## Acidity gradients shape the phylogenetic structure of odonate communities across three biomes

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## Abstract

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Environmental filtering and competitive exclusion can act simultaneously to shape the structure of communities, but disentangling them has proved difficult. Specifically, environmental filtering may restrict establishment at a site to a set of species sharing particular traits permitting local persistence. Mutual exclusion of ecologically similar or phylogenetically related species can also dictate community composition. Patterns of phylogenetic structure allow assessment of the relative influence of these processes. Using phylogenetic patterns of community structure, this study aims to assess the predominant processes structuring odonate communities along a broad-scale environmental gradient in Quebec. Phylogenetic analyses of forty lentic (i.e. lake) odonate communities revealed that co-occurring species in temperate regions were more related than expected by chance, suggesting a predominant role of environmental filtering. Site-to-site variation in phylogenetic structure was related to pH. That is, the most alkaline lakes, found in temperate regions, were the most phylogenetically clustered, suggesting that pH acts as a main environmental filter of odonate communities. However, environmental filtering may not be the only important process. One alternative explanation is that temperate communities are phylogenetically clustered because damselflies are disproportionally diverse relative to dragonflies in this region. Specifically, the recent radiation of damselflies in temperate regions could have increased the diversity of this group in the temperate species pool, which could then shape local communities in that region. Nevertheless, further analyses suggested that environmental filtering along a pH gradient, rather than the evolutionary history of the species pool, shapes odonate communities in Quebec.

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#### Introduction

Elucidating the processes driving spatial variation in the structure of ecological communities remains a challenge. Specifically, assessing the relative importance of different community structure processes, and the contexts under which some prevail over the others, is a central focus of community ecology. Abiotic processes, such as environmental filtering, produce very similar communities under similar environmental conditions (Clements 1916, Chesson 2000). According to this view, community composition is determined by local environmental conditions and species-specific niche attributes. That is, species have different tolerances and requirements and therefore differ in their responses to environmental conditions; thus, their relative abundances vary along environmental gradients (Keddy 1992, Woodward and Diament 1991). However, community composition cannot always be predicted by environmental conditions alone. Stochastic processes, such as the sequence of species entering a community through time, random extinction events, and ecological drift, can create communities that cannot be predicted purely by local environmental conditions (Gleason 1926, Chase 2003, Vellend 2010). Biotic processes, such as competition, may also create mismatches between the environment and the species that occupy a community (Elton 1946, Webb et al. 2002). The Theory of Limiting Similarity posits that species must differ in some aspect of their niche in order to coexist with other species within a community (Hutchinson 1959). Since niches are often evolutionarily conserved, closelyrelated species usually have more similar niches and are prone to strong interspecific competition (Elton 1946, Webb et al. 2002). Competition and environmental filtering can act simultaneously to shape species composition, but disentangling them has proved difficult and is the focus of many ecological studies (Chesson 2000, Gravel et al. 2006, Leibold and McPeek 2006, Thompson and Townsend 2006, Chase and Myers 2011, Lessard et al. 2016).

The relative influence of competition and environmental filtering can be tested by comparing observed patterns of phylogenetic structure (Webb et al. 2002, Cavender-Bares et al. 2009, Lessard et al. 2012) to those generated using null models (Gotelli and Graves 1996).

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By studying species co-occurrences and comparing the relatedness of species within observed communities to randomly-assembled (i.e. null) communities, inferences can be made about the ecological processes shaping community composition. Phylogenetic analyses of community structure rely heavily on the assumption of niche conservatism, the idea that closely related species are ecologically similar (Kraft et al. 2007, Losos 2008). A community that is phylogenetically clustered, containing species that are more highly related than expected by chance, indicates that environmental filtering is the primary process structuring the community. On the other hand, low relatedness between species in a community, or phylogenetic evenness, indicates that competition is largely structuring the community, as closely-related species with similar traits are prevented from co-existing (Vamosi and Vamosi 2007, Machac et al. 2011, Hoiss et al. 2012). Phylogenetic structure may appear random if stochastic processes are at play, or if environmental filtering and interspecific competition are simultaneously shaping community structure (Mayfield and Levine 2010).

Very few studies to date have assessed how the phylogenetic structure of 'true' local communities varies along broad-scale environmental gradients spanning several biomes. That is, most studies either lack standardized data on the relative abundance and composition of species in local assemblages, or they lack the geographic scope that enables a generalization of the results (Lessard et al. 2012a). Odonates, which include dragonflies (Anisoptera) and damselflies (Zygoptera), are near ideal study organisms to assess the processes determining species composition of ecological communities along broad-scale gradients. Odonate communities are easy to sample and identify to species-level (Oertli 2008), meaning that one can rapidly document community structure at several sites encompassing broad geographical gradients. Moreover, a plethora of local studies have documented the factors that are potentially important for odonates (McPeek 1990, McPeek and Brown 2000, Turgeon and McPeek 2002, Turgeon et al. 2005, McCauley 2006, Siepielski et al. 2010). The predominant view is that odonate communities are shaped by a combination of evolutionary history of the species pool (McPeek and Brown 2000, Turgeon and McPeek 2002, Turgeon et al. 2005), stochastic processes (McCauley 2006, Siepielski et al. 2010), and fish predation (McPeek 1990). However, these studies either lacked high-resolution data on

community structure or geographical scope, such that the results can hardly be extrapolated. Moreover, they mostly focused on particular clades of odonates, rather than on the entire community. Finally, no study to date has simultaneously assessed the relative importance of evolutionary history, stochasticity and niche based-processes on odonate communities along a geographical gradient spanning several biomes.

Using the largest time-calibrated phylogenetic tree for North American odonate ever constructed and standardized data on the local community structure of 40 odonate communities spread across the temperate, boreal, and subarctic biomes, this study aims to assess the predominant processes structuring odonate communities. Specifically, we aim to assess the relative importance of competitive exclusion, or limiting similarity, (the co-occurrence of phylogenetically-unrelated species) and environmental filtering (the co-occurrence of phylogenetically-related species) on community composition. Then, we assess whether the relative importance of these structuring processes vary along broad-scale environmental gradients. Finally, we use a species pool framework (Lessard et al. 2012b, Carstensen et al. 2013) to assess the degree to which the evolutionary history of the regional biota might affect the structure of local communities.

#### **Materials and Methods**

#### Site Descriptions

Sampling was performed at 40 lakes across a large gradient encompassing 8.6 degrees latitude and three biomes (Figure 1). Lakes were selected based on ease of access, the presence of a well-developed littoral zone, canopy openness in the riparian zone, and minimal anthropogenic disturbance (Schindler et al. 2003, Remsburg et al. 2008, Siepielski et al. 2011). The riparian zone of each lake was mostly dominated by grasses and shrubs, often *Kalmia latifolia* (mountain laurel) and *Carex* (sedges). The surrounding forest in the southernmost sites (below 48 degrees latitude) was mixed temperate vegetation, dominated by *Abies balsamea* (balsam fir), *Betula alleghaniensis* (yellow birch), and *Acer saccharum* (sugar maple). The northern lakes were surrounded by boreal forest, with dominant species including *Picea mariana* (black spruce), *Picea* 

*glauca* (white spruce), *Abies balsamea* (balsam fir), *Betula papyrifera* (paper birch), and *Larix laricina* (tamarack). The most northern sites (above 53 degrees latitude) were in the subarctic region and were bordered by lichen, shrubs and sparse forest.

#### **Odonate Sampling**

We developed and implemented a standardized sampling protocol consisting of sampling adult odonates along a 1 km transect at each of the 40 sites. Transect methods have been shown to be effective sampling techniques for flying insects such as butterflies (Pollard 1977), yet our study is the first to apply such an approach to odonates. Standardized sampling allows estimating not only the species composition, but also the relative abundance of species at each site (Pollard 1977, Pollard and Yates 1993, Sutherland 2006, Raebel et al. 2010). At each lake, a sampling transect was set up in the riparian zone, which is the preferred odonate habitat (Bried and Ervin 2006, Butler and deMaynadier 2006). The transect ran along the perimeter of the least-shaded edge of the water body, which receives the most sun and will therefore be preferred by most species (Remsburg et al. 2008). The transect was also designed to account for habitat heterogeneity, that is, all habitat types within a site were included (Oppel 2006, Willigalla and Fartmann 2012).

Sampling was performed during the period of May-August 2015, which corresponds to the period of high activity of odonates in Quebec (Wissinger 1988, Giberson and Dobrin 2003, Paulson 2011). To ensure the independence of observations, 10 evenly spaced sampling stations were marked along the riparian transect, approximately 100m apart. Each sampling station consisted of a 20m × 20m area, extending into the emergent vegetation and into shallow waters. All adult odonates observed at each station along the transect were sampled with a hand-sweep net. The odonates that were observed but not caught were recorded to the species level, if possible. This data set is further referred to in the text as the 'observational data'. To standardize the sampling effort across all sampling stations, sampling was performed at each station for a period of ten minutes. The sampling trial was interrupted each time an individual was caught and resumed after the individual was stored.

odonate foraging. Sampling was performed between the hours of 10:00-17:00, when the air temperature site was greater than 13°C, cloud cover was less than 50% (can be greater when temperature is above 18°C), and with only weak winds (Pollard 1977, Pollard and Yates 1993, McCauley 2006, Van Swaay 2012). Odonates are sensitive to sunlight because they are ectothermic and rely on heat to warm their flight muscles (May 1976, Marden 1995).

The specimens were placed in glassine envelopes for several hours to allow the emptying of gut contents and then soaked overnight in 95% ethanol for color preservation (Paulson 2011). Individuals were later identified to species level using taxonomic keys (Westfall and May 1996, Needham et al. 2000). A total of 1052 adult specimens were collected. Species lists were compiled for each of the 40 sampled sites, along with calculations of species richness with relative abundance measures. In addition, 681 adults were observed and noted, but not caught. Identification of the observed specimens was to the highest degree possible, ideally species-level, but often to family or genus-level.

This study focused primarily on the collection of adult odonates, as species-level identification of larvae is challenging and time-consuming. However, larvae were sampled at half of the sites (Method S1). Since there is no standardized protocol for the assessment of community structure in odonates, we first aim to assess whether sampling only adult odonates can efficiently and accurately estimate community composition. As odonates have both an aquatic larval stage and a flying adult phase, with different ecological traits and responses to changing environmental conditions, the observed community composition may vary with the sampled life stage (Stoks and Cordoba-Aguilar 2012). Although adult odonates are easier to sample and identify, they are more sensitive to weather fluctuations and display specific phenologies, whereas larvae may be less sensitive to weather conditions and seasonality, but are more time-consuming to sample and more difficult to identify to species-level (Wissinger 1988, Oertli 2008). By sampling and comparing the composition of both life stages across different sites, we aim to determine whether adult sampling yields an accurate estimate of odonate community composition.

#### Measuring Habitat Characteristics

Environmental variables which have previously been shown to affect odonate species richness and composition were measured at each site. The perimeter and surface area of each water body were estimated using Google Earth, as area has been found to be a strong explanatory variable of odonate species richness and is an example of the classic species-richness-area relationship (Dijikstra and Lempert 2003). Using handheld probes (WTW kit, model Multi 3420 SET G), water temperature, pH, dissolved oxygen, and conductivity were measured, along with visual categorical estimations of canopy openness (0, 10, 25, 50, 75, 100%), percent cover of macrophytes (0, 1, 10, 25, 50, 75, 100%), and the level of human disturbance (none, low, moderate, high) (McPeek 1990, Samways and Steytler 1996, Rychla et al. 2011, Siepielski et al. 2011). Annual mean temperature, annual precipitation, temperature seasonality, actual evapotranspiration (AET), and potential evapotranspiration (PET), were also collected from WorldClim using the geographic centroid coordinates of each site (Hijmans et al. 2005). The characteristics of each site are listed in Table A1.

## Analysis of Community Data

To compare and contrast the odonate species composition of the communities, a nonmetric multidimensional scaling (NMDS) was performed on the community abundance matrix using the VEGAN package v2.3-1 in R (Oksanen et al. 2015). NMDS is an ordination technique that uses a Bray-Curtis dissimilarity matrix to rank order species in communities and then plot the sites in a multidimensional space with a reduced number of dimensions. This technique allows visualization of the similarity in species composition between sites (Kindt and Coe 2005, Borcard et al. 2010). It also provides the ability to distinguish between distinct assemblages of species (Carstensen et al. 2013). The ordination plot was used to determine if there were two distinct regional assemblages, representing separate temperate and boreal species pools (Lessard et al. 2012b).

An NMDS was also performed with the data to assess whether genus-level composition of adult and larval communities differed at the sampled sites. The

communities were examined separately for Anisoptera and Zygoptera. The adult community was analyzed first by excluding the observational data and then by including it, along with the captured species data. Sampling data for the two suborders at each site is given in Table A2. Then, richness and evenness of the genus-level adult and larvae communities and the species-level adult communities were examined using accumulation curves (Soberon and Llorente 1993), which were plotted using the VEGAN package (Oksanen et al. 2015). Accumulation curves show the cumulative species (or generic) richness as a function of sampling effort (the number of samples). Plotting the curves allows for estimation of the number of additional species (or genera) that would be discovered through more sampling (Soberon and Llorente 1993).

Additionally, the average values of the measured environmental variables were calculated for each species and are listed in Table A3.

## Phylogeny Construction

#### 1. Genetic Sequence Collection

Six candidate loci that have been broadly sampled and successfully used in odonate phylogenetics at both lower and higher taxonomic levels were identified (Dumont et al. 2005, Bybee et al. 2008, Dumont et al. 2010, Letsch et al. 2016). These six loci included two mitochondrial genes, CO1 (cytochrome oxidase subunit 1) and CO11 (cytochrome oxidase subunit 11), and four nuclear genes, 18s (18s ribosomal RNA), 16s (16s ribosomal RNA), ITS1 (internal transcribed spacer 1), and ITS2 (internal transcribed spacer 2). These loci are the most commonly used genes in higher-level and large-scale phylogenetic analyses of odonates (Dumont et al. 2005, Bybee et al. 2008, Dumont et al. 2010, Letsch et al. 2016).

As there was no phylogeny available to use as a reference tree for creating a phylogeny of the Quebec odonate species, a full North American phylogeny was first constructed and was later pruned to contain only the Quebec species. A North American species list was compiled from the online database hosted by the Slater Museum of Natural History (Slater Museum of Natural History) and GenBank searches were conducted both manually and with the phyloGenerator program (Pearse and Purvis 2013) to collect sequence data for these species for the 6 loci mentioned above. The search yielded sequence information for 242 out of 463 North American odonate species (52%), including 104 out of 146 Quebec species (71%). Not all species had sequences for all loci; a list of the sequenced species with corresponding GenBank accession numbers is given in Table A3. The sequence alignment for each locus, along with the editing and assembly of the concatenated alignment of all loci, was done using Geneious R9 (www.geneious.com, Kearse et al. 2012). Sequence alignments were done using the global alignment algorithm MUSCLE (Edgar 2004).

#### 2. Phylogenetic Reconstruction and Divergence Time Estimation

Phylogenetic relationships for North American odonates were inferred from the nucleotide data using maximum likelihood (ML), and Bayesian inference (BI). Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using the CIPRES Science Gateway v.3.3 (www.phylo.org, Miller et al. 2010). ML analyses were conducted using the default parameters in GARLI v.2.01 (Zwickl 2006). One thousand bootstrap (BS) replicates were conducted using the same parameters that were applied for ML searches. BI was performed using MrBayes v.3.2.3 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Alteker et al. 2004). All BI analyses were run for 15,000,000 generations with four chains in four parallel runs, sampling every 1000 generations.

Best fitting models of sequence evolution for each locus were determined using the Corrected Akaike Information Criterion (AICc) in jModeltest v.2.1.4 (Guindon and Gascuel 2003, Darriba et al. 2012). The AICc was used for model selection based on its ability to outperform other model-selection criteria. The details of the model selected for each locus are given in Table A4. Proper mixing was determined using Tracer v.1.6 (Rambaut et al. 2014) and 25% of trees were discarded as burn-in prior to constructing a majority rule consensus tree. Both ML and BI analyses were topologically constrained at the family level. The family-level odonate tree was constructed by referring to the phylogenetic trees generated by Dumont et al. (2010) and Letsch et al. (2016). Finally, the family-level tree was uploaded to Phylomatic (V3) (Webb and Donoghue 2005) to obtain a family-level constraining tree for North American odonates to use in ML and BI analyses. Finally, the resulting maximum likelihood tree served as the input phylogram for the subsequent age estimation analyses.

A Bayesian method, implemented in the program BEAST v.1.8.2 (Drummond et al. 2006), was used to estimate the phylogeny and divergence times simultaneously. Rates and ages were estimated from our sequences, modeling fossils as lognormal priors. The data set was partitioned by gene, estimating separate rates and ratechange parameters for each partition. The underlying model of molecular evolution for each of the individual genes was set to be GTR + I + Γ. The UCLN model was also used and allows for rates of molecular evolution to be uncorrelated across the tree. BEAST also allows for uncertainty in the age of calibrations to be represented as prior distributions rather than as strict/fixed calibration points. Therefore, the minimum ages of several of the clades in the tree were constrained to prior probability distributions. For each analysis, two independent Markov chain Monte Carlo (MCMC) analyses were initiated from starting trees with branch lengths that satisfied the priors on divergence times. A starting tree with branch lengths satisfying all fossil prior constraints was created using the program r8s version 1.7 (Sanderson 2003) using NPRS. For each MCMC analysis, two independent chains were run for 200 million generations and convergence and stationarity of each chain to the posterior distribution were assessed using Tracer v.1.3 (Rambaut and Drummond 2005) and by plotting time series of the log posterior probability of sampled parameter values. After stationarity was achieved, each chain was sampled every 500 steps until an effective sample size (ESS) of more than 200 samples was obtained. If convergence between the independent chains was evident, the samples from each run were combined using the program LogCombiner v.1.8.2 (part of the BEAST distribution, Drummond et al. 2006).

#### 3. Fossil constraints

All fossils were treated as minimum age constraints, with the exception of the root node, which was set to a uniform distribution between 237 Myr (minimum age of angiosperms) and 242 Myr (most recent common ancestor (MRCA) of odonates) (Shcherbakov 2008, Kohli et al. 2015). All other fossil constraints were modelled as lognormal distribution with different means and standard deviations (Table A5). Fossil constraints were further

applied on eight deep nodes (e.g. families). Deep node calibration fossils for age estimation in Odonata at the family level were recently evaluated by Kohli et al. (2015) according to the recommendations of Parham et al. (2012) for the best practices for justifying fossil calibrations (Table A6). Recently these fossils were also used for divergence time estimation of Odonates (Letsch et al. 2016).

The final phylogenetic tree for the North American odonates is shown in Figure S5. The phylogenetic tree pruned for the Quebec species is shown in Figure S6.

#### Community phylogenetic analysis

Patterns of community phylogenetic structure were examined across a latitudinal gradient using the constructed phylogeny and an index of phylogenetic structure, namely mean phylogenetic distance (MPD) (Webb et al. 2002). MPD estimates the average relatedness between all pairs of species in a community. To allow comparisons of phylogenetic structure between sites, net relatedness index (NRI) was used as a measure of the standard effect size of MPD. This index is commonly used to compare the average phylogenetic distance within observed communities to the average distance within randomly generated null communities and is standardized by the standard deviation of the phylogenetic distances in the null communities (Webb et al. 2002). Positive values of NRI (>1.96) indicate significant phylogenetic clustering, meaning the observed communities are more related than expected by chance, whereas negative values (<1.96) indicate significant phylogenetic evenness, or communities which are less related than expected by chance (Webb et al. 2002, Lessard et al. 2016).

Several species pool definitions were considered while generating null communities. A species pool should be designed in such a way as to include only species that can disperse to and persist at a given site (Lessard et al. 2012b). The North American species list was trimmed to include only the species that occur east of the Great Lakes and within the temperate and boreal biomes, as these are the species with the potential to occur in Quebec. This eastern North American species pool is further referred to as the "full species pool" (173 species). The full pool was then used to prune the North American phylogeny and abundance-weighted NRI values were calculated for all communities using the R package picante v1.6-2 (Kembel et al. 2010)

and phylogeny.pool as the null model for the ses.mpd function. This model randomizes the community matrix by drawing species from the phylogeny pool with equal probability.

The sampled community matrix was then separated by biome (boreal or temperate) and NRI values were again calculated for each site using the full species pool. The analysis was then repeated after separating the full species pool into boreal (Canadian species) and temperate (US species) species pools and subsequently pruning the full phylogeny to separate boreal and temperate phylogenies. The boreal phylogeny included 128 species; the temperate phylogeny contained 168 species. NRI was calculated separately for the boreal (sites above 48 degrees latitude) and temperate communities (sites below 48 degrees latitude) using the respective phylogenies. The mean NRI values from the analyses using the full species pool were calculated for each region (full, boreal, and temperate) and the mean NRI values from the analyses using the biome-specific species pools were also calculated for each region (boreal and temperate). This allowed us to determine if the phylogenetic structure of odonate communities is sensitive to species pool definitions (Kraft et al. 2007, Lessard et al. 2012). When using the full species pool to calculate NRI, if the mean NRI values of the communities differ between boreal, temperate, and full datasets, then separating the species pools is appropriate. If the mean NRI values of the boreal and temperate communities differ when using the biome-specific pools in the null model as opposed to the full species pool, then the evolutionary histories of the species pools may be affecting odonate community structure (Lessard et al. 2012). A ttest was used to see if the mean NRI value of any of the groups (full, boreal, temperate) differed significantly from zero, representing the null. If the mean NRI value is significantly greater than zero, then the communities are, on average, phylogenetically clustered. If the mean NRI value is significantly less than zero, then communities are phylogenetically evenly dispersed. If the mean NRI value does not differ significantly from zero, then the communities are either structured by a combination of different processes or by random factors.

#### Multiple Regression Analysis

With the calculated NRI values for each site, a multiple regression analysis was used to determine which of the measured environmental variables were most strongly correlated with phylogenetic structure. A forward stepwise regression was performed using a regular linear model and the function step(Im()) in R with the following variables: surface area, perimeter, annual mean temperature, temperature seasonality, annual precipitation, AET, PET, dissolved oxygen, conductivity, pH, disturbance level, macrophyte cover, and canopy cover. A forward stepwise regression starts with a null model and sequentially adds factors until the addition of factor does not improve model fit relative to the null model (i.e. AIC of the null is smaller than a model with an additional factor) (Burnham and Anderson 2002). The step function looks at AIC values, "criterion values", and if there is an increase in AIC after some variables have been added to the null model, it function stops and the model with the lowest AIC score is the model that best explains the data (Yamashita et al. 2007). Two measures were used to compare models: delta AIC and Akaike weights. Delta AIC ( $\Delta$ AIC) is a measure of each model relative to the best model and the Akaike weight (w) is the ratio of the  $\Delta AIC$  value of each model relative to the whole set of candidate models. Models that have low  $\Delta AIC$ values can be considered better compared to those with high  $\Delta AIC$  values: values of 0– 2 =very strong support; 3–4=strong support; 5–9=less support; >10=essentially no support. The Akaike weight of each model can be interpreted as the probability that the model is the best among the set of candidate models, where the best model will have the highest probability (Burnham and Anderson 2002). Analyses were performed for the full community data set and then separately for the boreal and temperate community data sets.

## Results

## **Odonate Diversity**

The number of individuals caught at a site ranged from 1 to 47, the number of species collected ranged from 1 to 10, and the number of genera ranged from 1 to 9. Species

richness was related to latitude, with the most species-rich sites sampled in southern Quebec and the most species-poor sites in northern Quebec ( $R^2 = 0.2142$ , p = 0.003). The ratio of the number of anisopteran species collected relative to the number of zygopteran species increased linearly with latitude ( $R^2 = 0.130$ , p = 0.024), meaning damselflies were over-represented relative to dragonflies in the temperate region whereas the opposite was true in the north. Moreover, zygopteran assemblages had more species per genus than the anisopteran assemblages in the temperate region (means = 1.371, 1.012 respectively, *paired t* = -2.254, n = 28, p = 0.028). The temperate zygopteran assemblages also had significantly more species per genus than the boreal zygopteran assemblages (means = 1.371, 1.000 respectively, *paired t* = -2.138, n = 28, 11 respectively, p = 0.043). The number of species per genus did not differ significantly between anisopterans and zygopterans in the boreal region (means = 0.917, 1.000 respectively, paired t = -0.419, n = 11, p = 0.680) or when comparing anisopterans between the temperate and boreal regions (means = 1.012, 0.917 respectively, paired t = -0.511, n = 28, 11 respectively, p = 0.614). The damselfly species that occurred at the highest number of sites in the temperate biome were *Ischnura verticalis* (n = 20), *Enallagma ebrium* (n = 17), and *Enallagma hageni* (n = 13). The most common temperate dragonflies were Sympetrum obtrusum (n = 9), Ladona julia (n = 7), and *Leucorrhinia frigida* (n = 5). In the boreal biome, the most commonly occurring damselfly species were *Enallagma boreale* (n = 8) and *Coenagrion interrogatum* (n = 3), and the most common dragonflies were Cordulia shurtleffi (n = 5), Leucorrhinia hudsonica (n = 4), Somatochlora albicincta (n = 3). Three species which are rarely found in southern Quebec were collected during the sampling period at their northern range limits: Enallagma civile, Enallagma traviatum, and Lestes vigilax.

#### Analysis of Community Data

The NMDS of the larval and adult communities showed a distinction between assemblages. Overall, there appears to be high similarity in composition between the sampled adult and larval assemblages (Figure S1a). However, when the observational data is included in the adult community dataset along with the captured specimen data

(Figure S1b), the compositional similarity between adult and larval assemblages is lower.

The accumulation curves (Figure S2) show that adult sampling was fairly complete at the genus-level, but sampling could be improved at the species-level. The larvae sampling at the genus-level does not seem to be quite complete and could benefit from more samples.

The NMDS of the regional assemblages also revealed a separation between the boreal and temperate community composition (Figure S3). Although there were fewer boreal communities sampled than temperate communities, the boreal communities were much more similar to each other than to the temperate communities.

#### Community phylogenetic analysis

We tested whether the phylogenetic structure of odonate communities was, on average, different from a null expectation using one-sample *t*-tests. When the entire species pool was used to calculate NRI for all communities, and then separately for the temperate and boreal communities, the temperate region had a mean NRI that was significantly greater than zero (mean = 0.633, *t* = 2.365, n = 27, *p* = 0.026), indicating phylogenetic clustering, or higher relatedness between species in the communities than expected by chance (Figure 2). The full community data set as well as the boreal communities did not have mean NRI values significantly different from zero (full: mean = 0.376, *t* = 1.649, n = 36, *p* = 0.108, boreal: mean = -0.390, *t* = -1.157, n = 9, *p* = 0.281). The local phylogenetic analyses of the biomes, using the biome-specific species pools to calculate NRI, revealed the same pattern, where only the temperate biome had a significant and positive mean NRI (mean=0.621, *t* = 2.346, n = 27, *p* = 0.023). The local boreal biome analysis was not significant (mean = -0.333, *t* = -0.961, n = 9, *p* = 0.365). *Multiple Regression Analysis* 

Using a stepwise multiple regression with a forward selection procedure and using the NRI values of the full community data set as a response variable, the only predictor variable retained in the model was pH (AIC = 20.1, p = 0.023) (Figure 3a.). When the multiple regression analysis was repeated with the NRI values for the boreal biome, no

variables were significant. However, the best model for the multiple regression of the temperate biome retained AET, temperature seasonality, perimeter, disturbance, surface area, pH, conductivity, and dissolved oxygen (AIC = 6.77, p = 0.007). Finally, pH was also found to be related to latitude (R<sup>2</sup> = 0.203, p = 0.006), with acidic sites being concentrated in the north and more basic sites concentrated in the temperate region. The details of the multiple regression model selection for the full and temperate community analyses are available in the Appendix (Table A7).

#### Discussion

Environmental filtering seems to be the process having the strongest influence on the composition of odonate communities in temperate regions, as species in temperate communities were more related than expected by chance. Two different processes could be the cause of this phylogenetic clustering: contemporary environmental filtering or evolutionary historical processes. However, the temperate communities retained their phylogenetic structure when using the temperate species pool, suggesting that contemporary niche-based processes, rather than the evolutionary history of the species pool, have a stronger influence on community composition (Lessard et al. 2012a, Lessard et al. 2012b). Phylogenetic structure across all odonate communities was found to be most strongly correlated with pH. Specifically, environmental filtering was stronger at the less acidic sites, which also happened to be concentrated in the temperate region. This correlation of pH with phylogenetic community structure provides a possible explanation for why we see stronger environmental filtering among temperate communities.

Consistent with the hypothesis that the recent evolutionary radiation of damselfly species in the temperate region may have given rise to communities dominated by recently diverged and closely related species (McPeek and Brown 2000, Turgeon and McPeek 2002, Turgeon 2005), the ratio of the number of zygopteran species relative to the number of anisopteran species was found to be negatively related to latitude. The evolutionary history of odonate species in Quebec could therefore be affecting the phylogenetic structure of communities. However, if this was the case, then accounting

for the influence of the species pool should have changed the results; that is, by using a null model constructed with the biome-specific species pool as opposed to the full species pool, any features of community structure arising from the influence of evolutionary history should have led to a change in the mean NRI (Lessard et al. 2012a, Lessard et al. 2012b). As we saw phylogenetic clustering when using both species pool definitions, it is likely that contemporary niche-based processes, such as environmental filtering, have a stronger effect on community composition than evolutionary history.

Phylogenetic structure across all odonate communities was also found to be most strongly correlated with pH. Low relatedness among species, or stronger competition, was found to be more likely among species in acidic environments, whereas environmental filtering, producing highly related communities, seemed to be more common in basic environments. PH was also correlated with latitude, with more northern regions tending to be more acidic. This is consistent with our finding that temperate odonate communities seem to be more structured by environmental filtering and also provides a possible explanation as to why we see this pattern. These results are also consistent with previous research on the response of dragonflies to variations in acidity. Although most aquatic invertebrates are negatively affected by changes in pH, odonates are relatively resistant (Rychla et al. 2001). Species richness of odonates is not related to pH (Pollard and Berrill 1991), however compositional turnover and species replacements are. This is likely due to differential responses among odonate species to changes in acidity (Rychla et al. 2001). In fact, Siepielski and colleagues (2011) found pH to be one of the water chemistry variables which structures beta diversity patterns of odonate communities in New England. This is in line with our finding that differences in acidity between lakes can affect compositional structure among odonate communities.

Differences in odonate community composition in response to changes in pH may be due to a combination of three mechanisms. Odonate species have different sensitivities to water conditions due to differences in egg porosity and nymph respiration rates. The eggs of some dragonfly species absorb more water than others and are therefore more sensitive to water chemistry (Hudson and Berrill 1986). Similarly, young nymphs vary in their tolerance to water conditions and species vary in their ability to

maintain efficient respiration rates in acidic environments (Correa et al. 1985, Hudson and Berrill 1986). Another mechanism driving differences in odonate communities could be the avoidance of fish predation. As fish are the primary predators of odonates and are intolerant of acidic waters, some odonate species may prefer acidic lakes as a way to avoid predators, while larger odonates that are less affected by fish predation are able to survive in more neutral waters (Bendell and McNicol 1987, Johansson and Brodin 2003, Rychla et al. 2011). Finally, pH affects the vegetation structure in and around water bodies. More advanced successional vegetation stages are usually found in less acidic waters (Solski and Jedrczak 1990, Pietsch 1996). Adult odonates can occupy a wide range of environments and species differ in their dependence on vegetation type and structure (Toivonen and Huttunen 1995, Pietsch 1996). Odonates that require dense, well-developed vegetation will likely be found in less acidic environments, whereas species that can live in sparse vegetation may be more likely to tolerate more acidic water bodies (Rychla et al. 2011). Testing the relative influence of these processes on structuring odonate communities could be an avenue for future research.

Our understanding of the processes underlying odonate community composition and phylogenetic structure remains limited as previous research on the topic has produced conflicting hypotheses and has been relatively scarce. There is mixed evidence for the importance of environmental filtering in structuring odonate communities, yet geographic variation in the relative importance of structuring processes had not yet been examined before this study. Previous work by Siepielski et al. (2010) on the damselfly genus Enallagma suggested that neutral processes are the main drivers of community composition at the species level, as species are ecologically equivalent and did not exhibit species-specific responses to spatial variation in ecological conditions. Siepielski and McPeek (2013) further suggested that although Enallagma species are ecologically similar, weak ecological filters may act on communities to create spatial variation, due to ecological drift and weak dispersal abilities. However, previous work on the mechanisms of odonate community structure has focused mainly on damselflies, which make up less than half of the order Odonata and have different ecological traits than their dragonfly counterparts (Paulson 2011, Raebel et al. 2012). In addition, these studies did not examine communities over a broad environmental gradient, as only 20 natural lakes were sampled and thus, the generality of the results may be limited. Our study provides insight into the relative influence of community structuring processes across a broad environmental gradient. This study is also one of the first to establish a standardized protocol for quantifying the diversity of odonates. By using a standardized sampling method, we were able to quantify the relative abundance of odonates at each of the sampled sites. Transect sampling methods have been well developed for other groups of organisms, such as butterflies (Pollard 1977, Pollard and Yates 1993, Thomas 2005), but have never before been systematically applied to odonates.

One issue with ectotherms, like insects, is that they are highly sensitive to daily fluctuations in sunlight (May 1976, Marden 1995). As such, it has been suggested that larval sampling might be a more accurate way to quantify community structure (Wissinger 1988, Oertli 2008). However, larval specimens are difficult to identify past the genus-level whereas adults are much easier. Here, adult and larval assemblage composition was highly similar at the generic level, especially for the anisopteran communities. Differences in generic composition arise when the observational data is included in the adult dataset, suggesting the need for more effective adult sampling in order to get a complete understanding of community composition. However, as large adults are extremely hard to sample due to their speed and agility, improvements could be made in observational identification so that species-level observational data can be included in the dataset along with the captured adult data. Another caveat is the difference between larval and adult assemblage composition among zygopterans. This could be due to the fact that not many damselfly larvae were captured. On the other hand, damselfly adults are easy to capture and therefore, including the adult observational data did not change the results much. What can be taken from this result is that larvae sampling techniques could be improved so that small damselfly larvae can be captured more efficiently. However, despite the potential sampling improvements, there were many compositional similarities between the larval and adult assemblages and our data still revealed interesting patterns.

In eastern Canada, where freshwater environments are currently threatened and lake acidification is a concern, the relationship between pH and odonate community structure has important conservation implications. It is imperative that we monitor any changes in odonate communities, which could have effects on the rest of the freshwater ecosystem (Bouchard 1995, Jeffries et al. 2003). We can predict that increased acidity in lakes may cause odonate communities to become less phylogenetically clustered thereby increasing phylogenetic diversity. In turn, increased phylogenetic diversity could have top-down consequences on the rest of the ecosystem and affect could potentially ecosystem functioning (Flynn et al. 2011, Thompson et al. 2015). With our knowledge of odonates' sensitivity to temperature and precipitation patterns, we can predict that future climatic changes may also have effects on odonate communities and species distributions could be altered (Keil et al. 2008). In this study, we collected three species at the northern edges of their range boundaries and this number might increase with further monitoring (Hickling et al. 2005, Hassall and Thompson 2008). Furthermore, we found that although the temperate and boreal biomes had many species in common, different species were dominant in different regions. For example, zygopteran species were much more abundant in temperate regions, whereas anisopteran species became more abundant in boreal regions. Relative abundances, as well as species distributions, could be altered by changes in climate and thus, monitoring both distribution and abundance patterns of odonates, using standardized sampling protocols, could help to detect the impacts of climate change (Oertli 2008).

Future research regarding the drivers of odonate community composition should sample a broader pH gradient to see if the pattern we observed in phylogenetic structure applies to a larger pH range. Additionally, fish presence should be included in observational data, as it has been shown to be a factor influencing odonate community composition (McPeek 1990, Johansson and Brodin 2003). The presence of fish was not accounted for in this study as it proved very difficult to assess during the brief time we spent at each lake and has been found to have a negligible effect on community structure of odonates at broad spatial scales (Siepielski et al. 2010). Moreover, parasite load may have an effect on odonate community composition as it may differentially affect odonate fitness (Mlynarek et al. 2014, Mlynarek et al. 2015). A next step for this study would be to quantify parasite load along the environmental gradient and relate it to changes in odonate composition (Forbes and Robb 2008). Although some improvements and additions could be made in future studies of odonate community composition, our results contribute valuable insight to the growing body of knowledge regarding the mechanisms of community structure.

In sum, this study provides a standardized sampling protocol for adults and larvae, a phylogenetic tree for North American odonate species, and useful knowledge about the processes driving odonate community composition across three biomes. Such a comprehensive and thorough empirical approach sheds new light on the forces shaping odonate communities. Taken together, our results suggest that contemporary processes, such as environmental filtering along a pH gradient, seem to have a stronger influence on odonate community composition than contemporary competitive interactions, stochastic processes, or the evolutionary history of the regional species pool.

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## **Tables and Figures**

Figure 1: Map of the 40 sampled sites.



**Figure 2:** Box plots of the mean NRI values for the odonate community data. NRI values were calculated across the full sampling gradient and then separately for the boreal and temperate communities. The regions with means significantly different from zero are marked with an asterisk (\*\*).



**Figure 3:** Results from the multiple regression analysis performed with the NRI values for the whole community matrix against the measured environmental variables. PH was the most strongly correlated variable with NRI (AIC = 20.1,  $R^2 = 0.145$ , p = 0.023,). The grey dotted line represents the null expectation for phylogenetic relatedness (NRI = 0). The open dots represent the boreal sites and the filled dots represent the temperate sites.



## Appendix

Table A1: Site characteristics. Sites are	e ordered by their	r centroid latitudinal	coordinates.
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Site Name	Latitude	Longitude	Annual Mean Temperature (°C)	Temperature Seasonality (°C)	Annual Precipitation (mm)	AET	PET	рН	Conductivity (µs/cm)	Water Temperature (°C)	Dissolved Oxygen (mg/L)
Lac Selby	45.093	-72.801	5.8	10.369	1025	695	843	7.1	66.7	23.8	7.9
Lac Long Pond	45.248	-72.328	5	10.462	1066	694	826	7.1	98.2	25.6	8.1
Lac Gale	45.27	-72.695	5.3	10.488	1056	687	819	6.9	34.6	25.1	8.1
Lac Parker	45.326	-72.313	4.9	10.53	1076	691	819	7.2	98.3	23.8	7.7
Lac Waterloo	45.333	-72.518	5.2	10.525	1054	686	818	7.1	97.2	22.5	7.8
Saint Francois	45.537	-72.037	4.7	10.536	1067	682	811	7.3	103.9	23	8
Lac McGill	45.589	-71.287	3.7	10.329	1035	658	763	7.1	101.1	25.9	8
Lac du Pointe Au Chene	45.686	-74.756	4.9	10.806	972	631	779	6.8	38.2	24.3	8.1
Lac Thor	45.777	-71.231	4	10.416	1018	649	762	7.2	78.9	23.1	7.9
Lac Des lles	45.79	-71.188	3.9	10.439	1011	647	760	6.9	40.2	22.5	8.3
Lac Jerome	45.798	-73.972	5.3	10.75	974	641	815	7.1	88.4	25.5	8.2
Lac Egan	45.812	-71.209	3.9	10.443	1021	649	758	6.9	38.8	24.7	8.1
Lac Des Ours	45.85	-71.19	3.9	10.387	1015	646	755	6.9	35.8	23	8.2
Lac aux Atocas	45.860	-71.177	3.8	10.418	1022	648	754	6.9	44.5	22.3	8.7
Lac Cornu	45.881	-73.999	4.5	10.74	1020	640	787	6.7	44.8	21.5	8.8
Lac Cromwell	45.939	-73.997	4.6	10.755	1017	643	793	6.7	42.8	21.5	8.7
Lac A l'Ours	45.958	-74.058	4	10.709	1052	641	766	6.8	41.3	21.3	9
Lac Thibault	45.977	-74.02	3.7	10.699	1069	644	764	7	71.2	22.6	7.8
Lac Triton	45.988	-74.006	3.7	10.693	1066	642	767	7.2	78.9	22.7	8.3
Lac Croche	45.993	-74.009	3.7	10.693	1066	642	767	6.7	44.8	21.5	8.8
Lac Paquin	46.002	-74.238	3.6	10.66	1087	640	748	7.2	103	24.3	7.6
Lac Mousseau	46.545	-74.927	3.3	11.085	1026	626	744	7.3	103.1	24.3	7.7
Lac Howard	46.597	-75.663	3.3	11.17	950	617	767	6.9	44.1	25.3	7.9

Site Name	Latitude	Longitude	Annual Mean Temperature (°C)	Temperature Seasonality (°C)	Annual Precipitation (mm)	AET	PET	рН	Conductivity (µs/cm)	Water Temperature (°C)	Dissolved Oxygen (mg/L)
Lac du Bois Franc	46.921	-76.394	2.4	11.382	963	600	731	6.9	36.4	23.2	8.3
Lac Marin	47.085	-76.524	2.2	11.464	964	595	722	7	98	25.4	8
Lac Sylans	47.324	-76.882	1.9	11.468	961	592	717	7.1	67.6	23.4	7.9
Lac Ronan	47.65	-77.28	1.3	11.625	970	592	707	6.9	58.7	23.8	7.4
Lac Ben	48.006	-77.598	1	11.726	937	580	695	6.6	36.4	21.9	8.7
Lac Joannes	48.192	-78.683	1.5	11.876	897	581	711	7	89.9	24.7	8
Lac Celeron	48.86	-77.933	0.5	12.009	899	571	684	6.6	31.7	21.7	8.7
Km 88	50.739	-77.711	-1.2	12.431	827	522	623	7.2	101.5	24.7	7.6
Km 284	51.544	-77.436	-1.8	12.503	777	483	584	6.3	38.6	20.8	8.5
Lac Mirabelli	51.871	-77.371	-2	12.528	750	470	573	7.1	101.1	25.1	8
Km 456	52.656	-77.382	-2.7	12.603	716	440	533	6.3	28.6	20.6	8.6
Km 580	53.115	-77.47	-3.2	12.646	703	423	511	6.6	35.5	20.9	9.1
Lac Desaulniers	53.573	-77.562	-3.2	12.701	669	412	504	6.9	41.4	21.9	8.5
La Grande	53.691	-78.107	-3.3	12.525	638	400	494	7	94.2	22.3	7.7
Km 485	53.751	-77.62	-3.5	12.668	666	405	490	6.6	38	20.1	8.9

Km 456

Km 580

La Grande

Km 485

TOTAL

Lac Desaulniers

**Table A2:** Site sampling data for adult individuals. Sites are ordered by their centroid latitudinal coordinates.

**Table A3:** Characteristics of sampled species. The bioclimatic zones listed are the regions in which the species are known to occur. S = Subarctic, B = Boreal, M = Mixed Temperate Forest, F = Broadleaf Temperate Forest. Lowercase letters indicate rarity in the region.

Species Name	Bioclimatic Zone	Total Abundance	Total Occurrences	Average Annual Mean Temperature (°C)	Average Annual Precipitation (mm)	Maximum Temperature (°C)	Minimum Temperature (°C)	Average pH	Average Conductivity (µs/cm)	Average Dissolved Oxygen (mg/L)
Aeshna canadensis	BMF	10	4	1.43	948.25	23.70	-26.60	7.08	82.23	7.73
Argia fumipennis	bMF	33	8	4.64	1027.50	26.00	-17.60	6.96	55.65	8.23
Argia moesta	MF	1	1	3.70	1066.00	24.10	-18.00	6.70	44.80	8.80
Boyeria vinosa	MF	1	1	4.00	1018.00	23.90	-16.90	7.20	78.90	7.90
Calopteryx maculata	BMF	1	1	4.70	1067.00	25.20	-16.90	7.30	103.90	8.00
Celithemis elisa	F	1	1	3.90	1011.00	23.90	-16.90	6.90	40.20	8.30
Chromagrion conditum	BMF	29	6	3.48	1043.50	25.10	-23.20	6.97	65.98	8.13
Coenagrion interrogatum	SBMF	14	3	-1.50	793.00	22.90	-28.00	6.50	35.27	8.77
Cordulia shurtleffii	SBMF	9	8	0.16	847.75	25.10	-28.10	6.94	72.70	8.16
Didymops transversa	bMF	3	3	4.10	997.67	25.80	-20.00	7.00	67.90	7.97
Dorocordulia libera	SBMF	4	4	3.65	1039.50	24.40	-20.00	6.98	64.90	8.08
Enallagma boreale	SBMF	55	11	-0.55	824.27	25.20	-28.10	6.84	63.21	8.28
Enallagma civile	mf	5	1	4.70	1067.00	25.20	-16.90	7.30	103.90	8.00
Enallagma ebrium	BMF	128	18	3.75	1007.44	26.00	-23.60	7.01	67.99	7.99
Enallagma exsulans	mF	9	2	4.30	1041.00	25.60	-19.60	7.10	68.85	7.90
Enallagma geminatum	mF	1	1	5.80	1025.00	26.00	-15.40	7.10	67.90	7.80
Enallagma hageni	SBMF	96	13	4.34	1025.92	26.00	-20.00	7.06	73.18	8.02
Enallagma traviatum	f	3	2	5.55	1040.50	26.00	-16.00	7.00	51.25	7.95
Enallagma vernale	bMF	35	7	2.73	958.57	25.20	-27.90	7.03	64.37	8.14
Epitheca canis	BMF	3	3	4.20	1042.33	25.80	-18.00	7.17	90.10	8.03
Gomphus exilis	BMF	8	3	3.03	1012.00	24.40	-22.90	7.00	78.07	8.20
Gomphus spicatus	BMF	13	4	3.20	973.00	25.80	-16.10	6.90	58.85	8.15
Hagenius brevistylus	MF	1	1	3.60	1087.00	23.70	-17.90	7.20	103.00	7.60

Species Name	Bioclimatic Zone	Total Abundance	Total Occurrences	Average Annual Mean Temperature (°C)	Average Annual Precipitation (mm)	Maximum Temperature (°C)	Minimum Temperature (°C)	Average pH	Average Conductivity (µs/cm)	Average Dissolved Oxygen (mg/L)	
Ischnura verticalis	BMF	126	22	4.03	1021.27	26.00	-24.00	6.97	64.05	8.18	
Ladona julia	BMF	19	7	3.99	1032.71	25.80	-20.00	6.97	67.60	8.19	
Lestes congener	BMF	7	4	3.83	1022.25	23.90	-17.20	6.95	56.15	8.28	
Lestes disjunctus	SBMF	40	6	3.57	1018.67	25.60	-23.20	6.98	71.57	7.95	
Lestes vigilax	f	13	1	5.80	1025.00	26.00	-15.40	7.10	67.90	7.80	
Leucorrhinia frigida	MF	20	5	4.08	1005.80	25.10	-20.00	6.86	47.02	8.12	
Leucorrhinia glacialis	SBMF	5	2	1.35	931.50	23.40	-24.00	6.80	64.85	8.35	
Leucorrhinia hudsonica	SBMF	9	5	-1.34	803.00	22.90	-28.10	6.60	40.50	8.52	
Leucorrhinia intacta	MF	4	2	5.35	1050.50	26.00	-16.60	7.15	83.10	7.75	
Leucorrhinia patricia	SBmf	5	3	-1.27	795.00	22.70	-28.10	6.73	63.83	7.87	
Leucorrhinia proxima	SBMF	3	3	1.17	866.67	26.00	-28.10	6.90	65.77	8.10	
Libellula incesta	F	2	2	5.10	1014.00	25.60	-16.60	6.85	36.40	8.10	
Libellula quadrimaculata	SBMF	4	3	2.50	972.67	24.20	-24.00	6.83	49.00	8.13	
Nehalennia irene	BMF	24	10	4.49	1031.50	26.00	-22.20	6.98	63.29	8.11	
Somatochlora albicincta	SBMf	16	3	-3.33	669.00	18.50	-28.10	6.73	55.90	8.57	
Somatochlora forcipata	SBMF	1	1	-1.20	827.00	21.60	-26.60	7.20	101.50	7.60	
Somatochlora williamsoni	BMF	1	1	3.90	1021.00	23.90	-17.00	6.90	38.80	8.10	
Sympetrum costiferum	BMF	8	4	4.08	1010.00	25.10	-17.20	6.93	56.00	8.28	
Sympetrum obtrusum	BMF	47	9	3.62	1008.44	25.60	-23.20	6.94	61.50	8.10	
Sympetrum semicinctum	MF	2	1	3.90	1011.00	23.90	-16.90	6.90	40.20	8.30	
Sympetrum vicinum	MF	18	7	3.67	999.71	26.00	-23.20	6.96	63.27	7.96	

**Table A4:** Species GenBank accession numbers used in building the North American phylogeny. 242 species and 6 loci were used.

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Aeshnidae	Aeshna	canadensis	KM533915.1					
Aeshnidae	Aeshna	constricta	KM530356.1					
Aeshnidae	Aeshna	eremita	KM529685.1					
Aeshnidae	Aeshna	interrupta	KM537132.1					
Aeshnidae	Aeshna	juncea	AB708581.1		AB707647.1	AF461231	AB706690.1	AB706690.1
Aeshnidae	Aeshna	subarctica	AB711460.1		AB711435.1		AB706700.1	AB706700.1
Aeshnidae	Aeshna	umbrosa	JN419304.1					
Aeshnidae	Anax	junius	KM536275.1	EU055328.1	AY749829.1	AB706705.1	AB706705.1	
Aeshnidae	Basiaeschna	janata	JN419315.1					
Aeshnidae	Boyeria	grafiana	JN419346.1					
Aeshnidae	Gomphaeschna	furcillata			EU477638.1	FJ010016.1		
Aeshnidae	Rhionaeschna	multicolor		EU055343.1	EU055053.1	EU055147.1		
Calopterygidae	Calopteryx	aequabilis	JN419428.1	EU055325.1	AF170961.1	AJ308360.1	AJ308360.1	AJ308360.1
Calopterygidae	Calopteryx	amata				AJ458977.1	AJ308361.1	AJ308361.1
Calopterygidae	Calopteryx	dimidiata			AF170959.1			
Calopterygidae	Calopteryx	maculata	JN419454.1		AF170960.1	U65108.1	AJ459198.1	AJ459198.1
Calopterygidae	Hetaerina	americana		EU055327.1	AF170951.1	FJ010010.1	AJ458989.1	AJ458989.1
Calopterygidae	Hetaerina	titia					AJ458990.1	AJ458990.1
Coenagrionidae	Acanthagrion	quadratum				FJ010037.1		
Coenagrionidae	Amphiagrion	abbreviatum	KM534075.1	EU055371.1	EU055079.1	EU055174.1		
Coenagrionidae	Amphiagrion	saucium				FJ009998.1		
Coenagrionidae	Argia	agrioides			FJ592218.1			
Coenagrionidae	Argia	alberta			FJ592211.1			
Coenagrionidae	Argia	anceps			FJ592233.1			
Coenagrionidae	Argia	apicalis			FJ592212.1			
Coenagrionidae	Argia	barretti			JX173251.1			
Coenagrionidae	Argia	cuprea			FJ592227.1			
Coenagrionidae	Argia	emma			FJ592228.1			

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Coenagrionidae	Argia	extranea			FJ592231.1			
Coenagrionidae	Argia	fumipennis		AF064987.1	FJ592230.1			
Coenagrionidae	Argia	harknessi			FJ592199.1			
Coenagrionidae	Argia	hinei			FJ592207.1			
Coenagrionidae	Argia	immunda			FJ592214.1			
Coenagrionidae	Argia	lacrimans			FJ592216.1			
Coenagrionidae	Argia	leonorae			FJ592226.1			
Coenagrionidae	Argia	lugens			FJ592215.1			
Coenagrionidae	Argia	moesta	JN419311.1		JX121202.1	FJ009997.1		
Coenagrionidae	Argia	munda			FJ592223.1			
Coenagrionidae	Argia	nahuana		EU055417.1	FJ592225.1	EU055221.1		
Coenagrionidae	Argia	oenea			FJ592217.1			
Coenagrionidae	Argia	pallens			FJ592224.1			
Coenagrionidae	Argia	pima			FJ592208.1			
Coenagrionidae	Argia	plana			FJ592196.1			
Coenagrionidae	Argia	rhoadsi			FJ592206.1			
Coenagrionidae	Argia	sabino			FJ592202.1			
Coenagrionidae	Argia	sedula		AY179159.1	FJ592209.1			
Coenagrionidae	Argia	tarascana			JX121177.1			
Coenagrionidae	Argia	tezpi			FJ592220.1			
Coenagrionidae	Argia	tibialis			FJ592203.1			
Coenagrionidae	Argia	tonto			FJ592204.1			
Coenagrionidae	Argia	translata			FJ592210.1			
Coenagrionidae	Argia	vivida			FJ592201.1	AY121144.1		
Coenagrionidae	Chromagrion	conditum	JN419473.1			FJ009995.1		
Coenagrionidae	Coenagrion	angulatum	KM528305.1	KM659942.1	KM659995.1	KM660057.1		
Coenagrionidae	Coenagrion	interrogatum	KM529381.1	KM659946.1	KM659999.1	KM660058.1	FN356065.1	FN356065.1
Coenagrionidae	Coenagrion	resolutum	KM532805.1	JQ966636.1	KM659997.1	KM660077.1	FN356069.1	FN356069.1
Coenagrionidae	Enallagma	anna		AF064990.1				
Coenagrionidae	Enallagma	antennatum	AF064991.1	AF064991.1				
Coenagrionidae	Enallagma	aspersum		AF064994.1		DQ087506.1		

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Coenagrionidae	Enallagma	basidens		AF064995.1				
Coenagrionidae	Enallagma	boreale		AF064997.1				
Coenagrionidae	Enallagma	carunculatum		AF064998.1				
Coenagrionidae	Enallagma	civile	KM532500.1					
Coenagrionidae	Enallagma	clausum		AF065001.1				
Coenagrionidae	Enallagma	concisum		AF065002.1				
Coenagrionidae	Enallagma	daeckii		AF065007.1				
Coenagrionidae	Enallagma	davisi		AF065008.1				
Coenagrionidae	Enallagma	divagans	KM534165.1					
Coenagrionidae	Enallagma	doubledayi	KM531115.1					
Coenagrionidae	Enallagma	dubium		AF065013.1				
Coenagrionidae	Enallagma	durum		AF065014.1				
Coenagrionidae	Enallagma	ebrium	KM537311.1					
Coenagrionidae	Enallagma	exsulans	KT708111.1					
Coenagrionidae	Enallagma	geminatum	KT708001.1					
Coenagrionidae	Enallagma	hageni	KM537733.1					
Coenagrionidae	Enallagma	laterale		AF065022.1				
Coenagrionidae	Enallagma	minusculum		AF065023.1				
Coenagrionidae	Enallagma	pallidum		AF065024.1				
Coenagrionidae	Enallagma	pictum		AF065026.1				
Coenagrionidae	Enallagma	pollutum		AF065028.1				
Coenagrionidae	Enallagma	praevarum		AF065029.1				
Coenagrionidae	Enallagma	recurvatum		AF065030.1				
Coenagrionidae	Enallagma	signatum	KT708225.1					
Coenagrionidae	Enallagma	sulcatum		AF065034.1				
Coenagrionidae	Enallagma	traviatum		AF065035.1				
Coenagrionidae	Enallagma	vernale		AF065037.1				
Coenagrionidae	Enallagma	vesperum		AF065038.1				
Coenagrionidae	Enallagma	weewa		AF065040.1				
Coenagrionidae	Hesperagrion	heterodoxum		AF067674.1				
Coenagrionidae	Ischnura	barberi		AF067663.1	EU055042.1	EU055136.1		

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Coenagrionidae	Ischnura	cervula		AF067665.1		FN356101.1	FN356101.1	FN356101.1
Coenagrionidae	Ischnura	damula		AF067666.1				
Coenagrionidae	Ischnura	demorsa		AF067667.1				
Coenagrionidae	Ischnura	denticollis		AF067668.1	GU812265.1	FN356102.1	FN356102.1	FN356102.1
Coenagrionidae	Ischnura	erratica		AF067671.1				
Coenagrionidae	Ischnura	gemina		AF067672.1	GU812267.1			
Coenagrionidae	Ischnura	hastata		AF067673.1				
Coenagrionidae	Ischnura	kellicotti	KT708070.1	AF067675.1				
Coenagrionidae	Ischnura	perparva		AF067676.1	GU812263.1	FN356106.1	FN356106.1	FN356106.1
Coenagrionidae	Ischnura	posita	JN419853.1	AF067678.1				
Coenagrionidae	Ischnura	prognata		AF067679.1				
Coenagrionidae	Ischnura	ramburii		AF067680.1		FN356108.1	FN356108.1	FN356108.1
Coenagrionidae	Ischnura	verticalis	KM530329.1	AF067682.1				
Coenagrionidae	Leptobasis	vacillans				KT324242.1		
Coenagrionidae	Nehalennia	gracilis	GQ256040.1	GQ256054.1	GQ256017.1	FJ009994.1		
Coenagrionidae	Nehalennia	irene	KT708088.1	GQ256062.1	GQ256023.1			
Coenagrionidae	Telebasis	byersi		AF064986.1				
Coenagrionidae	Telebasis	salva		EU055369.1	EU055077.1	EU055172.1		
Cordulegastridae	Cordulegaster	bilineata				AY082597.1		
Cordulegastridae	Cordulegaster	diastatops				AY082601.1		
Cordulegastridae	Cordulegaster	dorsalis		EU055376.1	EU055084.1	EU055179.1		
Cordulegastridae	Cordulegaster	erronea	GQ329628.1		EU477690.1	AY082599.1		
Cordulegastridae	Cordulegaster	maculata	JN419645.1		EU477689.1	AY082600.1		
Cordulegastridae	Cordulegaster	obliqua			EF631533.1			
Cordulegastridae	Cordulegaster	sayi				AY337235.1		
Cordulegastridae	Cordulegaster	talaria				AY337236.1		
Corduliidae	Cordulia	shurtleffii		EU055377.1	EU055085.1	EU055180.1		
Corduliidae	Dorocordulia	libera	KM528893.1					
Corduliidae	Epitheca	canis	KM533859.1		EU477712.1			
Corduliidae	Epitheca	costalis			EU477713.1			
Corduliidae	Epitheca	cynosura	KM536481.1		EU477709.1			

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Corduliidae	Epitheca	princeps			EU477710.1			
Corduliidae	Helocordulia	uhleri	JN419849.1		EF631544.1			
Corduliidae	Neurocordulia	obsoleta			EF631509.1			
Corduliidae	Somatochlora	elongata	KM528142.1					
Corduliidae	Somatochlora	franklini	GU013661.1					
Corduliidae	Somatochlora	minor	JN420265.1					
Corduliidae	Somatochlora	sahlbergi				FN356167.1	FN356167.1	FN356167.1
Corduliidae	Somatochlora	semicircularis	KM529041.1					
Corduliidae	Somatochlora	tenebrosa			EF631532.1	FJ010028.1		
Corduliidae	Somatochlora	williamsoni	KM531663.1					
Gomphidae	Arigomphus	cornutus				DQ008188.1		
Gomphidae	Arigomphus	villosipes				KT324336		
Gomphidae	Dromogomphus	spinosus			EU477662.1	DQ008189.1		
Gomphidae	Gomphus	abbreviatus	JN419828.1					
Gomphidae	Gomphus	adelphus	JN419830.1			FJ010019.1		
Gomphidae	Gomphus	descriptus	JN419840.1					
Gomphidae	Gomphus	exilis	KM534754.1		EU477656.1	DQ008187.1		
Gomphidae	Gomphus	externus			EU477655.1	DQ008184.1		
Gomphidae	Gomphus	graslinellus	KM529170.1					
Gomphidae	Gomphus	spicatus	KM534994.1					
Gomphidae	Hagenius	brevistylus	JN419844.1		EU477667.1	DQ008193.1		
Gomphidae	Lanthus	parvulus	JN419943.1					
Gomphidae	Lanthus	vernalis				KT324307.1		
Gomphidae	Ophiogomphus	carolus	JN420082.1					
Gomphidae	Ophiogomphus	mainensis	JN420156.1					
Gomphidae	Ophiogomphus	severus			EU477673.1	DQ008192.1		
Gomphidae	Phyllogomphoides	albrighti			EU477675.1			
Gomphidae	Phyllogomphoides	stigmatus				KT324305.1		
Gomphidae	Progomphus	borealis		EU055370.1	EU055078.1	EU055173.1		
Gomphidae	Progomphus	obscurus	KJ873212.1		EU477676.1	AY749909.1		
Gomphidae	Stylogomphus	albistylus	JN420310.1		EU477665.1			

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Gomphidae	Stylurus	amnicola			EU477657.1	DQ008186.1		
Gomphidae	Stylurus	intricatus	KJ873219.1		EU477658.1	DQ008185.1		
Lestidae	Archilestes	grandis		EU055382.1	EU055090.1	EU055185.1		
Lestidae	Lestes	alacer			JX121132.1			
Lestidae	Lestes	congener	KM530687.1					
Lestidae	Lestes	disjunctus	KM529836.1					
Lestidae	Lestes	dryas	KM537254.1		AB707358.1		AB706408.1	AB706408.1
Lestidae	Lestes	eurinus				KT324298.1		
Lestidae	Lestes	rectangularis	KM536047.1			FJ010011.1		
Lestidae	Lestes	unguiculatus	KM533933.1					
Libellulidae	Brachymesia	gravida			EF640392.1			
Libellulidae	Brechmorhoga	mendax	KJ873229.1		EF631502.1			
Libellulidae	Celithemis	elisa	KM531025.1		DQ021425.1	FJ010031.1		
Libellulidae	Celithemis	eponina			EF640393.1	AF461233.1		
Libellulidae	Crocothemis	servilia	AB711448.1	DQ166789.1	KF256856.1		AB707065.1	AB707065.1
Libellulidae	Dythemis	fugax			EF631503.1			
Libellulidae	Erythemis	collocata			EF640422.1			
Libellulidae	Erythemis	simplicicollis	KM536722.1		AF037191.1			
Libellulidae	Erythrodiplax	fusca			EF640424.1			
Libellulidae	Erythrodiplax	minuscula		EU055340.1	EU055050.1	EU055144.1		
Libellulidae	Erythrodiplax	umbrata			EF640426.1			
Libellulidae	Ladona	deplanata	AF195740.1		AF037187.1			
Libellulidae	Ladona	exusta	AF195742.1		AF037188.1			
Libellulidae	Ladona	julia	KM534905.1		EF631536.1			
Libellulidae	Leucorrhinia	frigida	KM534739.1					
Libellulidae	Leucorrhinia	glacialis	KM535316.1		EF631523.1			
Libellulidae	Leucorrhinia	hudsonica	KM529732.1		EF640395.1			
Libellulidae	Leucorrhinia	intacta	JN419952.1		EF640396.1			
Libellulidae	Leucorrhinia	patricia	KM537513.1					
Libellulidae	Leucorrhinia	proxima	KM532321.1		EF640397.1			
Libellulidae	Libellula	auripennis	AF195734.1		AF037176.1			

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Libellulidae	Libellula	axilena	AF195735.1		AF037175.1			
Libellulidae	Libellula	comanche	AF195736.1		AF037182.1			
Libellulidae	Libellula	composita	AF195737.1		AF195727.1			
Libellulidae	Libellula	croceipennis	AF195738.1		AF037183.1			
Libellulidae	Libellula	cyanea	AF195739.1					
Libellulidae	Libellula	flavida	AF195743.1		AF195728.1			
Libellulidae	Libellula	forensis	AF195744.1		EF640399.1			
Libellulidae	Libellula	incesta	AF195746.1		AF037179.1			
Libellulidae	Libellula	jesseana	AF195747.1		AF037174.1			
Libellulidae	Libellula	luctuosa	KT707326.1		AF037178.1			
Libellulidae	Libellula	needhami	AF195751.1		AF195730.1			
Libellulidae	Libellula	pulchella	KM535999.1		AF037180.1	U65109.1		
Libellulidae	Libellula	quadrimaculata	JN419954.1		KF256841.1	AB707092.1	AB707091.1	AB707091.1
Libellulidae	Libellula	saturata	AF195755.1	EU055326.1	AF037181.1	AY338717.1		
Libellulidae	Libellula	semifasciata	AF195756.1		AF037171.1			
Libellulidae	Libellula	vibrans	AF195758.1		AF037172.1			
Libellulidae	Macrodiplax	balteata		EU055332.1	EF640459.1	EU055134.1		
Libellulidae	Miathyria	marcella			EF640449.1			
Libellulidae	Micrathyria	aequalis			EF631508.1			
Libellulidae	Micrathyria	didyma			DQ021421.1			
Libellulidae	Nannothemis	bella			EF640388.1			
Libellulidae	Orthemis	discolor			DQ021417.1			
Libellulidae	Orthemis	ferruginea	AF195760.1		EF631581.1			
Libellulidae	Pachydiplax	longipennis	KM532021.1		EF640433.1			
Libellulidae	Paltothemis	lineatipes			EF640455.1			
Libellulidae	Pantala	flavescens	KR011198.1	DQ166791.1	KF256865.1	EF680326.1	AB707211.1	AB707211.1
Libellulidae	Perithemis	intensa		EU055337.1	EU055047.1	EU055141.1		
Libellulidae	Perithemis	tenera			EF640409.1	FJ010032.1		
Libellulidae	Plathemis	lydia	JN420199.1		AF037184.1			
Libellulidae	Plathemis	subornata	AF195757.1		EF640406.1			
Libellulidae	Pseudoleon	superbus			EF640435.1			

Family	Genus	Species	COI	COII	16S	18S	ITS1	ITS2
Libellulidae	Sympetrum	ambiguum	EF636300.1		EF631548.1	EF636418.1		
Libellulidae	Sympetrum	corruptum	KM529511.1		JQ964129.1	EU055135.1		
Libellulidae	Sympetrum	costiferum	EF636249.1		JQ772596.1	EF636368.1		
Libellulidae	Sympetrum	danae	KM529124.1		AB708177.1	EU243994.1	AB707228.1	AB707228.1
Libellulidae	Sympetrum	illotum			EF640441.1			
Libellulidae	Sympetrum	internum	KM535858.1		JQ772605.1	EF636423.1		
Libellulidae	Sympetrum	madidum	KM529557.1		JQ772608.1	JQ772560.1		
Libellulidae	Sympetrum	obtrusum	EF636324.1		EF640443.1	EF636432.1		
Libellulidae	Sympetrum	pallipes	KM534409.1		JQ772610.1	EF636419.1		
Libellulidae	Sympetrum	rubicundulum	EF636333.1		JQ772613.1	EF636442.1		
Libellulidae	Sympetrum	semicinctum	EF636273.1		EF640446.1	EF636396.1		
Libellulidae	Sympetrum	signiferum	EF636280.1		JQ772614.1	EF636414.1		
Libellulidae	Sympetrum	vicinum	EF636289.1		JQ772617.1	JQ772568.1		
Libellulidae	Tholymis	citrina	KJ994784.1		DQ021423.1			
Libellulidae	Tramea	calverti			EU477750.1			
Libellulidae	Tramea	lacerata	AB709202.1	EU055368.1	AB708258.1	EU055171.1	AB707308.1	AB707308.1
Libellulidae	Tramea	onusta			EF631593.1			
Macromiidae	Didymops	transversa			EF631549.1	FN356079.1	FN356079.1	FN356079.1
Macromiidae	Macromia	alleghaniensis				FN356122.1	FN356122.1	FN356122.1
Macromiidae	Macromia	illinoiensis	JQ780892.1		EF631524.1	FJ010027.1	FN356124.1	FN356124.1
Macromiidae	Macromia	magnifica			EF640463.1	FN356125.1	FN356125.1	FN356125.1
Macromiidae	Macromia	taeniolata			EU477695.1			
Petaluridae	Tachopteryx	thoreyi	KJ873230.1		KJ856847.1	KJ856866.1	FN356173.1	FN356173.1
Petaluridae	Tanypteryx	hageni		EU055367.1	EU055075.1	KJ856877.1		
Platystictidae	Palaemnema	domina	KF369473.1		KF369820.1			
Protoneuridae	Neoneura	aaroni				FJ009982.1		
Protoneuridae	Neoneura	amelia				KT324246.1		
Protoneuridae	Protoneura	cara				KT324245.1		
		TOTAL	113	66	150	86	26	25

**Table A5:** The six genes used for phylogeny construction. The length of each gene (number of base pairs) is listed, as well as the models selected with JModelTest v.2.1.4 (Guindon and Gascuel 2003, Darriba et al. 2012).

Gene	Length (bp)	Selected model
CO1	999	TVM+I+G
CO11	662	GTR+I+G
ITS1	268	TrN+G
ITS2	333	TVM
18S rRNA	1983	GTR+G
16S rRNA	582	TVM+I+G

**Table A6:** Results of the divergence time estimations, including mean ages, as well as upper and lower bounds of the highest posterior density (HPD). Additional bootstrap support values (BS) as obtained in the maximum likelihood reconstructions, as well as fossils used for calibrating the odonate phylogeny, with minimal ages assigned to the according clades.

Fossil	Clade	Age (Ma*)	Reference		Estimated age (Ma)		
				Mean	95% HPD lower	95% HPD upper	
Triassolestodes asiaticus	Odonata (crown)	237	Pritykina 1981	238.1	237.27	239.02	
Mersituria ludmilae	Zygoptera (crown)	152.2	Vasilenko 2005	153.30	152.45	154.21	
Sinacymatophlebia mongolica	Anisoptera (crown)	168	Nel and Huang 2009	169.13	168.25	170.04	
Gomphaeschna inferna	Aeshnidae (crown)	139.8	Pritykina 1977	140.91	140.07	142.2	
Proterogomphus renateae	Gomphidae (crown)	150	Bechly 1998	151.08	150.25	152.2	
Epophthalmia biordinata	Macromiidae (crown)	15.5	Lewis 1969	16.70	15.76	17.99	
Croatocordulia platyptera	Corduliidae (crown)	12.7	de Charpentier 1843	14.43	12.90	16.60	
Tauriphila cerestensis	Libellulidae (crown)	29.2	Nel and Paicheler 1993	31.87	29.45	37.15	

**Table A7:** Model selection details for the forward stepwise multiple regression analyses performed with the measured environmental variables as predictor variables and the NRI values as the response variables for the full community data set (a) and the temperate community data set (b). The models are listed in descending order and the model with the lowest AIC score was chosen for each region. Delta AIC ( $\Delta$ AIC) is a measure of each model relative to the best model and w is the Akaike weight, which represents the probability of the model being the best among the whole set of candidate models. The details of the multiple regression analysis performed with the boreal community data set are not listed as none of the models were significant.

#### a. Full Community Data Set

Model	AIC	ΔΑΙϹ	W	Р
Start	23.68	3.58	-	-
pН	20.1	0	1	0.023

#### b. Temperate Community Data Set

MODEL	AIC	ΔΑΙϹ	w	Ρ
Start	18.22	11.44	-	-
AET	14	7.22	0.011	0.017
AET + Temperature Seasonality	11.53	4.75	0.039	0.009
AET + Temperature Seasonality + Perimeter	10.74	3.96	0.059	0.008
AET + Temperature Seasonality + Perimeter + Disturbance	10.54	3.76	0.065	0.010
AET + Temperature Seasonality + Perimeter + Disturbance + Surface Area	9.93	3.15	0.088	0.010
AET + Temperature Seasonality + Perimeter + Disturbance + Surface Area + pH	9.73	2.95	0.097	0.011
AET + Temperature Seasonality + Perimeter + Disturbance + Surface Area + pH + Conductivity	8.13	1.35	0.216	0.008
AET + Temperature Seasonality + Perimeter + Disturbance + Surface Area + pH + Conductivity + DO	6.78	0	0.424	0.007

## **Supplementary Material**

## Supplementary Methods

Method S1: Larvae Sampling Technique.

A total of 216 larval individuals were sampled at a subset of sites (23 sites) and were identified to the genus-level. A 500micron mesh D-net was used to sample larvae at each of the ten sampling stations along the site transect. At each station, a 2m x 2m area was defined in the water adjacent to the adult sampling station and two successive sweeps were performed with the D-net (Bright and Lewington 1999, Worthen and Horacek 2015). The net was emptied into a tray after each sweep and the contents of the two sweeps were then searched for ten minutes. Larval individuals were stored in tubes of 95% ethanol and were later identified to the genus-level.

## Supplementary Results

The NMDS plot of the larval and adult communities showed a distinction between assemblages. When looking at the whole community dataset (Figure S1a), it seems that there is some similarity in composition between the sampled adult and larval communities. When the observational data is included in the adult community data (Figure S1b), the difference in composition between the adult and larval communities is greater. The results are similar when the community data is separated into Anisopteran (Figure S1c and d) and Zygopteran (Figure S1e and f) communities: including the adult observational data leads to greater differences between the adult and larval communities. The difference between the adult and larval assemblages is greater for Zygopteran communities than Anisopteran communities, with more sites having compositional differences between the two life stages.

The accumulation curves (Figure S2) show that adult sampling was fairly complete at the genus-level, as the curve seems to be levelling-off, but sampling could be improved at the species-level by collecting individuals from more sites. The larvae sampling at the genus-level does not seem to be quite complete and could benefit from more samples. The predicted species-level larvae accumulation curve also shows that more samples would improve the sampling completeness.

Supplementary Tables and Figures (see next page)

**Figure S1:** NMDS plots of larval (open dots) and adult (solid dots) communities (genuslevel). Figures in the left column show only sampled larvae and sampled adults whereas figures in the right column show the sampled larval communities compared to adult communities comprised of sampled and also observational data. Figures a and b are ordinations of the whole odonate community matrix, figures c and d show the Anisopteran communities, and figures e and f show the Zygopteran communities.



**Figure S2:** Accumulation curves for larval generic richness, adult generic richness, and adult species richness, as well as a predicted curve for the larval species richness. The green line represents the adult species, the red line is the adult genera, and the blue line is the larvae genera. The black dotted line is the predicted larvae species richness.



Sites

**Figure S3:** NMDS plot of the boreal (open dots) and temperate (solid dots) lake communities.



NMDS1

**Figure S4:** Phylogenetic tree for the North American odonate species for which genetic sequences were available. The tree includes information for six different loci across 242 species. The branch colors depict the level of Bayesian support: black => 0.9, dark grey => 0.75, light grey => 0.5.



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**Figure S5:** Phylogenetic tree for the Quebec odonate species for which genetic sequences were available. The tree includes information for six different loci across 104 species. The branch colors depict the level of Bayesian support: black => 0.9, dark grey => 0.75, light grey => 0.5.

