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BENTHIC INVERTEBRATE COMMUNITY STRUCTURE

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Benthic (bottom-dwelling) macroinvertebrates are the basis for most biomonitoring programs currently in operation worldwide (Rosenberg and Resh 1993), and the reasons underlying the use of these organisms are compelling (Rosenberg and Resh 1996). In the United States, regional programs tend to be local modifications of national programs (*e.g.* Plafkin *et al.* 1989), and national programs in both developed (*e.g.* Australia: Parsons and Norris 1996) and developing countries (*e.g.* India: Sivaramakrishnan *et al.* 1996) may be modifications of ones developed elsewhere.

Fundamental to the scientific method is the use of controls or control conditions against which results obtained under test conditions are compared. In field studies, all variables cannot be controlled and replicates cannot be randomly assigned to treatments, so an attempt is made to choose test and control conditions (often represented by different sites) that are as similar as possible; the variable of interest is then manipulated, and uncontrolled variables are assumed to fluctuate similarly. Traditionally, this problem has been solved in aquatic studies by choosing adjacent sites in streams (*i.e.* upstream and downstream comparisons, Norris *et al.* 1982), by dividing lakes into halves (Schindler 1974), or by using mesocosms (Graney *et al.* 1984). Such approaches have several problems (Cooper and Barmuta 1993); a major issue in streams is confounded designs (Eberhardt 1978), often called "pseudoreplication" (Hurlbert 1984).

A recent development in water quality monitoring has been the attempt to describe **reference conditions** (Reynoldson *et al*. 1997) based on pre-established criteria that exist at a wide range of sites rather than relying on information from one or a few control sites. These reference conditions then serve as the control against which test-site conditions are compared. The notion of reference condition is really one of **best available condition** and it is represented by information from numerous sites.

The concept of a reference condition is a critical element in approaches now being developed for biomonitoring and bioassessment of aquatic resources. For example, the reference condition is central to currently accepted ideas of "biocriteria" being developed by the US Environmental Protection Agency (EPA) (Davis and Simon 1995). The same approach has been used in the United Kingdom for river classification and water quality assessment (Wright 1995). It is currently being used in Canada to develop biological sediment guidelines for the Great Lakes (Reynoldson *et al.* 1995) and is the basis for the National River Health Program in Australia (Parsons and Norris 1996).

The reference-condition approach using benthic invertebrates is well-suited to large-scale biomonitoring programs because sites serve as replicates, and reference sites can be scattered throughout a catchment (Reynoldson *et al.* 1997). Local knowledge, published information or simple reconnaissance trips can be used to identify sites that represent best available condition for use in building the reference-site models. Several approaches to establishing reference-site conditions exist (Figure 1); based on comparisons of these methods (Reynoldson *et al.* 1997) we are currently using the BEAST (BEnthic Assessment of SedimenT), which was developed in Canada (Reynoldson *et al.* 1995). However, the efficacy of the other approaches will be assessed further.

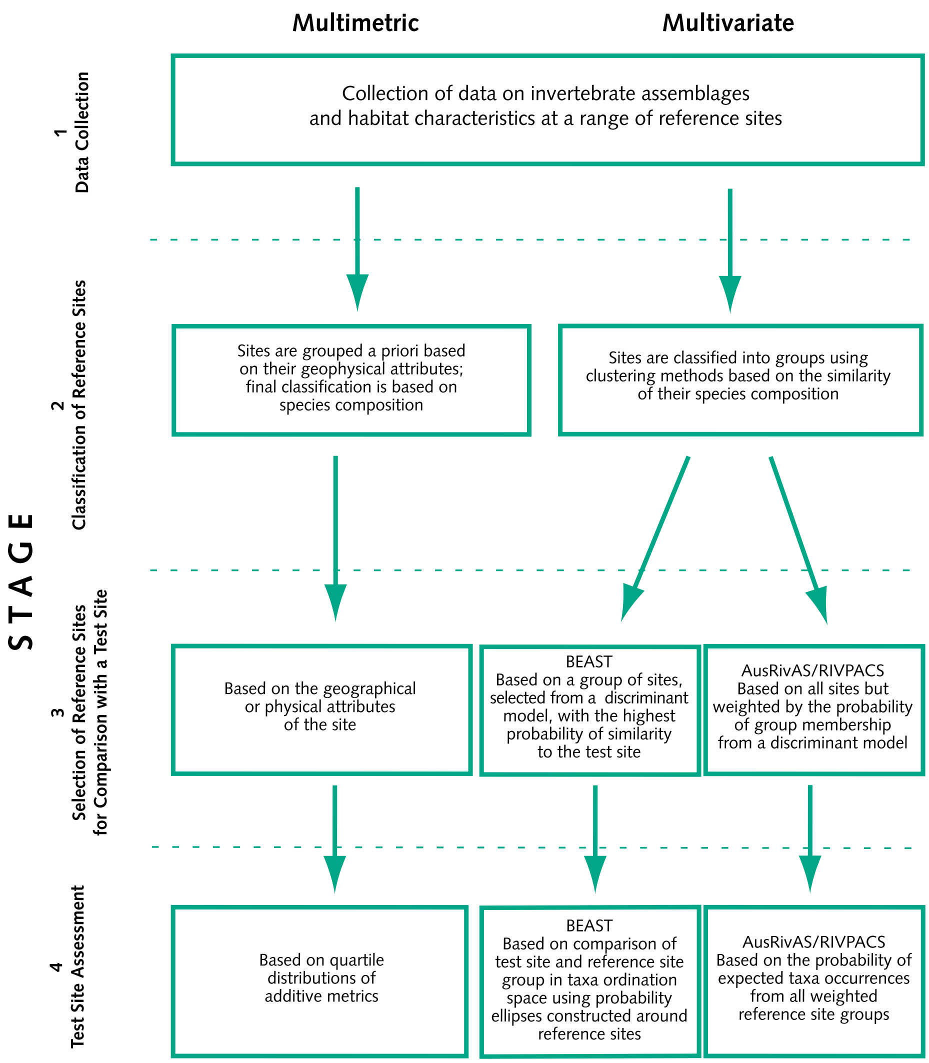

Figure 1. *Flowchart of assessment methods using multimetric and multivariate approaches.*

AUSRIVAS = AUStralian RIVer Assessment Scheme; BEAST = BEnthic Assessment of SedimenT; RIVPACS = River InVertebrate Prediction And Classification System (from Reynoldson et al. *1997).*

We used the reference-condition approach to develop a permanent database for biomonitoring water quality in the Fraser River catchment. In this report, we discuss key elements of a regional benthic monitoring programme developed for the entire catchment (234,000 km^2) of the Fraser River.

METHODS

Site selection

Our intent was to sample approximately 250 reference sites over three field seasons (1994–1996) to characterize variability and to build the appropriate predictive models. In addition, we included a smaller number of test sites (*i.e.*

those potentially impacted by human activity) to verify performance of the reference-site model during its development.

Site selection was based on two spatial scales (ecoregion and stream order) and two temporal scales (annual and seasonal).

Spatial scales

It is important to capture the range of conditions within the study boundaries. The diversity of sites was maximized by stratifying the basin in terms of both ecoregions and stream orders. The ecoregion scale ensured the inclusion of different climatic and landscape conditions, whereas the stream-order scale ensured the inclusion of a range of hydraulic conditions. Sites were located on up to seven stream orders (at a map scale of 1:250,000) in 23 subcatchments representing 11 ecoregions. In the selected subcatchments approximately equal numbers of sites were sampled for each stream order.

A series of workshops with local experts identified impacted and unimpacted subcatchments. Unimpacted catchments were those judged to have no or minimal human activity, such as logging, mining or agriculture. Reference and test sites were randomly selected from each sampled subcatchment.

Temporal scales

We examined how annual and seasonal variability affected the accuracy of the predictive models. We sampled nine sites in all three years of the field research to measure annual variability; the results are unavailable at this time. Seasonal variability was investigated by P. Dymond (Dymond *et al.* 1997). Analysis of data from eight sites sampled seasonally showed that there was considerable variation of the communities at the species level from the reference condition based on fall sampling. Using models based on family level taxonomic information it was observed that the seasonal samples frequently fell within the range of variation described by sites sampled only in the fall. However, at this time we would recommend that, where possible, site assessments be conducted based on fall sampling.

Environmental variables

Environmental variables were used in two ways: (1) to relate habitat conditions to subsets of reference sites grouped by similarities in invertebrate communities and (2) to build the predictive models for matching new sites to the appropriate subset of reference sites.

An optimum set of predictor variables cannot be determined *a priori*, so a maximum number of likely variables was chosen before sampling began. We compiled these variables from published information and reviewed and amended them at an initial workshop. Table 1 shows the final list for which data were collected.

Sites sampled

Figure 2 shows the distribution of reference and test sites sampled over the three years of the study. We sampled 37 reference sites and nine test sites in 1994, 90 reference and 11 test sites in 1995, and 97 reference and 26 test sites in 1996 for a total of 224 reference sites and 46 test sites. In addition, 21 sites were used for quality assurance at which additional samples were taken. At reference sites where additional quality assurance samples were collected, the first sample was used in the reference database, whereas the additional samples were used as test samples.

Benthic macroinvertebrate collection

We collected benthic invertebrates at erosional habitats (*i.e.* riffles) using a 38 x 38 x 38 cm kick net (400-mm mesh). We took one sample of three minutes duration per site. Two-hundred-organism subsamples were removed using a Marchant box (Marchant 1989). Details of the calibration study and procedures are given in Rosenberg *et al*. (in preparation). The leaf-pack assemblage was also sampled concurrently to

Table 1. Environmental variables measured.

MAP	SITE	CHANNEL	WATER
Latitude	Date	Wetted width	pH
Longitude	Velocity	Mean depth	Dissolved oxygen
Altitude	Discharge	Max. depth	Conductivity
Ecoregion	Flow	Bankfull width	Temperature
Stream order	Macrophyte cover	Slope	Total phosphorus
	Riparian vegetation	Substrate framework[1]	Nitrate
		Substrate matrix[2]	Alkalinity
		Substrate embeddedness[3]	Total suspended solids
		Mean particle size	
		Max. particle size	
		Periphyton biomass	
		Periphyton chlorophyll *a*	

[1] Estimated size of the dominant particle
[2] Estimated size of the surrounding material
[3] The degree to which the framework material is buried within the matrix

determine if the benthic invertebrate assemblage from this habitat provided additional information on the biological condition of a site. This study was the subject of a M.Sc. thesis by S. Sylvestre at the University of Western Ontario (Sylvestre 1997).

We were concerned about the need for non-specialists to identify macroinvertebrates, so we compared the efficacy of family-level versus lower-taxon (mostly genus) level identifications in the determination of site groups and in model development (see below).

Reference condition statistics

We used the four-stage procedure (Fig. 1) described in Reynoldson *et al*. (1997) for the Fraser River catchment data. The stages are as follows: (1) assemble data from multiple reference sites; (2) use cluster analysis (unweighted pair mean group average) to develop reference-site groups based on the invertebrate assemblage data; (3) use discriminant function analysis to relate the structure observed in invertebrate assemblages to environmental variables, and derive an

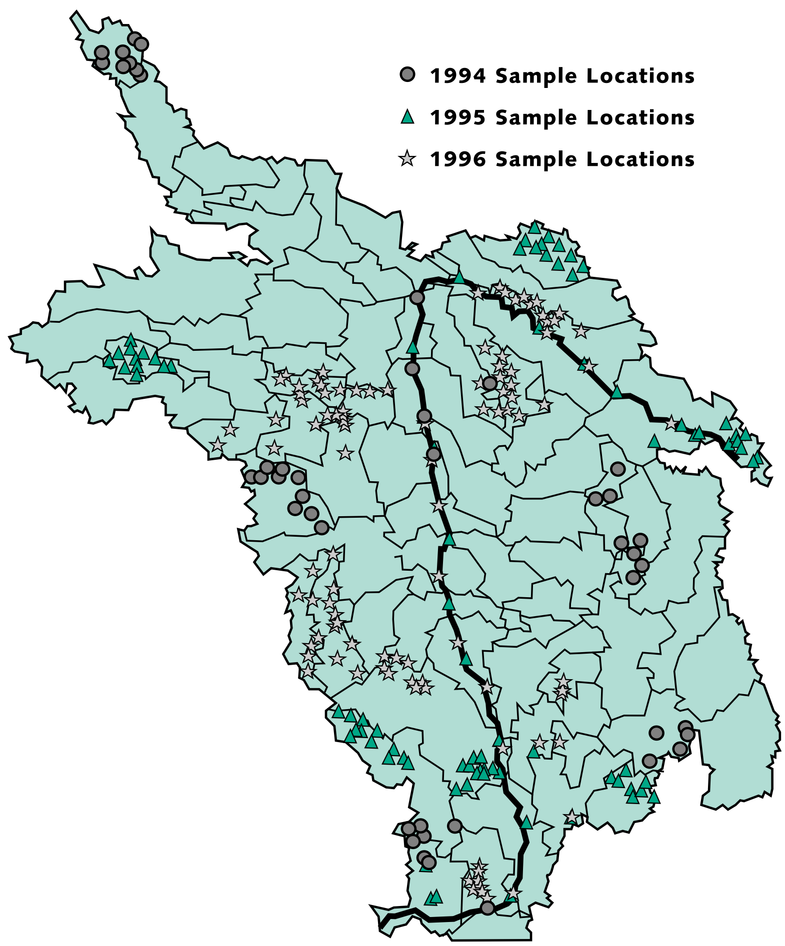

Figure 2. Fraser River Basin sampling locations, 1994 (circles), 1995 (triangles) and 1996 (stars), with the Fraser River and sub-basin boundaries indicated.

optimal set of variables to predict group membership; and (4) use hybrid multi-dimensional scaling ordination to assess the biological condition of new (test) sites in comparison to the reference group or groups that has/have similar values for predictor variables.

The following results describe models developed from a preliminary analysis of the data. Slight modifications in model performance may result from further analysis once the taxonomic data have been verified and final data analysis is complete. Furthermore, as new reference sites are added, the models will be re-calibrated on a regular basis.

RESULTS AND DISCUSSION

Site selection

Stage 1

The database currently consists of 322 separate records, each representing a sample taken from a discrete physical location and having complementary data on the benthic fauna and habitat descriptors. Each site has been categorized as either reference or test (Table 2).

Table 2. *Reference and test sites included in the Fraser River Basin database, 1994–1996.*

YEAR	REFERENCE	QUALITY ASSURANCE	TEST	
			POSSIBLE IMPACT	MAIN STEM
1994	37	[a]5 (20 samples)	5	4
1995	90	[b]7 (14 samples)	0	11
1996	97	[b]9 (18 samples)	15	11
Total	224	21 (52 samples)	20	26

[a] 5 replicated samples taken per site; 1 used as a reference site, 4 used as test sites.
[b] 3 replicated samples taken per site; 1 used as a reference site, 2 used as test sites.

Model building at the family level

Stage 2

Of 224 potential reference sites, 220 were included in the initial model building. Four sites were excluded because they were apparent outliers; they will be re-examined for later inclusion in the database. The cluster analysis and ordination diagrams show six macroinvertebrate groups (Fig. 3), formed from 74 families. Group determination is subjective and based upon a minimum group size of five sites. The six groups showed little geographic pattern (*i.e.* they were scattered among the subcatchments sampled) except for Group 6 sites, which were largely located on the western side of the basin.

Stage 3

Principal axis correlation, a multiple regression method, was used to relate the habitat descriptor matrix to the taxa matrix. Twenty habitat variables were identified to be significantly related ($P<0.01$) to the ordination structure of the benthic fauna at the family level: ecoregion, altitude, latitude and longitude (map variables); water velocity (maximum and average), per cent grasses, and macrophytes (site variables); bankfull width, channel width, channel depth (average and maximum), and three substrate measures (channel variables); and Kjeldahl nitrogen, nitrate-nitrite, total phosphorus, pH, and alkalinity (water variables).

Stepwise discriminant analysis selected nine variables to describe the six groups identified by cluster analyses (Fig. 3) of the family-level invertebrate data. The relationship of these nine variables in ordination space

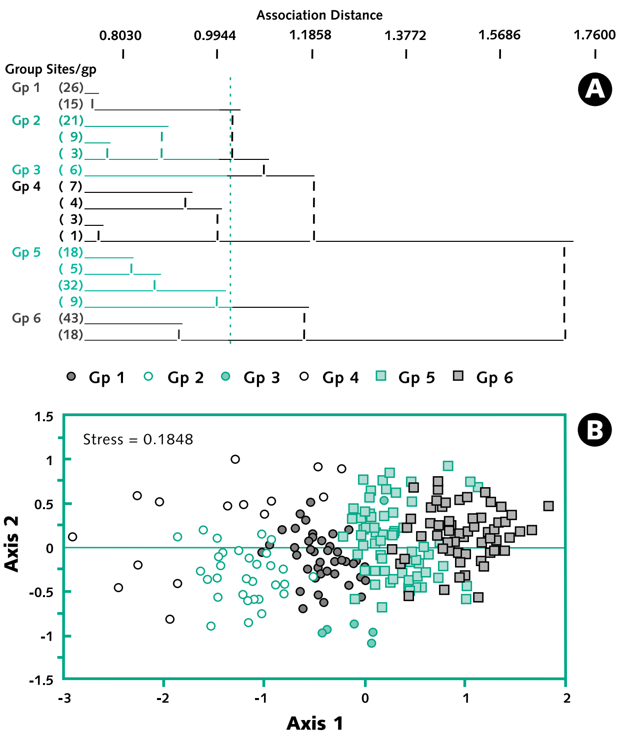

Figure 3. Family-level classification of 220 reference sites in the Fraser River Basin, 1994–1996.

(A) Cluster diagram. Vertical dotted line indicates cut-off point for group formation. (B) Ordination of six groups.

to the centroids of each of the site groups is shown in Figure 4, and the mean values of these environmental variables for each group are shown in Table 3.

Using discriminant analysis (Table 4), a number of predictive models were tested. The performance of different models was tested by their ability to classify sites, using a set of habitat variables, to the group they had been originally assigned to by cluster analysis of the invertebrate assemblage. The error rate for each model was calculated as the average of the percentage of sites per group incorrectly assigned. Three models appeared to perform well. The optimal model used 27 variables (error rate = 34.5%), the stepwise model using nine variables had an error rate of 42.9 per cent, and a model based on stepwise discriminant analysis using 19 variables performed similarly to the optimal model (error rate = 36.4%).

The same analysis is being done at the lower-taxon level. At the species/genus level preliminary analysis has produced nine groups with an error rate of 32.7 per cent using 27 environmental variables. Details of the lower-taxon level analysis for the complete

Table 3. Mean values of predictor variables in six reference groups.

PREDICTOR VARIABLES	GROUP					
	1	2	3	4	5	6
Grasses present (% sites in group)	22	6	17	7	48	69
Alkalinity (mg/L)	32.3	26.0	23.9	45.6	44.0	62.3
Channel width (m)	23.8	23.9	15.3	77.4	12.3	15.0
Bankfull width (m)	57.1	59.8	95.8	126.3	26.6	30.2
Framework (cm)[1]	11.3	22.1	24.3	4.8	7.7	3.6
Average velocity (cm/s)	0.50	0.59	0.43	0.36	0.41	0.37
Maximum channel depth (cm)	45.9	49.7	33.0	37.3	34.7	31.8
pH	7.6	7.3	7.3	7.7	7.7	7.7
Embeddedness (%)[1]	30.0	33.2	29.2	46.8	28.0	26.8

[1] *Defined in Table 1*

Table 4. Prediction of family groups from three discriminant models.

	TO GP 1	TO GP 2	TO GP 3	TO GP 4	TO GP 5	TO GP 6	AVERAGE ERROR RATE
Reference sites per group	41	33	6	15	64	61	
Sites correctly predicted by:							
Stepwise model (9 variables)	16	20	5	8	28	38	42.9%
Enhanced stepwise model (19 variables)	15	21	6	11	26	41	36.4%
Optimal model (27 variables)	15	18	6	11	35	45	34.5%

data set will be presented in Reynoldson *et al.* (in prep.).

These models were developed with the taxonomic database before final revision and with species level data for 1994 and 1995 only. Finalised models for three taxonomic levels (species, genus and family) are presented in the final technical report (Rosenberg *et al.* 1999).

Stage 4

Next, we assessed test sites against the reference-site groups. We used a multivariate method that incorporates the entire invertebrate assemblage. In this method, the reference and matching test sites are ordinated and plotted in ordination space using hybrid multi-dimensional scaling. The distribution of the reference sites provides the normal range of variation in unimpaired communities. The expected assemblage at a test site is determined by predicting the site to one of the reference-site groups using the predictive models constructed in stage 3. The actual assemblage at a test site can then be compared to this normal variability with a given probability of belonging to the reference group using probability ellipses built around the reference sites. The greater the departure from reference state, as measured in ordination space, then the greater the impact. However, determining actual degree of impact and what departure from the reference state defines an unacceptable impact is ultimately a subjective decision.

An extensive river water quality survey was conducted in the United Kingdom in 1990. The survey provided the impetus for the development of methods to circumscribe the continuum of responses into a series of bands representing grades of biological quality (Wright *et al.* 1991). The method is a simplification of what is a description of the continuum of site responses ranging from good to poor biological quality. The method is appropriate for obtaining a simple statement of biological quality allowing broad comparisons in either space or time that would be useful for management purposes.

We used a similar approach based on probability ellipses (Figure 5) constructed around a ref-

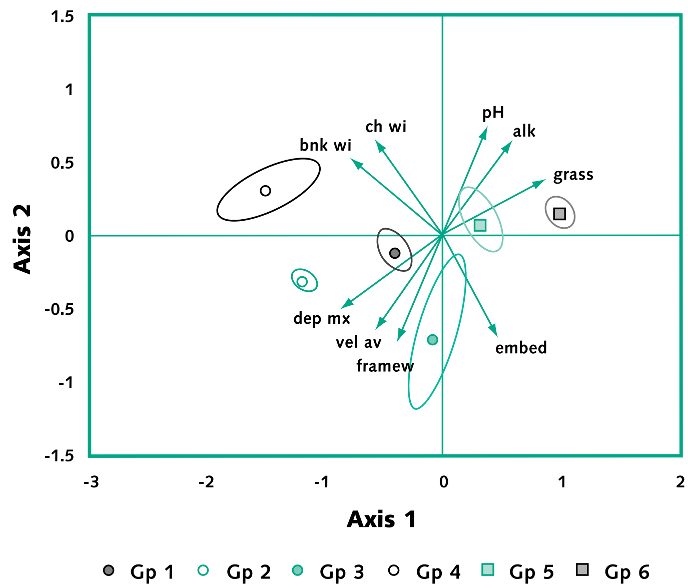

Figure 4. Principal axis correlation vectors for predictor variables and their relationship to six family-level groups for Fraser River Basin reference-site data, 1994 to 1996.

Ninety per cent confidence ellipses represent the ordination space occupied by the centroid of each group. (alk. = alkalinity, bnk.wi. = bankfull width, ch.wi. = channel width, embed. = embeddedness, dep. mx. = maximum channel depth, framew. = framework, grass = presence of grasses in riparian zone, vel. av. = average velocity).

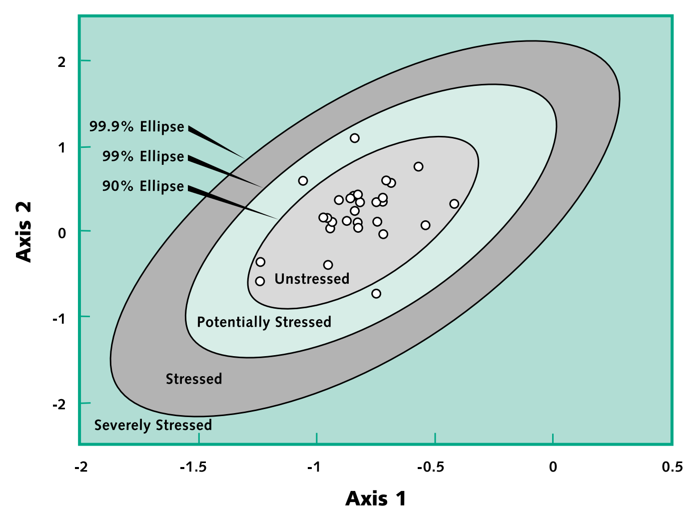

Figure 5. Example of probability ellipses calculated for reference sites to indicate potentially stressed (90%), stressed (99%) and severely stressed (99.9%) invertebrate assemblages at test sites.

Note that some reference sites fall naturally outside the ellipses.

erence-site group. Test sites within the smallest ellipse (90% probability) would be considered **unstressed**; sites between the smallest and next ellipse (99% probability) would be considered **potentially stressed**; sites between the 99 per cent probability and the largest ellipse (99.9% probability) would be considered **stressed**; and sites located outside the 99.9 per cent ellipse would be designated as **severely stressed**.

A test site is assigned to a reference group using a discriminant model, and the assemblage at the test site is compared to the assemblage of the matching reference sites. A data file is constructed that includes the taxa counts for the appropriate reference sites and the test site. The data are ordinated so that a matrix is calculated for both reference and test sites. The sites can then be plotted in ordination space showing the ordination dimensions that synthesize the biological attributes of the sites (Fig. 5). Probability ellipses are calculated for the reference sites only. The location of the test sites relative to the reference sites can then be determined. The assessment of stress is based on site locations on the ordination axes, and the overall assessment is based on the most stressed band to which a site belongs. In using such a probability based method there is a likelihood that some reference sites will naturally fall outside the ellipses (*e.g.* 10% of the reference sites are likely to be outside the 90% ellipse). In Figure 5 this can be seen for three of the 29 reference sites.

To demonstrate this approach, a selection of sites representing potential impacts from agriculture, logging and mining were sampled (Fig. 6). The observed responses are summarized below. Water quality in the Fraser River main stem was also assessed to determine if there were large spatial responses to urban activities and cumulative impacts on the river from basin activities.

Detecting impacts from agriculture, logging and mining

The methods described above were used to assess the ability of the reference-condition approach to detect and differentiate between different types of impact. To assign test sites to a reference-site group we tested all three models described above (Table 4) and found high concordance (87.2%) among the groups to which a test site was predicted. To illustrate the method for assessing individual test sites we have presented detailed results from those assessed sites predicted to belong to one reference group (Group 6, Fig. 7). A complete summary for all test sites is shown in Table 5.

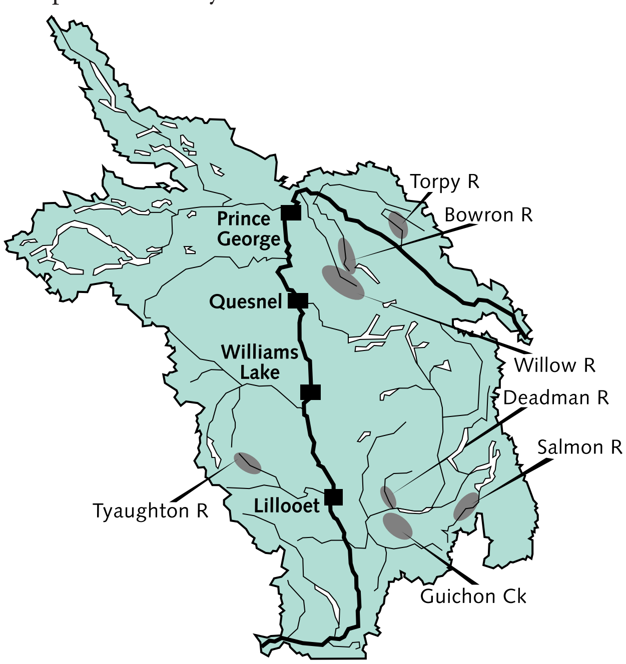

Figure 6. *General location of sites in the Fraser River catchment used to test for stress caused by human activity.*

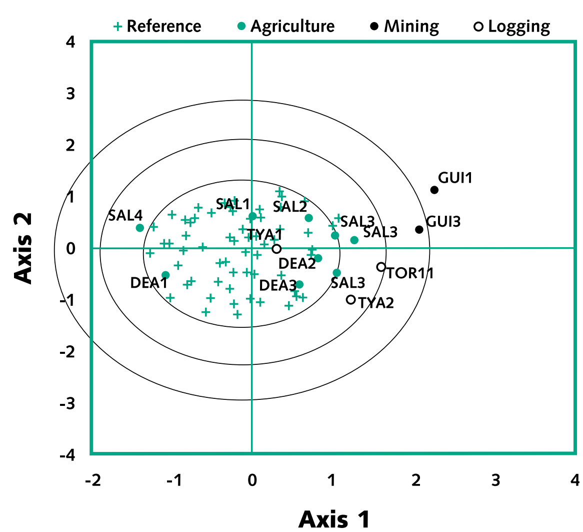

Figure 7. *Assessment of macroinvertebrate assemblage structure at test sites predicted to Group 6 using the BEAST model from the Fraser River Basin.*

Sites were located in the Deadman (DEA), Salmon (SAL), Torpy (TOR), Tyaughton (TYA) and Guichon (GUI) catchments.

Table 5. Assessment of test sites exposed to either agricultural (A), mining (M) or logging (L) activities. Sites were located in the Deadman (DEA), Salmon (SAL), Bowron (BOW), Torpy (TOR), Tyaughton (TYA), Willow (WIL) and Guichon (GUI) catchments.

SITE	POTENTIAL STRESS	PREDICTED TO GROUP	PROBABILITY OF BELONGING TO PREDICTED GROUP	ASSESSMENT
DEA1	A	6	0.90	Unstressed
DEA2	A	6	0.89	Unstressed
DEA3	A	6	0.92	Unstressed
SAL1	A	6	0.98	Unstressed
SAL2	A	6	0.75	Unstressed
SAL3	A	6	0.93	Unstressed
SAL3	A	6	0.93	Potentially stressed
SAL3	A	6	0.93	Potentially stressed
SAL4	A	6	1.00	Potentially stressed
BOW13	L	5	0.74	Unstressed
BOW14	L	1	0.43	Potentially stressed
TOR10	L	1	0.51	Unstressed
TOR11	L	6	0.74	Potentially stressed
TYA1	L	6	0.79	Unstressed
TYA2	L	6	0.92	Potentially stressed
TYA5	L	5	0.55	Unstressed
WIL1	L	5	0.62	Unstressed
WIL4	L	5	0.64	Unstressed
GUI1	M	2/6	0.68/0.090	Severely stressed/severely stressed
GUI3	M	2/6	0.81/0.94	Stressed/stressed
WIL2	M	5	0.46	Unstressed
WIL3	M	5	0.52	Unstressed

Sites potentially impacted by agriculture were sampled on the Deadman (DEA1–3) and Salmon (SAL1–4) rivers (Fig. 6). At one of these sites (SAL3), three replicates were taken for quality assurance purposes and were assessed individually. The sites on the Deadman River showed no effects of agricultural activity. They were within the 90 per cent probability ellipse for the reference sites (Figure 7), and are therefore assessed as being unstressed (equivalent to reference). Field notes and photographs taken from these three sites at the time of sampling showed little evidence of physical disturbance, grazing or other agricultural activity. On the Salmon River, only the two downstream sites showed indications of potential stress (SAL3 and SAL4 in Fig. 7). The effects on the invertebrate assemblage are different at SAL3 and SAL4 based on their location in ordination space (Fig. 7). All three samples from SAL3 were located to the right of the reference group. This site showed evidence of physical disturbance and bank instability, and the response in the assemblage was one of reduced abundance of organisms. Site SAL4 moved in the opposite direction in ordination space because of increases in numbers of organisms. This site was located adjacent to a small paddock with grazing stock and demonstrates the effects of nutrient enrichment.

Four streams were sampled that had, based on visual inspection, varying degrees of logging activity. Two sites were sampled on the Bowron River (BOW13, 14), two sites on the Torpy River (TOR10, 11), three sites on the Tyaughton River (TYA1, 2, 5), and two sites on the Willow River (WIL1, 4). Although at each of these sites logging had occurred over the past few years, in most cases there was a well-developed riparian buffer zone. Only three sites indicated potential stress (Table 5, Figure 7): BOW14, TOR11, and TYA2 (BOW14 is not shown in Figure 7 because it was a Group 1 site). All three sites had generally lower numbers and/or taxa than the reference sites, which indicates physical disturbance. Site TOR11 had been channelized and TYA2 was just upstream of a large culvert.

Guichon Creek and Willow River were sampled for potential mining impacts. One Guichon site (GUI1) was a drainage ditch from a tailings pond, and the creek channel had no flow. The second site (GUI3) was a residual channel in the original water course; however, it was fed by a small pipe and the substrate had extensive mineral encrustation. Both sites were identified as impaired: GUI1 was severely stressed and GUI3 was stressed. The two sites on the Willow (WIL2 and WIL3) were located downstream of Jack of Clubs Lake, which has received extensive tailings from historic mining activities in the Barkerville area (Mudroch *et al.* 1993). Site WIL3 was approximately two km from the lake outlet and WIL2 was a further 12 km downstream. Neither site showed any visual evidence of impairment and both had well-developed riparian zones. Both sites were assessed as unstressed.

From these results, it is evident that the reference-condition approach can detect invertebrate assemblage responses to all three stress categories. It is also noteworthy that the type of stress can be inferred by the trajectory of the site away from the reference-site swarm (Fig. 7). Responses to physical disturbance and enrichment resulted in sites moving in different directions away from the reference swarm.

Changes along the main stem of the Fraser River

A subsidiary objective of the study was to examine changes in invertebrate communities along the length of the Fraser River from the headwaters to the lower boundary of the study area at Agassiz. A total of 28 sites were located approximately every 50 km along the river. Four sites were sampled in 1994, and alternate sites were sampled in 1995 (even-numbered) and 1996 (odd-numbered). In addition, three sites were sampled in each of the study years (14, 16 and 28) and two sites (6 and 20) were replicated in 1995, to examine annual and site-scale variability.

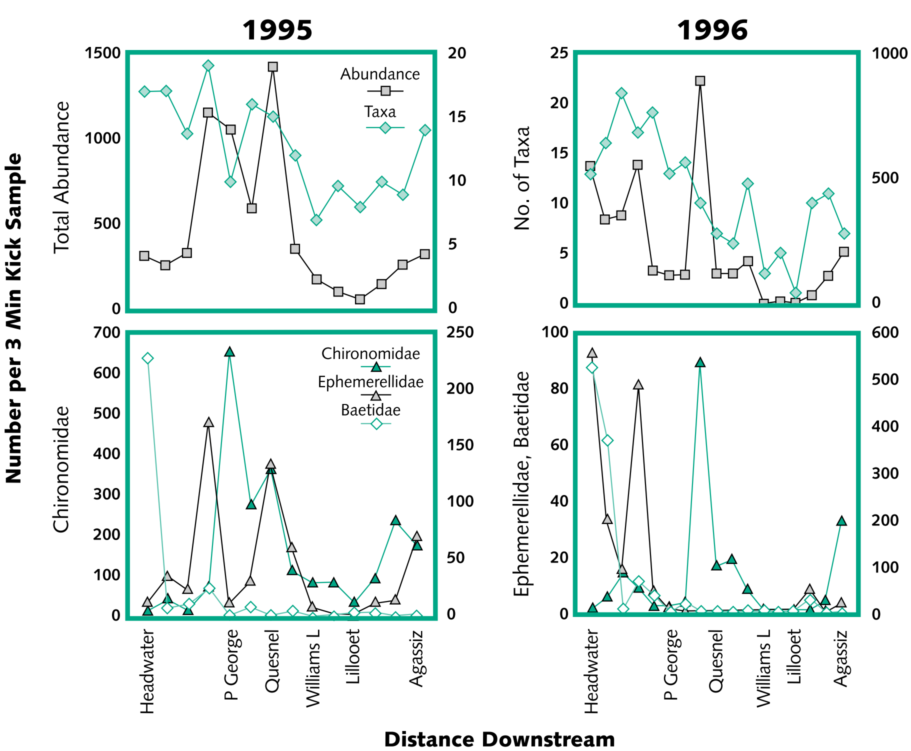

Figure 8. *Downstream changes in abundance of invertebrates in the Fraser River main stem, 1995 and 1996.*

There are similar trends in 1995 and 1996 in overall abundance and in the major families (Fig. 8). There is a general trend of increasing abundance from the headwaters to Quesnel (Site 14), followed by a region of low abundance to Lillooet (Site 23) and then a gradual increase in abundance to the last site (28). Diversity (number of taxa) decreases from the headwaters to the lower reaches. The changes in abundance and diversity are partially reflected by the major families. The upstream sites, particularly in the headwaters, are dominated by mayfly families.

The baetid mayflies occur primarily in the headwaters and the ephemerellid mayflies occur in the headwater and upstream sections (Figure 8). Increased total abundance between Prince George and Quesnel is primarily the result of increased abundance of chironomid midges (Fig. 8).

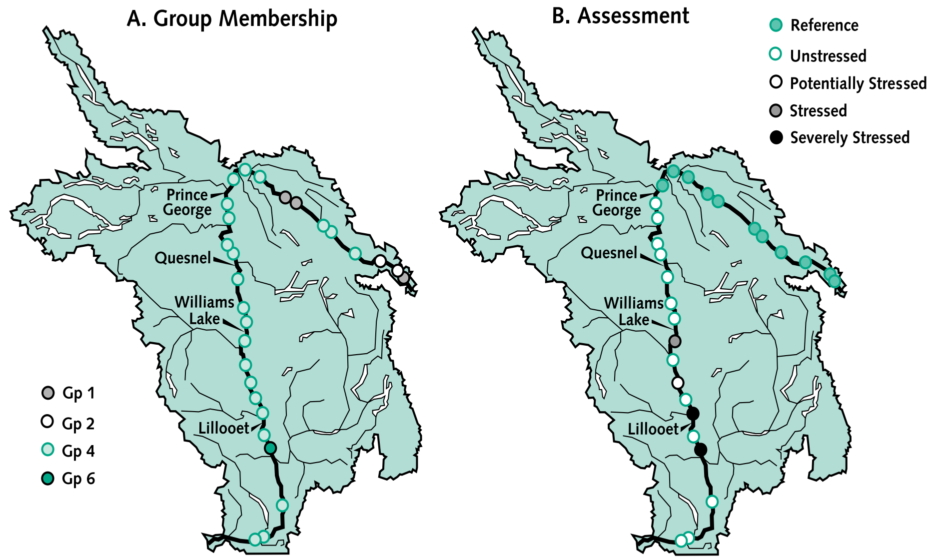

Figure 9. *Location of 28 sites on the main channel of the Fraser River.*
(A) Group membership of reference and test sites. (B) Assessment of test sites.

We compared the main stem invertebrate assemblages to the reference-site database to determine if the above pattern of change in invertebrates indicates changes other than those associated with natural variation. The main stem sites were separated into those included as part of the reference data set used for model building and those tested to determine if there were any impacts occurring in the river from point or non-point sources (Fig. 9).

Sites upstream of Prince George (FRA1–11) were considered to be part of the reference data set because of insignificant anthropogenic activity in the area. Sites downstream of Prince George (FRA12–28) were tested to determine whether or not they could be considered as equivalent to reference. The results are summarized in Table 6.

Most sites on the main stem are either members of Group 4 (Fig. 9A) or are predicted to be members of Group 4. Of the sites downstream of Prince George, most were assessed as unstressed (Fig. 9B). In 1996, four sites from downstream of Williams Lake to immediately downstream of Lillooet diverged from the reference state. There are no significant industrial discharges to the river in this area, and in both study years total abundance and number of taxa were low in this river reach (Fig. 8). There are three possible explanations for the assessment of these sites as being stressed in 1996. First, there is some stressor other than point source discharges to which the assemblages are responding. Second, the low numbers observed in 1996 are at the extreme of the normal range and thus, by chance, are outside the probability ellipse. Third, the reference-site group is an inappropriate match for these test sites. We suspect that the third explanation is the most probable one, because the four sites in question are in a canyon with quite different characteristics compared to other main stem sites. Furthermore, reference Group 4 is small (15 sites) and includes mainly main stem sites upstream of Prince George. The applicability of the reference-site database to this reach of the river will be investigated further.

The assessment shows no response in the invertebrate assemblages in the vicinity of the pulp and paper discharges at Prince George and Quesnel. The stimulation of invertebrate growth as a result of exposure to pulp mill effluent from Prince George, as observed under experimental conditions (Culp and Lowell 1999), is not sufficient to distinguish these sites from the normal variation found in the ambient river environment. The effect may be too small to be detected by our reference-condition approach but it should be noted that the closest sites were 17 km downstream of the discharges.

Table 6. Assessment of Fraser River main stem sites, 1994–1996.

SITE	PROBABILITY OF BELONGING TO PREDICTED GROUP	ASSESSMENT
FRA6-1995	1.00	Unstressed
FRA6-1995	1.00	Unstressed
FRA12-1995	1.00	Unstressed
FRA13-1994	1.00	Unstressed
FRA13-1996	1.00	Unstressed
FRA14-1994	1.00	Unstressed
FRA14-1995	1.00	Unstressed
FRA14-1996	1.00	Unstressed
FRA15-1996	0.95	Unstressed
FRA16-1994	1.00	Unstressed
FRA16-1995	1.00	Unstressed
FRA16-1996	1.00	Unstressed
FRA17-1996	0.99	Unstressed
FRA18-1995	1.00	Unstressed
FRA19-1996	1.00	Stressed
FRA20-1995	0.84	Unstressed
FRA20-1995	0.98	Unstressed
FRA20-1995	1.00	Unstressed
FRA21-1996	0.99	Potential stress
FRA22-1995	1.00	Unstressed
FRA23-1996	0.81	Severe stress
FRA24-1995	1.00	Unstressed
FRA25-1996	0.90	Severe stress
FRA26-1995	1.00	Unstressed
FRA27-1996	0.87	Unstressed
FRA28-1994	0.96	Unstressed
FRA28-1995	1.00	Unstressed
FRA28-1996	0.99	Unstressed

All sites were predicted to Group 4, except FRA25-1996, which was predicted to Group 6.

Replicated samples were taken for quality assurance at selected locations (FRA6, 20). These samples were assessed separately and showed consistent prediction and assessment. Four sites that were sampled for two or three years (FRA13, 14, 16, 28) showed no annual variation in either the predicted assemblage or the assessment, suggesting the database and predictive models are resilient to short-term annual variability.

CONCLUSIONS

Macroinvertebrate assemblages, and habitat and water chemistry variables were measured using 224 reference samples and 98 test, repeat or quality assurance samples from three field seasons. The sites sampled encompass 23 subcatchments, including the Fraser main stem. This database represents the most comprehensive sampling of benthic macroinvertebrate assemblages for the Fraser River catchment.

We have developed family-level predictive models that use six reference groups of benthic invertebrates and up to 27 predictor variables. Our error rates are 34.5 per cent to 42.9 per cent, for predicting reference sites to a group. For a preliminary lower-taxon predictive model, based on 1994–1996 data and using nine groups and 27 predictor variables, the error rate was 32.7 per cent. The most significant predictors of benthic macroinvertebrate assemblages at the family level are simple site descriptors such as presence of grass in the riparian zone, channel and bankfull width, channel depth, substrate measurements such as the size of the largest particles (framework) and the degree of embeddedness, and water chemistry descriptors such as alkalinity and pH. The importance of these variables suggests that at the family level large-scale processes are structuring benthic communities.

We have completed assessments on 46 test sites, which indicate that effects from logging, mining and agricultural activities can be evaluated. The analytical technique, using multivariate assessment methods, allows a judgment on the likely cause for the impairment (*e.g.* physical disturbance versus enrichment).

The reference database, models, and software (being developed) form the basis of an effective tool for assessing impairment of *in-situ* benthic invertebrate assemblages occurring in the Fraser River catchment. The tool can be used as is, or it can be added to over time, thus enhancing its application to the basin. We would encourage future studies to adopt the reference-condition approach and the protocols developed during this study to allow for further development.

REFERENCES

Cooper, S. D. and L. A. Barmuta. 1993. Field experiments in biomonitoring. Pages 399–441 in: D. M. Rosenberg and V. H. Resh, eds. *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, New York.

Culp, J. and R. Lowell. 1999. Pulp Mill Effluent Impacts on Benthic Communities and Selected Fish Species in the Fraser River Basin. In: C. Gray and T. Tuominen, eds. *Health of the Fraser River Aquatic Ecosystem: A Synthesis of Research Conducted Under the Fraser River Action Plan*. Environment Canada, Vancouver, B.C. DOE FRAP 98-11.

Davis, W. S. and T. P. Simon. 1995. Introduction. Pages 3–6 in: W.S. Davis and T.P. Simon, eds. *Biological Assessment and Criteria. Tools for Water Resource Planning and Decision Making*. Lewis Publishers, Boca Raton, Fla.

Dymond, P. F., J. S. Richardson, T. B. Reynoldson and D. M. Rosenberg. 1997. *Seasonal Changes of Benthic Macroinvertebrate Assemblage Structure and the Implications for Biomonitoring*. Abstract of paper presented at the 45th Annual Meeting of the North American Benthological Society, San Marcos, Texas, May 26–30, 1997. Bulletin of the North American Benthological Society 14: 95.

Eberhardt, L. L. 1978. Appraising variability in population studies. *Journal of Wildlife Management* 42: 207–238.

Graney, R. L., D. S. Cherry and J. Cairns. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam *(Corbicula fluminea*) in laboratory artificial streams. *Water Research* 18: 833–842.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54: 187–211.

Marchant, R. 1989. A subsampler for samples of benthic invertebrates. *Bulletin of the Australian Society of Limnology* 12: 49–52.

Mudroch, A., G. E. M. Hall, J. Azcue, T. A. Jackson, T. Reynoldson and F. Rosa. 1993. *Preliminary Report on the Effects of Abandoned Mine Tailings at Wells, B.C. on the Aquatic Ecosystem of Jack of Clubs Lake. Part I: Reconnaissance Study*. NWRI Report 93-23.

Norris, R. H., P. S. Lake and R. Swain. 1982. Ecological effects of mine effluents on the South Esk River, northeastern Tasmania. III. Benthic macroinvertebrates. *Australian Journal of Marine and Freshwater Research* 33: 789–809.

Parsons, M. and R. H. Norris. 1996. The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology* 36: 419–434.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross and R. M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers. Benthic Macroinvertebrates and Fish*. EPA/444/4-89/001. Office of Water Regulations and Standards, US Environmental Protection Agency, Washington, DC.

Reynoldson, T. B., R. C. Bailey, K. E. Day and R. H. Norris. 1995. Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. *Australian Journal of Ecology* 20: 198–219.

Reynoldson, T. B., R. H. Norris, V. H. Resh, K. E. Day and D. M. Rosenberg. 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society* 16: 833–852.

Reynoldson, T. B., V. H. Resh and D. M. Rosenberg. In prep. *Design of a Regional Benthic Biomonitoring Programme: II. Development of Predictive Models of Invertebrate Assemblages Using Multimetric and Multivariate Approaches in the Fraser River Catchment, British Columbia.*

Rosenberg, D. M. and V. H. Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: D.M. Rosenberg and V.H. Resh, eds. *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, New York.

Rosenberg, D. M. and V. H. Resh. 1996. Use of insects in biomonitoring. Pages 87–97 in: R.W. Merritt and K.W. Cummins, eds. *An Introduction to the Aquatic Insects of North America*. 3rd ed. Kendall/Hunt, Dubuque, Iowa.

Rosenberg, D.M., T.B. Reynoldson and V.H Resh. 1999. *Establishing Reference Conditions for Benthic Biomonitoring in the Fraser River Catchment, British Columbia, Canada.* Fraser River Action Plan, Environment Canada, Vancouver, B.C. DOE FRAP 1998-32.

Rosenberg, D. M., T. B. Reynoldson and V. H. Resh. In prep. *Design of a Regional Benthic Biomonitoring Programme: I. Development of Protocols for Establishing Reference Conditions in the Fraser River Catchment, British Columbia*.

Schindler, D. W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. *Science* 184: 897–899.

Sivaramakrishnan, K. G., M. J. Hannaford and V. H. Resh. 1996. Biological assessment of the Kaveri River catchment, South India, using benthic macroinvertebrates: applicability of water quality monitoring approaches developed in other countries. *International Journal of Ecology and Environmental Sciences* 22: 113–132.

Sylvestre, S. 1997. *Stream Bioassessment Using Benthic Macroinvertebrate Communities from the Fraser River Basin, British Columbia, Sampled at Two Habitat Scales: riffles and leaf packs*. M.Sc. Thesis. University of Western Ontario, London, Ont.

Wright, J. F. 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* 20: 181–197.

Wright, J. F., M. T. Furse, P.D. Armitage and D. Moss. 1991. *Testing and Further Development of RIVPACS. IFE Interim Report to the National Rivers Authority, December 1991*.