Development and Testing of a Fish-based Index of Biological Integrity to Quantify the Health of Grassland Streams in Alberta

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EXECUTIVE SUMMARY

Management synopsis

We developed a fish-based index of biological integrity (IBI) to aid resource managers with assessing the health of grassland streams in Alberta. The IBI consisted of five metrics reflecting structural and functional attributes of a fish assemblage comprising only five species. With the aid of a simple spreadsheet, formulas provided within this report, and information on stream size, land managers and researchers in east-central Alberta can quickly calculate stream IBI scores using data collected on their fish assemblages. The IBI may be a valuable tool for resource management when it is used to compare biotic integrity among sites, and for making regional assessments of stream health. In our study, scores approaching a perfect score of 50 would suggest high-quality conditions appropriate for maintaining biological integrity. High-quality sites (i.e., IBI scores > 30) include a relatively high proportion of older fish, high numbers of ≥1 yr fathead minnow, the presence of white sucker but low numbers of young-of-year, and a relatively low proportion of fish with disease and deformities. Below a disturbance threshold however, the environmental conditions and streamside activities are no longer capable of supporting systems displaying biological integrity. For example, low-quality sites (i.e., IBI scores < 20) in streams may be linked to human disturbance, particularly activities contributing to phosphorus inputs. However, we acknowledge uncertainty in correctly identifying healthy versus unhealthy conditions for sites with mid-range IBI scores (i.e., 20-30), and recommend that resource managers, whenever possible, incorporate data on other biota and the physical and chemical attributes of a site.

Research synopsis

Grassland stream ecosystems are widely impacted by agriculture and other anthropogenic activities that can alter the abundance of fish and structure of stream communities. Variable hydrological conditions, however, coupled with inherently low species richness and assemblages dominated by tolerant species, make assessments of fish communities and stream health challenging. We developed a fish-based index of biological integrity (IBI) and compared its use with a multivariate reference-condition approach (RCA) to assess the ecological condition of streams in east-central Alberta. Data on fish assemblages collected via electro-fishing was combined with stream and

watershed-scale environmental variables for 69 wadeable stream reaches (basin area range 73 to 1,845 km^2) that reflected a range of levels and types of agricultural disturbance and human landuse. Stream fish assemblages were numerically dominated by brook stickleback (*Culaea inconstans*) (46% of all individuals captured) and fathead minnow (*Pimephales promelas*) (49%); lake chub (*Couesius plumbeus*) and white sucker (*Catostomus commersoni*) were detected at many sites but were seldom numerically dominant. We developed the IBI by screening candidate metrics for redundancy and sensitivity to disturbance. Of 10 candidates, which were either new metrics or a modification of metrics in Karr (1981), we selected five for the final IBI. These metrics were related to reproductive guilds, trophic position, fish abundance, tolerance to disturbance and individual condition of fish. Specifically, we used occurrence of white sucker, number of ≥1 yr fathead minnow, number of young-of-year, % ≥1 yr fish and % DELTs (deformities or disease, fin erosion, lesions and tumors). IBI validation showed that scores were unrelated to watershed area but particularly responsive to changes in total nitrogen and phosphorus and negatively related with an integrative disturbance index. This disturbance index was based on the ranking of 11 environmental variables measuring water quality, hydrological conditions, riparian structure, and landscape converted to cropland and pasture, and general anthropogenic activities in watersheds. As the first step of the RCA, we used the integrative disturbance index to identify (*a priori*) the best available reference sites (n = 20) and identified these sites to be minimally to moderately impacted for the purpose of our study. The reference condition examined two types of fish assemblages (brook stickleback vs. fathead minnow dominated) and assemblage type differed with stream width and depth based on discriminant function analyses. Based on reference ranges of fish assemblage scores in ordination space, 63 and 37% of large stream test sites failed to match the 90 and 99% reference condition, respectively. Failure rates for test sites were considerably lower for smaller streams (40% fell outside the 90% reference range whereas none failed at the 99% level). Results also showed that the reference condition may be dependent on stream size, possibly because of groundwater influences on small streams, and should be carefully defined in highly modified landscapes such as the Alberta grasslands. Using IBI and RCA approaches, we proposed that only a small proportion of regional streams (approximately 23%) clearly support living systems capable of maintaining biological integrity but acknowledge uncertainty in correctly identifying healthy versus unhealthy conditions for sites with mid-range IBI scores

(i.e., 20-30 of 50). To correctly interpret our IBI and improve its application for bioassessments of grassland streams, we recommend further evaluation of our IBI, particularly across a broader disturbance gradient using independent datasets from other watersheds, and better biological knowledge of the definition of metric types and their responses to human disturbance. Our pilot study has created a potentially useful multimetric fish-based index for the conservation of grassland streams and the services they provide Albertans.

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1.0 INTRODUCTION

In November 2003, the Alberta Government announced "Water for Life: Alberta's Strategy for Sustainability" as part of an ongoing commitment to sustainable resource management and maintenance of the biological integrity of provincial waters. Biological integrity can be broadly defined as "a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region" (Karr and Dudley 1981). Unfortunately, the biological integrity of ecosystems throughout North America is widely compromised by anthropogenic activities through their influences on energy sources, water quality and quantity, habitat structure, flow regime and biotic interactions. In particular, prairie streams, including those in Alberta, are part of a highly impacted ecosystem of which remaining native fragments may not be large enough to encompass a significant, functional watershed (Dodds et al. 2004). Most sub-basins are heavily impacted by agriculture and to moderate extents, urbanization and oil/gas exploitations, a situation that creates a formidable challenge for managers when assessing current levels of impairment or forecasting biological integrity as human activities continue to expand.

Furthermore, resource managers in many parts of Canada, including Alberta, lack the tools needed to evaluate biological integrity, even though biological monitoring is increasingly important and the concept of bioassessment (determining the health of an environment based on its biotic composition) is not new (Karr 1981, Karr and Dudley 1981). The development of monitoring programs is hampered because bioassessment and monitoring approaches are generally designed for specific ecoregions thus, their performance may be unreliable elsewhere (Fausch et al. 1984, Hughes et al. 1998, Angermeier et al. 2000). There also remains considerable debate about how to best design and implement bioassessments, and the specific taxa on which a bioassessment should focus (Hughes et al. 1998, Bowman and Somers 2005, Mazor et al. 2006). Historically, bioassessment programs for streams have been developed using a variety of taxa, including fish, macroinvertebrates, and benthic algae (e.g., Reynoldson et al. 1995, Griffith et al. 2005, Santucci et al. 2005). Bioassessment of streams and rivers based on resident fish assemblages has become a widely used and effective tool for managing aquatic resources in North America (Daniels et al. 2002, Mebane et al. 2003,

Bramblett et al. 2005). Because governmental water standards explicitly call for fish protection (e.g., U.S. Clean Water Act, Canadian Fisheries Act, Canada's National Park Act), direct assessment of fish assemblages are often more relevant than surrogate approaches based on invertebrates and abiotic criteria alone. In addition, fish are a highly visible component of aquatic environments and are relatively easy to sample in the field.

Review of recent literature reveals that a popular method for bioassessment of aquatic systems that focuses on fish is the index of biotic integrity (IBI, Appendix 1). IBI was originally developed by Karr (1981) as a cost-effective and standardized method to assess environmental degradation in Midwestern U.S. streams based on the characteristics of their fish assemblages. The IBI is a composite index that incorporates structural, ecological, trophic and reproductive attributes of fish assemblages at multiple levels of organization. This approach has been successfully applied to streams and occasionally lakes throughout much of the United States (Appendix 1, Karr and Chu 1999), but only one fish-based IBI has been created for Canadian streams and rivers (Steedman 1988). One potential factor limiting the use of such a bioassessment tool at northern latitudes, particularly in the Canadian prairies, is the depauperate and relatively tolerant (of harsh environmental conditions) ichthyofauna of these ecosystems (Bramblett et al. 2005). For example, the mean number of species used in the development of 14 recent, fish-based IBIs was 59 (18 – 137 range), whereas assemblages in relatively small lakes and streams in many regions of Alberta generally comprise far fewer species (<5 species) (Nelson and Paetz 1992, Scrimgeour et al. 2002, 2003, Tonn et al. 2003). The low species richness in Alberta systems reduces the number of potential candidate metrics to characterize fish assemblages. In addition, the biota of prairie streams, in particular, are thought to be dominated by fish species characterized as habitat, trophic and reproductive generalists adapted to unstable flow regimes with harsh, fluctuating environmental conditions, including high summer temperatures and suspended sediment concentrations making it more tolerant than the biota of more stable systems (Dodds et al. 2004, Bramblett et al. 2005).

Given the lack of established bioassessment tools for resource managers in Alberta, our primary objective was to conduct a pilot study near Three Hills, Alberta to assess whether an effective fish-based IBI could be developed to characterize the ecological

condition of small (i.e., wadeable) streams in the Alberta grasslands. We built anthropogenic disturbance models predicting IBI scores using 69 reaches within five sub-basins of the Red Deer River, and compared scores on a subset of paired stream reaches reflecting low and high livestock grazing intensities to validate the application of the IBI and to better understand potential threats to biological integrity. Our second objective was to compare bioassessments based on an IBI approach with those based on a Reference Condition Approach (RCA) using the same fish-environment database. The RCA is a multivariate method; specifically we focus on the 'Benthic Assessment of Sediment' design where stream impairment is identified as change in community composition as reflected in location in ordination space. Although fish-based assessments using an RCA approach are applied less frequently than assessments using an IBI approach (Appendix 1, Tonn et al. 2003), the RCA method is well recognized as a viable alternative to IBI for bioassessment (Reynoldson et al. 1995, Reynoldson et al. 1997, Bailey et al. 2004). Using both RCA and IBI methods, our third objective was to identify regional levels of impairment for grassland streams. This work is part of on-going provincial efforts to develop tools for measuring stream health and represents the first published account of developing and evaluating an IBI in Alberta.

2.0 STUDY AREA

Our study was conducted near the town of Three Hills (51°42'N,113°15' W) in a 7,000 km^2 region of east-central Alberta (Figure 1). This region is part of the grassland climatic regime (or eco-province) and includes mixed grassland, fescue grassland, and aspen parkland eco-regions (Strong and Leggatt 1992). However, majority of the landscape has been converted to agriculture, with cropland being the dominant landuse type (64%) followed by pasture for livestock (8%) (Strong and Leggatt 1992). The remaining landscape components included native grassland, wetland and urban cover. The landscape encompasses seven villages (300-1000 people) and one town (Three Hills; population approximately 3,500) that lie within close proximity (<10 km) and upstream of our sampling locations. The terrain is undulating and consists of numerous low-order streams with occasional small, temporary beaver dams on parts of Kneehills and Michichi creeks. One man-made dam (Bigelow Reservoir) lies approximately 10 km upstream of our study site on Three Hills Creek; no permanent

man-made dams were located between our sampling locations and the Red Deer River. All study sites were located on wadeable streams (i.e., typically < 1.0 m depth). For the purposes of our study, we defined a study site as a stream reach of approximately 60-80 m long depending on stream size; study reach length increased with decreasing stream size to ensure adequate fish catches for analyses. Basin size varied among stream reaches, ranging from 73 to 1,845 km^2 (mean ± SE = 629 ± 50 km^2), and stream discharge ranged from 0.001 to 0.591 m^3/sec (mean ± SE = 0.127 ± 0.013 m^3/sec).

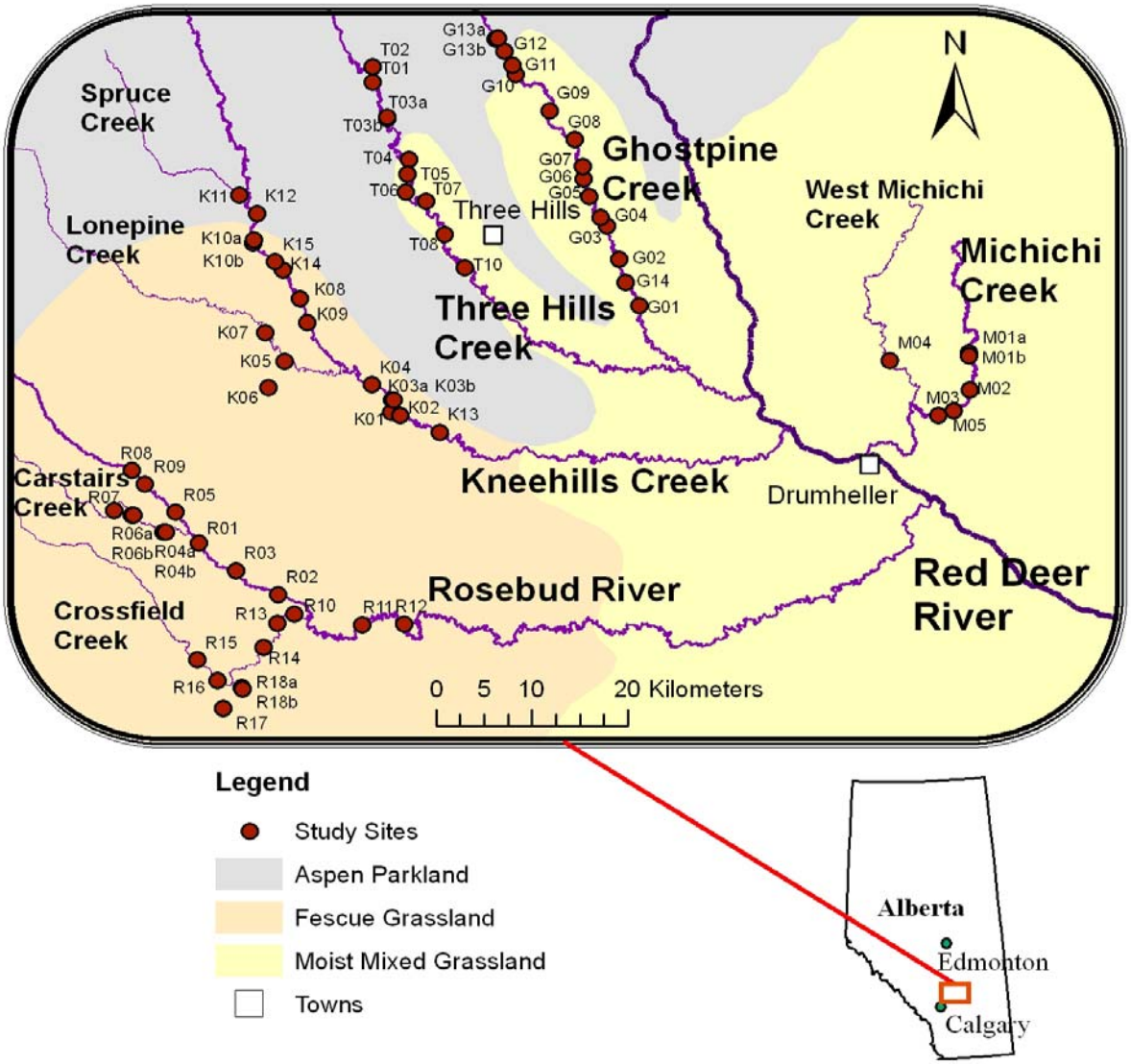

Figure 1. Location of 69 study sites (stream reaches) in the five sub-basins of the Red Deer River that were used to assess the health of grassland streams: Rosebud River, Kneehills Creek, Three Hills Creek, Ghostpine Creek and Michichi Creek. Note that 6 sites are on small, unnamed tributaries and appear disconnected from the stream network in the above map (R17, R18a, R18b, K01, K05, and K06).

3.0 METHODS

3.1 Scoping of study sites

We first conducted a 3-day reconnaissance of the study area in early September to select study sites representing a range of human disturbance levels and types from multiple sub-basins, and to determine the most appropriate scale (i.e., sample size and geographic extent) to complete the IBI assessment based on travel time among study locations and the availability of road access to streams. This ensured that data on fish and habitat was not only statistically robust for IBI development but that data could be effectively gathered from field work given our time constraints, specifically the approaching onset of significant changes in weather (i.e., winter). In total, 69 study sites were haphazardly selected from five sub-basins of the Red Deer River (Basin area = 24,800 km^2 at Drumheller, Alberta). The sub-basins included the Rosebud River, Kneehills Creek, Three Hills Creek, Ghostpine Creek, and Michichi Creek (Figure 1). To minimize potential spatial pseudoreplication in the response of fish assemblages to disturbance, most study reaches were >2 km apart. Disturbance, herein, refers to a suite of anthropogenic activities at various spatial scales that may affect fish assemblages through changes in water quality, habitat structure, and stream connectivity. Activities include livestock grazing of riparian zones, conversion to cropland and its associated practices (e.g., fertilizers, pesticides), urbanization, point-source pollution, and general human landuse patterns at the watershed-level. Geographical Universal Transverse Mercator (UTM) coordinates of study sites are provided in Appendix 2.

3.2 Fish and habitat sampling

Fish assemblages were determined by single-pass electrofishing surveys of stream reaches using a Smith-Root™ Type 12 pulsed DC-backpack electrofisher (350 V, 20 Hz, 12% duty cycle). Shock time was recorded during each survey session and used in calculating catch-per-effort as number of fish captured per 100 sec. Our effort ranged from 198 to 577 sec per site (60-80 m long by <3 m wide). All streams were surveyed within an 8-day period in early fall 2005 (27 September to 4 October), a year in which high precipitation events during both early spring and September resulted in above normal baseline flows during our electrofishing period. Following capture, all fish

were identified to species and a sub-sample was measured for total length (TL), examined for anomalies, and released into the stream reach from which they were captured. Anomalies are standard metrics in bioassessments and are often described as DELTs, which include deformities, sign of disease, eroded fins, lesions, and tumors (e.g., Steedman 1988, Lyons et al. 2001, Daniels et al. 2002, Mebane et al. 2003). We also included the incidence of black spot parasitism caused by the trematode parasite *Neascus* spp. (Blouin et al. 1984), as a DELT and indicator of individual fish condition (see Steedman 1988). Using natural breaks in histograms of TL distributions of our capture and length information on age classes in the literature (Smiley 1972, Chen and Harvey 1999, Mills et al. 2000), we assigned individual fish to either young-of-year (YOY) or ≥1 yr categories (Figure 2). Brook stickleback < 50 mm, fathead minnow < 35 mm, lake chub < 75 mm and white sucker < 100 mm were considered YOY. We were unable to complete a thorough historical survey of regional ichthyofauna through interviews with local landowners due to limited resources. However, such data may aid in the development of new IBI metrics given the long history of human settlement and agriculture (>100 yrs) in east-central Albert, and the potential for recent extirpation because of these long-term activities.

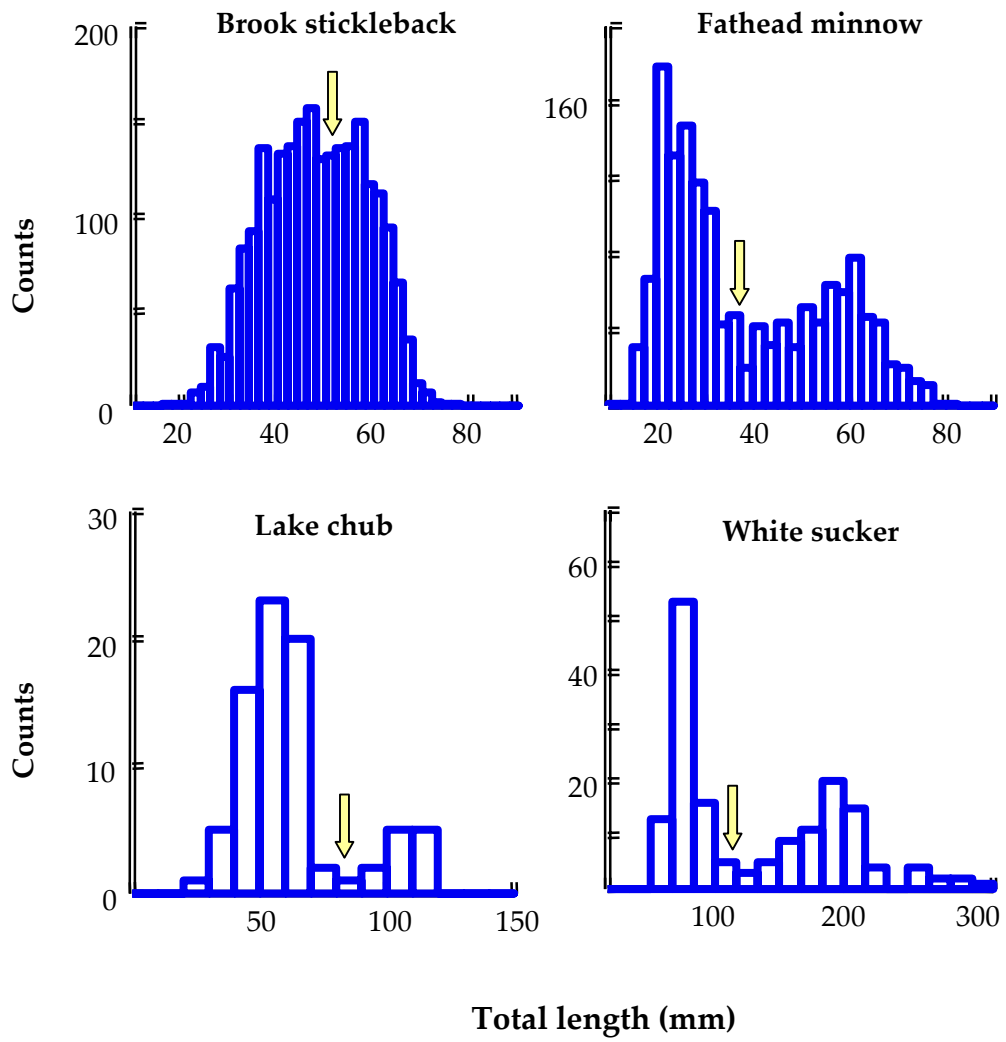

Figure 2 . Histograms of total length (mm) for brook stickleback, fathead minnow, lake chub and white sucker. Arrows mark size length separating young of the year (YOY) (i.e., small fish) from fish ≥1 year in age (i.e., large fish). Criteria for size thresholds were based on natural breaks and size per age information in the literature

To link patterns in fish assemblages with human landuse, we also collected data on a suite of riparian zone landuse types, including livestock, agriculture (cropland and pasture) and road density. To assess impacts of livestock, we divided each reach into 10-m sections, measured the grazing intensity in each section and calculated a mean reach value (reach score) using data from the three sections. Measures of grazing intensities included 1) % unstable shoreline with no vegetation, 2) % non-wood cover, 3) % exposed soil, and 4) the occurrence of cattle trails (<10 m of the shoreline) (Fitch and Adams 1998). In addition, we qualitatively estimated livestock grazing intensities on the landscape per stream-side (within 200 m of shoreline) using a 4-point scale as i) 0 = idle pasture or native grassland, ii) 1 = minimally grazed, iii) 2 = moderately grazed, and iv) 3 = intensively grazed, and summed the two stream-side scores from each bank for each site. We calculated % cropland, % agriculture (both cropland and pasture combined) and road density (m/ha) within 500 m wide by 5 km long sections of riparian zones at each study site using the spatial analyst tools in ArcGIS. Data on landuse were obtained from LANDSAT 7 (2001) imagery collected at a 30-m spatial resolution by the Prairie Farm Rehabilitation Administration (PFRA; see http://www.agr.gc.ca/pfra/gis). Watershed basins were identified through a digital elevation model (DEM) based on data from the 1:50000 National Topographic Data Base.

Additional information on habitat and water quality was provided by the collection of water samples during 14-17 October 2005. We measured turbidity (NTU) using a Hach Turbidimeter Model 2100A and total nitrogen (TN) with a CHN analyzer. Total suspended solids (TSS), total phosphorus (TP) and colour were measured using standard procedures (see Prepas and Rigler 1982, Cuthbert and del Giorgio 1992). We conducted field measurements of conductivity and dissolved oxygen with a YSI Model 85 DO Meter and pH with an Oakton® pHTestr 10. Percentage of fine sediments in the stream substratum was estimated by feeling the texture of substrate while walking the length of a study reach (waters were too turbid for visual assessments). We also measured a number of hydrological parameters, including stream wetted width,

maximum depth, and velocity from the center of stream, all of which were measured every 10 m and presented as a mean value per stream reach (note that wetted width was estimated during electrofishing). A Model FP101 Global Flow Probe was used to measure stream velocity. Stream discharge was determined at one location per site by dividing the cross-section into 5 portions if a small stream (<3 m) or 10 portions if a large stream (>3 m); the area and current velocity was measured for each portion individually and the resulting products were summed for a total discharge. All readings of current velocity were taken at 0.6 times the water depth from the top of the water column. Information on the occurrence of fish refuges during fall and winter low flow periods (e.g., beaver ponds and springs), was beyond the scope of this work and was not collected.

Our next step was to use the above mentioned landuse and water chemistry variables to identify the best available reference sites. We created an integrative disturbance score for each site based on the sum of 11 ranked disturbance variables (Table 1). The selection of 11 ranked disturbance variables was based on the *a priori* assumption that fish assemblages in streams were strongly influenced by water chemistry and surrounding landuse at multiple spatial scales (Allan 2004). Specifically, the disturbance score was based on ranking of:

- one variable reflecting varying hydrological conditions (% unstable shoreline),
- two variables reflecting local riparian condition (non-wood cover and bare soil),
- two metrics measuring grazing intensity by livestock and upstream cropland at a landscape scale,
- two metrics indicative of general anthropogenic activities at a watershed-scale (% agriculture in basin, and road density in basin), and
- four water chemistry variables (conductivity, turbidity, total nitrogen and total phosphorus) commonly used in developing IBIs (Hughes et al. 1998, Bramblett et al. 2005) based on their strong relationship with human landuse patterns (Johnson et al. 1997, Carpenter et al. 1998, Allan 2004).

Dissolved oxygen was omitted because levels during our sampling period were relatively high and likely non-responsive to anthropogenic disturbance. We were also

skeptical of the accuracy and precision of our estimates of % fine sediments, thus omitted this variable from the integrative disturbance score and only present the raw data (see Results section). Seventeen sites with scores within the lowest quartile (<309) were designated as reference sites. We also added three sites to this reference group that were slightly above the lowest quartile score because of a natural break in scores bringing the total number of reference sites to 20. We acknowledge that our reference sites may be moderately impacted given that the proportion of agriculture in our reference watersheds was relatively high at 53%, and beyond the 50% threshold used to identify unhealthy streams in forested watersheds in the United States (see Wang et al. 1997, and review by Allan 2004). However, we believe this ecological threshold is likely to be higher in grassland watersheds given the dynamic and disturbance-driven characteristics of prairie stream ecosystems (Dodds et al. 2004). Our definition of reference sites herein refers to minimally to moderately impacted sites, which was the best available condition of streams in our study area, as determined by the integrative disturbance score.

Table 1. List of 11 variables used to describe watershed disturbances of the 69 study reaches of grassland streams in east-central Alberta.

Disturbance variable
Percent unstable shoreline of reach
Percent wood cover in riparian zone
Percent bare soil in riparian zone
Grazed landscape index
Conductivity (μS/cm)
Turbidity (NTU)
Total phosphorus (TP; μg/L)
Total nitrogen (TN; μg/L)
Percent (upstream) landscape in cropland
Percent agriculture in basin
Road density in basin (m/ha)

3.3 Overview of bioassessment methods

Examination of the health of grassland streams was completed in four main steps summarize below: Step 1) development and evaluation of IBI, Step 2) classification of impaired sites using RCA, and Step 3) comparison of bioassessment methods; and Step 4) identification of regional levels of impairment of stream environments. A brief description of each step is provided below.

Step 1. IBI.

a) Candidate metrics were proposed for IBI by considering fish assemblages likely to occur in the region, local knowledge and published information on species habitat requirements and life history, and by reviewing the applicability of metrics that were successfully used in previously published IBIs (if necessary, proposed metrics were revised to account for structure and type of fish data that was collected, for example low capture rates for a species).
b) Candidate metrics were screened for redundancy and responsiveness to anthropogenic disturbance using correlation analyses. Significance levels for correlations were based on those in published IBIs.
c) If a screened metric showed a correlation with watershed area, it was then adjusted for watershed area by calculating residuals of observed versus predicted metric scores per site using regression models (linear or non-linear) from reference sites only (note if final IBI showed a correlation with watershed area even after adjustment of metrics, additional non-adjusted metrics were calibrated for watershed area).
d) Scores per metric were linearly scored but with an upper ceiling identified for positive-scoring IBI metrics (95th percentiles) and a lower floor set for negative-scoring IBI metrics (5th percentiles), such that scores above or below these thresholds, respectively, received a score of 10. Metric scores were then summed per site such that in our study, a perfect score was 50 (because we used 5 metrics for the final IBI).
e) IBI was validated by examining its relationship with landscape and physico-chemical variables correlated with human disturbance, including the integrative disturbance index, using univariate and multivariate regression techniques.

Step 2. RCA.

a) Fish assemblage types inhabiting reference sites (i.e., sites with the lowest quartile of an integrative disturbance score) were first identified using clustering techniques, followed by modeling habitat variables unrelated to anthropogenic disturbance that best discriminated between sites supporting different fish assemblages. Using habitat variables, such as stream size, in models generated by discriminant function analyses, test sites (i.e., non-reference streams) were assigned to a particular fish assemblage type.
b) Fish assemblages in reference sites were plotted in ordination space using site scores based on relative abundance data examined with non-metric multidimensional scaling. Test sites were passively plotted in this space to determine if their fish assemblage fell within the range of assemblage composition recorded for reference sites. Test sites outside the 90% reference range were considered to differ from the reference condition, and to be potentially degraded or impaired. Test sites outside the 99% reference range were considered as being severely degraded.
c) Failure rates were summed per fish assemblage (or stream habitat) type.

Step 3. RCA vs. IBI.

a) The percentages of high IBI-scoring sites (>25) that failed based on RCA, and of low IBI-scoring sites (<25) that passed based on RCA were calculated for all sites and per fish assemblage (or stream habitat) type to evaluate concordance between the two bioassessment methods.
b) If there was evidence that the reference condition was incorrectly defined, for example one of two fish assemblage types had higher failure rates than the other in RCA, the reference condition per habitats associated with each fish assemblage group was evaluated. Specifically, the relationship of IBI scores with landscape and physico-chemical correlates of disturbance, including the integrative disturbance index, was examined using univariate and multivariate regression techniques per habitat type (e.g., stream size).

Step 4. Regional levels of impairment.

a) Levels of impairment were assessed by examining the distribution of IBI scores that failed and passed RCA and by setting thresholds of IBI scores for differentiating streams as unhealthy, questionable or healthy; the latter condition being that able to sustain biological integrity according to Karr and Chu (1999).

3.4 IBI development

Because of the low richness of fish species, we derived an IBI based on an initial evaluation of only 10 candidate metrics. Positive scoring metrics (i.e., those that should increase with increasing biological integrity) included number of fish species, occurrence of white sucker, number and proportion of ≥1 yr fathead minnows, number and proportion of ≥1 yr fish. Potential negative scoring metrics (i.e., those that should decrease with increasing biological integrity) included the proportion of brook stickleback, proportion of DELTs, total number of fish, and number of young-of-year. Some of these candidate metrics were modifications of those described by Karr (1981). For example, the occurrence of white sucker replaced the number of sucker species, as only white sucker occurs in small streams in Alberta (Nelson and Paetz 1992, Scrimgeour et al. 2002, 2003). White sucker is a litho-obligate breeder and previous research has demonstrated that this reproductive guild is particularly sensitive to human disturbance (Steedman 1988, Bramblett et al. 2005). Litho-obligate species breed on rock and gravel and have benthic larvae that hide under stones making successful reproduction challenging in highly turbid waters caused by erosion. Karr's (1981) feeding guild metric of proportion of top carnivore was modified and replaced with abundance indices of ≥1 yr fathead minnow. Although an omnivore, the adult fathead minnow has a larger gape and a larger maximum body size than brook stickleback (W. Tonn, unpublished data), and is potentially most responsive to changes in the composition (e.g., size) of macroinvertebrates in prairie streams (also see Zimmer et al. 2001). In contrast, the proportion of brook stickleback was considered a measure of disturbance because this species is especially tolerant of harsh abiotic conditions such as low dissolved oxygen levels (Klinger et al. 1985). As such, it is considered as a modification of Karr's (1981) metric of "proportion of sunfish (Centrachids)". Abundance indices of ≥1 yr fish (i.e., longer-lived individuals) were expected to increase with the permanence of suitable habitat, connectivity to other populations and

decreased levels of anthropogenic disturbance causing mortality. Like Karr (1981) and many subsequent IBI studies, we examined the proportion of individuals with disease and DELTs. Total number of individuals and catch of YOY were considered for evaluation because they may be indicators of levels of primary production and nutrients from nearby urban and agricultural sources (Steedman 1988, Schleiger 2000). However, these two metrics may measure opposite features of integrity and the number of individual metric, in particular, may be unreliable in an IBI (Karr and Chu 1999).

Given that one of the major criticisms of the IBI approach is the use of redundant metrics (Minns et al. 1994 , Hughes et al. 1998), we constructed an IBI from metrics that were only weakly correlated with each other. We first screened the 10 above-mentioned metrics using correlation analyses to eliminate those metrics that were redundant ($r > 0.6$; see Lyons et al. 2001). If possible, decisions to include or exclude a metric were based on whether a similar metric was incorporated in a published IBI for prairie streams (see Bramblett et al. 2005). In addition, we only considered metrics that showed a relationship ($r > 0.2$) to at least one of the measured correlates of anthropogenic disturbance in our study that were consistently negative or positive (McCormick et al. 2001, Daniels et al. 2002, Drake and Pereira 2002). Next, raw values of IBI metrics were corrected for basin area if they showed a correlation with this variable ($r > 0.2$; Karr and Chu 1999). Raw scores of unadjusted metrics were also calibrated if the final IBI showed a correlation with basin area. Calculations necessary for correction were based on linear or non-linear models, which were evaluated with scatterplots and built using data from reference sites only (i.e., sites that were least affected by anthropogenic activities in our study area). Finally, scores were predicted for all sites and the associated residuals were calculated and transformed to positive values for simplicity, by adding a constant equal to the predicted value for the largest basin area (approximately 200,000 ha).

Each metric was scored on a continuous scale from 0 to 10 and with using lower and upper expectation limits (Minns et al. 1994, Hughes et al. 1998, Bramblett et al. 2005). For a positive metric (i.e., a metric negatively correlated with the level of human disturbance), the upper expectation (ceiling) was the value corresponding to the 95th percentile of the distribution of the metric for all reaches. Sites with a metric value

above the ceiling received a score of 10 whereas all other values were linearly scaled along the range by dividing raw scores with the value at the 95th percentile and multiplying the result by 10. For a negative metric (i.e., a metric positively correlated with the level of human disturbance), the lower expectation (floor) was the value at the 5th percentile of the distribution of all reaches. Metric values below the floor received a score of 10 and remaining values were linearly scaled by dividing the value at the 5th percentile with raw scores and by multiplying the results by 10. As a result, the total IBI score was a sum of all metrics with a perfect score of 50 reflecting conditions appropriate for maintaining biological integrity. Below a disturbance threshold however, the environmental conditions or streamside activities are no longer healthy or capable of supporting systems displaying biological integrity (Figure 3, Karr and Chu 1999).

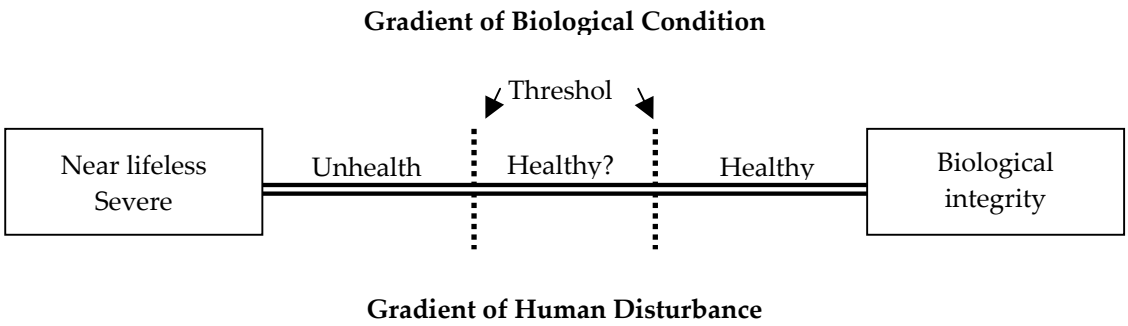

Figure 3. At one end of the continuum of human influence on biological condition, severe disturbance eliminates all or most life. At the other end of the gradient are pristine streams that possess biological integrity. Below a threshold, the conditions or activities may no longer support living systems capable of maintaining biological integrity. Figure adapted from Karr and Chu (1999).

3.5 IBI evaluation

To validate our IBI we first examined its relation with an integrative disturbance score using linear regression. Next, the influence of stream size on IBI scores was examined by adding watershed area to the linear regression model, and the statistical significance of this addition was assessed through examination of P-values. Specifically, $P > 0.15$ indicated a poor predictor of IBI scores and warranted removal of stream size from the model (see stepwise procedure outlined below). We also assessed whether the addition of watershed basin identity (5 major basins outlined above) as a random effect improved the fit of the integrative disturbance model and accounted for potential spatial autocorrelation in IBI scores (Crawley 2002). To compare models, we conducted a likelihood ratio test and chose either the model that had more explanatory power or the simpler model without random effects if differences in likelihoods were non-significant.

A predictive model identifying significant relationships between the 11 disturbance metrics and IBI scores was also constructed through multiple regression and stepwise forward-selection techniques. Using this approach, a variable was entered into the model if its alpha value was < 0.15 and was removed from the model if its alpha value was > 0.15. Prior to constructing the multivariate models, Pearson correlations were also used to identify potential problems of multicollinearity among the 11 variables. If $r > 0.8$, one of the two highly correlated variables was removed from analyses. Finally, we constructed scatterplots of IBI scores and selected water chemistry parameters against study stream position to detect trends in IBI scores and water chemistry based on basin and longitudinal effects related to point-source pollution from villages and towns (Scrimgeour and Chambers 2000), and to natural changes in geo-morphology (Mebane et al. 2003, Allan 2004).

3.5.1 Paired comparison of grazing effects

To evaluate the hypothesis that livestock grazing negatively impacts fish assemblages through local changes to habitat quality, we examined IBI scores and physico-chemical properties on a paired subset ($n = 8$) of the 69 study reaches. Paired reaches consisted of a site heavily impacted by livestock and a nearby site (200-500 m) minimally impacted by livestock. We used ANOVA with site location as an error term to compare both IBI

scores and correlates of disturbance between grazing levels. We accepted statistical significance at the 5% level for all analyses.

3.5.2 Reference condition approach (RCA)

The main steps in an RCA are the selection of reference sites, grouping reference sites into biologically similar types, and comparing the biota of potentially impacted test sites to the biota expected based on the reference condition (Bailey et al. 2004). In our study, we defined reference sites as those with integrative disturbance scores within the lowest quartile of observed values (n = 20). These reference streams may be moderately impacted by anthropogenic activities, as noted earlier, but are the best available reference condition in our study area. Next, multivariate methods were used for classifying reference sites and matching test sites with the appropriate reference group (Reynoldson et al. 1995, Reynoldson et al. 1997). Reference sites were clustered in SYSTAT 11 using both a non-hierarchical K-means method (2 groups) and an agglomerative hierarchical, unweighted pair-groups method with arithmetic averages (UPGMA) on fish species relative abundance (%) data, excluding rare taxa occurring at < 5% of sites (Bowman and Somers 2005). To predict the reference group (i.e., reference fish assemblage) to which a test site was expected to belong, we used Discriminant Function Analyses (DFA; in SYSTAT 11) and environmental variables that were unlikely to be strongly influenced by anthropogenic activity, namely variables that measured stream sizes such as basin area, stream discharge, velocity, wetted width and maximum depth. These variables were included in a stepwise DFA to identify the key variables that differentiate the fish assemblage types defined previously through clustering techniques. A variable was entered into the model if its alpha value was <0.15 and was removed from the model if its alpha value was >0.15.

To determine if the composition of fish assemblages at test sites were within the range of assemblage composition in reference condition, we first ordinated fish assemblage types at reference sites separately and then compared the test site location in ordination space with the assemblage type with the highest probability of membership using data on fish species relative abundance (%) only (Reynoldson et al. 1995). Reference site assemblages were ordinated in PC-ORD using NMDS under the following conditions: Bray-Curtis distance, stability criterion = 0.00001, iterations to evaluate stability = 50, maximum number of iterations = 150, and step length = 0.2 (Mazor et al. 2006). An

initial analysis of 2000 runs with random starting coordinates was used to determine the number of stable dimensions and to provide a solution of starting coordinates for a second analysis of 500 runs, which provided the final configuration. Ordination scores for each of the test sites were determined using the NMDS Scores procedure in PC-ORD. This procedure uses the reference sites and their ordination scores to predict ordination scores for the test sites. It also allows for insertion of new test sites into an ordination without altering the configuration of the original reference group.

We used sample (Ell) plots in SYSTAT 11 for assemblages ordinated in 2 dimensions and designated sites falling outside the 0.9 probability ellipse around reference sites (i.e., the 90% reference range) as different from reference sites and potentially degraded. Test sites outside a 0.99 probability ellipse were designated as severely degraded and with fish assemblages differing dramatically from reference sites (Reynoldson et al. 1995). The reference ranges for assemblages in only 1 dimension were calculated as mean score ± 1.6449·SD (for 0.9 probability range) and mean score ± 2.5758·SD (for 0.99 probability range) (Whitley and Ball 2002).

We compared bioassessment outcomes by examining histograms of IBI scores of sites that failed and passed the RCA. High failure rates in RCA for test sites with high IBI scores or low failure rates for test sites with low IBI scores suggests poor concordance in assessments based on the two approaches. To determine if failure rates varied among fish assemblage types linked to stream size, because for example, the effect of anthropogenic disturbance on fish assemblages is dependent on spatial-scale (Allan 2004), we conducted separate linear regressions of the integrative disturbance index versus IBI scores per fish assemblage type identified using the DFA model described previously.

Finally, we assessed regional levels of stream health using two methods. One method was based on an IBI generated by other authors (e.g., see Hughes et al. 2004) for regions like ours where agricultural landuse occurred at all study sites, including sites used as a reference. The % agriculture in basin ranged 53-84% in our study. To separate streams in healthy versus unhealthy states, we used the IBI score at the 25th percentile for reference sites (Hughes et al 2004). The second method examined the distribution of IBI scores for test sites that failed and passed based on the RCA and 90% reference range.

We determined that an IBI score below the value at the 10th percentile of sites that passed the RCA was indicative of a site in poor condition or health. We considered streams to be in good condition or health if their IBI scores were above the value at the 90th percentile of sites that failed the RCA. This novel method acknowledged a degree of uncertainty, particularly for mid-ranging IBI scores that might easily pass or fail the RCA. IBI scores in this range were identified as being in questionable health or as having an uncertain health status.

4.0 RESULTS

4.1 Characteristics of study sites

The physical characteristics of the 69 study sites varied appreciably among sites (Table 2). For instance, we observed substantial gradients in % of unstable shorelines, % bare soil, % grazed vegetation within riparian zones, water chemistry, including conductivity and concentrations of total phosphorus, total nitrogen, and water turbidity. Landuse practices and road densities also varied appreciably among sites (Table 2).

Table 2. Summary of hydrological, riparian, landscape and watershed characteristics of the 69 study reaches of grassland streams in east-central Alberta.

Variable	Mean	SE	Min	Max
Percent unstable shoreline of reach[1]	12.1	1.92	0	77
Percent wood cover in riparian zone[1]	10.1	1.56	0	58.8
Percent bare soil in riparian zone[1]	10.2	1.31	0	45.8
Percent grazed vegetation in riparian zone	39.2	4.20	0	100
Cattle trail index in riparian zone	0.51	0.043	0	1
Grazed landscape index[1]	3.6	0.224	0	6
Percent fine sediments	62.7	3.51	5	100
Color (mg/L Pt-Co)	134.6	12.10	31.5	554.8
Dissolved Oxygen (DO; mg/L)	11.6	0.19	8.1	16.1
Conductivity (μS/cm)[1]	1237	40	674	2111
Total Suspended Solids (TSS; mg/L)	29.7	4.03	2	166
Turbidity (Nephelometric turbidity unit; NTU)[1]	19.0	2.94	1.5	110
Total phosphorus (TP; μg/L)[1]	218.9	11.7	77.7	467.7
Total nitrogen (TN; μg/L)[1]	2073	59.4	843	3553
Percent landscape in cropland[1]	44.7	2.5	4.6	90.3
Percent agriculture in basin[1]	71.7	0.9	53	84.3
Road density in basin (m/ha)[1]	11.1	0.2	6.8	14.1
Watershed basin area (ha)	61893	4873	7300	184974
Stream discharge (m^3/sec)	0.127	0.013	0.001	0.591
Stream velocity (m/sec)	0.114	0.008	0.006	0.276
Stream wetted width (m)	5.8	0.32	0.79	12.5
Stream maximum depth (cm)	52.3	2.3	17.94	104.1

[1]Used in calculating integrative disturbance score for each site.

4.2 Total catch, DELT occurrence, and relative abundance of fish species

In total, 433 min of electrofishing on 69 stream reaches resulted in the capture of five species and 5,758 fish, most of which were fathead minnow (2,854) and brook stickleback (2,651) (Table 3). Substantially fewer white sucker (159), lake chub (91) and pearl dace (3) were captured. In addition, top predators, such as northern pike (*Esox lucius*), were absent from our catch, and of the approximately 10 landowners in our study who had historical knowledge of local fish abundance, none had ever heard of or encountered northern pike or other large-bodied fish species in the study waters. Deformities, fin erosion, lesion, and tumors were visible on 3.9% of all fishes captured. Blackspot parasite (*Neascus* spp.) was the DELT most frequently observed (75% of all DELTS) and was particularly prevalent in Three Hills Creek where 78% of all fish with blackspot were observed. The proportion of larger and presumably older individuals in our sample was 55% for fathead minnow, 41% for brook stickleback, and 47% for white sucker, but only 15% for lake chub.

Table 3. Total catch and frequency of occurrence of individual fish species captured during electrofishing at 69 reaches of grassland streams in east-central Alberta. Species abbreviations are provided in parentheses.

Species	Catch	Percentage of sites occupied	Trophic classification[c]	Reproductive guild[d]
Brook Stickleback (BRST) – *Culaea inconstans*	2651	100.0 [a]	Planktivore-invertivore; particulate feeder	Ariadnophils (glue-making nesters)
Fathead Minnow (FTMN) – *Pimephales promelas*	2854	88.4 [a]	Detritivore-invertivore; particulate feeder	Hole nesting Speleophils
Lake Chub (LKCH) – *Couesius plumbeus*	91	27.5	Invertivore-planktivore	Open rock and gravel Lithopelagophils
Pearl Dace (PRDC) – *Margariscus margarita*	3	1.4	Invertivore-carnivore	-
White Sucker (WHSC) – *Catostomus commersonii*	159	34.8	Invertivore-detritivore; benthic/filter feeder	Open rock and gravel Lithophils
Total fish	5758			

[a]49% of sites was occupied by both BRST and FTMN only;
[b]75% was those with black spot parasites.
[c]Goldstein and Simon (1999)
[d]Simon (1999)

4.3 Developing the IBI

Correlation analyses indicated moderate levels of collinearity among several of the candidate IBI metrics (Appendix 3). As a result, we modified our initial models to exclude variables that were highly related ($r > 0.8$) to other variables (Table 4). In addition, because of the composition of fish assemblages at our study sites and that only 4% of sites sampled had 1 species whereas 49% of sites had 2 species, we modified the species richness metric to the occurrence of ≥3 species. For richness and the nine other proposed metrics, only % DELT and % BRST exhibited weak correlations with other metrics ($r < 0.6$); correlation analyses (Pearson and Spearman) identified six redundant relationships ($r > 0.6$). Three of these were between total number of fish captured (per 100 sec) and total number of YOY, ≥1 yr fish, and ≥1 yr FTMN (per 100 sec). Thus, total number of fish was deemed to be redundant and removed from the IBI. We chose the metric of % ≥1 yr fish rather than number of ≥1 yr fish as a candidate metric because it was responsive to a higher number of disturbance correlates (8 versus 4), and because Bramblett et al. (2005) successfully included this metric in an IBI for streams in the northwestern Great Plains, USA. We also excluded % ≥1 yr FTMN as a candidate metric due to its correlation with % ≥1 yr fish ($r > 0.6$). We selected the metric of occurrence of white sucker rather than occurrence of ≥3 species because it was responsive to a higher number of variables related to disturbance (3 versus 1; see Table 4). Percent BRST was negatively correlated with % agriculture in basin ($r = -0.22$), but also positively correlated with conductivity and % upstream cropland (range of correlations = 0.31 - 0.46), and was therefore excluded from the final IBI as an ambiguous metric generating both positive and negative scores. Of the remaining non-redundant metrics, all were correlated with either a landscape variable or physico-chemical measure related to disturbance. Thus, the five IBI metrics used in our bioassessment were: 1) % ≥1 yr fish, 2) WHSC occurrence, 4) number of ≥ 1 yr FTMN (per 100 sec), 4) % DELTs, and 5) number of YOY (per 100 sec) (Tables 4 and 5).

Table 4. Summary of candidate and final IBI metrics for bioassessment of grassland streams in east-central Alberta. IBI metrics were screened for redundancy and responsiveness to disturbance (see Appendix 3).

Type	Candidate Metrics	Karr's (1981) original metric	Description	In final IBI?	Redundancy
Taxa richness	1. No. of species [a]	Same metric	Often decreases with degradation	No	Yes (5)
Tolerance, intolerance	2. % brook stickleback	% (by no. of individuals) that are tolerant species	Species is particularly tolerant to low levels of dissolved oxygen	No[c]	None
	3. % ≥1 yr fish	None	Older fish indicative of suitable habitat, reduction in anthropogenic disturbance, stream connectivity	**Yes**	--
	4. No. of ≥1 yr fish	None	Similar to above	No	Yes (10, 7)
Reproductive (habitat) guild	5. Occurrence of white sucker	Number of sucker species[b]	Lithophilous – require silt-free substrates for spawning in interstitial spaces of coble and gravel where embryos develop	**Yes**	--
Trophic guild	6. % ≥1 yr fathead minnow	% top carnivores	Relatively large-gaped omnivore (particulate feeder) that has strong effects on aquatic food webs in prairies	No	Yes (3)
	7. No. of ≥1 yr fathead minnow	Same as above	Similar to above	**Yes**	--
Individual health	8. DELTs	% diseased individuals	Individuals with deformities, disease, parasites, fin erosion, lesions or tumors, reflecting stress often caused by pollution	**Yes**	--
Abundance	9. No. of YOY	No. of individuals	Positively respond to eutrophic conditions or be indicative of suitable habitat, reduction in disturbance, connectivity, etc.	**Yes**	--
	10. Total no. of individuals	Same metric	Positively respond to eutrophic conditions or be indicative of suitable habitat, reduction in disturbance, connectivity, etc.	No	Yes (4, 7)

[a]Later modified to occurrence of ≥3 species because of distribution of species richness among study sites.
[b]Number of sucker species was originally used to measure number of pool-benthic insectivores.
[c]Excluded from IBI due to positive and negative relations with correlates of anthropogenic disturbance.

Table 5. IBI metrics, including watershed area models that were used to calculate residuals of IBI metrics and the minimum and range of residuals and raw values of YOY required for standardizing metrics (0-1).

Metric	Type	Metric Transfor.[1]	Basin Area Model	Min	Range
% ≥1 yr	+ve scoring	NA	$y=57.0292+(6.58E\text{-}15 \times \text{basin area}^3)$	-52.9	95.8
WHSC occurrence	+ve scoring	NA	$y=\frac{e^{[-7.96+(6.98E\text{-}5 \times \text{basin area})+2.75\,(\text{Group 1 or 2})]}}{1+e^{[-7.96+(6.98E\text{-}5 \times \text{basin area})+2.75(\text{Group 1 or 2})]}}$	-0.98	1.88
≥1 yr FTMN / 100 sec	+ve scoring	Square-root	y=7.06-3.03 (Group 1 or 2)	-4.03	9.61
% DELTs	-ve scoring	Square-root	$y=0.4549+(5.8697E\text{-}11 \times \text{basin area}^2)$	-1.94	8.57
YOY / 100 sec	-ve scoring	NA	NA	0	59.5

[1]Transformation required to reduce heteroskedasticity, NA = not applicable.

4.4 Relations between IBI metric scores and measures of human disturbance

We evaluated the responsiveness of the IBI to environmental variables related to anthropogenic disturbance using a suite of univariate and multivariate models (Table 6). Our IBI was negatively related to an integrative disturbance index (univariate linear regression $t_{67} = -5.03$, $P < 0.001$, $R^2 = 0.27$; Figure 4). Analyses also showed that basin area was a poor predictor of IBI scores ($t_{66} = -0.91$, $P = 0.367$). Similarly, the addition of sub-basin identity as a random effect did not improve the overall fit of the disturbance index model (LR = 1.02, P = 0.331), nor change statistical inferences obtained from the relation between the disturbance index and IBI ($P < 0.001$ in both models). To identify correlates of disturbance that were important predictors of IBI scores, we used multivariate techniques and stepwise selection of 11 variables used to construct the disturbance index, plus basin area (Table 6). The final linear regression model included TN, TP and road density in basin ($R^2 = 0.41$). Of these correlates of disturbance, only TN ($t_{65} = -2.6$, $P = 0.011$) and TP ($t_{65} = -3.1$ $P = 0.003$) were significant predictors of IBI scores. A non-significant relationship was noted between road density and IBI scores ($t_{65} = -1.9$, $P = 0.058$).

Table 6. Summary of univariate and multivariate models predicting IBI scores with an integrative disturbance score and basin area, including results from a forward stepwise regression of 11 disturbance parameters and basin area (see Table 1), and from a mixed-effect model with the disturbance score as the fixed effect and basin identity as the random effect. Small and large streams were identified using stream width and depth dimensions in models identified through stepwise DFA of pre-defined fish assemblages.

Models	R^2	Effect	Coefficient	T	P
Small and Large Streams (n = 69)					
Univariate-Disturbance	0.27	Disturbance Index	-0.039	-5.03	<0.001
Univariate-Disturbance with random effect (basin)	NA[a]	Disturbance index	-0.035	-4.22	<0.001
Complete Multivariate - Disturbance and Basin Area	0.28	Disturbance index	-0.041	-5.1	<0.001
		Basin area	1.71E-5	-0.91	0.367
Stepwise Multivariate – basin area and 11 disturbance variables	0.41	TN	-0.0043	-2.6	0.011
		TP	-0.026	-3.1	0.003
		Road density in basin	-0.733	-1.9	0.058
Small Streams (n = 40)[b]					
Univariate- Disturbance	0.18	Disturbance Index	-0.034	-2.9	0.006
Stepwise Multivariate – basin area and 11 disturbance variables	0.47	Conductivity	-0.004	-1.5	0.132
		TP	-0.038	-4.2	<0.001
		Road density in basin	-0.672	-1.5	0.147
Large Streams (n = 29)[c]					
Univariate- Disturbance	0.47	Disturbance Index	-0.046	-4.9	<0.001
Stepwise Multivariate – basin area and 11 disturbance variables	0.58	% non-wood cover	-0.184	-2.7	0.014
		Cattle trail index	-3.72	-1.6	0.131
		Turbidity	-0.149	-2.4	0.022
		% upstream cropland	-0.121	-2.7	0.014

[a]NA = not available.
[b] Mean (±SE) width = 4.2 ± 0.26 m and depth = 42.9 ± 1.9 cm.
[c] Mean (±SE) width = 7.9 ± 0.43 m and depth = 65.3 ± 3.6 cm..

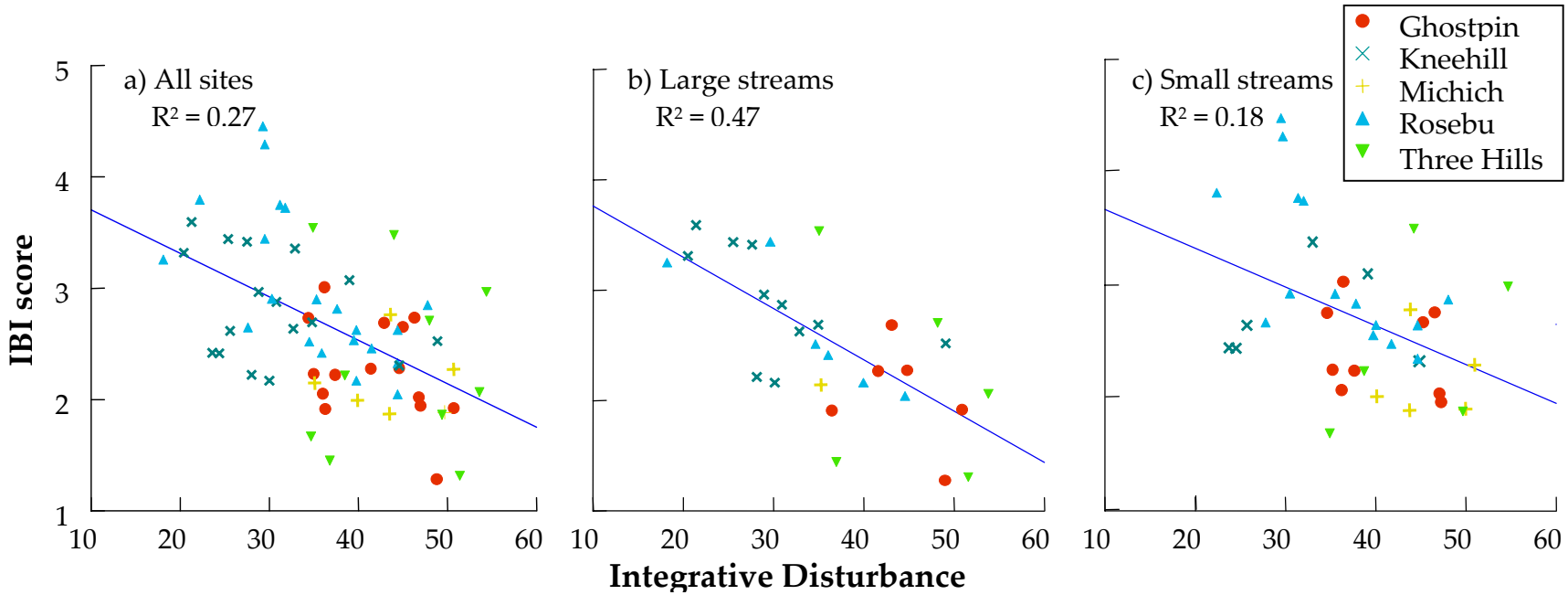

Figure 4. Relationships between IBI scores and the integrative disturbance index for all study sites (a; n = 69), large streams only (b; n = 29) and small streams only (c; n = 40) in five sub-basins of the Red Deer River. R^2 values and lines were estimated using linear least squares regression. The integrative disturbance index was comprised of 11 variables (see Table 1). Small and large streams were identified using stream width and depth dimensions in models identified through stepwise DFA of pre-defined fish assemblages.

The effects of sub-basin and longitudinal position on turbidity, TN, TP and IBI scores were visually assessed using scatterplots for sites on the main stem of the respective study stream. In general, the responses of turbidity, TN, and TP varied appreciably among the major study basins (Figure 5). For example, turbidity (Turb) remained relatively unchanged along most study creeks but increased in Michichi Creek with increasing distance downstream while TN decreased with increasing distance downstream in the Rosebud River. Longitudinal trends in TN and TP were not apparent in the Kneehills, Three Hills and Ghostpine creeks. Water chemistry patterns on Michichi Creek were particularly variable for TP. Of the human settlements in the regions, only Trochu was a potential point-source of pollution and a possible contributor to low IBI scores on Ghostpine Creek (Figure 5). Overall, TN, TP and turbidity levels were highest and IBI scores were lowest on Michichi creek compared to other sub-basins (Figures 5 and 6). The opposite was true of Kneehills Creek, which had relatively high IBI scores and low levels of TN, TP and turbidity (Figures 5 and 6). With the exception of Michichi Creek, IBI scores were in general, highly variable among stream locations within a sub-basin. In addition, average IBI scores were higher in the Rosebud and Kneehills basins compared to sites in the Three Hills, Ghostpine and Michichi basins (Figure 6).

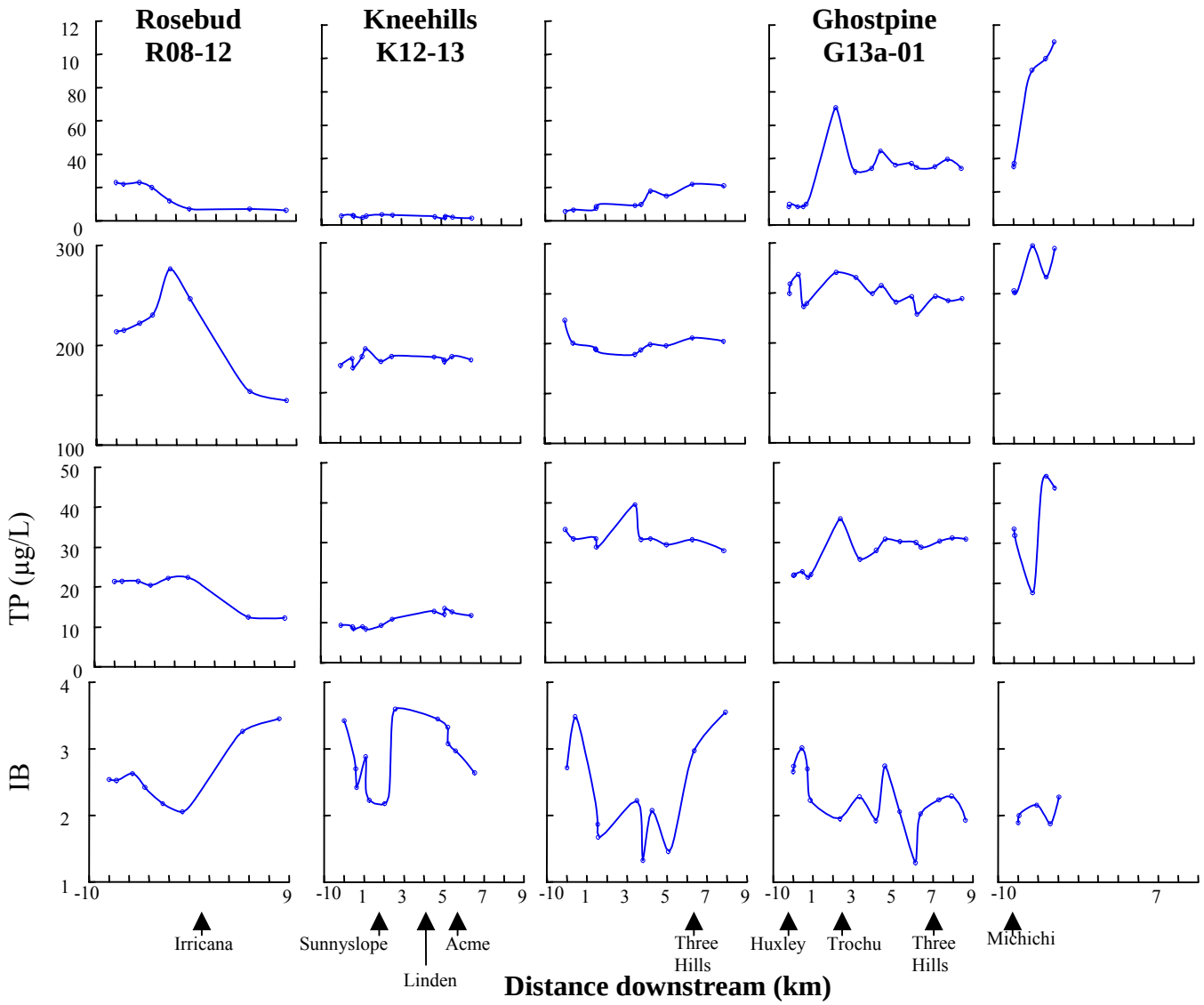

Figure 5. Scatterplots showing relationships between select physico-chemical variables and IBI scores versus distance downstream in Rosebud, Kneehills, Threehills, Ghostpine and Michichi creeks. Arrows indicate locations of human settlements.

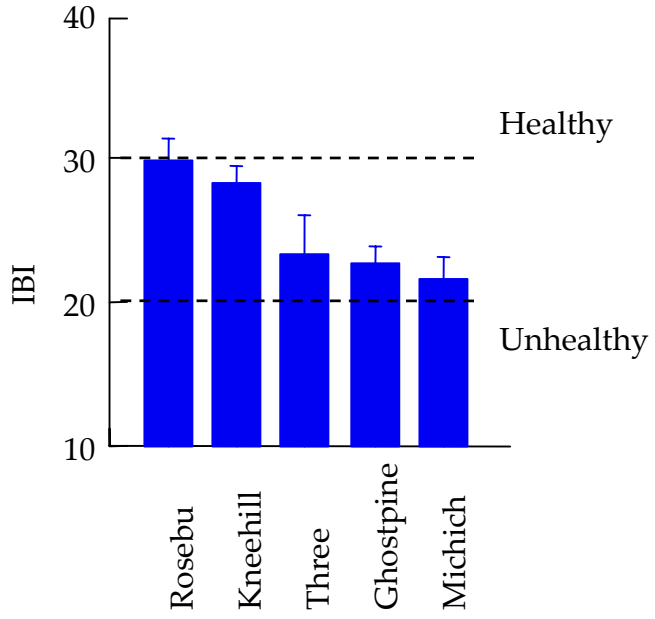

Figure 6. Mean (±SE) IBI scores and IBI thresholds for identifying healthy vs. unhealthy sub-basins of the Red Deer River in east-central Alberta. Order of sub-basins is based on their longitudinal location from west to east.

4.5 Paired site comparison of grazing effects on IBI

We used ANOVA to test for differences in physico-chemical variables and IBI scores between sites that were heavily impacted by livestock with nearby sites that were minimally impacted by livestock (Table 7). These sites were a subset of those used to construct the IBI. The ANOVA showed that the structure and composition of riparian zones varied between the two site types (Table 7) confirming our initial observations of riparian condition during site selection. The heavily impacted sites had greater lengths of unstable shoreline and cattle trails, and greater areas of exposed soil and grazed vegetation, including evidence of intense livestock grazing on the surrounding landscape compared to minimally grazed sites (df = 1,7, $P < 0.05$). Percentage of non-wood cover, a potential indicator of long-term grazing did not differ ($P > 0.05$) between the two site types. Of the water chemistry variables, only TP differed significantly between minimally and heavily grazed sites. On average, TP concentrations were 4%

higher on heavily grazed sites than minimally grazed sites ($F_{1,7}$ = 6.1, P = 0.043). Although the IBI score was 10% higher at minimally grazed sites than heavily grazed sites, scores did not differ statistically (P = 0.12) between the two site types (Table 7).

Table 7. Riparian and water chemistry characteristics of stream reaches (8 pairs) under minimal and intense grazing pressure by livestock. Sites are a subset of those used to construct the IBI. P-values reflect differences between sites as determined by a 1-way ANOVA with site location as a random effect.

Variable	Minimally grazed		Heavily grazed		Percentage change	$F_{1,7}$	P
	Mean	SE	Mean	SE			
Percent unstable shoreline	1.9	0.67	27.5	7.84	1377	9.9	0.016*
Percent non-wood cover	88.8	6.25	95.8	2.28	8	1.3	0.296
Percent exposed soil	3.0	1.28	20.8	5.89	587	8.1	0.025*
Percent grazed vegetation	8.3	3.12	83.2	5.37	900	183.3	<0.001*
Cattle trail index	0.19	0.064	0.75	0.120	287	20.5	0.003*
Grazed land index	2.88	0.409	5.75	0.189	100	37.4	<0.001*
Percent fines	68.0	9.71	69.6	8.21	2	0.0	0.879
Colour (mg/L Pt-Co)	105.9	19.4	107	19.4	1	0.9	0.383
DO (mg/L)	12.3	0.62	12.5	0.52	1	1.5	0.264
Conductivity (μS/cm)	1254	149.9	1250	148.2	0	1.2	0.311
TSS (mg/L)	13.13	4.011	14.25	4.288	9	0.8	0.411
Turbidity (NTU)	9.70	4.067	9.39	3.769	-3	0.7	0.432
TP (μg/L)	170.6	33.0	178.2	34.8	4	6.1	0.043*
TN (μg/L)	1839	192.2	1868	186.2	2	0.5	0.505
IBI	28.1	2.74	25.4	1.63	-10	3.1	0.12

*Statistically significant at P < 0.05

4.6 RCA and variation in the reference condition

The second step in the RCA after identifying reference sites was to group sites based on fish assemblage characteristics. We identified two distinct fish assemblages in our reference sites (number of sites per group were n = 9 and n = 11) using both UPGMA and K-means clustering (Figure 7). Fish assemblage in Cluster 1 (assemblage 1)

comprised all four species and was dominated by fathead minnow (FTMN, 70%) followed by brook stickleback (BRST, 23.9%), white sucker (WHSC, 5.3%) and lake chub (LKCH, 0.4%) (Table 8). LKCH was absent from Cluster 2 (assemblage 2), which was dominated by BRST (80.5%) followed by FTMN (18.4%) and WHSC (1.1%). A stepwise DFA showed that stream depth and wetted width discriminated the two types of fish assemblages ($F_{2,17}$ = 5.8, P = 0.012, eigenvalue = 0.682, canonical correlation = 0.637, % correctly classified based on classification matrix = 85%). Assemblage 1 (large stream assemblage) was found in streams that were, on average (±SE), 8.5 ± 0.9 m wide and 0.64 ± 0.08 m deep while assemblage 2 (small stream assemblage) were in streams that were 4.4 ± 1.0 m wide and 0.42 ± 0.03 m deep (Figure 7). The DFA classification function for large streams was -8.479 + (width x 0.728) + (depth x 0.147), and the function for small streams was: -3.64 + (width x 0.356) + (depth x 0.101). These functions assigned 19 test sites to the large stream fish assemblage and 30 test sites to the small stream fish assemblage.

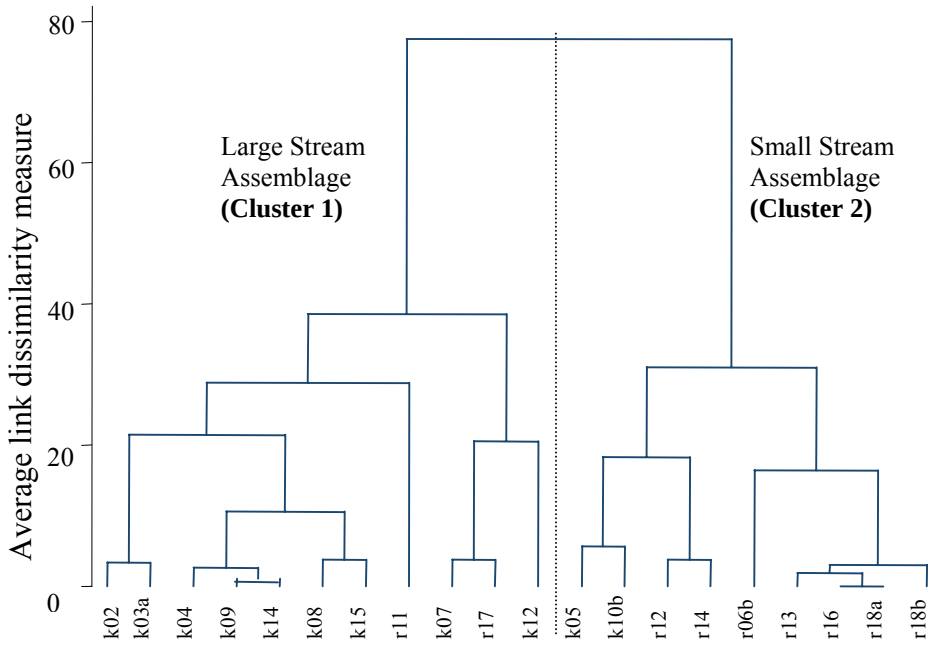

Figure 7. Dendrogram from an unweighted pair-groups cluster (UPGMA) using relative abundance of species from reference sites. Note that a K-means cluster (2 groups) identified the same groups of 9 and 11 sites as being unique fish assemblages whose characteristics are provided in Table 8. The dashed line separates fish assemblages from large streams compared to those from small streams as determined by DFA.

Table 8. Calibration scores and fish composition for reference streams used in the NMDS Scores procedure. Small and large streams were identified using stream width and depth dimensions in models identified through stepwise DFA of pre-defined fish assemblage types (see Figure 7). BRST = Brook stickleback, FTMN = fathead minnow, LKCH = lake chub and WHSC = white sucker.

Site ID	Axis 1	Axis 2	Stream	Percentage composition			
				BRST	FTMN	LKCH	WHSC
Cluster 1							
k02	-0.5211	1.2422	Large	2.83	85.87	2.12	9.19
k03a	-0.3492	1.2992	Large	4.66	86.75	2.05	6.53
k04	0.0755	0.4177	Large	18.97	79.31	0.00	1.72
k07	0.1566	-1.6463	Large	45.83	44.44	0.00	9.72
k08	0.3497	-0.0109	Large	27.66	72.34	0.00	0.00
k09	0.1975	0.5277	Large	18.50	80.92	0.00	0.58
k12	0.3961	-0.6137	Large	38.00	62.00	0.00	0.00
k14	0.1988	0.5391	Large	18.23	81.77	0.00	0.00
k15	0.284	0.1578	Large	25.00	75.00	0.00	0.00
r11	-0.7576	-0.3874	Large	20.00	60.00	0.00	20.00
r17	-0.0302	-1.5253	Large	42.86	46.43	0.00	10.71
Mean				***23.87***	***70.44***	***0.38***	***5.31***
Cluster 2							
k05	1.5112	NA	Small	60.00	38.46	0.00	1.54
k10b	1.5112	NA	Small	64.52	35.48	0.00	0.00
r06b	-1.6275	NA	Small	100.00	0.00	0.00	0.00
r12	0.465	NA	Small	72.00	24.00	0.00	4.00
r13	-0.5812	NA	Small	88.24	10.29	0.00	1.47
r14	0.465	NA	Small	75.00	21.88	0.00	3.13
r16	-0.5812	NA	Small	88.89	11.11	0.00	0.00
r18a	-0.5812	NA	Small	88.89	11.11	0.00	0.00
r18b	-0.5812	NA	Small	86.96	13.04	0.00	0.00
Mean				***80.50***	***18.38***	***0.00***	***1.13***

NA= not applicable

4.7 Pass or fail in the RCA

To determine whether test sites supported similar species abundances to expected assemblages based on reference condition, we first defined the reference condition in ordination space using NMDS, which identified 2 axes for large streams and 1 axis for small streams under our stability criterion (Table 8). Based on a 90% probability ellipse constructed from ordination scores of reference sites, 63% of large stream test sites failed to contain a fish assemblage predicted by the reference condition (Figure 8). Further, 37% of large stream test sites fell outside the 99% probability ellipse. Failure rates for individual test sites were considerably lower for small stream test sites (40% fell outside the 90% reference range whereas none failed at the 99% level) than for large test sites (Figure 8). Overall, 49% of test sites failed the 90% reference range criterion and 14% (all on large streams) failed the 99% reference range.

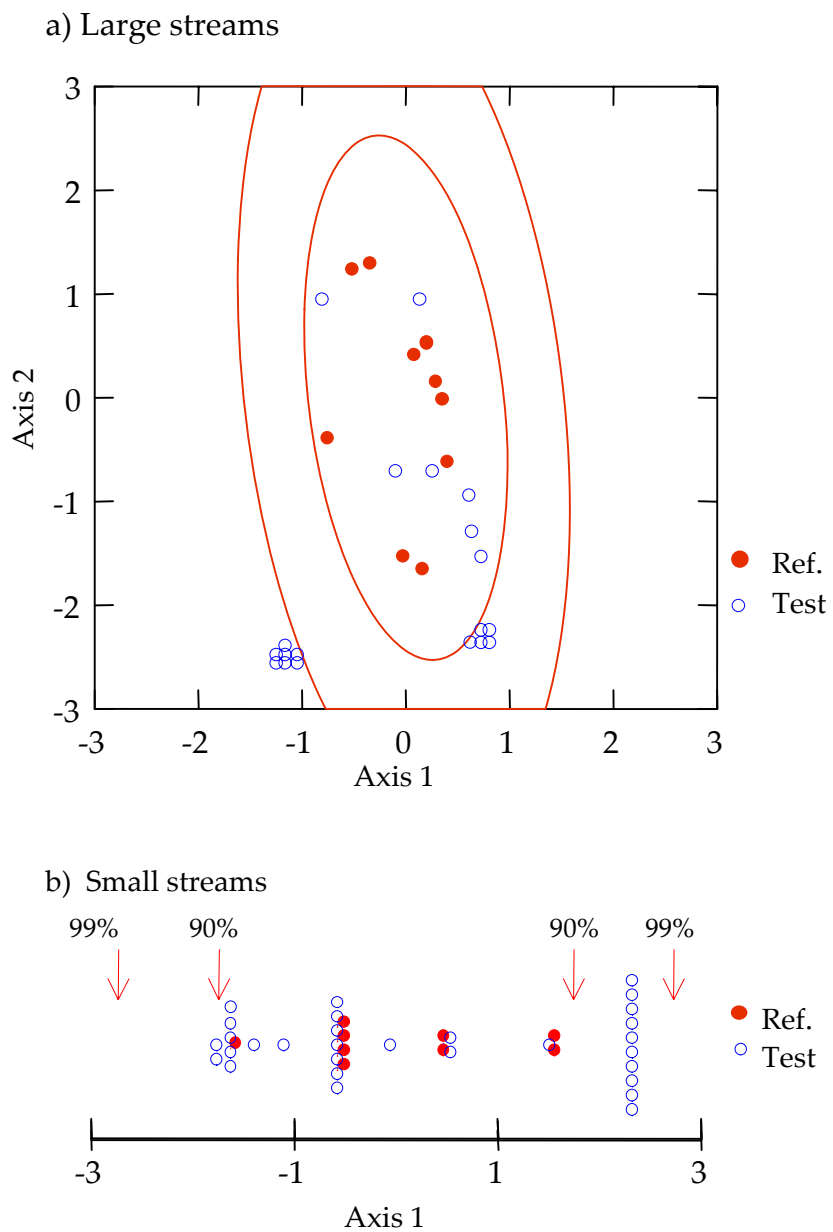

Figure 8. Ninety and 99% reference ranges of fish assemblages in ordination space for large streams (a) and small streams (b) with test sites passively plotted in their ordination space using NMDS Scores procedure. See Table 8 for details on the reference range identified using NMDS. Test sites outside the reference range had fish assemblages that were different and potentially indicative of degraded streams unable to maintain biological integrity.

4.8 RCA considerations for assessments of streams in east-central Alberta

An overall evaluation of RCA test site results using the 90% reference range versus IBI score, suggested poor concordance between the two bioassessment methods: 48% of sites with low IBI scores (<25) passed and 45% of sites with high IBI scores (>25) failed. Interestingly, compared to large stream test sites, a relatively high proportion of small stream test sites (66 vs. 25%) that met the 90% reference range criterion had a low IBI score (<25) (Figure 9). The proportion of high IBI-scoring test sites (>25) that failed was also slightly higher on small streams (47%) than on large streams (43%). These results, in addition to the overall low failure rates of small streams in the RCA, suggest that the criteria for identifying reference sites may be flawed for small streams.

To aid with identification of the reference condition, we constructed disturbance models to predict IBI scores on small and large streams separately (see Table 7). Sites were assigned to a particular stream size using DFA functions mentioned previously. Results clearly show that both the IBI measurement of human disturbance and criteria for the reference condition are dependent on stream width and depth. For example, on small streams (n = 40), a univariate model predicting IBI scores using the integrative disturbance index resulted in a low R^2 value of 0.18 versus 0.47 for the model for large streams (n = 29) (see Figure 4). A stepwise multivariate regression of basin area and 11 physico-chemical parameters (from the disturbance rank) showed that conductivity, TP and road density in basin were important predictors of IBI scores in small streams ($R^2 = 0.41$). However, only TP was significantly related with the IBI ($t_{36} = -3.1$, $P = 0.003$). On large streams, a different suite of disturbance correlates were identified as important predictors of IBI scores. Specifically, % non-wood cover, cattle trail index, turbidity and % upstream cropland were chosen for the final model ($R^2 = 0.58$), and of these variables, % non-wood cover ($t_{24} = -2.7$, $P = 0.014$), turbidity ($t_{24} = -2.4$, $P = 0.022$) and % upstream cropland ($t_{24} = -2.7$, $P = 0.014$) were significantly related with IBI scores on large streams.

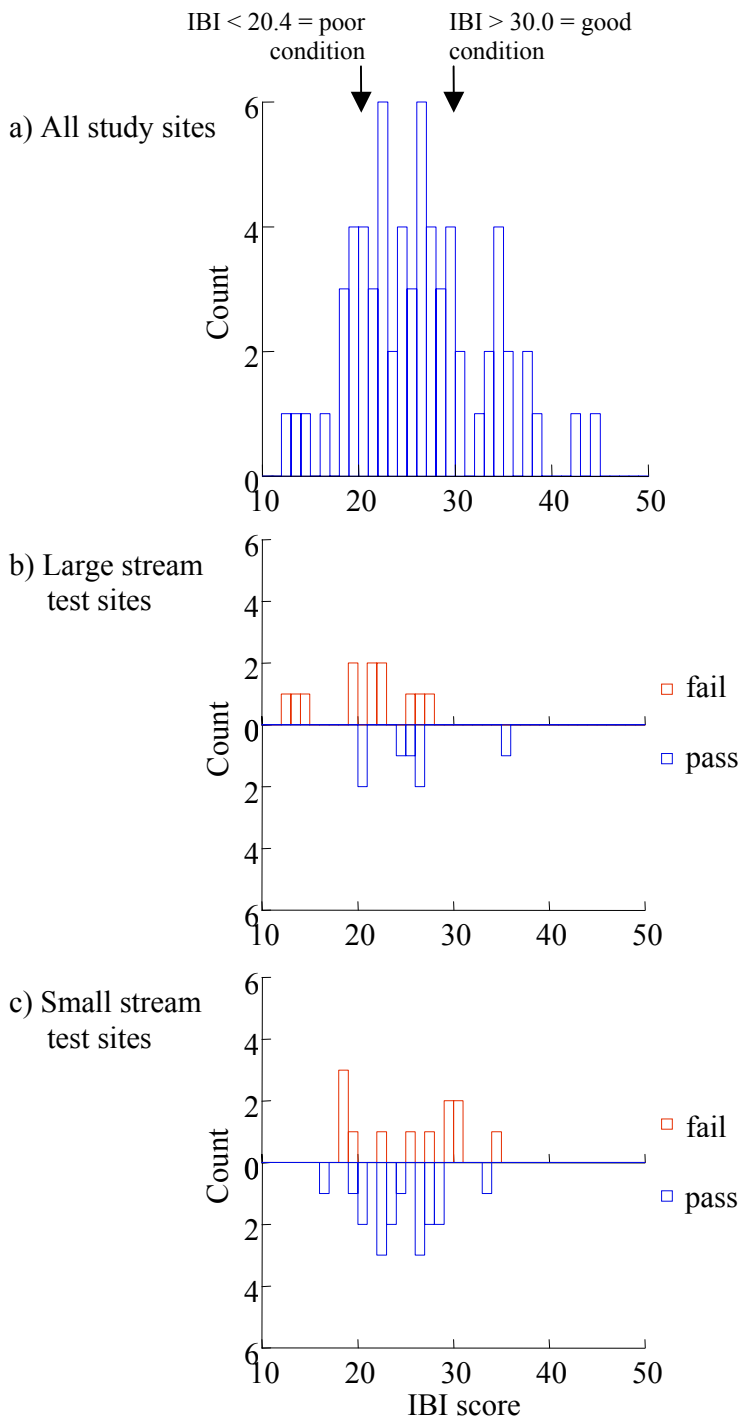

Figure 9. Histogram of IBI scores for all sites (a), and for test sites that failed and passed the RCA on large (b) and small streams (c) according to 90% reference range (also see Figure 6). We used high (90th) and low (10th) percentiles of IBI scores from test sites that failed and passed reference range, respectively, to assess levels of impairment for IBI.

4.9 Identifying levels of impairment using both IBI and RCA

Our method used high and low percentiles of IBI scores from test sites that failed and passed 90% reference ranges, respectively, to assess levels of impairment connected to IBI scores. Specifically, calculation of the 90th percentile of IBI scores for failed test sites in the RCA indicated that a score above 30 characterized a site in good health. In contrast, calculation of the 10th percentile for test sites that met the reference condition criteria revealed that sites with IBI scores below 20.4 should be considered in poor health. An alternative method of identifying levels of impairment, which used the score at the 25th percentile of reference sites, found that 26.5 was the threshold for distinguishing between healthy and unhealthy conditions.

5.0 DISCUSSION

5.1 A novel IBI to assess the integrity of grassland streams

Our pilot study developed a potentially useful IBI for bioassessment of northern grassland streams using a fish fauna consisting of only five species, including species generally considered tolerant of disturbance, such as fathead minnow and white sucker (Lyons et al. 1996, Lyons et al. 2001, Drake and Pereira 2002, Bramblett et al. 2005). The successful development of a fish-based IBI for our system counters general opinions expressed in the published literature, particularly comments on the type of assemblage required to construct an IBI (Mebane et al. 2003, Bramblett et al. 2005). In deed, no previous researchers have developed an IBI for an assemblage similar to that encountered in our study (see Appendix 1). Bramblett et al. (2005) proposed that their IBI, which was developed for Montana prairies streams and used 42 species of fish, could be applied in other semiarid regions, and yet some of their metrics, e.g., number of benthic invertivores, could not be calculated for our study streams because the group was absent. An admitted shortcoming of our study included the lack of appropriate reference conditions, and an independent dataset for further evaluation of the IBI, which is required to support the wide application of our IBI to prairie and northern ecoregions with depauperate faunas and even larger rivers where the same species might occur in richer assemblages.

Interestingly, our IBI detected changes in fish assemblages linked to human disturbance even in a system characterized by large natural fluctuations in physico-chemical conditions, such as stream flow and temperature (Dodds et al. 2004). A possible explanation may be the high level of agricultural disturbance in our study watersheds (53–84%), which may have overwhelming effects on nutrient and sediment measures in streams (Johnson et al. 1997, Allan 2004). The IBI in our study was responsive to an index integrating various landscape and physico-chemical correlates of disturbance at various spatial scales. We also showed that TP and TN were particularly important variables affecting IBI scores. Although the mechanisms are unclear, high nutrients may alter food webs through changes in the composition of basal algal resources, create harmful algal blooms in slow moving waters (Kalff 2002) or promote eutrophication and high numbers of snails which are the first intermediate host for parasites (e.g., *Neascus* spp.) that later encyst as black-spots in fish (Blouin et al. 1984). Non-point sources of these nutrients in our study may include manure from grazing livestock and fertilizers (also see review by Carpenter et al. 1998). The inclusion of road density in the final model predicting IBI scores may be a result of its association with large farming operations, such as feedlots, and point-source pollution from these operations. Fragmentation is also possible but almost all road crossings we encountered in the study area were passable and not a threat to fish movements.

The high proportion of agricultural land in watersheds (53-84%) may be of particular concern for the health of grassland streams in Alberta and is considerably higher than that reported for other prairie ecoregions (x = 28% reported for Great Plains streams in Montana, Bramblett et al. 2005), and beyond the proposed 50% threshold of land in agriculture where streams in forested watersheds can remain healthy (see Wang et al. 1997, and review by Allan 2004). However, further research encompassing a broader range of agricultural disturbance than that documented in our study is needed to determine if this 50% threshold applies to the biotic integrity of grassland watersheds. Interesting to note was the varying response of IBI scores to disturbance between small and large streams and the potential problem this presents when defining an *a priori* reference condition for streams in highly modified landscapes such as ours.

5.2 RCA in east-central Alberta

Although the use of the RCA in environmental assessments is becoming more prevalent, it remains untested against the more commonly-used IBI method, nor have the limitations in the use of RCA been thoroughly examined. One of the unanswered questions in the RCA method is what criteria or accepted method should be used for identifying reference conditions (also see Bowman and Somers 2005). Because streams can be affected by multiple, interacting anthropogenic disturbances, the identification and selection of reference sites could potentially be problematic in highly modified landscapes. We identified two types of fish assemblages occurring in reference streams of east-central Alberta. One community was dominated by brook stickleback and found in temporary habitats, specifically streams that were narrow and shallow. The second community consisted predominantly of fathead minnow and was found in more permanent habitats with streams that were wide and deep. These fish assemblage patterns may reflect dispersal abilities and species-specific resilience to stress caused by varying hydrological conditions. That the failure rate of small-stream test sites was considerably lower than that of large streams suggests that the small-stream fish assemblage was more resilient to disturbance, or alternatively, that the reference condition was incorrectly identified for streams that are relatively narrow and shallow. Supporting the latter possibility were results from regression models predicting IBI scores with the integrative disturbance rank, which was also used in selecting the best available reference streams prior to testing. Specifically, the fit of the large stream model was almost twice as good as that of the model using small stream data only. At least in our study, the small stream reference condition should be better defined and should differ from that used for larger stream sites to reflect differences in groundwater influences on TP concentrations (Hancock 2002), which was the only variable significantly related to IBI scores in the small stream model. Although agricultural landuse patterns often have an overwhelming effect on nutrient (and sediment) measures in streams (Johnson et al. 1997, Allan 2004), we must acknowledge that variations in TP could reflect covariation of anthropogenic and natural landscape features, such as soil or geo-morphological characteristics of watersheds. Other sources of variation in IBI scores may be refuges for winter and drought, such as natural springs and beaver ponds, which were not accounted for in this study and may be particularly important for fish in smaller streams.

5.3 A regional assessment of stream impairment

The IBI may be a valuable tool for resource management when it is used to compare biotic integrity among sites. The IBI can also be used to make regional assessments based on criteria of what IBI scores represent streams in either "healthy" or "degraded" states. However, criteria concerning the condition of sites vary from study to study illustrating the subjectivity in making such decisions (Hughes et al. 2004). For their IBI, Hughes et al. (2004) proposed using scores less than values at the 25th percentile of reference sites for identifying impairment (i.e., <60 IBI score of 100) given that their reference sites were in agricultural valleys and potentially of poor quality.

Like Hughes et al. (2004), we believed our reference sites to be of questionable health but proposed two options for regional bioassessment. The first used a traditional method and the value at the 25th percentile of reference study sites (IBI score= 26.5) based on criteria in Hughes et al. (2004). Approximately 45% of our study sites had values above this threshold and were therefore, potentially in good condition and health. The other method was an evaluation of the distribution of IBI scores of sites that passed and failed in RCA. Specifically, a threshold based on the value at the 10th percentile of test sites that matched the reference condition was an IBI score of 20 (out of a total score of 50; 19% of streams were impaired) and a threshold based on the value at the 90th percentile of test sites that failed was an IBI score of 30 (out of a total score of 50; 23% of streams were healthy). The health of approximately 58% of wadeable streams in east-central Alberta was questionable. Although percentiles and thresholds were subjectively determined, this second option for regional assessment is potentially more rigorous than previous IBIs in that we have set impairment levels using results from another bioassessment technique (RCA). In addition, this second option acknowledges a degree of uncertainty in whether streams with mid-ranging scores are unhealthy, as determined by RCA.

5.4 Review of temporal stability of IBIs

A potential flaw in any IBI is high variability in scores among seasons and years and a general lack of precision (Hughes et al. 1998). Unfortunately we were unable to test temporal variability in sites scores within and among seasons and years. However, previous IBIs have shown little intraseasonal variation in scores despite differences in

flows, temperature, and crews when sites were resampled (Hughes et al. 1998, McCormick et al. 2001). In addition, Bramblett et al. (2005) contend that prairie stream habitats, although temporally variable, can have reasonably stable IBI scores within and between years as long as water is present (also see Fausch and Bramblett 1991).

5.5 Conclusions and caveats

Our study demonstrated that it is possible to develop a multimetric fish-based index for northern grassland streams. Our IBI incorporated functional metrics based on relatively few species but included those that are widely distributed in the prairies and boreal forest (Nelson and Paetz 1992). The IBI was also unrelated to watershed area, reducing the upstream-downstream variability of scores, but was responsive to the effects of both livestock grazing in riparian zones and upstream landscape in cropland, particularly on larger streams (maximum basin size in our study = 1,845 km^2). With the aid of a simple spreadsheet, formulas provided in Table 5 and information on stream size, land managers and researchers in east-central Alberta can quickly assess stream habitats using data collected on their fish assemblages. We also recommend that assessments incorporate data on other biota, such as macroinvertebrates, and additional information on physical and chemical attributes whenever possible. Our IBI will be most useful when it complements rather than replaces other measures of environmental quality and biotic integrity. However, whether the IBI in this study can be useful for bioassessment of streams in other watersheds and of fish assemblages with true piscivores and higher trophic elements remains unknown. A generalization of our method to the grassland ecoregion is possible but only by collecting new data particularly in other watersheds reflecting a broader range of agricultural disturbance, and by recalibrating models. We acknowledge that our methodology could be improved by a better understanding of underlying mechanisms of fish assemblage patterns and metric responses to human disturbance. The correct application and interpretation of IBIs will aid in the conservation of grassland streams and the services they provide to Albertans.

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7.0 APPENDIX

Appendix 1. Summary of recent studies evaluating the ecological health of stream and lakes ecosystems using a fish-based index of biological integrity (IBI) (14 studies) or reference condition approach (1 study) between 2000 and 2005. Studies were identified based on a review of three dominant journals publishing the results of studied aquatic ecosystems [i.e., Canadian Journal of Fisheries and Aquatic Sciences (CJFAS), Transactions of the American Fisheries Society (TAFS), North American Journal of Fisheries Management (NAJFM)]. The search query used "fish".

	Journal	Study Region	Habitat Type	No. spp.	IBI metrics / candidates	Metrics 'new' or from previous study?	Major disturbance in basins
Index of Biotic Integrity (IBI)							
Bramblett et al. 2005	TAFS	Great Plains, Montana	Warmwater prairie streams (2nd -7th order)	42	10/32	Karr 1981 and other published work	Agriculture, natural gas extraction
Santucci et al. 2005	NAJFM	Fox River, Illinois	Warmwater river (2435-4453 km^2 drainage area)	30	12/12	Karr 1981	Dam impoundments
Drake & Valley 2005	NAJFM	Central Minnesota	Small lakes (46-203 ha)	56	16/16	Drake and Pereira 2002	Agriculture, urbanization
Hughes et al. 2004	TAFS	Western Oregon, Washington	Coldwater streams (0.1-160 km^2 drainage area)	38[a]	8/109	New	Agriculture, urbanization, roads
Mebane et al. 2003	TAFS	Pacific Northwest, USA	Cold water rivers (>4th order)	30	10/16	Previous[b]	Agriculture
Wang et al. 2003	TAFS	Wisconsin, Minnesota	Small, cold streams (3.5-153.1 km^2 drainage area)	18	10/10	5 from Lyons et al. 1996; 5 new	Agriculture, urbanization
Daniels et al. 2002	TAFS	Northeastern USA	Forest streams (19-1903 km^2 drainage area)	>19	12/35	Previous[c]	Agriculture and urbanization
Drake & Pereira 2002	NAJFM	Central Minnesota	Small inland lakes (111-125 ha)	53	16/30	New	Agriculture, urbanization
Goldstrein et al. 2002[d]	NAJFM	Minnesota, Wisconsin, Michigan	Small streams (1st- 3rd order; 1-458 km^2 drainage area)	NA	5/5	Lyons et al. 1996 (71 spp., 22 candidate metrics)	Forestry, agriculture

McCormick et al. 2001	TAFS	Mid-Atlantic Highlands, USA	Low-order (1st-3rd) streams	137	9/58	New and modified metrics from Karr 1981	Agriculture, forestry, mining
Smogor & Angermeier 2001	TAFS	Virginia	Warm streams (3rd-6th order; 7-454 km^2 drainage area)	134	14/14	Angermeier et al. 2000 (14 spp, 62 candidate metrics)	Mining, urbanization, forestry
Lyons et al. 2001	TAFS	Wisconsin	Large, warm rivers (order and drainage area = NA)	90	10/26	New	Impoundments, industrial-municipal waste
Stauffer et al. 2000	CJFAS	Minnesota River basin	Streams (246-813 km^2 drainage area)	49	12/12	Karr 1981	Agriculture
Schleiger 2000	TAFS	West-central Georgia	Streams (1st-5th order; 66-87936 ha drainage area)	36	12/12	Karr 1981	Agriculture, forestry, urbanization
Reference Condition Approach (RCA)							
Tonn et al. 2003	TAFS	Boreal Plains, Alberta	Lakes (14-206 ha)	10	NA	NA	Forestry

NA = not available.

[a]Includes 12 amphibian species; (1981).

[b]Candidate metrics chosen from IBIs in government reports, dissertations, unpublished literature, etc.

[c]Selected metrics from potentially 35 IBI metrics including 12 IBI metrics of mid-western streams (Karr 1981), 14 IBI metrics of Virginia streams (Angermeier et al 2000) and 9 IBI metrics of mid-Atlantic highland streams (McCormick et al. 2001).

[d]Used both a warmwater and coldwater IBI but only coldwater metrics reported here.

Appendix 2. Easting and northing UTMs of 69 study sites near Three Hills, Alberta.

Site	EAST	NORTH	Major sub-basin	Creek or river name
G01	358286	5721534	Ghostpine	Ghostpine Creek
G02	356571	5726758	Ghostpine	Ghostpine Creek
G03	355571	5730461	Ghostpine	Ghostpine Creek
G04	355028	5731505	Ghostpine	Ghostpine Creek
G05	354008	5733846	Ghostpine	Ghostpine Creek
G06	353587	5735785	Ghostpine	Ghostpine Creek
G07	353608	5737209	Ghostpine	Ghostpine Creek
G08	352996	5740166	Ghostpine	Ghostpine Creek
G09	350643	5743501	Ghostpine	Ghostpine Creek
G10	347494	5747748	Ghostpine	Ghostpine Creek
G11	347224	5748754	Ghostpine	Ghostpine Creek
G12	346482	5750364	Ghostpine	Ghostpine Creek
G13a	345710	5751802	Ghostpine	Ghostpine Creek
G13b	345952	5751850	Ghostpine	Ghostpine Creek
G14	357041	5724174	Ghostpine	Ghostpine Creek
K01	331476	5712041	Kneehills	unnamed tributary
K02	332417	5711680	Kneehills	Kneehills Creek
K03a	331712	5713434	Kneehills	Kneehills Creek
K03b	331862	5713383	Kneehills	Kneehills Creek
K04	329738	5715234	Kneehills	Kneehills Creek
K05	320832	5718563	Kneehills	Lonepine Creek
K06	318898	5715792	Kneehills	unnamed tributary
K07	319110	5721802	Kneehills	Lonepine Creek
K08	323013	5725236	Kneehills	Kneehills Creek
K09	323559	5722584	Kneehills	Kneehills Creek
K10a	318786	5731988	Kneehills	Kneehills Creek
K10b	318625	5731654	Kneehills	Kneehills Creek
K11	317657	5736985	Kneehills	Spruce Creek
K12	319320	5734818	Kneehills	Kneehills Creek
K13	336377	5709472	Kneehills	Kneehills Creek
K14	321482	5728459	Kneehills	Kneehills Creek
K15	320770	5729433	Kneehills	Kneehills Creek
M01a	392162	5713634	Michichi	Michichi Creek
M01b	392120	5713308	Michichi	Michichi Creek
M02	391868	5709556	Michichi	Michichi Creek
M03	388387	5707058	Michichi	Michichi Creek
M04	383863	5713468	Michichi	West Michichi Creek
M05	390022	5707464	Michichi	Michichi Creek
R01	310337	5699532	Rosebud	Rosebud River
R02	318098	5693228	Rosebud	Rosebud River
R03	313943	5696207	Rosebud	Rosebud River
R04a	306731	5701000	Rosebud	Carstairs Creek

R04b	306935	5700942	Rosebud	Carstairs Creek
R05	308156	5703048	Rosebud	Rosebud River
R06a	303574	5703148	Rosebud	Carstairs Creek
R06b	303766	5703074	Rosebud	Carstairs Creek
R07	301814	5703763	Rosebud	Carstairs Creek
R08	303952	5707963	Rosebud	Rosebud River
R09	305254	5706364	Rosebud	Rosebud River
R10	319639	5690928	Rosebud	Crossfield Creek
R11	326610	5689186	Rosebud	Rosebud River
R12	330977	5688916	Rosebud	Rosebud River
R13	317777	5690061	Rosebud	Crossfield Creek
R14	316140	5687643	Rosebud	Crossfield Creek
R15	309106	5686797	Rosebud	Crossfield Creek
R16	311010	5684350	Rosebud	Crossfield Creek
R17	311347	5681308	Rosebud	unnamed tributary
R18a	313479	5683389	Rosebud	unnamed tributary
R18b	313529	5683279	Rosebud	unnamed tributary
T01	332548	5748114	Three Hills	Three hills Creek
T02	332623	5749792	Three Hills	Three Hills Creek
T03a	333694	5744244	Three Hills	Three Hills Creek
T03b	333748	5744045	Three Hills	Three Hills Creek
T04	335616	5739396	Three Hills	Three Hills Creek
T05	335340	5737865	Three Hills	Three Hills Creek
T06	334967	5735920	Three Hills	Three Hills Creek
T07	336998	5734696	Three Hills	Three Hills Creek
T08	338653	5730970	Three Hills	Three Hills Creek
T10	340450	5727204	Three Hills	Three Hills Creek

Appendix 3. Correlation matrix of candidate IBI metrics and environmental parameters. IBI metrics that were moderate-highly correlated with one another (r > 0.6, in bold) were considered redundant. Environment-metric relationships were identified at r > 0.2, and high correlations between environmental parameters (r > 0.8) were potential contributors to multi-collinearity (in bold). Columns and rows in grey were final IBI metrics screened for redundancy and responsiveness to anthropogenic disturbance. % graz. = % grazed.

	≥3spp occ1	total	YOY	>1yr	% >1yr	%> 1yr FTMN	>1yr FTMN	% DELT	% BRST	WHSC occ1	% unst. shore	% non-wood	cattle trail	% exp. soil	% graz.
total	0.20	1.00													
YOY	0.02	0.73	1.00												
>1yr	0.25	**0.81**	0.19	1.00											
%>1yr	0.27	-0.05	-0.54	0.40	1.00										
%>1yr FTMN	0.22	0.07	-0.28	0.35	**0.60**	1.00									
>1yr FTMN	0.21	**0.77**	0.15	**0.98**	0.38	0.35	1.00								
%DELT	0.19	-0.06	-0.04	-0.05	0.04	-0.06	0.00	1.00							
%BRST	-0.35	-0.53	-0.32	-0.48	-0.25	-0.17	-0.54	-0.27	1.00						
WHSC occur[1]	**0.81**	-0.11	-0.22	0.03	0.31	0.06	0.07	0.45	-0.31	1.00					
%unstable shore	-0.04	0.14	**0.34**	-0.08	**-0.21**	-0.19	-0.05	0.11	-0.06	-0.09	1.00				
%non-wood	**-0.38**	**-0.34**	-0.17	**-0.34**	0.04	-0.02	**-0.37**	0.16	0.16	**-0.24**	0.21	1.00			
cattle trail	0.13	0.03	0.11	-0.05	-0.06	0.09	-0.07	**0.20**	-0.03	0.06	0.38	0.05	1.00		
%exposed soil	0.06	0.06	0.17	-0.06	-0.10	-0.07	-0.05	**0.22**	-0.09	0.01	0.69	0.11	0.63	1.00	
%grazed	0.02	-0.16	-0.08	-0.17	0.00	0.10	-0.18	**0.27**	0.13	0.04	0.43	0.32	0.55	0.58	1.00
%fines	0.17	0.10	-0.02	0.16	0.05	0.10	**0.20**	0.06	-0.11	0.18	0.09	0.02	-0.09	-0.03	-0.03
colour	0.00	0.16	**0.37**	-0.09	**-0.40**	**-0.24**	-0.07	-0.17	-0.08	**-0.27**	0.08	-0.40	0.13	0.13	-0.24
DO	-0.15	-0.16	-0.15	-0.10	0.12	-0.09	-0.14	-0.08	**0.31**	-0.12	0.16	0.35	-0.01	0.09	0.20
TSS	0.07	0.00	0.19	-0.17	**-0.37**	-0.11	-0.18	-0.18	0.18	-0.17	-0.01	-0.17	-0.02	0.10	-0.16
Cond.	-0.16	-0.10	0.13	**-0.26**	**-0.54**	**-0.36**	**-0.29**	-0.14	**0.46**	**-0.37**	0.12	-0.18	0.05	0.15	0.01
NTU	0.07	0.10	**0.34**	-0.16	**-0.41**	**-0.23**	-0.16	-0.16	0.01	-0.19	0.02	-0.31	0.01	0.14	-0.21
TN	0.05	0.07	**0.27**	-0.13	**-0.53**	**-0.30**	-0.14	-0.12	0.14	-0.19	0.02	-0.17	0.11	0.06	-0.15
TP	0.02	-0.04	**0.23**	**-0.26**	**-0.55**	**-0.38**	**-0.25**	**0.23**	0.19	-0.17	0.04	-0.12	0.10	0.20	-0.05
Grazed land	0.01	-0.16	-0.13	-0.12	0.08	0.11	-0.11	**0.26**	0.07	0.06	0.39	0.27	0.60	0.58	**0.80**

%upstream crop	-0.05	-0.13	0.01	**-0.20**	**-0.23**	**-0.21**	**-0.20**	0.13	**0.32**	-0.10	-0.03	0.04	-0.19	0.04	0.06
%agr. in basin	-0.02	0.04	-0.05	0.10	0.20	0.16	0.14	0.14	**-0.22**	0.05	0.00	-0.21	-0.02	0.08	0.00
Roads in basin	-0.20	0.04	-0.13	0.16	0.13	-0.06	0.13	0.16	-0.03	0.03	0.00	0.31	-0.13	-0.13	0.07
Dist. index	-0.12	-0.13	0.16	**-0.33**	**-0.48**	**-0.33**	**-0.33**	**0.30**	**0.28**	-0.27	0.44	0.22	0.46	0.58	0.48
IBI	0.34	-0.05	**-0.40**	**0.28**	**0.80**	**0.55**	**0.28**	-0.15	-0.17	**0.57**	-0.12	-0.03	-0.08	-0.10	-0.06
Basin area	**0.28**	**0.34**	-0.04	**0.53**	**0.40**	**0.30**	**0.54**	0.08	**-0.44**	**0.33**	-0.10	-0.16	-0.03	0.00	-0.10
Discharge	**0.27**	0.12	-0.16	**0.32**	**0.49**	**0.36**	**0.33**	0.05	**-0.31**	**0.40**	-0.13	-0.09	-0.07	-0.08	-0.08

[1]Spearman correlations whereas all others were Pearson correlations.

Appendix 3 Continued.

	% fines	Colour	DO	TSS	Cond.	NTU	TN	TP	Grazed land	% upstr. crop	% agr. in basin	Roads in basin	Dist. index	IBI	Basin area
Colour	-0.09	1.00													
DO	0.17	-0.35	1.00												
TSS	-0.07	0.62	-0.05	1.00											
Cond.	-0.06	0.37	0.12	0.45	1.00										
NTU	-0.11	**0.87**	-0.21	**0.88**	0.42	1.00									
TN	-0.01	0.66	-0.07	0.71	0.56	0.70	1.00								
TP	-0.18	0.48	-0.21	0.54	0.51	0.59	0.64	1.00							
Grazed land	-0.02	-0.22	0.18	-0.25	-0.05	-0.28	-0.23	-0.14	1.00						
%upstream crop	-0.26	0.07	0.06	0.29	0.27	0.27	0.19	0.45	-0.25	1.00					
%agr. in basin	-0.20	0.06	-0.62	-0.32	-0.11	-0.13	-0.44	-0.14	0.07	-0.11	1.00				
Rds in basin	-0.14	-0.60	0.23	-0.36	-0.03	-0.54	-0.19	-0.16	0.10	-0.05	-0.11	1.00			
Dist. index	-0.21	0.27	0.05	0.40	0.60	0.38	0.54	0.68	0.36	0.40	-0.14	0.05	1.00		
IBI	0.12	-0.29	0.04	-0.35	-0.46	-0.35	-0.54	-0.57	0.11	-0.27	0.21	-0.06	-0.52	1.00	
Basin area	0.01	-0.16	0.04	0.01	-0.29	-0.09	-0.01	-0.13	-0.14	-0.06	-0.07	0.25	-0.23	0.03	1.00
Discharge	0.00	-0.33	0.11	-0.10	-0.44	-0.23	-0.22	-0.27	-0.09	-0.05	-0.11	0.19	-0.38	0.21	**0.83**

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