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Authors: Orlofske, Jessica M., and Baird, Donald J.

Source: Freshwater Science, 33(3) : 978-994

Published By: Society for Freshwater Science

URL: https://doi.org/10.1086/676912

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A geometric morphometric approach to establish body-shape trait criteria for aquatic insects

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Abstract: Body shapes of aquatic insect larvae reflect phenotypic responses to complex environmental conditions and can be used to infer habitat properties and indicate natural and anthropogenic perturbations in river ecosystems. Investigation of relationships between body shape and physical-habitat characteristics has been restricted by a lack of an objective schema for quantitative characterization of body-shape variation. We present a functional ecological framework for body-shape classification based on defined criteria. We applied a geometric morphometric (GM) approach to the general classification of body shape in 4 morphologically diverse orders, Ephemeroptera (E), Plecoptera (P), Trichoptera (T), and Odonata (O) collected from 3 sites with contrasting hydrological and hydraulic characteristics. We describe a robust classification of body shapes for E, P, and O, which possess a compartmentalized body plan, and suggest a preliminary classification for T. We compared GM body shapes with body-shape trait states available in trait databases and found discordance between the 2 classifications. We explored the value of GM body shapes to describe taxon shape structure of reference sites and to detect variation reflecting physical properties of the sites. GM body-shape classes can augment the trait states already available and enhance inference regarding habitat status. Patterns in the shape strategies of aquatic insects, particularly EPO taxa, can be used to extrapolate shape information for other taxonomic groups. GM provides a stable shape classification that can contribute to the description of different ecological strategies of aquatic insects. Expanding the scope of shape information available for many taxonomic groups can improve our understanding of how organism phenotype relates to environmental conditions and supports traits-based assessment.

Key words: trait states, trait variation, biomonitoring, Ephemeroptera, Plecoptera, Trichoptera, Odonata, ecohydraulics

Body shape or form is an important phenotypic character of aquatic organisms and reflects morphological adaptation linked to life-history strategies in the 3-dimensional volume of aquatic habitats (Purcell 1977, Vogel 1994). Body shape has been studied extensively for aquatic vertebrates, especially fish, with a focus on morphological properties that contribute to feeding and locomotion (Webb 1984, Haas et al. 2010, Farré et al. 2013). Body shape is also a valuable functional attribute of aquatic insects, but it has received only sporadic attention, generally focused on a limited selection of families, genera, and species (Sheldon 1980, Smith and Dartnall 1980, Hogue and Hawkins 1991, Sites et al. 1996, Arnqvist and Johansson 1998, Funk et al. 2008, Giacomini and de Marco 2008, Lee and Lin 2012, Müller-Peddinghaus and Hering 2013) with little integration among studies regarding the influence of environmental characteristics on aquatic insect shape.

Aquatic systems present insects with physical conditions that can impose significant fitness challenges on physiological and morphological adaptations, i.e., the habitat templet (Southwood 1977) influences both taxonomic and trait-occurrence patterns (Townsend and Hildrew 1994, Poff 1997). In lotic environments, body shape affects how aquatic insects cope with complex micro- and mesoscale flow environments (Smith and Dartnall 1980, Statzner and Holm 1982, 1989, Statzner 1988, 2008). Mediated through other morphological traits, such as body size, body shape interacts with flow forces including turbulence, fluid viscosity (Reynolds numbers), boundary layers, and shear stress (Smith and Dartnall 1980, Statzner and Holm 1982, Statzner 1988, Peckarsky et al. 1990, Sagnes et al. 2008, Oldmeadow et al. 2010). Body shape couples fluid properties with morphological characteristics and may correspond to behavioral responses, including movement patterns and

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DOI: 10.1086/676912. Received 14 July 2013; Accepted 15 November 2013; Published online 16 May 2014.
Freshwater Science. 2014. 33(3):978–994.

orientation to flow and suspended materials. Positive responses to water movement may increase respiration rates or accelerate seston delivery to filter feeders (Davis 1986, Lacoursière 1991, Vogel 1994, Statzner 2008). Negative responses may arise through abrasion from sediment in the water column or detachment from the substrate (Davis 1986, Peckarsky et al. 1990, Oldmeadow et al. 2010). Thus, body shape has implications for how aquatic insects use their habitat and the inferences that can be drawn from their occurrence in a stream (Smith and Dartnall 1980, Oldmeadow et al. 2010).

Body-shape data have been used to assess ecological consequences of natural and anthropogenic perturbations in aquatic systems, particularly hydrological alteration (Horrigan and Baird 2008, Brooks et al. 2011), sedimentation (Dolédec et al. 2006), or multiple stressors associated with land use and industrial development (Dolédec et al. 1999, 2006, 2011). Body shape generally was just one of many traits used in these analyses to form the basis for traits-based metrics, which can support biomonitoring program objectives. Public databases are repositories of aquatic-insect shape-trait information (e.g., US Environmental Protection Agency [EPA] Freshwater Biological Traits Database, http://www.epa.gov/ncea/global/traits/; Taxa and Autecology Database for Freshwater Organisms, http://www.freshwaterecology.info; Tachet et al. 1991, Usseglio-Polatera et al. 2000a, b). In these databases, body shape/form information typically is compiled from species descriptions and taxonomic revisions and only rarely from ecological studies (Poff et al. 2006, Vieira et al. 2006, Culp et al. 2011). Taxonomic sources of body-shape information often describe an idealized form of the organism and provide little data regarding ontogenetic variation or phenotypic plasticity within or between habitats across the geographic range of a taxon. Such variation in body shape could inform ecoevolutionary dynamics as has been hypothesized for other traits (Berg and Ellers 2010, Bolnick et al. 2011).

Availability of body-shape trait records is limited for many taxa. The absence of body-shape trait records may cause loss of taxa that could be used for analysis, and such losses can have detrimental effects (e.g., Pakeman et al. 2013). The gaps in body-shape trait records may be so extensive that they cannot be used in some traits-based analyses. Body-shape trait states used in databases (henceforth, database trait states) are defined primarily on the basis of expectations regarding how the organism experiences drag from fluid movement (Smith and Dartnall 1980, Davis 1986). However, these categories are difficult to assign to previously uncharacterized taxa and may not fully express the shape variation in the aquatic insect community. Important evidence of ecosystem alteration could be lost when analyses of body-shape patterns in aquatic insect communities exclude a significant portion of taxa or specimens because of missing trait-state records or omit shape information completely because of a lack of trait data. The deficiencies in current body-shape trait descriptions indicate a need to develop explicit shape criteria for aquatic insects and to explore shape patterns in natural assemblages.

Body shape is multidimensional, and several methods have been proposed to evaluate it quantitatively. Traditional morphometric approaches (sensu Rohlf and Marcus 1993) rely on measurements of distances between morphological features, including specific body regions or lengths of structures, to describe shape and infer ecological or evolutionarily relationships. Measurement-based approaches have been applied to a limited number of larval (Sheldon 1980, Funk et al. 2008, Giacomini and de Marco 2008, de Paula Paciencia et al. 2012) and adult (Hogue and Hawkins 1991, Ribera and Nilsson 1995, Müller-Peddinghaus and Hering 2013) aquatic insect taxa. A geometric morphometrics (GM) approach for describing organismal shape uses configurations of key morphological points or landmarks to delineate the shape of individual structures (e.g., animal skulls; Rohlf et al. 1996) or entire body forms (e.g., Haas et al. 2010, Farré et al. 2013). The coordinates for each landmark, rather than measurements between points, provide shape information (Zelditch et al. 2004). This approach has several advantages over earlier methods. GM provides shape variables that are appropriate for rigorous statistical analysis (Rohlf and Marcus 1993). The landmark coordinates can be visualized easily to enable interpretation of body form (Rohlf and Marcus 1993). GM has become a standard procedure for examining organismal shape in evolutionary and developmental studies (Adams and Otárola-Castillo 2013) and has been used to resolve evolutionary and systematic questions for aquatic insects (Arnqvist and Johansson 1998, Lee and Lin 2012). GM techniques also have potential for application in conservation (Haas et al. 2010, Perry et al. 2013) and biodiversity studies (Farré et al. 2013).

We used a simplified GM approach to examine patterns in basic shape morphology for selected orders of aquatic insects with diverse ecological characteristics. We described major patterns of shape variation and derived classes of quantitative shape-trait information for ecological analyses of aquatic insect trait data. Our goal was to develop criteria that could facilitate consistent, objective body-shape definitions for a diverse assemblage of aquatic insects.

METHODS

Study sites

We collected aquatic insects from sites in the Miramichi River basin (New Brunswick, Canada). The Miramichi basin covers 13,800 km^2 and drains into the Gulf of St Lawrence (Swansburg et al. 2004). We sampled at 1 site on the South Branch Renous River (SBREN) and 2 sites on the Dungarvon River that were ~23.4 km apart (mid-stream, DUNMR; downstream, DUNDS). We selected sites to represent a gradient of flow and substrate conditions created by the high natural variability of these rivers (Table 1). Sites were assessed in 2010 as being in reference or near-reference condition (least impacted) in New Brunswick (Canadian Aquatic Biomonitoring Network [CABIN] criteria; Environment Canada 2012).

Benthic sampling

We collected benthic macroinvertebrate samples according to the CABIN protocol (Environment Canada 2012). We used a 3-min traveling kicknet (mesh size = 400 μm) procedure in a zig-zag pattern across a riffle to collect each sample. This approach provides an integrated sample across the primary microhabitats available in each reach. We collected all samples in late autumn (2 November 2007) as recommended in the CABIN protocol. We preserved samples in 10% buffered formalin and transferred them to 70% ethanol after 24 to 48 h.

Specimen processing

We extracted specimens representing Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO) from each sample, identified them to the lowest possible taxonomic level (family or genus; Merritt et al. 2008), and photographed them digitally with the aid of a stereomicroscope (Leica Mz 16 A, Wetzlar, Germany; QImaging MicroPublisher 5.0 RTV, Surrey, British Columbia) attached to a Leica 10446261 0.63× extension tube. We collected size information (total body length from the anterior margin of the head to posterior tip of the last abdominal segment) for each specimen from the digital photographs and used size as a covariate in our analyses, where appropriate.

GM analysis

We used a GM approach to describe aquatic insect shape. In a GM approach, the form of each specimen is summarized by a configuration of consistent landmark positions (coordinates) independent of size (Zelditch et al. 2004, Claude 2008). We selected landmark locations for T separately from EPO (Fig. 1A, B, Table 2) because the strongly divergent morphologies of these orders make consistent landmark observations impossible without damaging specimens. We selected landmarks categorized as Type II (maxima, minima, or endpoints of a structure) and Type III (extremal points of morphological structures relative to other features), following the approaches of Bookstein (1991) and Zelditch et al. (2004). Together, these landmarks were suitable for a taxonomically broad (64 families/genera), coarse GM analysis to characterize overall patterns in shape variation. We identified 15 regular landmarks and

Table 1. Summary of water chemistry and channel characteristics obtained during biomonitoring sampling at 3 locations in the Miramichi basin on 2 November 2007 (from Orlofske and Baird 2013).

Variables	South Branch Renous	Dungarvon Mid-reach	Dungarvon downstream
Site			
Abbreviation	SBREN	DUNMR	DUNDS
Latitude	46.79287	46.70777	46.81393
Longitude	−66.48058	−66.15686	−65.91795
Water chemistry variables			
Temperature (°C)	3.59	5.42	6.60
Dissolved O_2 (mg/L)	13.32	14.82	16.56
Conductivity (μS/cm)	36	38	26
pH	7.25	6.39	6.96
Channel characteristics			
Depth (cm)			
Mean	33.5	33.1	42.5
Maximum	48.0	56.0	67.0
Velocity (m/s)			
Mean	0.32	0.63	0.56
Maximum	0.49	0.81	0.71
Width (m)			
Bankfull	33.3	34.4	42.3
Wetted	21.7	24.7	28.0
Discharge (m^3/s)	2.34	5.12	6.64

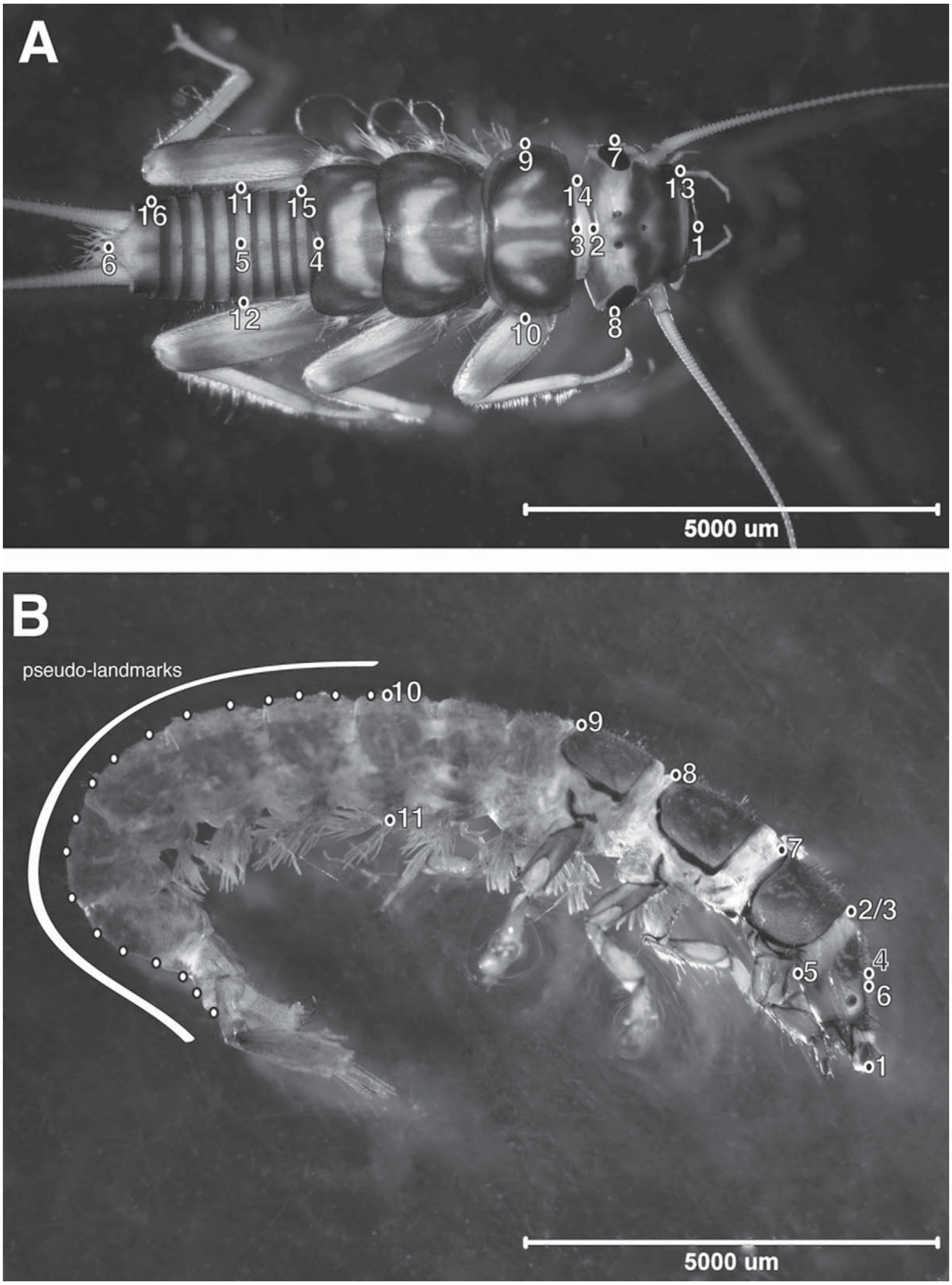

Figure 1. A.—Dorsal view of Perlidae specimen showing positions of 16 landmarks used to define the shape of Ephemeroptera, Plecoptera, and Odonata specimens. A sliding landmark (5) was used to correct for variation in bent specimens and was not included in final statistical analysis. B.—Lateral view of Hydropsychidae specimen showing positions of 11 landmarks and relative positions of pseudolandmarks used to define the shape of Trichoptera specimens. Pseudolandmarks were used along the abdomen of Trichoptera to aid in measurement. Figure originally published by Orlofske and Baird (2013).

Table 2. Morphological landmarks used to characterize shapes of larval aquatic insect orders (Ephemeroptera, Plecoptera, Odonata [EPO], and Trichoptera [T]). Separate landmarks were used to characterize EPO and T. Annotation is based on Merritt et al. 2008. A sliding landmark was used to correct for variation in bent specimens and was not included in final statistical analysis. Pseudolandmarks were used along the abdomen of Trichoptera to aid in measurement. See text for explanation of Type II and III landmarks.

Landmark	Description	Type
Ephemeroptera, Plecoptera, Odonata (dorsal view)		
1	Median point on the distal margin of the labrum	II
2	Intersection of the cervix and the median posterior margin of the head	II
3	Anterior median margin of the pronotum (can be the same point as 2)	II
4	Posterior median margin of the metanotum	II
5	Variably positioned along the dorsal median line of the abdomen	Sliding
6	Dorsal apex of the supraanal process	II
7, 8	Left and right distal margins of the head corresponding to the point of maximum width	II
9, 10	Left and right distal margin of the thoracic region corresponding to the point of maximum width	II
11, 12	Left and right distal margin of the abdominal region corresponding to the point of maximum width	II
13	Midpoint of the left distal margin of the head between landmarks 1 and 7	III
14	Junction of the left posterior margin of the head and the left anterior margin of the pronotum	III
15	Junction of the left posterior margin of the metanotum and the left anterior margin of the 1st abdominal tergum	III
16	Junction of the left anterior margin of tergum 10 (terminal segment) and the left posterior margin of abdominal tergum 9	III
Trichoptera (lateral view)		
1	Distal margin of the labrum	II
2	Intersection of the posterior margin of the head and the cervix (or pronotum if head is not protruding) along the dorsal edge	II
3	Intersection of the anterior margin of the pronotum and the cervix (or posterior margin of head if head is not protruding) along the dorsal edge (2 and 3 may be the same if head is not protruding)	II
4	Dorsal margin of the head corresponding to the point of maximum depth	II
5	Ventral margin of the head corresponding to the point of maximum depth	II
6	Midpoint of the distal dorsal margin of the head between landmarks 1 and 2	II
7	Union of the posterior margin of the prothorax and the anterior margin of the mesothorax along the dorsal edge	II
8	Union of the posterior margin of the mesothorax and the anterior margin of the metathorax along the dorsal edge	II
9	Union of the posterior margin of the metathorax and the anterior margin of the 1st abdominal segment along the dorsal edge	II
10	Dorsal margin of the abdominal region corresponding to the point of maximum depth	III
11	Ventral margin of the abdominal region corresponding to the point of maximum depth	III
12	Multiple landmarks along the dorsal median line of the specimen extending from landmark 10 to the base of the anal proleg of abdominal segment 10 to account for abdomen curvature	Pseudo

1 sliding landmark (used to "unbend" distorted specimens) for a total of 16 landmarks for EPO (Fig. 1A) and 11 landmarks plus a series of pseudolandmarks for T. We used pseudolandmarks to account for the extreme curvature of some T specimens (e.g., Hydropsychidae; Fig. 1B). We used a separate subset method to digitize each body region (head, thorax, and abdomen) to reduce the effect of nonshape variation resulting from the position of each body segment in the photograph (Adams and Rohlf 2000). Coordinates recorded on each body segment were recombined before further analysis. The calibrated digital photographs were used to digitize landmark locations in the package *jpeg* in R (version 0.1-2; Urbanek 2011) and R Studio (version 0.96.304; R Project for Statistical Computing, Vienna, Austria; http://www.rstudio.org/). We stored landmark coordinates directly as R objects, which we exported into data formats appropriate for analysis using TPS morphometric software packages (available from F. J. Rohlf, State University of New York Stony Brook; life.bio.sunysb.edu/morph/index.html). We used a Procrustes analysis (super-

imposition) to eliminate the effects of nonshape variation resulting from rotation, translation, and scaling (*tpsSplin*). The values obtained from this analysis were used to calculate a weight matrix composed of partial warp scores (nonuniform, nonaffine shape components) and uniform, affine shape components (*tpsRelw*). The weight matrix provides the shape variables appropriate for statistical analysis. The weight matrix was converted to a comma-separated values (csv) file (*tpsUtil*) and exported to R for statistical procedures. Analysis of shape data was done in R using the *asbio* (Aho 2011) and *cluster* (Maechler et al. 2012) packages.

Shape-trait classification based on GM data

Body-shape categories were established by using a multivariate cluster analysis (*cluster*) of the weight matrix to identify the patterns of landmark configurations that produced the optimal explained variance and number of distinguishable shape classes (*PAM* function). Classifications for EPO and T were produced separately because of the difference in the initial landmark configurations. Taxonomic identity was compared to the resulting shape classes to assess whether shape classes were evolutionarily linked. Individual specimens were classified into a single shape class. Each taxon was assigned to the shape class having the highest frequency of its constituent specimens. A principal components analysis (PCA) of the multivariate shape variables (weight matrix) was used to visualize patterns in shape variability.

Availability of shape-trait data

Body-shape trait data for North America are available in the US EPA Freshwater Biological Traits Database. Body-shape trait states include *bluff* (blocky), *dorsoventrally flattened*, *round* (humped), *streamlined* (fusiform), and *tubular*. We compiled body-shape trait records from the database for each taxon at the family or genus level. We obtained family-level summaries from data records for genera found only in Atlantic Canada from the CABIN database (http://www.ec.gc.ca/rcba-cabin/default.asp?lang=En&n=4A1D6389-1).

Shape-trait suitability for detecting differences among reference sites

We compared the distribution of individuals and taxa classified according to the database trait states and GM shape classes. We used χ^2 tests to compare the proportions of database trait states and GM shape classes among sites separately for EPO and T in R.

RESULTS

Three biomonitoring samples yielded 4723 intact specimens appropriate for shape analysis. Identifications included 28 families and 44 genera (Table 3), but 9 specimens could not be identified below order level and were excluded from further analysis. Therefore, a total of 64 unique 'lowest taxonomic units' occurred among the samples (Table 3).

GM classification of body shape

The cluster analysis of GM shapes identified 4 EPO shape classes (Fig. 2) distinguished primarily by the width of the head relative to the thorax and abdomen, the widest point of the thorax, the length of the abdomen, and the bluntness of the head and abdomen (Fig. 2). Simple ratios of sizes of body regions could be used to differentiate EPO shape classes (Table 4). Individuals in EPO shape class *narrow head* possessed a narrow head relative to thorax and abdomen width, whereas individuals in EPO shape class *wide head* possessed a wide head relative to thorax and abdomen width. Individuals in EPO shape class *slender* possessed elongated, parallel-sided body forms, and individuals in the EPO shape class *stout* possessed shorter, more compact body shapes (Fig. 2).

Analysis of the T landmarks revealed 4 shape classes (Fig. 3) distinguished by the length and angle of the head, and the relative lengths of each thoracic segment (Fig. 3). The T shape classes were *short head*, *concave head*, *long head*, and *convex head* (Fig. 3). T shape classes were superficially more similar to one another than to EPO shape classes.

GM shape classes are applied to individual specimens, in contrast with the database traits states, which can be applied only to taxonomic units. A taxon's GM shape class can be identified based on the frequency of specimens in a particular shape class. Specimens in the same family may occur in different shape classes because shapes vary among genera within families (e.g., Heptageniidae, Gomphidae, Perlidae, and Perlodidae). However, in our study, 15 to 56% of specimens in 4 genera (*Isonychia*, *Rhithrogena*, *Ephemerella*, *Agnetina*) were distributed among >1 shape classes. We tested for differences in specimen size among classes for each of these genera as a test for allometry, a change in body shape or proportion with development or age. We used 4 separate unbalanced analyses of variance (ANOVAs; one for each genus) to compare log(x)-transformed body length of specimens classified into each shape class. Three of the 4 tests gave preliminary evidence that shape-class membership was related to size (*Isonychia*: $n = 190$, $F = 8.9895$, df = 1, $p = 0.003$; *Agnetina*: $n = 35$, $F = 12.045$, df = 1, $p = 0.001$; *Ephemerella*: $n = 100$, $F = 4.3822$, df = 1, $p = 0.04$; *Rhithrogena*: $n = 93$, $F = 1.2699$, df = 1, $p = 0.3$) indicating that for some taxa, body shape, in this case GM shape class, may depend on specimen size.

The shape classes allowed us to attribute shape information to all specimens and, therefore, all taxa in our samples. We classified each taxon (genus and family) into the shape class that contained most of the specimens of the taxon. This process adequately summarized the shape properties of the taxon, even in instances of potential allometry. Each shape class included several taxa, often a mixture of taxonomic groups, except for EPO shape class *wide head,* which included only individuals of the family Heptageniidae (Ephemeroptera) and T shape class *long head*, which contained only a portion of Leptoceridae (Trichoptera). Genera tended to be grouped with families. However, notable exceptions occurred for EPO (e.g., *Rhithrogena* and Heptageniidae; *Lanthus* and Gomphidae; *Agnetina* and Perlidae; *Isoperla* and Perlodidae) and T (e.g., *Cheumatopsyche* and Hydropsychidae; *Ceraclea*, *Mystacides*, and Leptoceridae). The EPO shape class *narrow head* had the highest taxonomic diversity (14) and included families and genera from both Plecoptera and Odonata. The T shape class *short head* had the highest taxonomic diversity (10) and included both free-living and case-making taxa. The T shape class *concave head* possessed only case-making taxa, whereas the T shape class *convex head* contained only free-living taxa.

Correspondence of body-shape trait states and GM shapes

We compared assignment of body-shape information for EPTO taxa between the database trait states and GM shape classes. Body-shape trait-state information was absent from the EPA traits database for 41% of taxa (n = 26) constraining our comparison of specimen records to 1141 individuals of 38 taxa (Table 3). All T taxa were described as *tubular* in the EPA traits database, whereas they were distributed among 4 GM classes (Table 3). EPO taxa possessed several database trait states, but these states did not correspond directly to our GM shape classes (Table 5). The 2 classification systems were not consistent, and descriptions of taxon body shape are not interchangeable between schemas.

We examined the arrangement of specimens according to their shape variables in multivariate space to identify patterns in the shape structure of the EPO assemblage and assessed how well each classification scheme resolved the primary trends. The 1st principal component of the PCA of the weight matrix explained 49% of the variation in the EPO shapes and corresponded to differences in head width relative to thorax and abdomen width (Fig. 4A). Shape classes *narrow head* and *wide head* were extremes along this gradient. The 2nd principal component accounted for an additional 18% of the shape variation and represented a gradient from shorter, more compact body shapes (shape class *stout*) to longer, more slender body forms (shape class *slender*). The database trait states were unable to resolve these patterns in shape among the EPO specimens because the database trait states blend across both PCA shape-gradient axes (Fig. 4B). This result supports our previous assertion that the 2 classification schemes do not provide the same information about organism shape.

Detecting differences among reference sites

Categorical shape data can be used to compare shape composition among sites. We evaluated the body-shape structure of the aquatic insect community based on database trait states and GM shape classes (Fig. 5A–C). We examined the GM shape classes for EPO and T separately (Fig. 5B, C). The community structure represented by the proportion of database trait states differed significantly among sites (χ^2 = 306.2904, df = 6, $p < 2.2 \times 10^{-16}$; Fig. 5A). However, this structure was based on only 69% of the available data. The trait state *flat* predominated at all 3 sites (Fig. 5A). Sites DUNDS and SBREN had similar proportions of the trait state *bluff* as the 2nd most frequently occurring trait state (Fig. 5A), but DUNMR had a higher proportion of trait state *tubular* than *bluff* (Fig. 5A). The trait state *streamline* occurred with similar frequency among all sites (Fig. 5A). The proportions of each GM shape class applied to taxonomic groups as states differed significantly among sites for EPO (χ^2 = 362.1095, df = 6, $p < 2.2 \times 10^{-16}$; Fig. 5B) and T taxa (χ^2 = 99.1524, df = 6, $p < 2.2 \times 10^{-16}$; Fig. 5C). The proportions of EPO shape classes *slender*, *stout*, and *wide head* differed among the 3 sites (Fig. 5B). DUNDS had the highest proportion of the *slender* class and the lowest proportion of the *wide head* class (Fig. 5B). SBREN had a lower proportion of the *slender* class and a higher proportion of the *stout* and *wide head* classes (Fig. 5B). DUNMR possessed an intermediate proportion for *slender*, *stout*, and *wide head* classes relative to the other 2 sites (Fig. 5B). All sites had a similar proportion of the *narrow head* class (Fig. 5B). All Trichoptera share a similar general body plan (*tubular*) according to the EPA database, but community structure based on GM shape classes for T differed strongly among sites (Fig. 5C). T shape class *long head* was absent from DUNDS and SBREN (Fig. 5C). DUNDS had the highest proportion of T shape class *convex head* among the 3 sites (Fig. 5C). SBREN had the highest proportion of T shape class *concave head* among the 3 sites, but the lowest proportion of T shape class *short head* (Fig. 5C). All T shape classes were present at DUNMR, including the highest proportion of T shape class *short head* (Fig. 5C).

DISCUSSION

Morphological characteristics of fish assemblages have been used to predict the ecological status of freshwater habitats (Haas et al. 2010, Farré et al. 2013), but we are the first to attempt this task using GM classification of aquatic insects. We derived a GM classification for aquatic insects at the assemblage level, whereas previous studies have been limited to detailed investigation of only 1 or 2 closely related taxa (e.g., Sheldon 1980, Hogue and Hawkins 1991, Sites et al. 1996, Arnqvist and Johansson 1998, Funk et al. 2008, Giacomini and de Marco 2008, Lee and Lin 2012, Müller-Peddinghaus and Hering 2013). We applied the

Table 3. Body-shape trait states for aquatic insect specimens collected from 3 sites in the Miramichi River basin on 2 November 2007 and identified to lowest practical taxonomic level. US Environmental Protection Agency (EPA) database body-shape trait states were bluff, flat, round, humped, streamlined, and tubular. Geometric morphometric (GM) shape classes were narrow head, wide head, slender, and stout. The 2 classification approaches are similar, but not interchangeable. GM shapes are available for all taxa recovered from our samples; however, trait database shapes are only available for 59% of taxa (n = 38).

Order	Family	Genus	Trait database shape	GM shape
Ephemeroptera	Baetidae		Streamline	Slender
		Acentrella	Flat	Slender
	Caenidae	*Caenis*	Flat	Slender
	Ephemerellidae		Bluff	Stout
		Attenella	–	Stout
		Ephemerella	Bluff	Stout
		Eurylophella	–	Stout
		Serratella	Bluff	Stout
	Heptageniidae		Flat	Wide head
		Epeorus	Flat	Wide head
		Maccaffertium	–	Wide head
		Rhithrogena	Flat	Slender
		Stenonema	Flat	Wide head
	Isonychiidae	*Isonychia*	Streamline	Slender
	Leptophlebiidae		–	Slender
		Choroterpes	–	Slender
		Paraleptophlebia	–	Slender
Odonata	Aeshnidae	*Boyeria*	Bluff	Slender
	Coenagrionidae		Flat	Narrow head
	Gomphidae		Bluff	Slender
		Lanthus	Flat	Narrow head
		Ophiogomphus	Bluff	Slender
Plecoptera	Capniidae		Flat	Narrow head
		Paracapnia	Flat	Narrow head
	Chloroperlidae		Flat	Narrow head
		Haploperla	Flat	Narrow head
		Suwallia	–	Narrow head
	Leuctridae		–	Narrow head
	Perlidae		–	Stout
		Acroneuria	–	Stout
		Agnetina	–	Narrow head
		Hansonoperla	–	Stout
		Paragnetina	–	Stout
	Perlodidae		–	Narrow head
		Isogenoides	–	Narrow head
		Isoperla	–	Stout
	Pteronarcyidae	*Pteronarcys*	–	Narrow head
	Taeniopterygidae		–	Narrow head
		Taenionema	–	Narrow head
Trichoptera	Apataniidae		Tubular	Concave head
		Apatania	Tubular	Concave head
	Brachycentridae		Tubular	Concave head
		Brachycentrus	Tubular	Concave head
		Micrasema	Tubular	Concave head

Table 3 (*Continued*)

Order	Family	Genus	Trait database shape	GM shape
	Glossosomatidae		Tubular	Short head
		Glossosoma	Tubular	Short head
	Helicopsychidae	*Helicopsyche*	Tubular	Short head
	Hydropsychidae		Tubular	Convex head
		Cheumatopsyche	Tubular	Short head
		Hydropsyche	Tubular	Convex head
	Hydroptilidae	*Hydroptila*	Tubular	Short head
		Oxyethira	Tubular	Short head
	Lepidostomatidae		Tubular	Concave head
		Lepidostoma	Tubular	Concave head
	Leptoceridae		Tubular	Long head
		Ceraclea	Tubular	Concave head
		Mystacides	Tubular	Short head
		Setodes	–	Long head
	Limnephilidae		–	Concave head
	Philopotamidae		–	Short head
		Chimarra	–	Short head
		Dolophilodes	–	Short head
	Polycentropodidae	*Neureclipsis*	–	Convex head
	Rhyacophilidae	*Rhyacophila*	–	Convex head

approach to a broad group of aquatic insects to explore its potential for use in biomonitoring.

Greater integration and improved resolution of aquatic insect body-shape information for more taxonomic groups is an essential step in evaluating aquatic habitats. Lack of available shape information for many taxa in trait databases can negatively affect traits-based assessments (e.g., Pakeman 2013), or may lead to exclusion of shape from traits-based analyses. Our analyses increased available shape information for many taxonomic groups at the family and genus level and provided a template for studies of shape at the specimen, taxon, or assemblage scale. Our landmark configurations captured key morphological characteristics and enabled us to identify combinations of features indicative of general, 2-dimensional body forms.

Simple morphometric measurements, such as head width or maximum abdomen width, could be used to attribute our GM shape classes to additional EPO taxa, and potentially to other taxonomic groups, without repeatedly undertaking the entire GM procedure. A set of criteria, arranged in the form of a dichotomous key, such as the model we provide in Table 4, could be used to classify the shape of individual specimens or taxonomic groups in a similar fashion to taxonomic identifications. We propose that aquatic insect orders with body plans that superficially resemble those of EPO (Hemiptera and aquatic Coleoptera adults) taxa could be analyzed with a similar set of landmarks.

Body forms of and cases produced by many T larvae present challenges for GM shape assessment. The lack of unambiguous landmarks on the abdomen because of the flexible, expandable cuticle necessitates concentration of landmarks on the highly sclerotized head and thorax region. The locations of these landmarks provide the description and resolution of shape for the larva and can influence the interpretation of the organisms' response to the environment. This response can be modified by the presence or absence of cases, nets, retreats, and other structures for many taxa. In a case study of T genera in the UK, Orlofske et al. (2013) found that the presence of a case and the type of material used to construct the case differed among taxa classified into different flow groups based on the Lotic-Invertebrate Index for Flow Evaluation (Extence et al. 1999), which represents a gradient of velocity and hydraulic conditions. The differences in proportion of cases and case types among flow groups suggest that behavioral characteristics of organisms that produce and maintain these structures and physical properties of the structure can modify how the larvae select and experience the physical environment (Orlofske et al. 2013).

Shape configurations were more easily resolved for EPO taxa than for T taxa, a result suggesting that a conservative set of flexible landmarks is required to account for differences in the general body plan of larvae in particular orders. If we modify the landmarks for T and use con-

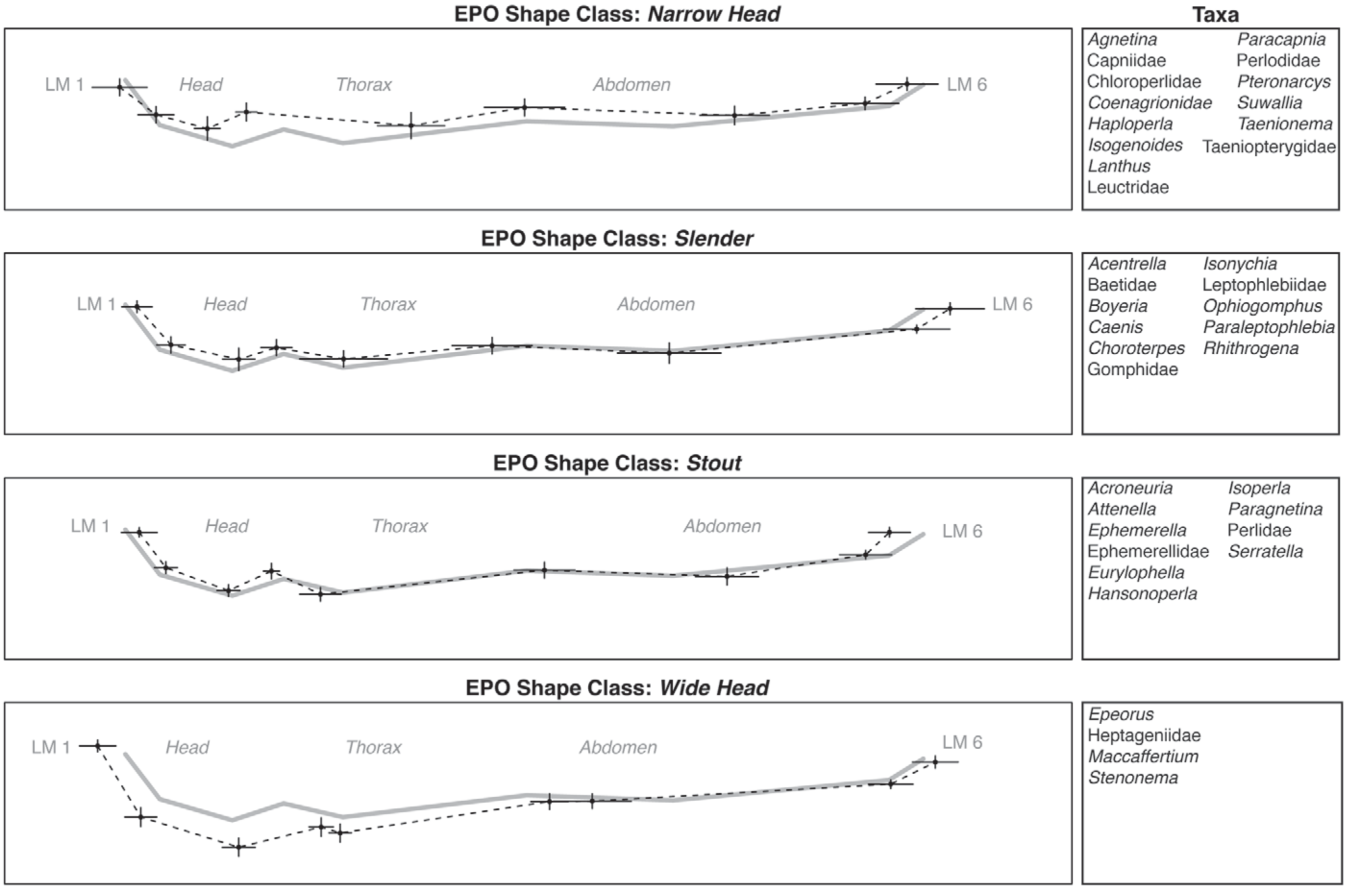

Figure 2. Diagrams of morphometric shape classes applied to Ephemeroptera, Plecoptera, and Odonata (EPO) taxonomic groups. Gray line represents the average shape for all EPO specimens as a reference. LM = landmark.

current analysis of cases, we might be able to describe T larval morphology in a way that would better describe the relationship between larvae and physical-habitat properties, including hydraulic forces. Such improvements could be applied to other orders, especially the diverse and abundant Diptera and Coleoptera whose larvae share the vermiform shape of Trichoptera and may construct cases (e.g., Chironomidae).

GM shape information is gathered at the level of individual specimens but can be summarized at the taxon level. Quantitative data at the specimen level revealed intra- and inter-taxonomic trait variation that carry implications for how organisms relate to environmental factors, particularly flow. Not all specimens in a taxon belonged to the same shape class. In the case of family-level taxa, this discrepancy was not surprising because constituent genera could belong to different shape classes. However, variability also occurred at the genus level for *Agnetina*, *Ephemerella*, *Isonychia*, and *Rhithrogena*. Species-level diversity might be an explanation, but we think that the variation was related to allometric changes in body proportions (Zelditch et al. 2004, Claude 2008) that could cause individuals to move from one shape class to another with development. Specimens obtained from biomonitoring samples can differ in size by multiple orders of magnitude within a taxon (Orlofske and Baird 2014). Size information is removed during GM analysis, but because we had the data, we were able to test for differences in size among specimens of the same taxon in different shape classes. In 3 cases, smaller specimens were classified into a different shape class than larger specimens of the same taxon. Tests for allometry can be confounded by growth-related impediments to tax-

Table 4. Dichotomous key to classification of Ephemeroptera, Plecoptera, and Odonata shape classes based on ratios of body regions.

1. Body length : head width ratio <3	**Wide head**
1'. Body length : head width ratio ≥3	2
2. Body length : head width ratio ≤4.2	**Stout**
2'. Body length : head width ratio >4.2	3
3. Body length : head width ratio ≤5.2	**Slender**
3'. Body length : head width ratio >5.2	**Narrow head**

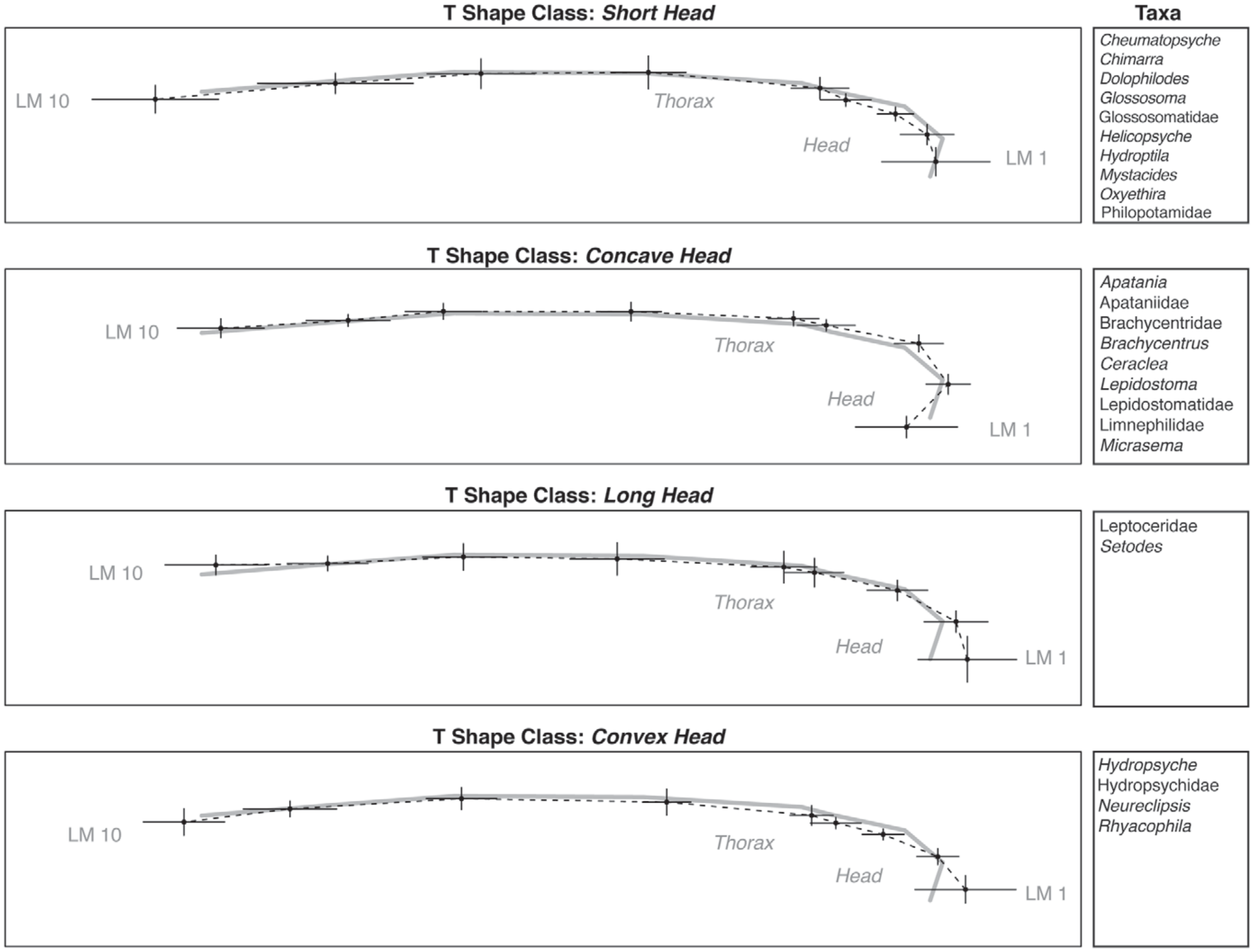

Figure 3. Diagrams of morphometric shape classes applied to Trichoptera (T) taxonomic groups. Gray line represents the average shape for all T specimens as a reference. LM = landmark.

onomic identification because genus-level identification was not always possible for the smallest specimens (Orlofske and Baird 2013). One exception is the monogeneric family Isonychiidae. In New Brunswick, all specimens in this family belong to the genus, *Isonychia.* We detected a significant difference in the size of *Isonychia* specimens distributed between 2 shape classes, a result suggesting that size and shape are interrelated for this taxon. Even in cases of isometry (static body proportions through growth), size is an important property that mediates the interaction of body form and environmental conditions, specifically hydraulic forces. Reynolds numbers, which describe the properties of the fluid surrounding an organism, are directly related to body length or size (Vogel 1988, 1994, Lancaster and Downes 2013). Thus, body-shape classification schemes may have to be scaled to organism size. An advantage of GM over the static body-shape trait states available in trait databases is that GM can provide shape variables for individual specimens that can be interpreted in the context of size.

Our GM shape classes bear some similarity to existing database trait states, but a comparison of GM shape classes and database trait states highlighted differences in trait designations for EPO taxa. The 2 approaches evaluate different aspects of organismal shape (2-dimensional GM vs 3-dimensional trait states), making precise comparison difficult and accounting for the lack of correspondence between schemes. Our GM approach was restricted to a 2-dimensional representation of an organism's shape. An ideal description of aquatic insect shape would consider the 3-dimensional properties of the organism because the forces of flow act on the entire body surface (Statzner 2008) and frontal area is particularly important for how flow moves over an organism (Vogel 1994). The shape descriptions used in the EPA trait database provide a proxy of 3-dimensional shape information implied by the trait

Table 5. Comparison of geometric morphometric (GM) shape classes and Environmental Protection Agency database trait states for Ephemeroptera, Plecoptera, and Odonata. Each classification considers different aspects of shape for the organism, and categories are not equivalent between classification systems.

	EPA database shape trait			
GM shape class	Bluff	Flat	Streamline	Unclassified
Narrow head		Capniidae		*Agnetina*
		Chloroperlidae		*Isogenoides*
		Coenagrionidae		Leuctridae
		Haploperla		Perlodidae
		Lanthus		*Pteronarcys*
		Paracapnia		*Suwallia*
				Taenionema
				Taeniopterygidae
Slender	*Boyeria*	*Acentrella*	Baetidae	*Choroterpes*
	Gomphidae	*Caenis*	*Isonychia*	Leptophlebiidae
	Ophiogomphus	Rhithrogena		*Paraleptophlebia*
Stout	*Ephemerella*			*Acroneuria*
	Ephemerellidae			*Attenella*
	Serratella			*Eurylophella*
				Hansonoperla
				Isoperla
				Paragnetina
				Perlidae
Wide head		*Epeorus*		*Maccaffertium*
		Heptageniidae		
		Stenonema		

state. For example, *flat* aquatic insects should have a lower profile than *streamline* or *bluff* forms, but these height differences are difficult to define and quantify without measurement. Newer technologies, expanded image capacity and analytical support may soon allow efficient GM characterization of insect body shape in 3 dimensions (Zelditch et al. 2004, Plyusnin et al. 2008). Our analysis provides an important proof of concept that GM, even in 2 dimensions may be ecologically informative for studies of aquatic insect communities. The GM classification provided more-precise criteria for defining shape classes and reflected alternative morphological strategies present in our aquatic insect assemblages.

Significant differences among sites in the proportion of shapes were observed for both GM shape classes and database shape states. The 2 approaches may provide different information related to site conditions, but without a clear framework for linking database shape states to physical conditions, the differences are difficult to interpret. GM classification may have greater potential than database shape states to indicate important facets of the habitat because it provides clear definitions of mutually exclusive categories. Variation in GM shape composition of EPO and T among sites may be related to physical-habitat variables, including temperature, hydraulic complexity, flow regime, and bed roughness. However, kick-net biomonitoring samples contain organisms from multiple microhabitats, and linking specimens in a multihabitat sample with microhabitat physical conditions is difficult (Davis and Barmuta 1989). Targeted sampling approaches paired with high-resolution physical data are needed to test mechanistic relationships between GM shape categories and relevant ecological factors.

GM provides a summarized, quantitative description of the complex morphologies of organisms that traditionally have been expressed in qualitative terms. GM shape variables facilitate analysis of morphological features that can vary in response to particular components of the habitat templet. Linkages among GM shape variables and environmental characteristics have been described for aquatic and semiaquatic vertebrates, including fish (Caldecutt and Adams 1998, Haas et al. 2010, Farré et al. 2013), turtles (Claude et al. 2004, Rivera 2008), and salamanders (Adams and Rohlf 2000, Arif et al. 2007, Church 2011), with recent

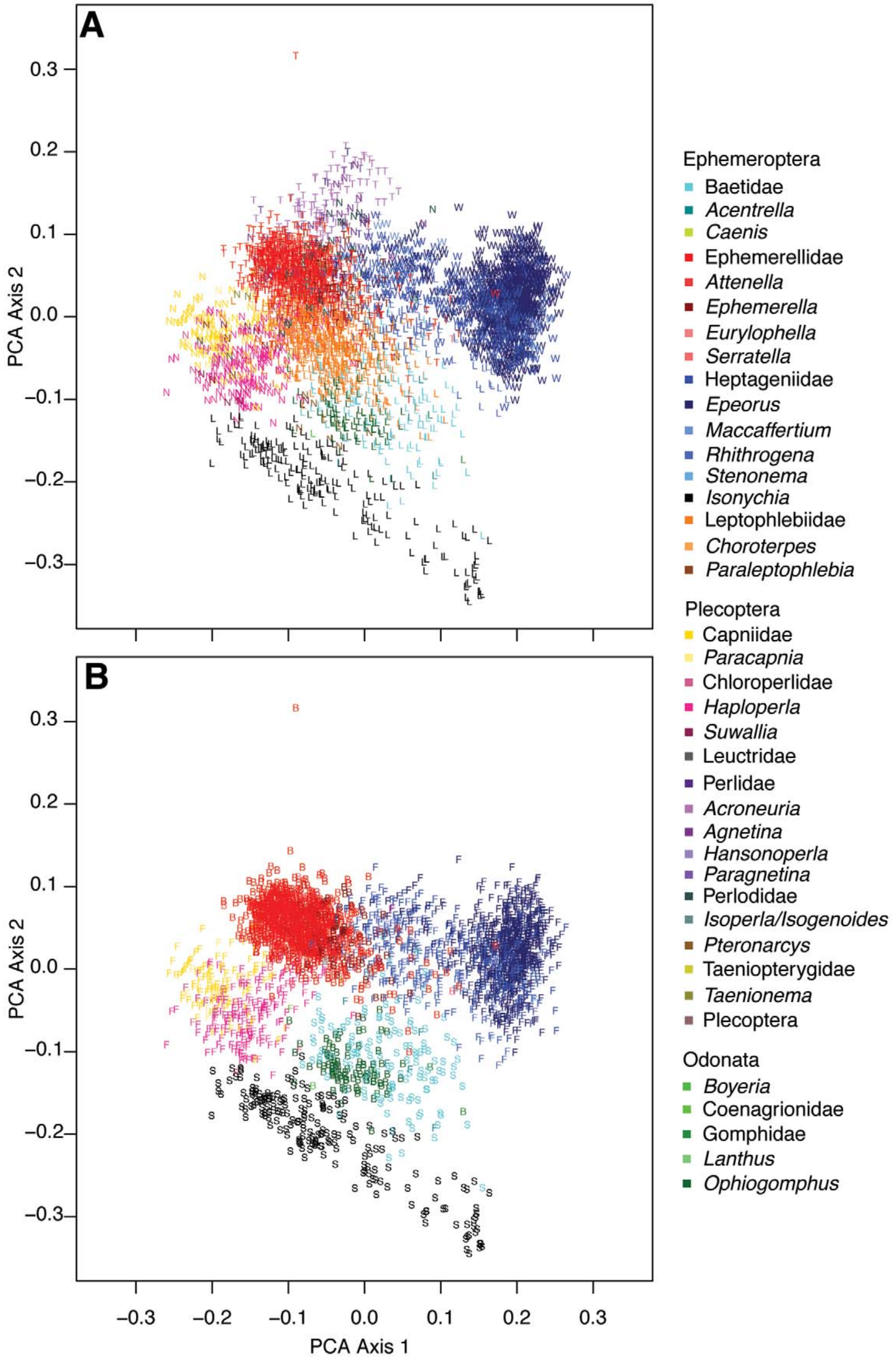

Figure 4. Principal Components Analysis (PCA) plots for Ephemeroptera, Plecoptera, Odonata (EPO) taxa and geometric morphometric shape classes (N = narrow head, L = slender, T = stout, W = wide head) applied at the lowest taxonomic level (A), and EPO taxa and trait database states (B = bluff, F = flat, S = streamline) (B). Colors indicate taxon, symbols indicate shape.

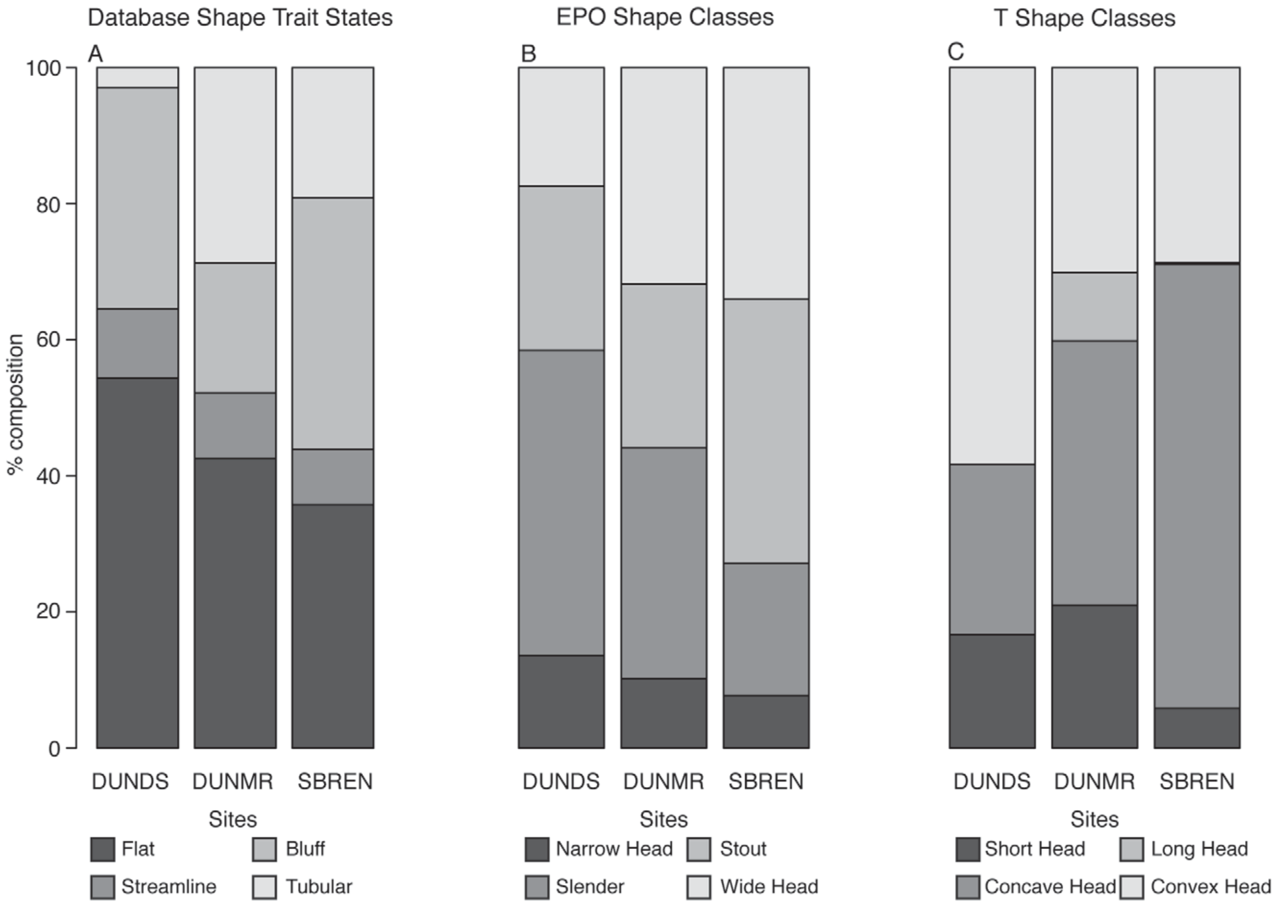

Figure 5. Shape structure of aquatic insect assemblages at sites (see Table 3 for codes) summarized as % composition of US Environmental Protection Agency trait database states (A), and geometric morphometric shape classes for Ephemeroptera, Plecoptera, Odonata (EPO) (B) and Trichoptera (T) (C). Shape classes applied as states to each taxon identified to the lowest taxonomic level.

work adding selected aquatic invertebrates (i.e., crayfish; Perry et al. 2013). These studies demonstrate the value of shape information for addressing basic and applied ecological questions.

Hydrologic and hydraulic forces are the prevailing physical features of lotic environments, and they strongly influence the composition and distribution of benthic macroinvertebrates (Davis and Barmuta 1989, Growns and Davis 1994, Bouckaert and Davis 1998, Robson et al. 1999, Orlofske et al. 2013). The GM approach is an alternative way to explore how hydraulic forces structure the stream benthos. Shape information may help explain sensitivities of aquatic insects to particular hydraulic conditions and could be used to support an integrated description of flow-indicator groups, such as the flow-exposure groups proposed by Growns and Davis (1994). Shape composition of aquatic insect assemblages could then be used to infer flow and substrate properties (e.g., Vaughn 1985, Franken et al. 2008, Statzner 2008, Orlofske et al. 2013) and other environmental stressors and contaminants (e.g., fluctuating asymmetry; Lenat 1993) that directly or indirectly interact with organism morphology. Thus, GM provides a way to establish body-shape criteria for diverse aquatic insect taxa that can be incorporated into ecomorphological studies and used to assess ecological condition of streams.

ACKNOWLEDGEMENTS

We appreciate contributions to this work from Colin Curry, Kristie Heard, Adam Bliss, Wendy Monk, Christopher Tyrrell, and Sarah Orlofske. We are grateful to reviewers and editorial staff for recommendations that improved the quality of this manuscript. We acknowledge funding support from the New Brunswick Museum Florence M. Christie Research Grant in the Natural Sciences to JMO, Natural Sciences and Engineering Research Council of Canada Discovery Grant to DJB, and Environment Canada program funds.

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