT taxa, but 24 taxa occurred at <5% of sites and were therefore excluded from the CA to determine macroinvertebrate classes. The indicator species analysis runs of 2–7 clusters of the 44 EPT taxa by 24 site data matrix showed that three clusters had the lowest average P value (P = 0.29098). NMS ordination plots that resulted from a 3-dimensional best fit solution (final stress = 11.30, final instability < 0.00001, 108 iterations) also supported grouping the sites into 3 classes based on the similarities in EPT taxa (Fig. 4). Therefore, three macroinvertebrate stream classes were used in subsequent analysis.

Class 1 macroinvertebrate streams contained 8 streams, class 2 macroinvertebrate streams had 9 streams, and class 3 macroinvertebrate streams had 7 streams (Fig. 4). Beaver Brook (SID 1236) and Chatfield Hollow Brook (SID 2334) had the most similar EPT taxa in macroinvertebrate stream class 1. EPT taxa lists from Day Pond Brook (SID 2304) and Muddy Brook (SID 2308) were the most similar for macroinvertebrate class 2 streams. The sites with the most similar EPT taxa in macroinvertebrate stream class 3 were Beaver Meadow Brook (SID 2296) and Early Brook (SID 2307).

There were 12 significant indicator taxa among the three macroinvertebrate stream classes (Table 4). *Isonychia*, a collector-gatherer mayfly, was the taxa most indicative of macroinvertebrate stream class 1. *Isonychia* had a highly significant (P = 0.0001) indicator value of 96.9%, showing that it occurred almost exclusively in macroinvertebrate class 1 sites and occurred at all class 1 sites.

A collector-filtering caddisfly, *Diplectrona*, was the taxon most indicative of macroinvertebrate class 2 sites, with an 81.1% indicator value (P = 0.0002). It is worth noting that the collection of *Diplectrona* from the least disturbed streams in this study represents 30% of its known occurrence in the CTDEP database.

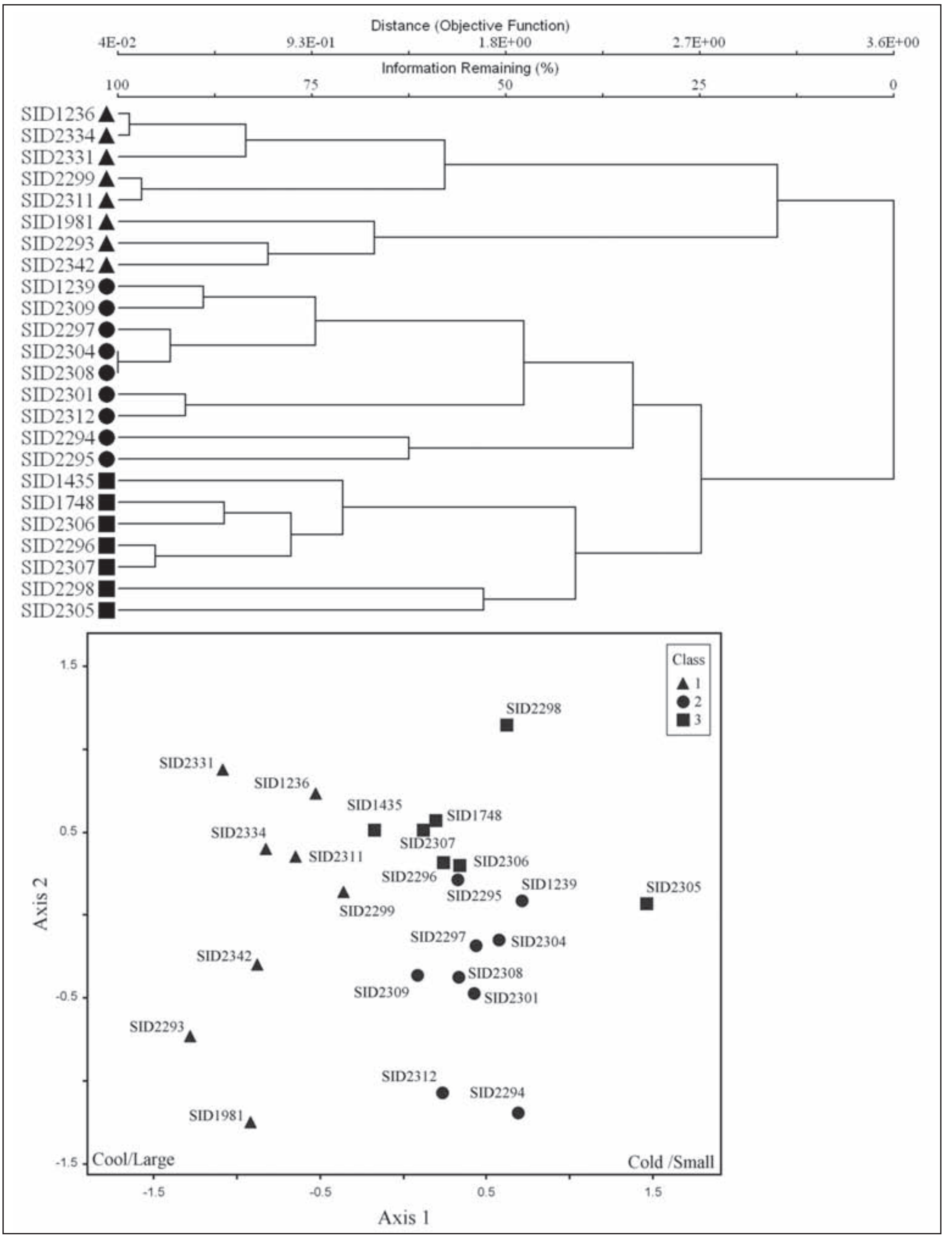


Figure 4. Dendrogram and ordination plot using nonmetric multidimensional scaling forming three macroinvertebrate stream classes (class 1 = triangles, class 2 = circles, class 3 = squares) using EPT taxa from 24 least disturbed streams. Refer to Table 1 for more information.

Acroneuria abnormis, a predatory Perlid stonefly, was the taxon most indicative of macroinvertebrate class 3, with a 54.2% indicator value (P = 0.0295).

A total of 27 fish species were collected from the 30 least disturbed watersheds (Appendix 2). Natural populations of *Salvelinus fontinalis* Mitchill (Brook Trout) and *Rhinichthys atratulus* Hermann (Blacknose Dace) were the most common fish species collected from the thirty least disturbed watersheds. Ninety percent of the least disturbed streams (27/30) sampled in this study contained Brook Trout, and the percent occurrence decreased to 28% in the 4.1–11.9% IC watersheds to 17% at >12% IC watersheds. Brook Trout densities ranged from 29 to 4902 individuals per ha (mean = 630, s.d. = 1003.53) from least disturbed watersheds, but were absent from Beaver Brook (SID 1236), Carse Brook (SID 1981), and Chatfield Hollow Brook (SID 2334). Blacknose Dace occurred at 87% of the least disturbed streams sites, but their occurrence at higher levels of human disturbance remained relatively constant (Appendix 2).

Two other fish species were notable since they occurred exclusively in least disturbed streams. *Lota lota* L. (Burbot), an endangered species in Connecticut (State of Connecticut 2004), was collected from one least disturbed stream. A species listed as endangered is any native species documented by biological research and inventory to be in danger of extirpation throughout all or a significant portion of the state, and to have no more than five occurrences in the state. *Cottus cognatus* Richardson (Slimy Sculpin) was collected from Mott Hill Brook (SID 2295) and is known to exist only in cold, high water-quality habitat (Edwards and Cunjak 2007).

Nine of 27 fish species that were collected in this study occurred in <5% of the samples and so were excluded from the CA to determine fish stream class. Despite our efforts to eliminate fry-stocked *Salmo salar* L. (Atlantic Salmon) from our pool of study sites, we collected Atlantic Salmon fry from

Table 4. Twelve macroinvertebrate taxa indicative of each least disturbed macroinvertebrate stream class as identified using indicator species analysis. Macroinvertebrate stream classes were determined using cluster analysis. P values < 0.05 were considered statistically significant.

Class	Taxa	Functional feeding group	Relative abundance (%)	Relative frequency (%)	Indicator value (%)	P value
1	*Isonychia*	Collector-gatherer	97	100	96.9	0.0001
1	*Paragnetina media*	Predator	89	63	55.7	0.0094
1	*Maccaffertium modestum* group	Scraper	64	75	47.7	0.0482
1	*Chimarra aterrima*	Collector-filterer	74	63	46.4	0.0356
2	*Diplectrona*	Collector-filterer	81	100	81.1	0.0002
2	*Ceratopsyche ventura*	Collector-filterer	97	78	75.2	0.0007
2	*Tallperla*	Shredder	69	89	61.5	0.0059
2	*Rhyacophila minora*	Predator	69	78	53.6	0.0211
3	*Acroneuria abnormis*	Predator	54	100	54.2	0.0295
3	*Brachycentrus appalachia*	Collector-filterer	100	43	42.9	0.0193
3	*Rhyacophila fuscula*	Predator	84	43	35.9	0.0462
3	*Oecetis persimilis*	Predator	89	43	37.9	0.0462

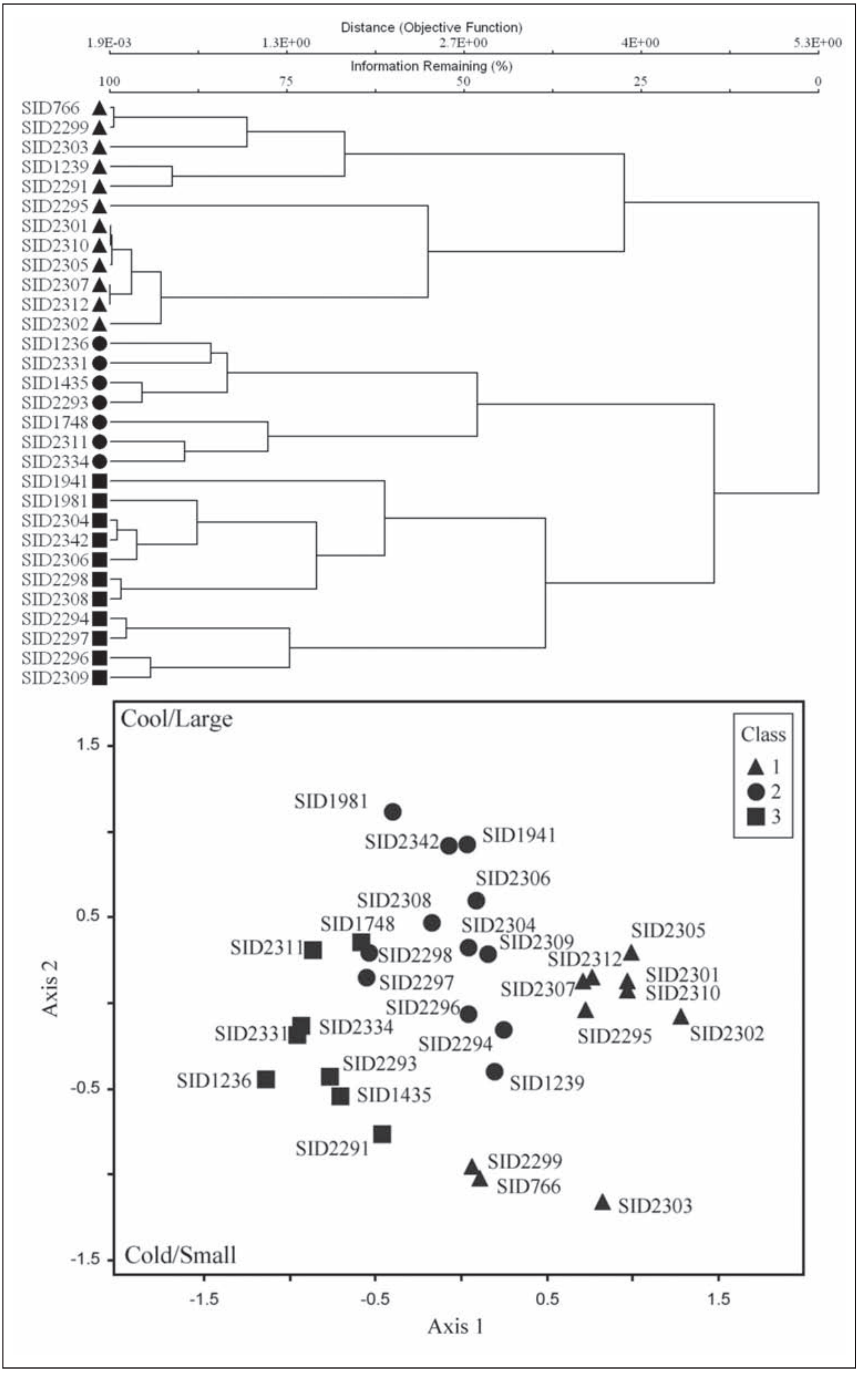
two streams in the Salmon River Basin—Day Pond Brook (SID 2304) and Flat Brook (SID 2306)—and Burnhams Brook (SID 1239) in the Eightmile River Basin. These incidental collections of Atlantic Salmon fry and any stocked adult salmonids (*Oncoryhchus mykiss* Walbaum [Rainbow Trout] and *Salmo trutta* L. [Brown Trout]) were eliminated from the analysis prior to grouping fish stream classes.

The indicator species analysis runs of 2–7 clusters of the 17 fish species by 30 site data matrix showed that three clusters had the lowest average *P* value ($P = 0.18059$). NMS ordination plots that resulted from a 3-dimensional best fit solution (final stress = 13.31, final instability < 0.00001, 139 iterations) also supported grouping the sites into 3 classes based on the similarities in fish species (Fig 5). Therefore, similar to the macroinvertebrate stream class analysis, three fish stream classes were used in subsequent analyses. Fish class 1 contained 12 streams, fish class 2 had 7 streams, and fish class 3 had 11 streams (Fig. 5).

There were nine significant indicator species among the three fish stream classes (Table 5). Brook Trout and *Notemigonus crysoleucas* Mitchill (Golden Shiner) were two fish species indicative of fish class 1 streams. Brook Trout, a fluvial specialist species, occurred in all fish class 1 streams (indicator value of 53.7%, $P = 0.0026$), but was also common in fish class 2 and fish class 3 streams. Golden Shiner, a macrohabitat generalist species, occurred exclusively in three fish class 1 streams and had an indicator value of 41.7% ($P = 0.0145$). In general, fish class 1 sites had fewer species per site than the

Table 5. Nine fish species indicative of each least disturbed fish stream class as identified using indicator species analysis. Fish stream classes were determined using cluster analysis. *P* values < 0.05 were considered statistically significant.

Class	Species	Habitat use	Relative abundance (%)	Relative frequency (%)	Indicator value (%)	*P* value
1	*Salvelinus fontinalis* (Brook Trout)	Fluvial specialist	54	100	53.7	0.0026
1	*Notemigonus crysoleucas* (Golden Shiner)	Macrohabitat generalist	100	42	41.7	0.0145
2	*Esox niger* (Chain Pickerel)	Macrohabitat generalist	100	71	71.4	0.0005
2	*Etheostoma olmstedi* (Tessellated Darter)	Fluvial specialist	100	71	71.4	0.0002
2	*Semotilus corporalis* (Fallfish)	Fluvial specialist	92	71	65.5	0.0005
2	*Lepomis macrochirus* (Bluegill)	Macrohabitat generalist	86	87	49.3	0.0105
2	*Micropterus salmoides* (Largemouth Bass)	Macrohabitat generalist	80	57	45.9	0.0100
2	*Luxilus cornutus* (Common Shiner)	Fluvial dependent	69	57	39.6	0.0316
3	*Semotilus atromaculatus* (Creek Chub)	Macrohabitat generalist	100	36	36.4	0.0372



Distance (Objective Function)
1.9E-03
1.3E+00
2.7E+00
4E+00
5.3E+00
Information Remaining (%)
100
75
50
25
0
SID766
SID2299
SID2303
SID1239
SID2291
SID2295
SID2301
SID2310
SID2305
SID2307
SID2312
SID2302
SID1236
SID2331
SID1435
SID2293
SID1748
SID2311
SID2334
SID1941
SID1981
SID2304
SID2342
SID2306
SID2298
SID2308
SID2294
SID2297
SID2296
SID2309
Cool/Large
Cold/Small
Class
1
2
3
Axis 2
Axis 1
1.5
0.5
-0.5
-1.5
SID1981
SID2342
SID1941
SID2306
SID2308
SID1748
SID2304
SID2305
SID2311
SID2298
SID2309
SID2312
SID2297
SID2301
SID2307
SID2310
SID2296
SID2331
SID2334
SID2302
SID2294
SID2295
SID2293
SID1236
SID1239
SID1435
SID2291
SID2299
SID766
SID2303

other two classes. Five sites in fish class 1 were most similar: Kettle Brook (SID 2301), Whiting Brook (SID 2310), Elbow Brook (SID 2305), Early Brook (SID 2307), and Jakes Brook (SID 2312) all contained Brook Trout and Blacknose Dace.

Fish class 2 streams had the highest species richness of the three fish classes and a mix of habitat-use requirements. Six species were significant indicators ($P < 0.05$) of fish class 2 streams. *Esox niger* Lesueur (Chain Pickerel) and *Etheostoma olmstedi* Storer (Tessellated Darter) both had indicator values of 71% and occurred exclusively in fish class 2 streams. Other indicator species of fish class 2 streams were *Semotilus corporalis* Mitchill (Fallfish), *Lepomis macrochirus* Rafinesque (Bluegill), *Micropterus salmoides* Lacepède (Largemouth Bass), and *Luxilus cornutus* Mitchill (Common Shiner).

The sites with the most similar fish species in class 3 were Day Pond Brook (SID 2304) and Brown Brook (SID 2342). Species richness from fish class 3 sites generally fell between class 1 and class 2. The only significant indicator species was *Semotilus atromaculatus* Mitchill (Creek Chub), a macrohabitat generalist, which had an indicator species value of 36.4% ($P = 0.0372$).

Neither macroinvertebrate stream classes nor fish stream classes were grouped in any noticeable geographic pattern (Fig. 6), suggesting that variables other than geographic location were more important in describing the distribution of macroinvertebrates within least disturbed watersheds. Drainage area, water temperature, alkalinity, hardness, chloride, ammonia, total nitrogen (TN), and total phosphorus (TP) were all significant variables ($P < 0.05$) between macroinvertebrate

Table 6. Median site characteristics for least disturbed macroinvertebrate site classes. The Kruskal-Wallis test was used to compare site characteristics between classes and those that showed significantly differences ($P < 0.05$) are noted with an asterisk.

Site characteristic	Class 1	Class 2	Class 3	P value
Drainage area (km^2)	16.07	3.84	6.31	0.004*
Stratified drift (%)	4.47	2.01	5.79	0.195
Road density (number per km^2)	8.40	7.43	8.62	0.500
Dam density (number per km^2)	1.64	0.93	0.95	0.147
Water temperature (ºC)	18.13	16.08	16.96	0.001*
Total suspended solids (mg/l)	3.0	2.0	2.0	0.339
Alkalinity (mg/l)	18.5	9.0	9.0	<0.001*
Hardness (mg/l)	25.0	11.0	14.0	<0.001*
Chloride (mg/l)	6.28	5.48	10.70	0.001*
Ammonia (mg/l)	0.011	0.008	0.015	<0.001*
Total nitrogen (mg/l)	0.328	0.264	0.357	0.044*
Total phosphorus (mg/l)	0.016	0.008	0.011	<0.001*
Macroinvertebrate MMI	65.50	80.00	69.00	0.011*

Figure 5 (opposite page). Dendrogram and ordination plot using nonmetric multidimensional scaling forming three fish macroinvertebrate stream classes (class 1 = triangles, class 2 = circles, class 3 = squares) using fish species from 30 least disturbed streams. Refer to Table 1 for more information.

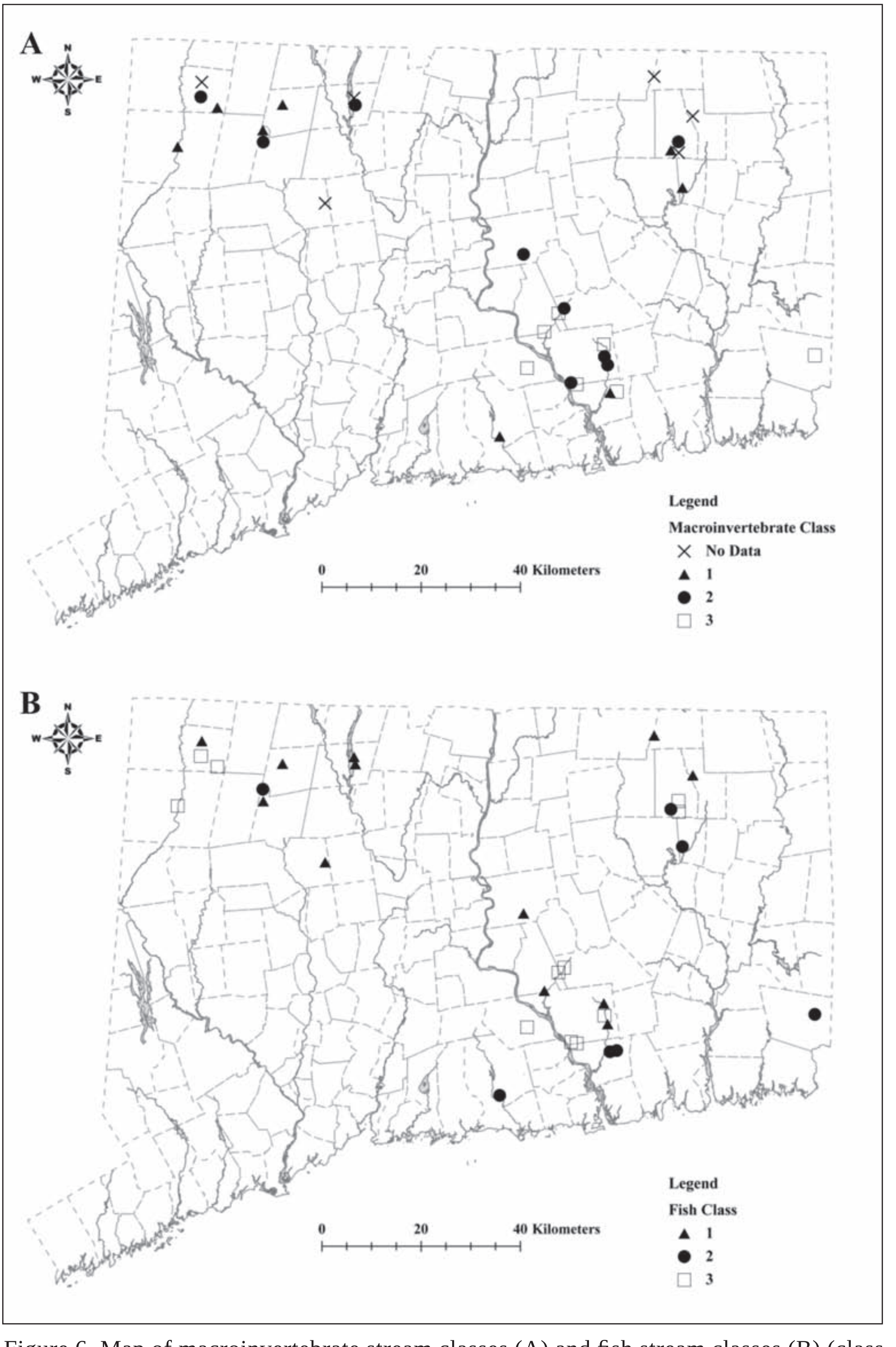


Figure 6. Map of macroinvertebrate stream classes (A) and fish stream classes (B) (class 1 = triangles, class 2 = circles, class 3 = squares) defined using cluster analysis. Refer to Table 1 for more information.

stream classes (Table 6). Macroinvertebrate stream class 1 sites were, in general, larger drainage basins with warmer water temperatures and higher alkalinity and hardness. Macroinvertebrate stream class 2 sites were the smallest and had the least amount of stratified drift, but with similar water temperatures to class 3. Macroinvertebrate stream class 3 sites were intermediate in drainage area and had the highest chloride concentrations. NMS ordination plots also showed a drainage area and temperature gradient for macroinvertebrate stream classes along axis 1 (Fig. 4). Axis 1 (r^2 = 0.404) and axis 2 (r^2 = 0.388) accounted for approximately about 80% of the variation present in the matrix based on of the Sorensen dissimilarities between all least disturbed sites.

Drainage area, stratified drift, dam density, water temperature, total suspended solids, alkalinity, hardness, ammonia, TN, and TP were all significant variables (P < 0.05) between fish stream classes (Table 7). Fish stream class 2 sites had larger drainage areas and warmer water temperatures than fish stream class 1 or 3. Fish stream class 1 sites were, in general, smaller drainage basins with low percentages of stratified drift, slightly cooler water temperatures, and the lowest alkalinity and hardness concentrations of the three fish classes. NMS ordination plots also showed a drainage area and temperature gradient for fish stream classes along axis 2 (Fig 5). Axis 1 (r^2 = 0.312) and axis 2 (r^2 = 0.248) accounted for approximately about 56% of the variation present in the matrix based on of the Sorensen dissimilarities between all least disturbed sites.

Discussion

This study is the first that we know of that identifies least disturbed streams in Connecticut. Identifying these 30 least disturbed streams and documenting the fish and macroinvertebrate communities, along with observations on some of the variables that influence their distribution, provides a necessary step to describing the biology of stream organisms under least disturbed conditions in Connecticut.

Table 7. Median site characteristics for least disturbed fish site classes. The Kruskal-Wallis test was used to compare site characteristics between classes and those that showed significantly differences (P < 0.05) are noted with an asterisk.

Site characteristic	Class 1	Class 2	Class 3	P value
Drainage area (km^2)	4.22	20.69	6.31	<0.001*
Stratified drift (%)	1.165	9.64	3.72	0.014*
Road density (number per km^2)	7.39	7.84	9.16	0.140
Dam density (number per km^2)	0.00	1.94	1.06	0.003*
Water temperature (ºC)	16.32	18.08	16.47	0.003*
Total suspended solids (mg/l)	2.0	2.0	3.0	0.595
Alkalinity (mg/l)	8.50	13.00	14.81	<0.001*
Hardness (mg/l)	12.00	16.00	21.00	<0.001*
Chloride (mg/l)	5.50	8.60	8.03	0.05
Ammonia (mg/l)	0.008	0.017	0.010	<0.001*
Total nitrogen (mg/l)	0.294	0.417	0.304	<0.001*
Total phosphorus (mg/l)	0.008	0.020	0.010	<0.001*

These 30 least disturbed streams were generally located in three groups in the northeast, northwest, and central Connecticut River valley. The least disturbed streams and their watersheds described in this study represent a subset of the "best of what's left" in Connecticut and are distributed in this pattern due to past land-use practices and human activities. Human activity—including town settlement, farming, forestry, canals, railroads, highways, mining, gristmills, factory mills, and urbanization—all have influenced Connecticut's landscape (Bell 1985). The areas that we have identified as least disturbed have not been subjected to land uses, such as urbanization, that can have potential long-term effects on biological communities (Foster 1992, Foster et al. 2003, Harding et al. 1998, Maloney et al. 2008, Wenger et al. 2008).

In addition, because these streams represent least disturbed conditions in Connecticut, those without existing land-protection practices could be targeted for protection and potential land acquisition. To this end, we did a cursory GIS analysis based on the best available data on a statewide scale. We calculated the percent of protected land (open space, preserved municipal land, state forests, state parks, and wildlife management areas) in the upstream drainage basin for the 30 least disturbed watersheds. We found that several of these watersheds may have opportunities for future preservation because they showed very low percentages of protected land at the scale we analyzed. It should be noted that a finer scale GIS analysis, which includes attributes such as town land records and data on local conservation and development areas, may reveal other opportunities for preservation and vulnerabilities in these watersheds, and we believe that it would be beneficial to assemble such a GIS layer to include in future analyses.

Our approach to identifying least disturbed streams by eliminating known anthropogenic stressors may be valuable for other programs that seek to identify least disturbed conditions. Our attempts to reduce or eliminate anthropogenic impacts, by selecting study streams using a GIS screening followed by site checks, incorporated many potential factors that impact biological integrity of streams, including measures of land use (% IC), stream flow and geomorphology (dams, diversions), habitat fragmentation (dams), and fish stocking (Bellucci 2007, Fausch 1988, Graf 1999, Poff et al. 1997, Wang et al. 2001). We recognize that in some cases, this approach could be viewed as restrictive (e.g., location of diversion is such that it does not impact the stream) or in other cases, there could be factors that are not captured by broad scale GIS (e.g., spills, natural disturbance) and therefore may not represent actual stream conditions.

Regardless, all but one of the streams in our study passed aquatic life goals (i.e., MMI > 55) using the macroinvertebrate MMI (Table 2) and, in general, plotted within the expected range of high MMI values given the level of disturbance (Fig. 3). In Hall Meadow Brook (SID 2311), the only least disturbed stream that had an inconclusive MMI score (50), the macroinvertebrate community could have been impacted by the low stream flow during the year prior to the macroinvertebrate index period. This period of low flow is a natural stressor that could not have been identified using GIS. Subsequent visits to this site on Hall Meadow Brook, using the same sampling methodology used in this study under average stream flow conditions, resulted in higher MMI scores (e.g., MMI of 74 collected on 4 November 2008).

It is unclear why MMI scores from Hall Meadow Brook could have been more impacted by low stream flow than the other least disturbed watersheds during our study period. Our hypothesis is that low stream flow, coupled with presence of a 0.40-km^2 wetland complex upstream of SID 2311, could have combined negative effects that resulted in lower macroinvertebrate abundance and diversity in the 2007 sample. SID 2311 was grouped by the CA into macroinvertebrate stream class 1 (Fig. 4), which consisted of larger drainage basins with warmer water temperatures (Table 6). Re-sampling the macroinvertebrates from least disturbed streams during additional years under varying flow conditions and adding a sampling site upstream of the wetland complex may help resolve this question.

Our data show that although most of the least disturbed streams in our study have MMI scores that meet aquatic life goals for Connecticut, there can be differences in the macroinvertebrate taxa and potential influencing abiotic factors that are worth noting. The three macroinvertebrate stream classes each had distinct indicator taxa; our data suggest that drainage area, water temperature, alkalinity, hardness, chloride, ammonia, TN, and TP may potentially be important variables that influence macroinvertebrate taxa distribution in least disturbed watersheds. Our analyses also show that drainage area, stratified drift, dam density, water temperature, total suspended solids, alkalinity, hardness, ammonia, TN, and TP may potentially be important variables that influence fish species distribution in Connecticut's least disturbed watersheds. These relationships do not show cause and effect relationships, but may help to identify parameters that could be important for monitoring least disturbed watersheds, and are worthy to consider for future monitoring efforts. Further data collection would help to confirm the importance of these variables on macroinvertebrate and fish communities and their influence in forming distinct community groups, such as the biological classes identified by the CA and indicator species analysis in this study.

Our results may also reflect our incomplete knowledge of how certain factors affect fish and macroinvertebrate species. On the one hand, variables such as drainage area consistently show a strong influence on macroinvertebrate and fish assemblages (Gerritsen and Jessup 2007, Kanno and Vokoun 2008, Vannote et al. 1980). On the other hand, our knowledge on the influence of dams on macroinvertebrate and fish assemblages is incomplete. For example, although we excluded watersheds with large dams and sampled at least 1.6 km downstream of small dams, macrohabitat generalist fish species were unexpectedly found to be indicator species in all three fish stream classes (Table 5). Brook Trout and Golden Shiner were two fish species indicative of fish class

1 streams. It was unexpected to have a species such as Golden Shiner, a macrohabitat specialist typically associated with ponds, as an indicator of least disturbed streams. This finding may reflect the unavoidable influence that mill dams have on aquatic biota in Connecticut. An interesting follow-up study would be to evaluate the changes in macroinvertebrate and fish species composition with increasing distance from a dam.

Our analysis of the percent occurrence of taxa from this study to higher levels of human disturbance indicate that least disturbed streams may offer important habitat for some aquatic species in Connecticut. Some macroinvertebrate taxa may occur exclusively in small, least disturbed streams in Connecticut (e.g., *Adicrophleps hitchcocki*), while others may be impacted by low levels of human disturbance (e.g., *Acroneuria abnormis, Diplectrona* spp.). Least disturbed streams may also be important habitat for fish species such as Burbot, Slimy Sculpin, and Brook Trout.

We believe that Brook Trout can be viewed as a sentinel species for small, healthy, least disturbed streams in Connecticut because they are the most important indicator fish species and are sensitive to landscape alterations (Kocovsky and Carline 2006, Stranko et al. 2008). Our study documents the occurrence of Brook Trout in 90% of the small, least disturbed streams and a decline in percent occurrence with an increase in human disturbance (Appendix 2). Similar to the use of the sentinel canary in a coal mine to warn miners of potentially lethal carbon monoxide concentrations in coal mines, monitoring shifts in age and size class of Brook Trout populations can warn natural resource managers of potential anthropogenic stress in healthy watersheds.

In an investigation that included 1184 streams in Connecticut that were representative of the entire BCG range, Kanno and Vokoun (2008) found that Brook Trout were indicators of small watersheds with cool water temperatures. Similarly, Brook Trout occurred in 90% of least disturbed study watersheds in our study and were a significant indicator of fish class 1 streams. An investigation (e.g., Cormier et al. 2000, Norton et al. 2009, Yuan and Norton 2004) to determine the cause for the absence of Brook Trout from three least disturbed watersheds in our study—Beaver Brook (SID 1236), Carse Brook (SID 1981), and Chatfield Hollow Brook (SID 2334)—could provide an opportunity to learn about important stressors to these least disturbed watersheds. Monitoring water temperature, total suspended solids, alkalinity, hardness, ammonia, TN, and TP, all significant variables in our fish stream analysis, would be an important component of such an investigation.

For decades, water programs were funded to support programs that focused on point-source pollution and impaired waters. This strategy has greatly improved the water quality in Connecticut and throughout the nation. However, we believe that the need for funding support to least disturbed streams is long overdue and a more holistic effort is needed to truly fulfill the requirements of the FCWA to *maintain*, as well as restore, the chemical, physical, and biological integrity of the nation's waters.

Decades of working on impaired waters has taught us that it is labor intensive, costly, and time consuming to identify, diagnose, and fix impaired waters. While these efforts must continue, we believe that a concurrent strategy to maintain least disturbed watersheds should be employed that involves evaluating their condition and using anti-degradation policies in the FCWA to hold the line and not allow these waters to degrade. This study is an important step in achieving this goal to ensure that we are maintaining the chemical, physical, and biological integrity of the "best of what's left" in Connecticut.

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Appendix 1. Percent occurrence and functional feeding group for macroinvertebrate taxa collected in this study (n = 24) compared to sites with higher levels of anthropogenic stress. The two additional categories along the human disturbance gradient were established using data collected by Connecticut DEP from wadeable streams in Connecticut 1995–2009 using similar sampling protocols used in this study. Sites were binned by percent impervious land cover (IC) as mid-level stress sites (IC 4.1-11.99%, n = 411) and high-level stress sites (IC values > 12%, n = 127). C-G = collector-gatherer, C-F = collector-filterer, SCR = scraper, SHR = shredder, and PRD = predator.

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Basommatophora	Ancylidae	*Ferrissia*	8.33	3.60	6.30	SCR
Basommatophora	Ancylidae	*Laevapex fuscus* C. B. Adams	8.33	15.10	7.90	SCR
Basommatophora	Lymnaeidae	*Pseudosuccinea columella* Say	4.17	0.20	0.00	C-G
Basommatophora	Physidae	*Physa*	4.17	7.10	11.80	C-G
Coleoptera	Dryopididae	*Helichus*	8.33	0.70	0.00	SCR
Coleoptera	Elmidae	*Macronychus glabratus* Say	8.33	8.30	8.70	SHR
Coleoptera	Elmidae	*Optioservus*	37.50	42.80	8.70	SCR
Coleoptera	Elmidae	*Optioservus ovalis* Leconte	50.00	15.10	3.10	SCR
Coleoptera	Elmidae	*Oulimnius*	54.17	10.00	3.90	SCR
Coleoptera	Elmidae	*Oulimnius latiusculus* Leconte	58.33	26.50	11.00	C-G
Coleoptera	Elmidae	*Promoresia*	4.17	1.90	1.60	SCR
Coleoptera	Elmidae	*Promoresia elegans* Leconte	4.17	1.50	0.00	SCR
Coleoptera	Elmidae	*Promoresia tardella* Fall	87.50	15.10	1.60	SCR
Coleoptera	Elmidae	*Stenelmis*	83.33	29.70	39.40	SCR
Coleoptera	Hydrophilidae	*Cymbiodyta*	4.17	0.00	0.00	N/A
Coleoptera	Psephenidae	*Ectopria*	66.67	3.90	3.90	SCR
Coleoptera	Psephenidae	*Psephenus herricki* DeKay	75.00	32.60	10.20	SCR
Coleoptera	Ptilodactylidae	*Anchytarsus bicolor* Melsheimer	33.33	3.20	0.00	SHR
Decapoda	Cambaridae	*Orconectes limosus* Rafinesque	4.17	0.50	1.60	C-G
Diptera	Athericidae	*Atherix*	12.50	1.20	0.00	PRD
Diptera	Ceratopogonidae	*Bezzia* group	25.00	1.90	0.80	PRD

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Diptera	Chironomidae	*Apsectrotanypus*	4.17	0.00	0.00	PRD
Diptera	Chironomidae	*Brillia*	4.17	4.90	8.70	SHR
Diptera	Chironomidae	*Corynoneura*	25.00	1.90	2.40	C-G
Diptera	Chironomidae	*Cricotopus*	8.33	7.10	14.20	SHR
Diptera	Chironomidae	*Diamesa*	4.17	4.60	2.40	C-G
Diptera	Chironomidae	*Eukiefferiella*	4.17	1.50	1.60	C-G
Diptera	Chironomidae	*Eukiefferiella claripennis* group Lundbeck	4.17	1.50	3.90	C-G
Diptera	Chironomidae	*Eukiefferiella devonica* group Edwards	4.17	3.60	0.00	C-G
Diptera	Chironomidae	*Eukiefferiella tirolensis* Goetghebuer	4.17	0.70	0.80	C-G
Diptera	Chironomidae	*Limnophyes*	16.67	0.20	0.80	C-G
Diptera	Chironomidae	*Lopescladius*	4.17	0.50	0.00	C-G
Diptera	Chironomidae	*Micropsectra*	50.00	2.40	2.40	C-G
Diptera	Chironomidae	*Micropsectra/Tanytarsus*	4.17	1.20	0.00	C-G
Diptera	Chironomidae	*Microtendipes pedellus* group De Geer	4.17	18.00	15.70	C-F
Diptera	Chironomidae	*Microtendipes rydalensis* group Edwards	8.33	3.60	0.00	C-F
Diptera	Chironomidae	*Nanocladius*	37.50	3.40	0.00	C-G
Diptera	Chironomidae	*Natarsia*	4.17	0.00	0.00	PRD
Diptera	Chironomidae	*Orthocladius (Symposiocladius) lignicola* Kieffer	4.17	0.00	0.00	C-G
Diptera	Chironomidae	*Parachaetocladius*	41.67	3.60	0.00	C-G
Diptera	Chironomidae	*Parametriocnemus*	66.67	13.40	10.20	C-G
Diptera	Chironomidae	*Paratanytarsus*	20.83	0.50	0.80	C-F
Diptera	Chironomidae	*Polypedilum*	12.50	5.80	8.70	SHR
Diptera	Chironomidae	*Polypedilum aviceps* Townes	54.17	10.90	11.00	SHR
Diptera	Chironomidae	*Polypedilum fallax* group Johannsen	8.33	1.00	1.60	SHR
Diptera	Chironomidae	*Polypedilum tritum* Walker	33.33	0.50	0.00	SHR
Diptera	Chironomidae	*Rheocricotopus*	8.33	2.70	5.50	C-G
Diptera	Chironomidae	*Rheotanytarsus exiguus* group Johannsen	16.67	18.20	15.00	C-F

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Diptera	Chironomidae	*Rheotanytarsus pellucidus* group Walker	16.67	4.60	3.10	C-F
Diptera	Chironomidae	*Stempellinella*	4.17	0.20	0.00	C-G
Diptera	Chironomidae	*Stenochironomus*	12.50	1.90	0.80	C-G
Diptera	Chironomidae	*Stilocladius*	12.50	1.70	0.80	C-G
Diptera	Chironomidae	*Tanytarsus*	20.83	4.10	10.20	C-F
Diptera	Chironomidae	*Thienemanniella*	12.50	2.40	4.70	C-G
Diptera	Chironomidae	*Thienemannimyia* group	37.50	11.20	18.10	PRD
Diptera	Chironomidae	*Tvetenia bavarica* group Kieffer	62.50	12.70	11.00	C-G
Diptera	Chironomidae	*Tvetenia vitracies* group Saether	4.17	18.50	18.90	C-G
Diptera	Empididae	*Hemerodromia*	25.00	18.00	37.80	PRD
Diptera	Simuliidae	*Simulium*	12.50	32.10	33.90	C-F
Diptera	Tabanidae	*Hybomitra*	8.33	0.00	0.00	PRD
Diptera	Tipulidae	*Antocha*	4.17	37.00	43.30	C-G
Diptera	Tipulidae	*Dicranota*	70.83	6.10	3.10	PRD
Diptera	Tipulidae	*Hexatoma*	75.00	2.90	0.80	PRD
Diptera	Tipulidae	*Limnophila*	8.33	0.50	0.00	PRD
Diptera	Tipulidae	*Limonia*	4.17	0.20	0.00	PRD
Diptera	Tipulidae	*Tipula*	87.50	20.20	27.60	SHR
Ephemeroptera	Baetidae	*Baetis*	16.67	26.80	19.70	C-G
Ephemeroptera	Baetidae	*Baetis flavistriga* McDunnough	4.17	5.40	6.30	C-G
Ephemeroptera	Baetidae	*Baetis pluto* McDunnough	16.67	4.40	1.60	C-G
Ephemeroptera	Baetidae	*Baetis tricaudatus* Dodds	16.67	6.10	9.40	C-G
Ephemeroptera	Baetidae	*Diphetor hageni* Eaton	4.17	0.00	0.00	C-G
Ephemeroptera	Baetidae	*Heterocloeon*	4.17	2.40	0.00	SCR
Ephemeroptera	Baetidae	*Procloeon*	4.17	0.00	0.00	C-G
Ephemeroptera	Ephemerellidae	*Ephemerella*	41.67	6.30	0.80	C-G
Ephemeroptera	Ephemerellidae	*Eurylophella funeralis* McDunnough	33.33	3.90	3.10	C-G

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Ephemeroptera	Ephemerellidae	*Serratella deficiens* Morgan	8.33	11.70	0.80	C-G
Ephemeroptera	Heptageniidae	*Epeorus*	12.50	5.10	0.00	SCR
Ephemeroptera	Heptageniidae	*Maccaffertium*	91.67	22.90	16.50	SCR
Ephemeroptera	Heptageniidae	*Maccaffertium modestum* group Banks	54.17	31.40	26.80	SCR
Ephemeroptera	Heptageniidae	*Maccaffertium terminatum* Walsh	8.33	0.20	0.00	SCR
Ephemeroptera	Heptageniidae	*Maccaffertium vicarium* Walker	25.00	1.00	0.00	SCR
Ephemeroptera	Isonychiidae	*Isonychia*	41.67	31.10	4.70	C-G
Hoplonemertea	Tetrastemmatidae	*Prostoma*	8.33	5.10	7.90	PRD
Lumbriculida	Lumbriculidae		66.67	17.30	15.00	C-G
Megaloptera	Corydalidae	*Corydalus cornutus* Linnaeus	4.17	10.90	4.70	PRD
Megaloptera	Corydalidae	*Nigronia serricornis* Say	100.00	27.30	20.50	PRD
Megaloptera	Sialidae	*Sialis*	8.33	3.20	1.60	PRD
Neotaenioglossa	Hydrobiidae	*Amnicola limosus* Say	4.17	4.60	0.80	SCR
Odonata	Aeshnidae	*Boyeria vinosa* Say	54.17	4.40	0.80	PRD
Odonata	Calopterygidae	Calopterygidae	8.33	0.50	0.00	PRD
Odonata	Cordulegastridae	*Cordulegaster*	4.17	0.20	0.00	PRD
Odonata	Cordulegastridae	*Cordulegaster maculate* Selys	4.17	0.00	0.00	PRD
Odonata	Gomphidae	*Lanthus*	20.83	0.50	0.00	PRD
Odonata	Gomphidae	*Lanthus parvulus* Selys	8.33	0.70	0.00	PRD
Odonata	Gomphidae	*Lanthus vernalis* Carle	4.17	0.00	0.00	PRD
Odonata	Gomphidae	*Ophiogomphus*	20.83	2.70	0.00	PRD
Odonata	Gomphidae	*Stylogomphus albistylus* Hagen	8.33	3.60	2.40	PRD
Odonata	Libellulidae		4.17	0.00	0.00	PRD
Plecoptera	Capniidae	*Paracapnia*	8.33	1.20	0.00	SHR
Plecoptera	Chloroperlidae	*Sweltsa*	29.17	1.50	0.80	PRD
Plecoptera	Leuctridae	*Leuctra*	33.33	1.50	0.00	SHR
Plecoptera	Peltoperlidae	*Tallaperla*	54.17	1.90	0.00	SHR

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Plecoptera	Perlidae	*Acroneuria*	25.00	7.10	1.60	PRD
Plecoptera	Perlidae	*Acroneuria abnormis* Newman	79.17	20.40	1.60	PRD
Plecoptera	Perlidae	*Agnetina capitata* Pictet	4.17	0.70	0.00	PRD
Plecoptera	Perlidae	*Eccoptura xanthenes* Newman	12.50	1.50	0.00	PRD
Plecoptera	Perlidae	*Paragnetina*	8.33	1.20	0.00	PRD
Plecoptera	Perlidae	*Paragnetina immarginata* Say	8.33	2.40	0.00	PRD
Plecoptera	Perlidae	*Paragnetina media* Walker	29.17	9.70	0.80	PRD
Plecoptera	Perlodidae		8.33	2.20	0.80	PRD
Plecoptera	Pteronarcyidae	*Pteronarcys*	16.67	0.70	0.00	SHR
Plecoptera	Taeniopterygidae	*Taeniopteryx*	4.17	12.90	5.50	SHR
Trichoptera	Apataniidae	*Apatania*	8.33	21.40	2.40	SCR
Trichoptera	Brachycentridae	*Adicrophleps hitchcocki* Flint	16.67	0.00	0.00	SHR
Trichoptera	Brachycentridae	*Brachycentrus appalachia* Flint	12.50	0.50	0.00	C-F
Trichoptera	Brachycentridae	*Micrasema*	37.50	9.50	3.90	SHR
Trichoptera	Glossosomatidae	*Agapetus*	4.17	0.00	0.00	SCR
Trichoptera	Glossosomatidae	*Glossosoma*	62.50	38.70	22.00	SCR
Trichoptera	Goeridae	*Goera*	12.50	1.50	0.00	SCR
Trichoptera	Helicopsychidae	*Helicopsyche borealis* Hagen	8.33	4.10	0.00	SCR
Trichoptera	Hydropsychidae	*Ceratopsyche sparna* Ross	41.67	39.90	28.30	C-F
Trichoptera	Hydropsychidae	*Ceratopsyche ventura* Ross	41.67	0.20	0.00	C-F
Trichoptera	Hydropsychidae	*Cheumatopsyche*	87.50	64.20	66.90	C-F
Trichoptera	Hydropsychidae	*Diplectrona*	79.17	4.40	3.90	C-F
Trichoptera	Hydropsychidae	*Hydropsyche*	70.83	29.40	33.10	C-F
Trichoptera	Hydropsychidae	*Hydropsyche betteni* Ross	50.00	56.40	55.90	C-F
Trichoptera	Lepidostomatidae	*Lepidostoma*	45.83	13.10	1.60	SHR
Trichoptera	Leptoceridae	*Mystacides sepulchralis* Walker	4.17	0.20	1.60	C-G
Trichoptera	Leptoceridae	*Oecetis persimilis* Banks	16.67	0.20	0.00	PRD

Order	Family	Taxon	% occurrence this study (n = 24 sites)	% occurrence mid-level stress (n = 411 sites)	% occurrence high-level stress (n = 127 sites)	Functional feeding group
Trichoptera	Limnephilidae		25.00	1.50	0.00	SHR
Trichoptera	Odontoceridae	*Psilotreta*	45.83	1.90	0.00	SCR
Trichoptera	Philopotamidae	*Chimarra aterrima* Hagen	29.17	39.20	22.80	C-F
Trichoptera	Philopotamidae	*Dolophilodes*	91.67	18.70	12.60	C-F
Trichoptera	Philopotamidae		4.17	3.20	0.80	C-F
Trichoptera	Polycentropodidae	*Polycentropus*	29.17	4.90	0.00	PRD
Trichoptera	Psychomyiidae	*Lype diversa* Banks	8.33	0.70	0.00	SCR
Trichoptera	Rhyacophilidae	*Rhyacophila*	62.50	9.00	2.40	PRD
Trichoptera	Rhyacophilidae	*Rhyacophila carolina* Banks	33.33	7.10	0.80	PRD
Trichoptera	Rhyacophilidae	*Rhyacophila fuscula* Walker	16.67	15.60	6.30	PRD
Trichoptera	Rhyacophilidae	*Rhyacophila manistee* Ross	12.50	1.90	0.00	PRD
Trichoptera	Rhyacophilidae	*Rhyacophila minora* Banks	41.67	1.20	0.00	PRD
Trichoptera	Uenoidae	*Neophylax*	4.17	6.60	0.80	SCR
Trombidiformes	Limnocharidae	*Rhyncholimnochares*	12.50	0.20	0.00	PRD
Trombidiformes	Sperchonidae	*Sperchon*	4.17	1.70	1.60	PRD
Tubificida	Tubificidae	Tubificidae with cap setae	4.17	0.00	0.80	C-G
Veneroida	Pisidiidae	*Pisidium*	8.33	0.50	0.80	C-F

Appendix 2. Percent occurrence, tolerance value, and stream flow guild for fish species collected in this study (n = 30) compared to sites with higher levels of anthropogenic stress. Species collected in this study were compared to 2 additional categories along the human disturbance gradient based on data collected by Connecticut DEP from wadeable streams in Connecticut 1995–2009 using similar sampling protocols used in this study. Sites were binned by percent impervious land cover (IC) as mid-level stress sites (IC 4.1-11.99%, n = 341) and high-level stress sites (IC values > 12%, n= 109). Tolerance values and stream preferences were taken from regional references (Armstrong et al. 2001, Halliwell et al. 1999, Whitworth 1996). I = intolerant, M = intermediate, T = tolerant; FS = fluvial specialist, FD = fluvial dependent, MG = macrohabitat generalist.

Family	Species	% occurrence this study (n = 30 sites)	% occurrence mid-level stress (n = 341 sites)	% occurrence high-level stress (n = 109 sites)	Tolerance value	Flow guild	Water temperature preference	Native
Anguillidae	*Anguilla rostrata* Lesueur	40.00	71.55	67.89	T	FD	W	Yes
Catostomidae	*Catostomus commersoni* Lacepède	40.00	82.40	77.06	T	FD	Cool	Yes
Centrarchidae	*Lepomis auritus* Linnaeus	3.33	38.12	25.69	M	MG	W	Yes
Centrarchidae	*Lepomis cyanellus* Rafinesque	16.67	9.09	9.17	T	FD	W	No
Centrarchidae	*Lepomis gibbosus* Linnaeus	13.33	40.76	33.03	M	MG	W	Yes
Centrarchidae	*Lepomis macrochirus* Rafinesque	20.00	48.39	43.12	T	MG	W	No
Centrarchidae	*Micropterus dolomieu* Lacepède	3.33	20.53	5.50	M	MG	Cool	No
Centrarchidae	*Micropterus salmoides* Lacepède	20.00	37.24	40.37	M	MG	W	No
Cottidae	*Cottus cognatus* Richardson	3.33	0.00	0.00	I	FS	Cold	Yes
Cyprinidae	*Luxilus cornutus* Mitchill	26.67	30.21	18.35	M	FD	Cool	Yes
Cyprinidae	*Notemigonus crysoleucas* Mitchill	16.67	16.13	17.43	T	MG	W	Yes
Cyprinidae	*Rhinichthys atratulus* Hermann	86.67	82.70	81.65	T	FS	Cool	Yes
Cyprinidae	*Rhinichthys cataractae* Valenciennes	30.00	46.92	49.54	M	FS	Cool	Yes
Cyprinidae	*Semotilus atromaculatus* Mitchill	13.33	21.70	19.27	T	MG	Cool	Yes
Cyprinidae	*Semotilus corporalis* Mitchill	23.33	35.78	13.76	M	FS	Cool	Yes
Esocidae	*Esox americanus* Gmelin	6.67	14.66	11.01	M	MG	W	Yes
Esocidae	*Esox niger* Lesueur	16.67	12.32	3.67	M	MG	W	Yes
Gadidae	*Lota lota* Linnaeus	3.33	0.00	0.00	M	FS	Cold	Yes

Family	Species	% occurrence this study (n = 30 sites)	% occurrence mid-level stress (n = 341 sites)	% occurrence high-level stress (n = 109 sites)	Tolerance value	Flow guild	Water temperature preference	Native
Ictaluridae	*Ameiurus natalis* Lesueur	3.33	5.57	0.92	T	MG	W	No
Ictaluridae	*Ameiurus nebulosus* Lesueur	13.33	15.54	9.17	T	MG	W	Yes
Percidae	*Etheostoma fusiforme* Girard	3.33	0.29	0.00	I	MG	W	Yes
Percidae	*Etheostoma olmstedi* Storer	16.67	59.82	57.80	M	FS	Cool	Yes
Percidae	*Perca flavescens* Mitchill	3.33	16.72	12.84	M	MG	Cool	Yes
Salmonidae	*Oncorhynchus mykiss* Walbaum	3.33	21.99	10.09	I	FS	Cold	No
Salmonidae	*Salmo salar* Linnaeus	10.00	6.45	1.83	I	FS	Cold	Yes
Salmonidae	*Salmo trutta* Linnaeus	16.67	29.33	20.18	I	FS	Cold	No
Salmonidae	*Salvelinus fontinalis* Mitchill	90.00	28.15	17.43	I	FS	Cold	Yes