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Natural flow regime, temperature and the composition and richness of invertebrate assemblages in streams of the western United States

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SUMMARY

1. We tested how strongly aquatic macroinvertebrate taxa richness and composition were associated with natural variation in both flow regime and stream temperatures across streams of the western United States.

2. We used long-term flow records from 543 minimally impacted gauged streams to quantify 12 streamflow variables thought to be ecologically important. A principal component analysis reduced the dimensionality of the data from 12 variables to seven principal component (PC) factors that characterised statistically independent aspects of streamflow: (1) zero flow days, (2) flow magnitude, (3) predictability, (4) flood duration, (5) seasonality, (6) flashiness and (7) base flow. *K*-means clustering was used to group streams into 4–8 hydrologically different classes based on these seven factors.

3. We also used empirical models to estimate mean annual, mean summer and mean winter stream temperatures at each stream site. We then used invertebrate data from 63 sites to develop Random Forest models to predict taxa richness and taxon-specific probabilities of capture at a site from flow and temperature. We used the predicted taxon-specific probabilities of capture to estimate how well predicted assemblages matched observed assemblages as measured by RIVPACS-type observed/expected (O/E) indices and Bray–Curtis dissimilarities.

4. Macroinvertebrate taxon richness was only weakly associated with streamflow and temperature variables, implying that other factors more strongly influenced taxa richness.

5. In contrast to taxa richness, taxa composition was strongly associated with streamflow and temperature. Predictions of taxa composition (O/E and Bray–Curtis) were most precise when both temperature and streamflow PC factors were used, although predictions based on either streamflow PC factors or temperature alone were also better than null model predictions. Of the seven aspects of the streamflow regime we examined, variation in baseflow conditions appeared to be most directly associated with invertebrate biotic composition. We were also able to predict assemblage composition from the conditional probabilities of hydrological class membership nearly as well as Random Forests models that were based directly on continuous PC factors.

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6. Our results have direct implication for understanding the relative importance of streamflow and temperature in regulating the structure and composition of stream assemblages and for improving the accuracy and precision of biological assessments.

Keywords: benthic invertebrates, classification, flow regime, streams, temperature

Introduction

A major goal of stream ecology is to understand the environmental factors that structure natural communities. Natural flows are thought to be critical to the maintenance of healthy stream ecosystems (Poff *et al.*, 1997; Bunn & Arthington, 2002), but currently less is known about the effects of flow on the distribution of stream invertebrates than that of temperature (e.g. Hawkins *et al.*, 1997; Poff & Zimmerman, 2009). Moreover, little is known about the relative or interactive effects of these two factors on stream invertebrates. The aspects of streamflow thought to influence ecological processes in rivers include: flow magnitude, duration, frequency, timing and rate of change (Poff, 1996; Poff *et al.*, 1997; Puckridge *et al.*, 1998). A large number of streamflow regime variables have been defined in efforts to quantify these factors (Richter *et al.*, 1996; Puckridge *et al.*, 1998; Snelder & Biggs, 2002; Sanz & Del Jalon, 2005; Sanborn & Bledsoe, 2006). In particular, Olden & Poff (2003) listed 171 flow variables assumed to be of ecological relevance.

Several researchers have developed catchment classifications based in whole or part on differences among streams in flow regime, often with the purpose of aiding understanding of how flow regime affects stream ecosystems and their biota (Snelder & Biggs, 2002; Sanborn & Bledsoe, 2006; Snelder *et al.*, 2009). Poff (1996) used 10 streamflow variables to classify 806 relatively undisturbed streams in the continental U.S.A. into groups of sites that differed in their flow regimes. The underlying assumption of his classification is that flow regime is an important aspect of the environmental template that influences the abundance and distribution of stream biota, hence such a classification could aid in explaining and predicting the distribution of different stream taxa.

Although understanding of how streams differ in terms of their flow regimes has greatly improved over the last 15 years, considerable uncertainty still exists regarding how different aspects of flow variation influence either specific stream biota or the overall ecological structure and function of streams (Poff & Zimmerman, 2009; Snelder & Lamouroux, 2009), particularly with respect to variation among streams within and across regions. Several studies have considered the general relevance of regional variation in streamflow regimes for stream ecosystems, but they have not directly quantified relationships between flow regime and biotic assemblages (e.g. Poff & Ward, 1989; Poff, 1996; Sanz & Del Jalon, 2005; Sanborn & Bledsoe, 2006; Snelder *et al.*, 2009). Other studies have tested specific hypotheses regarding the effects of differences in hydrological regimes on various ecological properties of streams (Poff & Allan, 1995; Clausen & Biggs, 1997; Konrad, Brasher & May, 2008; Monk *et al.*, 2008). However, such studies have often relied on aggregate biological measures, such as LIFE scores (e.g. Extence, Balbi & Chadd, 1999), to summarise biotic responses to differences in flow regime rather than more direct measures of taxonomic composition and richness. Furthermore, most of these studies have focused on the short-term response of stream biota to specific flow disturbances (e.g. Bickerton, 1995; Ilg *et al.*, 2009). These single-site or single-hydrological-event studies have established the importance of aspects of the streamflow regime for stream biota, but it remains difficult to draw general inferences as to how biotic assemblages will vary across large, hydrologically variable regions.

In this study, our goal was to understand how aquatic macroinvertebrate taxonomic composition and richness varied with differences in both streamflow regime and stream temperature at the subcontinental scale of the western United States. Our specific objectives were to quantify the variation in flow regime in terms of statistically independent flow-related factors, to classify streams to describe major aspects of hydrological variability based on these factors and to determine the relative and interactive importance of flow regime and stream temperature as predictors of the composition and richness of stream invertebrate assemblages.

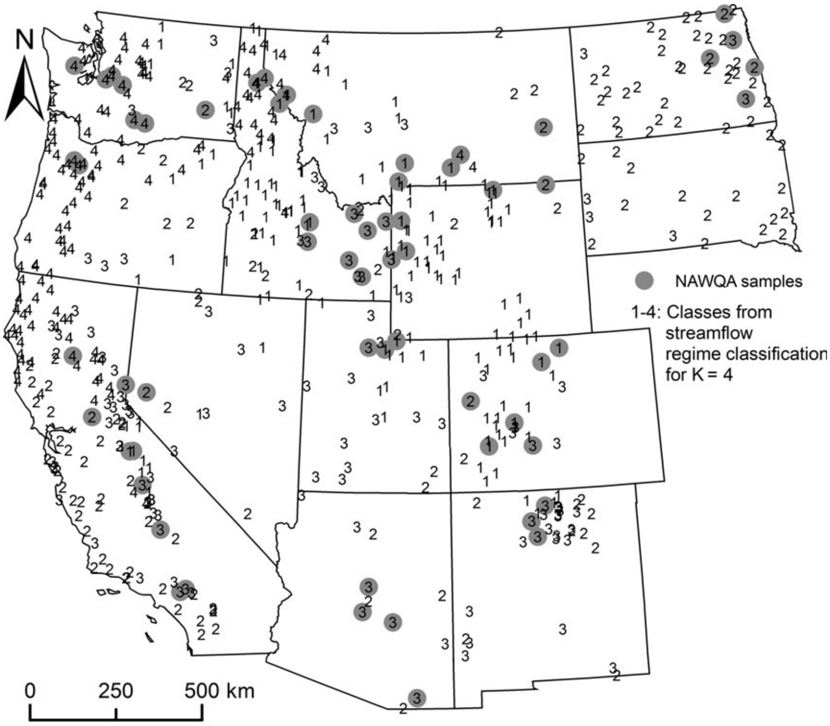

Fig. 1 Locations of 543 streamflow gauge sites used in this study. Five hundred and eleven sites are from the Hydro Climate Data Network (HCDN) and an additional 32 sites with benthic invertebrate data are from Carlisle *et al.* (2009). Numbers indicate regime class for $K = 4$ classification. Sites with NAWQA benthic invertebrate samples are also indicated.

Methods

General approach to characterising flow regimes

We characterised flow regimes across the western United States by analysing daily flow data from 543 relatively unimpaired streams in 13 western United States (Fig. 1). Catchment areas spanned 15–114 793 km^2. Based on a review of the literature and our own experience and judgment, we selected 12 flow variables (Appendix S1, Table 1) that we deemed sufficient to characterise those flow regime properties important to stream biota. We then used principal component analysis (PCA) to identify a set of derived variables (factors) that were statistically independent of one another. Factor scores from the PCA were used in a *K*-means cluster analysis to classify streams into 4, 5, 6, 7 and 8 streamflow regime classes.

Flow data

The Hydro Climatic Data Network (HCDN) is a streamflow data set for the U.S.A. that is relatively free from anthropogenic influences and has been developed for studying natural variations in surface-water conditions (Slack & Landwehr, 1992). The HCDN data cumulatively span the years 1874–1988, but the periods of record differ between sites and not all sites were considered by the United States Geologic Survey (USGS) (Slack & Landwehr, 1992) to be not significantly impacted by flow regulation for their entire record. Fifty-one HCDN sites within our study area were excluded for one or more of the following reasons: (i) closer examination revealed that they drained reservoirs, (ii) flows were unimpaired for less than 10 water years, or (iii) the HCDN database comments indicated that only monthly streamflow estimates were considered free of human influence. We included flow data from an additional 32 gauged sites at which benthic invertebrate samples were collected by the USGS and that Carlisle *et al.* (2009) indicated also had unimpaired streamflow. To calculate values of the 12 flow variables for each site, we used daily streamflow records only for the period identified as having unimpaired flows.

The record lengths for 540 sites ranged between 10 and 103 years with mean record length 43 years. However, three sites for which we had invertebrate samples had less than 10 years of data (6, 7 and

8 years). Because we were concerned that records <10 years in length would not adequately characterise long-term flow patterns (and hence biological associations), we conducted preliminary analyses both with and without these three sites. In fact, there were no significant differences between these two data sets in terms of model performance (described later), and we therefore present results based on the full data set.

At 34 sites with biological data, sampling was conducted outside (after) the period of record that was included in the HCDN database (that ended in 1988). We were concerned that the streamflow regime may have changed and thus influenced macroinvertebrates at these sampling sites. We checked this concern by computing streamflow regime variables for the periods where streamflow was designated by the USGS as unimpaired as well as for the subsequent period up to the most recent data available. The correlation coefficients between HCDN unimpaired and post-HCDN periods for 11 of the streamflow variables varied between 0.85 and 0.99. The correlation coefficient for ZERODAYS was somewhat lower (0.64), but these results generally allayed concern that potential changes in the streamflow regime might bias analyses.

Quantifying and classifying flow regimes

We used PCA (Jackson, 1991) with varimax rotation (Kaiser, 1958) based on the correlations between the 12 flow variables to identify the major statistically independent axes of hydrological variation across stream gauge sites. We followed the recommendations of Wilkinson, Blank & Gruber (1996) that the variables used in PCA should be normally distributed and not skewed prior to calculating principal components. Skewness can affect principal components and their interpretation (e.g. Reid & Spencer, 2009). Because the 12 hydrology variables exhibited various types and magnitudes of deviation from a normal distribution, we could not use a single, simple transformation (e.g. log values) to achieve near normal distributions for all variables. We therefore used the Box & Cox (1964) transformation with parameters chosen to maximise the W-statistic in a Shapiro–Wilks normality test (Royston, 1982).

PCA produces NV principal components (PC) where NV is the number of original variables. However, generally, a relatively small number of the NV possible PCs are associated with most of the variation exhibited by the raw variables. Selection of a subset of the PCs for further analysis can focus on either selecting those first $nv < NV$ PCs associated with most of the variability in the original raw variables or identifying those PCs that provide unique information. Traditionally, choice of the subset of PCs used in analyses has followed the first approach (Kaiser's rule: Lattin, Carroll & Green, 2003). However, we agree with Monk *et al.* (2007) that such traditional methods for PC selection may not represent all the important aspects of the streamflow regime. We therefore selected PCs based on how well they identified independent and unique aspects of the flow regime that we considered to be ecologically important.

We first chose a minimum number of PCs to work with by selecting those PCs with eigenvalues >1. We then used varimax rotation on the selected PCs to obtain factors such that each variable was maximally aligned with a single factor. We inspected the resulting factors to assess the degree to which they represented each of the 12 variables as quantified by the variable factor loadings. If one or more variables were not well represented (loading ≥0.6) in the initial set of rotated factors, we repeated the analysis after adding the PC from the original analysis with the next highest eigenvalue. This process was continued until we achieved a set of rotated PCs that included each of the 12 flow variables with loadings ≥0.6.

We used the R kmeans function (R Development Core Team, 2007) to conduct a K-means cluster analysis (Gordon, 1999) on the rotated PC factor scores to identify streamflow regime classes. K-means classification requires a priori specification of the number of classes, K. Because we had no a priori sense of how many classes would be optimal in terms of partitioning flow variability relevant to stream invertebrates, we examined a range of K values (K = 4–8). The number of classes we could examine was constrained by both resolution of flow information and sample size. $K < 4$ would not provide enough classes to discriminate all the streamflow regime characteristics of interest, whereas too few observations occurred per class when K was >8.

Temperature data

We included three measures of water temperature in our analyses: mean annual temperature (MAT), mean summer (June, July, August) temperature (MST) and mean winter (December, January, February) temperature (MWT), derived from stream temperature models (root mean square error = 0.86, 2.2 and 1.7 °C, respectively) developed for the western United States (see Appendix S2). Because aspects of flow and temperature may co-vary, we used backward stepwise multiple linear regression to assess the relationships between these three temperature measures and each of the rotated PC factors. We selected regression models based on those combinations of predictor variables (PC factors) that produced the lowest Akaike's Information Criterion (AIC) value (Burnham & Anderson, 2002). We also used ANOVA to assess how much variation in stream temperature was associated with the flow classes.

Invertebrate data

Between 1992 and 2003, USGS National Water-Quality Assessment Program (NAWQA) personnel collected benthic invertebrate samples at 63 of the 543 gauged sites (Fig. 1) we used to characterise flow regimes (see Appendix S3 for details regarding NAWQA sampling procedures and how we treated the sample data). Samples for 59 of these sites were collected between June and September. For four of the sites, samples were collected in October, December or January. Carlisle *et al.* (2009) indicated that streamflow was unimpaired at all these sites, but that the catchments of 30 sites were not in pristine condition, meaning that various land use alterations might have influenced macroinvertebrate assemblages through effects such as nutrient or sediment additions. These effects would emerge as unaccounted for variation in our analyses and thus potentially obscure variation in invertebrate richness and assemblage composition associated with differences in flow and temperature. However, by including these 30 non-reference sites, our results should be conservative with respect to the detection of biota relationships with both flow and temperature.

Assessing relationships between taxa composition, flow and temperature

We used RIVPACS-type niche models (Moss *et al.*, 1987; Hawkins, Cao & Roper, 2009) to assess the associations between macroinvertebrate taxonomic composition and both continuous measures of flow variability (PC factors) and flow classes. Predictive models like RIVPACS are frequently used in bioassessment programs to evaluate the degree to which observed taxonomic composition matches the composition expected under specific reference conditions (Moss *et al.*, 1987; Wright, Furse & Armitage, 1993; Hawkins, 2006). The RIVPACS approach generally consists of the following steps (Moss *et al.*, 1987): (1) classification of sites into groups based on their taxonomic composition (presence–absence data), (2) estimation of the frequencies of occurrence of different taxa within each group, (3) prediction of the probability of group membership for a site from environmental factors and (4) estimation of probabilities of capture of specific taxa as the taxon occurrence frequency within each group combined with probabilities of group membership.

We classified the 63 invertebrate sites based on their compositional similarity (Sørensen index). We used the flexible β hierarchical clustering method ($\beta = -0.5$) in the PC-ORD® software package (Mccune & Grace, 2002) to identify different biologically defined classes of sites. To facilitate interpretation of the resulting dendrogram, compositional dissimilarity between sites and groups of sites was scaled by Wishart's (1969) objective function expressed as the percentage of information remaining. Wishart's objective function is a measure of information loss as clustering proceeds. It is calculated as the sum of squares of the distances between the centroids of each group to the items in those groups. From the sample data in each group, we estimated the mean richness per group, the frequency of occurrence of each taxon within each group and the richness and composition expected at individual sites. We illustrated the biological differences among groups of sites by identifying the specific indicator taxa representative of each group following the method of Dufrêne & Legendre (1997) but applied to presence–absence data.

We used both Random Forests (RF) models (Breiman, 2001) and conditional probability (CP) models to predict the probabilities that a site belonged to each of the biologically defined groups (macroinvertebrate groups) as a function of three different combinations of continuous flow (PC factors) and temperature data and the 5 flow classifications. As described earlier, prediction of group membership is an intermediate step in the prediction of taxa composition in RIVPACS-type models (Moss *et al.*, 1987; Hawkins *et al.*, 2009). We used the randomforest package (Liaw & Wiener, 2002) in the R software (R Development Core Team, 2007) to develop the RF predictive models. RF models make no assumptions regarding the type of relationships (linear or nonlinear) between predictor and response variables, can use both continuous and categorical predictors and have been shown to perform well in a number of ecological settings (Prasad, Iverson & Liaw, 2006; Cutler *et al.*, 2007; Hawkins *et al.*, 2009). When RF models are used to predict class membership (classification mode), the importance of predictive variables is quantified by the Gini index score, a measure of the homogeneity at RF splits based on that variable (Breiman *et al.*, 1984). CP models predict the probabilities that a site belongs to different macroinvertebrate groups directly from a contingency table, which gives the number of sites in each macroinvertebrate group occurring in a given streamflow regime class. For a given streamflow regime classification, we computed the probability of a site belonging to different macroinvertebrate groups (P_b) directly from the contingency table.

Agreement between observed and predicted assemblage composition can be measured as either the O/E ratio (where O is the number of taxa observed in the sample that were predicted to occur and E is the number of predicted taxa, see Moss *et al.*, 1987) or by a Bray–Curtis (BC) type measure of compositional dissimilarity between observed and expected.

The performance of RIVPACS-type models is typically assessed by determining how well the taxa predicted to occur under reference conditions match the observed taxa (Hawkins, Olson & Hill, 2010). Agreement can be quantified in terms of either the number of predicted taxa that were observed (O/E, see Moss *et al.*, 1987) or by a BC type measure of compositional dissimilarity (Van Sickle, 2008). We used both measures to assess differences among models in their performance as influenced by the inclusion of different predictors. Comparison with a null model provides further context for evaluating whether the inclusion of predictor variables result in meaningful improvement in predictions of taxa composition (e.g. Van Sickle *et al.*, 2005). Because rare taxa cannot be predicted well, we included only those taxa with predicted probabilities of capture > 0.5 (Hawkins et al., 2000; Van Sickle, Larsen & Hawkins, 2007; Hawkins, Cao & Ropper, 2009). Null models predict the same taxonomic composition at all sites within a population of sites. The 10th percentile of the distribution of O/E values across sample sites is an arbitrary but useful measure of model precision that is less affected by outliers than estimates of the standard deviation (Van Sickle, 2008). Models that predict well should have O/E 10th percentile values close to 1. The 10th percentile values were used to assess model performance relative to the null model and to quantify how well flow and temperature variables predicted assemblage composition. When evaluating differences in models based on BC, 90th percentile values close to 0 (greater similarity) indicate a better fit between observed and predicted assemblages values (Van Sickle, 2008).

Assessing relationships between taxa richness, flow and temperature

We also used RF and CP models to predict taxa richness. In both cases, we developed models with the same set of predictors as used for predicting taxa composition. When RF models are used in regression mode, they predict the values of the response variable given different combinations of predictor variable values. In regression mode, RF models quantify the importance of each predictor variable by the percentage increase in the mean square error (MSE) when the variable is left out of the model. The fit between observed and expected values can be expressed as R^2, which describes the fraction of variance in the response variable associated with the predictor variables. To predict taxa richness with the CP models, we computed the probability of a site belonging to different macroinvertebrate groups (P_b) directly from the contingency table as we did for taxa composition. The expected taxa richness for a site was then estimated by $\sum_{b=1}^{B} e_b \cdot P_b$ (i.e. the weighted-average taxonomic richness across all the macroinvertebrate groups), where B is the total number of macroinvertebrate groups and e_b is the mean richness of macroinvertebrate group b.

Results

Independent components of flow variability

Our PCA identified seven statistically independent axes that represent different types of flow variation

Table 1 Streamflow variables and their varimax principal component (PC) factor loadings. High loadings are in bold font

Variable name	1	2	3	4	5	6	7
Base flow index, BFI	−0.299	0.006	−0.175	0.157	0.060	0.097	**0.895**
Daily coefficient of variation, DAYCV	0.045	−0.215	0.336	−0.125	−0.316	−0.210	**−0.769**
Average daily flow, Q mean	−0.091	**0.928**	−0.204	−0.017	0.152	0.141	0.207
Average number of zero flow days, ZERODAYS	**0.813**	−0.235	0.174	0.012	−0.221	−0.162	−0.408
Bank full flow, $Q_{1.67}$	−0.080	**0.951**	−0.120	−0.199	0.115	0.122	0.035
Flood duration, FLDDUR	0.002	−0.181	0.040	**0.967**	0.043	0.005	0.171
Colwell's index of predictability, P	0.078	−0.144	**0.930**	0.014	0.181	−0.152	−0.203
Colwell's index of constancy, C	0.127	−0.268	**0.822**	0.060	−0.350	−0.139	−0.272
Colwell's index of contingency, M	−0.157	0.225	−0.004	0.047	**0.927**	0.101	0.209
Average 7 day minimum flow, $\overline{7Q}_{\min}$	−0.200	**0.672**	−0.250	0.049	0.182	0.185	0.582
Average 7 day maximum flow, $\overline{7Q}_{\max}$	−0.071	**0.981**	−0.084	−0.086	0.080	0.089	0.005
Average number of flow reversals, $\overline{R}$	−0.135	0.274	−0.238	0.005	0.119	**0.885**	0.226
% variance explained by each factor	7.3	29.4	15.8	8.6	10.5	8.3	18.2
Interpretation	Zero flow days	Magnitude	Predictability	Flood duration	Seasonality	Flashiness	Baseflow

among sites (Table 1): (1) zero flow days, (2) magnitude, (3) predictability, (4) flood duration, (5) seasonality, (6) flashiness and (7) baseflow. Before varimax rotation, ten of the 12 variables had loadings on the first PC (50% of total variation) that ranged from 0.61 to 0.95, which made the interpretation of that PC extremely difficult. Following varimax rotation, the PC associated with the maximum proportion of the total variance (29.4%) was easily resolved as a flow magnitude component (Table 1). The seven rotated factors accounted for 98% of the total variance in the flow data. The traditional approach to PC selection based on Kaiser's rule (eigenvalues greater than 1) would have retained only the first three components (77% of the total variance in the flow data) and would have provided information on only the magnitude, flood duration and predictability aspects of the streamflow regime.

Hydrological classification

When conducting the K-means analyses, we found that as K was incremented from 4 to 8, each subsequent classification resulted in the addition of a new class while retaining classes with attributes very similar to the previous ones. Examination of the distribution of flow factors for each of these classes identified the dominant factors that characterised each class. We present factor distribution results from the $K = 8$ classification only, because it broadly encompasses the other classifications (Fig. 2). Other classification factor distributions are given in supplementary material (Appendix S4).

The distribution of values for each of the different flow factors within and among classes (Fig. 2) facilitated characterisation and interpretation of streamflow regime classes. The eight group classification consisted of (1) seasonal streams, (2) smaller predictable intermittent streams with low baseflow, (3) mid-size perennial streams with low seasonality (4) big streams with low predictability and short flood duration, (5) baseflow dominated streams, (6) big seasonal streams with high flood duration, (7) small unpredictable streams with high flood duration and (8) small flashy streams with high susceptibility to drying. Even though for a given class the dominant flow factors were similarly distributed for $K = 4$–8, other factors did differ in their distribution across K (Appendix S4).

Plots of the 5th, 50th and 95th percentiles of average daily flows in each month for the different classes illustrate some of the major differences in patterns of flow among the $K = 8$ classes (Fig. 3). The monthly mean values for the typical stream in each class (stream located closest to the centroid in the factor space of each class) illustrated similar, although not identical, patterns to class 50th percentiles (Fig. 3).

Spatial structure was evident in some, but not all, streamflow classes (Fig. 1 & 4). Sites in the first and the sixth classes occurred mostly in the Rocky Mountains and were characterised by high seasonality, but differed in magnitude. The second streamflow class dominated the relatively dry landscape of North and

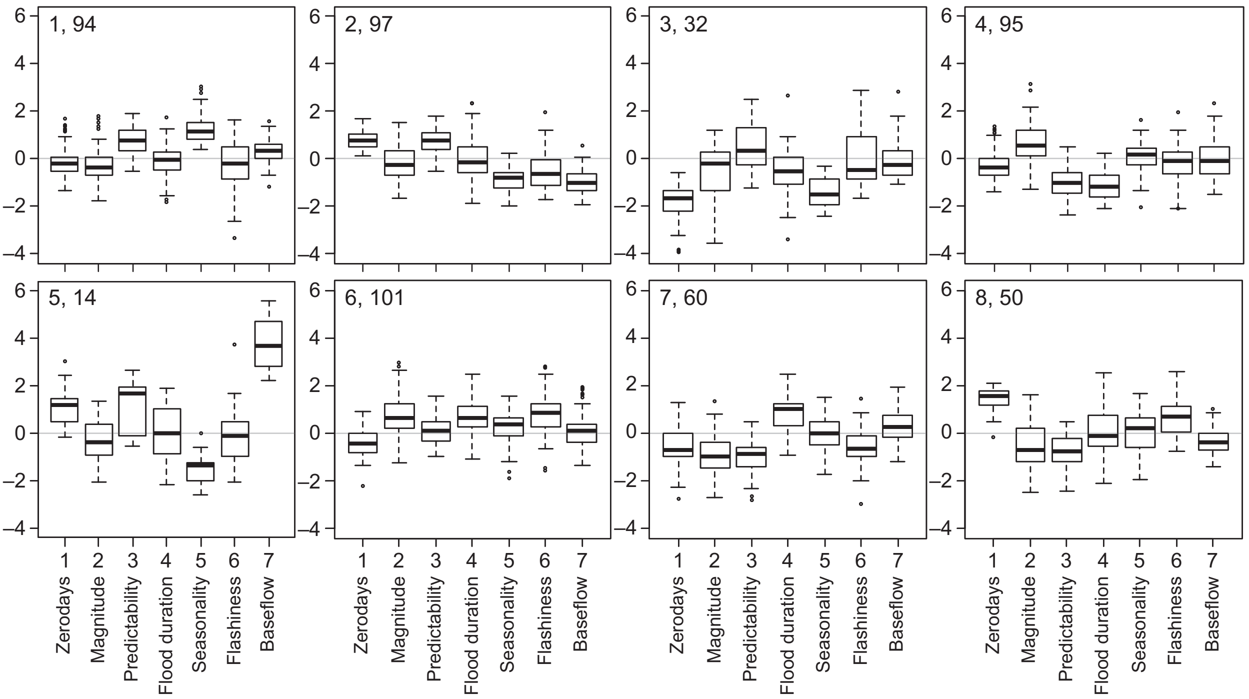

Fig. 2 Box plots showing the distribution of varimax-rotated principal component (PC) factors across different flow regime classes for $K = 8$ (the numbers on top of each plot represent the class number and the class size).

South Dakota and the coastal regions of central and southern California. The third class occurred in Arizona, New Mexico, the plains east of the Rocky Mountains, and some arid parts of California. The fourth class occurred mostly in the Washington, Oregon and northern California coastal ranges. Streams belonging to the seventh class occurred most frequently in the interior plateaus of Utah and Nevada and the plains east of the Rocky Mountains. Classes 5, 6 and 8 did not have an obvious regional structure.

Relationships between flow regime and stream temperature

The regression of stream temperature on flow regime factors showed that stream temperature co-varied with several aspects of flow. Mean annual stream temperature varied most strongly and negatively with flow seasonality (factor 5, standardised regression coefficient SRC = −0.66) and less strongly and negatively with baseflow (factor 7, SRC = −0.26) (adjusted $R^2 = 0.40$). Mean summer temperature varied negatively with five flow factors (seasonality, baseflow, predictability, flashiness and zero flow days) with seasonality showing the strongest single association (SRC = −0.77, −0.31, −0.26, −0.25 and −0.22, respectively, adjusted $R^2 = 0.56$). Mean winter temperature was less strongly related to streamflow regime factors, but increased with increasing predictability (factor 3, SRC = 0.39) and decreased with both increasing flood duration (factor 4, SRC = −0.36) and seasonality (factor 6, SRC = −0.39) (adjusted $R^2 = 0.37$). Flow regime classes showed similar associations with temperature. $K = 4$–8 classifications accounted for 42, 43, 44, 58 and 52% of the variation in mean annual temperature; 34, 39, 27, 52 and 44% of mean summer temperature; and 19, 27, 25, 40 and 38% of mean winter temperature, respectively.

Invertebrate composition and richness

Taxa composition and richness were highly variable among sites as illustrated by the cluster diagram (Fig. 5), the specific taxa representative of each group and the range of taxa richness values among sites. For modelling purposes, we identified six groups (see dashed line in Fig. 5) that represented a compromise between maximising average within-group compositional similarity and the number of sites per group. The

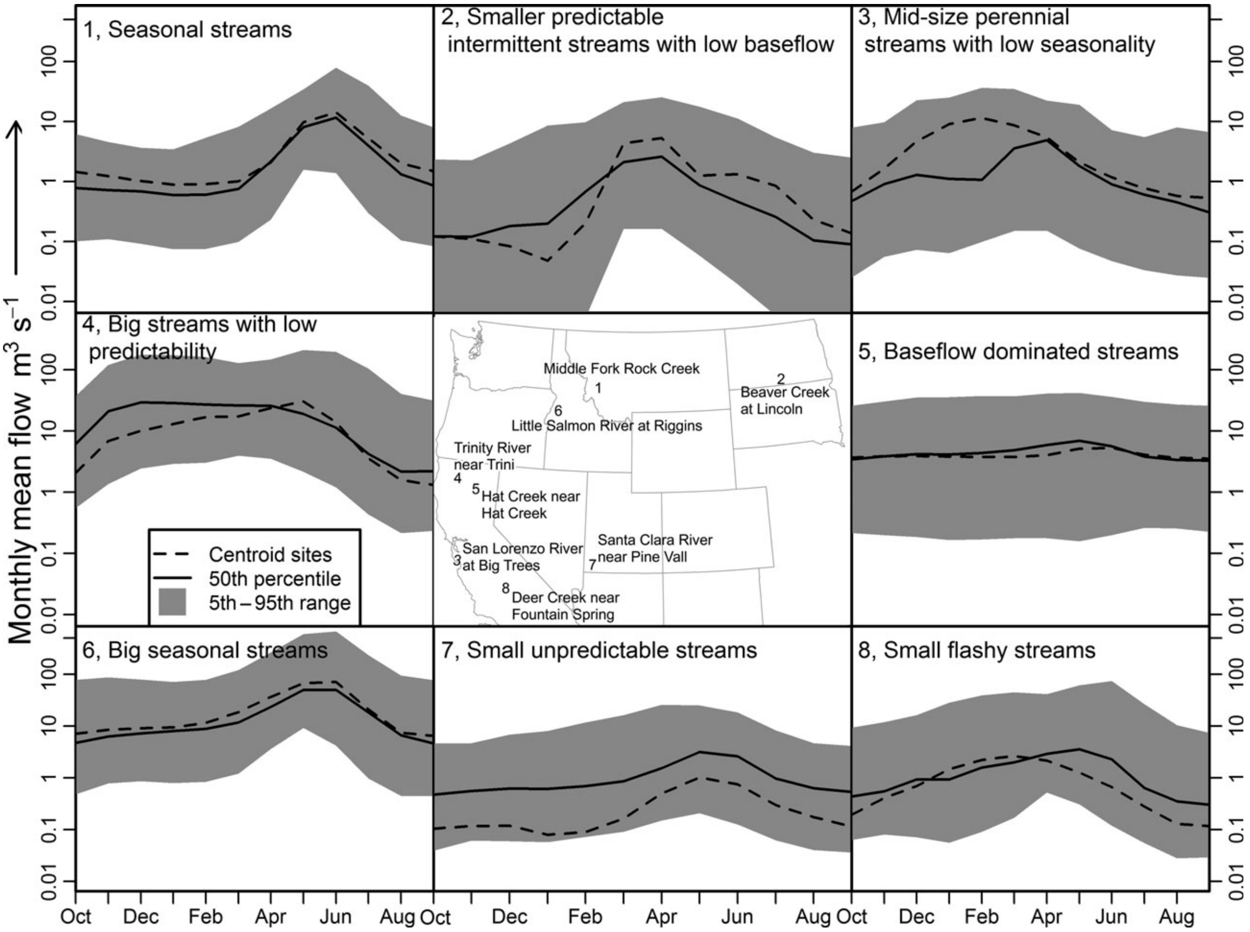

Fig. 3 Fifth, 50th and 95th percentiles of average monthly flows for each flow regime class and mean flow for streams closest to class centroids. The map shows the site nearest to the centroid of each flow regime class.

indicator species analyses showed that these groups were taxonomically and ecologically distinct from one another. Indicator taxa for groups A (*Micropsectra*, *Zapada*, Chloroperlidae, *Rhyacophila*, *Arctopsyche*, *Brillia*, *Heterlimnius*, *Epeorus*, *Cleptelmis*, Perlodidae, *Hesperoperla*, *Baetis*, Simuliidae) and B (*Lepidostoma*, *Claassenia*, Athericidae, *Drunella*, *Deuterophlebia*, *Acentrella*, *Hexatoma*, *Zaitzevia*) are known to require cold, fast-flowing water. Groups C (*Eukiefferiella*) and D (*Psephenus*, *Pteronarcys*, *Glossoma*, *Microcylloepus*, *Rheocricotopus*, *Protoptila*, Pyralidae, *Rheotanytarsus*, *Optioservus*, *Antocha*) indicator taxa require somewhat warmer, less swift streams, and indicator taxa for groups E (Dryopidae, *Hydropsyche*, *Chimarra*) and F (*Paratanytarsus*, *Dubiraphia*, *Caenis*, *Dicotendipes*, *Saetheria*, *Thienemannimyia*) are more typical of warmer, slowly moving streams. Taxonomic richness varied from 13 to 44 taxa per sample across the 63 study streams (mean = 31).

Contingency table analyses

Invertebrate-defined classes (Fig. 5) were non-randomly associated with flow regime classes for all classifications (Table 2, chi-square test $P < 0.00004$). This analysis showed that in many cases, the probability that a streamflow regime class was associated with a single macroinvertebrate group was >60%.

Associations between taxa composition, streamflow regime and temperature

All models predicting taxa composition performed substantially better than their respective null models as measured by both the 10th percentile of O/E values and the 90th percentiles of BC values (Table 3). Models incorporating both flow regime and temperature performed best. Streamflow variables alone performed better than the three temperature variables

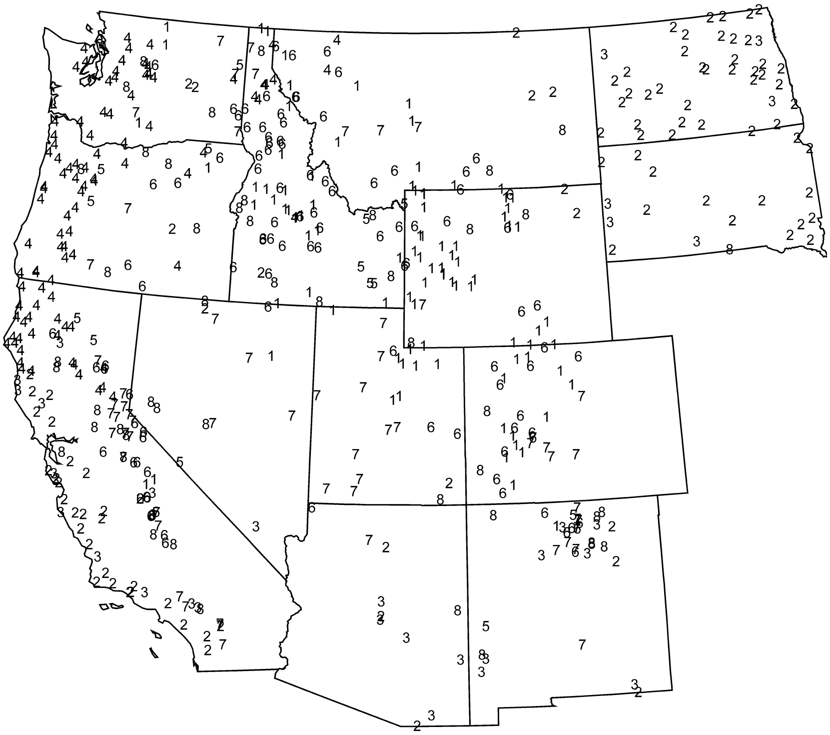

Fig. 4 Spatial distribution of sites within each flow regime classes for $K = 8$.

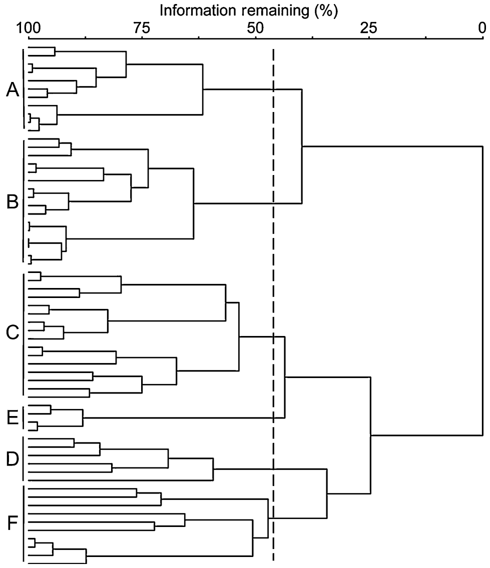

Fig. 5 Dendrogram produced by the hierarchical clustering showing dissimilarities between individual sites and groups of sites based on invertebrate taxonomic composition. The compositional distance between sites and groups of sites was scaled by Wishart's (1969) objective function expressed as the percentage of information remaining.

alone in terms of O/E, but temperature alone performed better than flow variables alone in terms of BC. RF predictions based on flow classes were generally slightly worse than those based on continuous flow variables. The importance scores of flow factors in predicting taxa composition differed from those for predicting richness (Fig. 6). Variation in baseflow was most useful in predicting composition followed by flow seasonality, flood duration, flow magnitude, flow predictability, zero flow day factor and flashiness. When flow and temperature predictors were combined, summer temperature was the most important variable followed by baseflow and mean annual temperature (Fig. 6f). Mean winter temperature, flow magnitude, flood duration, flow seasonality, flow predictability, flow flashiness and zero flow day factor (in that decreasing order) were less important to RF predictions. Predictions based on the conditional probability models were generally better than RF models based on only flow variables as measured by both O/E and BC measures of precision.

Associations between taxa richness, streamflow regime and temperature

The Random Forests models showed that taxonomic richness was only weakly (R^2 values <0.24, Table 3) associated with flow, temperature or streamflow class, although these values were statistically higher than that

Table 2 Probability that a site belongs to one of the macroinvertebrate groups given that its streamflow regime class is known. N_f is the number of sites in each streamflow regime class and N_b is the number of sites in each macroinvertebrate group. Probabilities of macroinvertebrate group membership >0.5 are highlighted in bold font. PS is the proportion of sites in each macroinvertebrate group, and e_b is the mean taxa richness in each streamflow regime class

		Macroinvertebrate groups							
K	Streamflow Class	A	B	C	D	E	F	N_f	e_b
4	1	0.22	**0.61**	0.17	0.00	0.00	0.00	18	35.6
	2	0.00	0.00	0.11	0.00	0.22	**0.67**	9	24.9
	3	0.09	0.09	0.43	0.09	0.17	0.13	23	28.8
	4	0.38	0.23	0.15	0.15	0.00	0.08	13	32.7
5	1	0.19	**0.69**	0.12	0.00	0.00	0.00	16	35.2
	2	0.00	0.00	0.11	0.00	0.22	**0.67**	9	24.9
	3	0.12	0.06	0.29	0.12	0.24	0.18	17	28.0
	4	0.36	0.21	0.21	0.14	0.00	0.07	14	32.7
	5	0.14	0.14	**0.71**	0.00	0.00	0.00	7	32.9
6	1	0.17	**0.75**	0.08	0.00	0.00	0.00	12	33.7
	2	0.00	0.00	0.12	0.00	0.25	**0.62**	8	25.3
	3	0.11	0.00	0.33	0.00	0.22	0.33	9	28.4
	4	**0.50**	0.20	0.00	0.20	0.00	0.10	10	33.1
	5	0.33	0.00	**0.67**	0.00	0.00	0.00	3	34.1
	6	0.10	0.24	0.43	0.10	0.10	0.05	21	31.3
7	1	0.18	**0.73**	0.09	0.00	0.00	0.00	11	33.1
	2	0.00	0.00	0.00	0.00	0.29	**0.71**	7	24.9
	3	0.00	0.00	0.12	0.00	**0.50**	0.38	8	25.0
	4	0.44	0.22	0.00	0.22	0.00	0.11	9	33.4
	5	0.33	0.00	**0.67**	0.00	0.00	0.00	3	34.0
	6	0.11	0.26	**0.53**	0.11	0.00	0.00	19	32.0
	7	0.33	0.17	0.33	0.00	0.00	0.17	6	32.0
8	1	0.22	**0.67**	0.11	0.00	0.00	0.00	9	32.3
	2	0.00	0.00	0.00	0.00	0.00	**1.00**	4	27.8
	3	0.00	0.00	0.14	0.00	**0.57**	0.29	7	25.1
	4	0.38	0.25	0.00	0.25	0.00	0.12	8	33.5
	5	0.33	0.00	**0.67**	0.00	0.00	0.00	3	34.0
	6	0.10	0.38	0.43	0.10	0.00	0.00	21	33.0
	7	0.25	0.00	**0.50**	0.00	0.00	0.25	4	33.8
	8	0.29	0.00	0.14	0.00	0.29	0.29	7	25.1
	PS	0.18	0.25	0.25	0.06	0.10	0.16		
	N_b	11	16	16	4	6	10		

of the null model. Seasonality of flow and the zero flow day factors were the most important flow regime predictors of invertebrate richness in the RF models when flow predictors were used alone, and mean summer temperature was the most important temperature predictor (Fig. 6a,b). When flow and temperature factors were used together, summer temperature followed by seasonality of flow and zero flow day factor were the most important predictors. Partial dependency plots (not shown) showed that richness decreased with increasing summer temperature, increased with seasonality and decreased with increasing values of the zero flow days factor. Conditional probability models for prediction of taxa richness based on 4, 5 and 7 classes accounted for slightly more (20 – 24%) of the variation. However, models based on 6 and 8 classes accounted for less (~11%) of the variation in taxa richness.

Discussion

Choice of streamflow variables and scaling magnitude-related variables

A critical step in quantifying which aspects of naturally occurring flow regimes most strongly affect

Table 3 Performance of the Random Forests (RF) and conditional probability (CP) models in predicting taxa richness and taxonomic composition. R^2 measures the strength of relationships between taxa richness and streamflow and temperature predictors. The 10th quantile of O/E values and the 90th quantile of Bray–Curtis (BC) values measure how well streamflow and temperature predict taxonomic composition

		Taxa richness	Taxa composition	
Model type	Predictors	R^2	10th quantile of O/E	90th quantile of BC
Null	–	0.000	0.576	0.460
RF	7 flow factors	0.142	0.725	0.418
RF	7 flow factors + 3 temperature variables	0.148	0.795	0.344
RF	3 temperature variables	0.108	0.665	0.398
RF	4 flow classes	0.145	0.638	0.449
RF	5 flow classes	0.097	0.696	0.406
RF	6 flow classes	−0.044	0.669	0.460
RF	7 flow classes	0.017	0.675	0.427
RF	8 flow classes	−0.028	0.678	0.412
CP	4 flow classes	0.237	0.756	0.391
CP	5 flow classes	0.223	0.753	0.389
CP	6 flow classes	0.111	0.749	0.422
CP	7 flow classes	0.198	0.755	0.390
CP	8 flow classes	0.115	0.742	0.397

stream ecosystems is identifying the flow variables that are most useful in understanding ecological patterns and processes from the many variables available. The streamflow variables that we selected influenced the subsequent quantification and classification of flow regimes. It was not, however, obvious from previous studies which variables should have been selected. We selected variables based on insights from previous studies, discussions with colleagues and our own experience. The number of variables was kept small (12) to facilitate interpretation. We concluded it would be increasingly difficult to interpret and understand the physical characteristics of classifications based on more variables. The use of PCA also reduced redundancy among the variables, which helped with both physical and ecological interpretations. We also treated magnitude-related streamflow variables differently than previous researchers. Previous work has often characterised flow magnitude in terms of unit discharge by scaling discharge variables either by catchment area or mean flow (e.g. Poff, 1996; Monk *et al.*, 2007). Because of this standardisation, previous classifications would potentially group small and large streams together. Our use of unscaled magnitude-related variables resulted in a magnitude factor (factor 2) that discriminated between small and large streams, which we showed was related to variation in invertebrate assemblage composition. Stream size, often measured as catchment area, is well known to be strongly associated with both variation in taxonomic composition and ecosystem processes (e.g. Vannote *et al.*, 1980).

Pre-treatment of data and use of PCA

We applied PCA in our analyses because we wanted to identify independent axes of flow variation. PCA is a statistical tool for reducing the dimensionality of multivariate data and facilitating interpretation of major aspects of variation within such data. However, the effectiveness of this approach presumes that the additional complexity involved with applying PCA provides more interpretable and robust results than analyses based on the original variables. It could be argued that a PCA that reduces 12 original variables to just seven derived variables might not accomplish much and might hinder interpretation rather than improve it. We therefore conducted additional flow regime classifications and invertebrate-flow regression analyses with the 12 original flow variables as independent variables and compared the results from these analyses with those derived from the PCA-based flow factors. We found that analyses based on the PCA-derived flow factors yielded both more easily interpretable flow regimes (classes) and better invertebrate-flow models. We provide the details of these additional analyses in Appendix S5.

Use of PCA does not strictly require that input variables be normally distributed, but the Pearson's

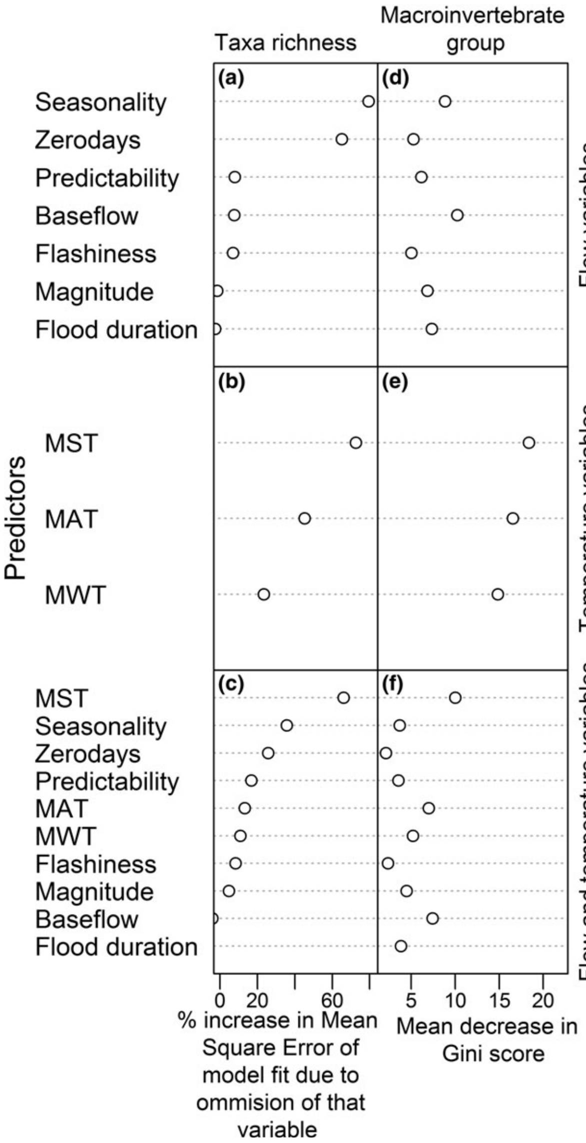

Fig. 6 Variable-importance plots from Random Forests models for predicting taxa richness (a, b and c) and macroinvertebrate group (d, e and f). Flow predictors only (a, d); temperature predictors only (b, e); and both flow and temperature predictors (c, f). Predictor variables are ordered in the same sequence for both taxa richness and macroinvertebrate group to facilitate comparisons. MAT, Mean Annual Temperature, MST, Mean Summer Temperature (Jun, Jul, Aug) and MWT, Mean Winter Temperature (Dec, Jan, Feb).

correlation coefficient used in PCA, and hence the principal components, is sensitive to non-normality, skewness and outliers (White, Richman & Yarnal, 1991). Use of non-normal variables may lead to erroneous interpretations of the PC data structure (Norris, 1971; Clark, 1973). Simple transformations such as log(x) can often produce sufficiently normal distributions for use in PCA, but such transformations can also increase skewness rather than reduce it (e.g. Reid & Spencer, 2009). In such cases, each variable may require a unique transformation. The Box–Cox transformation that we applied is useful in such situations, although the loss of a simple, universal transformation complicates understanding of how the transformed variables scale with one another.

The effectiveness of PCA in our study depended on how well it revealed ecologically meaningful variation in different aspects of flow. Simple PCA can be ineffective in revealing ecologically important variation in data if many uncorrelated variables load strongly on one PC. In our study, this approach to PC selection would have led to the identification of only three axes of streamflow variation and the use of only 77% of the information in the raw data. Our use of varimax rotation, a widely used orthogonal rotation (White *et al*., 1991), yielded a simpler, more interpretable structure (Wilkinson *et al*., 1996) that greatly improved interpretability, while reducing the dimensionality of the flow data. Without the rotation, we would have missed some important aspects of streamflow regime, and it would have been difficult to interpret each PC axis as a distinct characteristic of the streamflow regime.

Flow regime classifications

At the scale of the western U.S.A., climate has a major influence on the spatial structure of streamflow regime classes (Figs 1 & 4). However, streams belonging to different classes were also often found in close proximity to each other. Such close proximity of different stream types arose, in part, because magnitude was a factor in the classifications. This result implies that even though climate has a major influence on streamflow regimes, it will not be possible to identify geographically contiguous hydroregions (comparable to ecoregions) that are spatially discrete. Rather, stream segments will need to be individually characterised in recognition of the diversity of ecologically relevant flow regimes (or classes) that can occur within any climatic region.

Many previous studies have developed classifications of streamflow, but the classification presented here differs in the choice of underlying variables and the inclusion of flow magnitude as a factor in classification. When used to predict macroinvertebrate assemblage composition, Random Forests models with continuous streamflow factors appeared to be slightly better than models based on categorical variables (Table 3). However, predictions based on direct conditional probabilities derived from classifications also performed relatively well (e.g. model type CP in Table 3). The better performance of the continuous factors is probably due to the fact that some information is always lost when we collapse continuous factors into categorical classes. However, classifications are attractive to ecosystem managers because they are generally easier to communicate and implement. Our results showed that the use of flow regime classifications may not significantly compromise models when predicting taxonomic composition. Because the variables upon which this classification was based are relatively general descriptors of the stream environment, this classification may have applicability beyond macroinvertebrate composition.

Temperature and streamflow variables

Temperature is an important variable that regulates the local and regional composition of macroinvertebrates (e.g. Sweeney & Vannote, 1981; Hawkins *et al.*, 1997). Because temperature variables co-varied with some of the streamflow variables, it was difficult to differentiate the biological effects of one set of variables from the other. Although this co-variation confounds interpretation of the specific ecological importance of each variable, such co-variation implies that one type of variable might be used as a surrogate for the other type for predictive purposes. However, in our analysis, use of both hydrological and temperature variables resulted in the best predictions of taxonomic composition, which implies some degree of independent response of biota to both types of variables (Table 3). In general, such joint consideration of streamflow and temperature regimes should provide a more robust characterisation of the stream environment than either alone (e.g. Harris *et al.*, 2000) and thus allow more accurate predictions of the biological potential of different streams.

Relationships between flow regimes and biota

A primary goal of stream ecologists is to understand the independent and interactive affects of environmental factors on the structure and function of stream ecosystems (Allan & Castillo, 2007). With such understanding, it should be possible to predict the biota expected to occur under different environmental conditions and hence assess the degree to which anthropogenic alteration in those environmental conditions will affect the ecological condition of streams (e.g. Hawkins, 2006). Because the hydrological regime is a fundamental component of stream habitat, it is imperative to understand how it affects both populations and communities of stream organisms.

We used long-term flow variables to quantify hydrological regime. It is reasonable to ask whether macroinvertebrate richness and composition are more a function of recent short-term events than the predictor variables we used. If short-term variation in flow is important to biotic richness and composition, we might expect relatively poor associations between our long-term flow characterisations and the biota collected at a site. Such short-term effects may be one source of the unexplained variability in our results. However, despite uncertainties in the hydrological data, the marked variation in temperature among sites and the potential effects of land use alteration at some sites, the fact that we detected relationships between streamflow regime and biota (Table 3 and Fig. 6) supports our underlying hypothesis that long-term flow patterns are part of the hydrological template that influences which specific organisms can establish and persist in a specific stream. Certainly, further exploration of this issue is warranted as it has implications for both our understanding of the relative importance of short-term and long-term processes in structuring ecological communities and our ability to accurately specify the range of biotic conditions that represent natural reference states in bioassessments.

Our modelling focused on two aspects of stream invertebrate assemblages: taxa composition and richness. We observed reasonably strong relationships between the taxonomic composition at a site and both flow regime and temperature (90th percentile BC values in Table 3), perhaps because we focused on those taxa most common (estimated probabilities of detection >0.5) at each site. This result was encouraging because it has clear implications both for understanding the factors that regulate the specific taxa occurring in streams and the ability to assess the effects of landscape and waterway alteration on stream ecosystems. Although associations do not necessarily imply causation, two factors (temperature and baseflow) stood out as being important in predicting taxonomic composition (90th percentile BC values in Table 3). The role of temperature in structuring stream assemblages is well established, but we know less about which aspects of flow are critical in this regard. Several previous studies have focused on the role of flooding in structuring stream assemblages (e.g. Boulton *et al.*, 1992; Robinson, Uehlinger & Monaghan, 2004), but our results imply that future studies might profit by focusing on how the mechanisms associated with variation in baseflow affect assemblage composition.

Our results showed that overall taxa richness was not strongly associated with either flow regime or temperature in spite of the marked variation that occurred in the number of invertebrate taxa found at our study streams (Table 3). Other factors must therefore have been more important in regulating overall taxa richness in these streams (cf. Vinson & Hawkins, 1998). The fact that the models predicting taxa composition (as measured by O/E) performed well implies that they predicted the number of common (probabilities of capture >0.5) taxa well. The low R^2 values associated with the predictions of total taxa must therefore be largely driven by poor prediction of rare taxa. Assessments of the factors that influence total taxa richness may be of limited use in understanding assemblage structure of open ecosystems like streams where many of the observed taxa may be accidentals that have drifted into lower reaches from more suitable upstream habitats.

Our results are significant given that prediction of the taxa expected at a site is a critical component of bioassessment (e.g. Hawkins, 2006; Stoddard *et al.*, 2006; Paulsen *et al.*, 2008). The accuracies of the models developed here are comparable with those in use in many bioassessment programs. Use of direct estimates of 7 flow factors and 3 temperature variables produced a RF model of precision (10th percentile of O/E values = 0.80) similar to a much more sample rich (N = 729) model developed by Carlisle & Hawkins (2008) to assess the condition of western U.S.A.-wide invertebrate assemblages. The use of flow variables or the best classification alone resulted in only slightly less precise RF models (with 10th percentile of O/E values of 0.73 and 0.70, respectively). These values are also similar to the 10th percentile values reported for several other O/E indices (Hawkins, 2006). The use of direct measures of both flow and temperature should not only improve model accuracy and precision, but allow a more direct interpretation of the likely causes of biological impairment when it is observed. Improvement of the models used for bioassessment will require that we be able to estimate both the hydrological reference condition at ungauged sites (e.g. Sanborn & Bledsoe, 2006) in the same way that we estimated the expected thermal environment. The fact that use of both flow and temperature variables produced the best models of taxonomic composition is not surprising considering the frequent reference to these factors in the stream ecology literature (see Allan & Castillo, 2007). It is unclear, however, that their separate effects can be cleanly distinguished from one another. These issues notwithstanding the associations between stream biota, flow regime and temperature that we documented point to the need to refine the characterisation of both flow and thermal regimes as they influence stream biota and their ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Streamflow variables.

Appendix S2. Temperature regression model.

Appendix S3. Macroinvertebrate sampling procedure.

Appendix S4. PCA factors distribution for $K = 4$–7 classifications.

Appendix S5. Effectiveness of PCA-derived flow regime axes versus use of the original 12 streamflow variables for both classifying streamflow regimes and quantifying invertebrate-flow relationships.

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