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Biological trait analysis : application to the Waikato Regional Council Monitoring Programme

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Executive summary

As part of the Waikato Regional Council's Regional Ecological Monitoring of Streams (REMS) programme, metrics based on macroinvertebrate taxonomic composition are used to describe the ecological health of the region's aquatic resources. An alternative approach is to use the biological traits of macroinvertebrates, as these reflect changes in ecosystem function rather than just changes in macroinvertebrate composition.

In this project we determined the response of both metrics and traits to a gradient of pastoral development (% pastoral) for Waikato streams. We developed *a priori* hypotheses of likely responses of traits to these stressors. We then compared the effectiveness of metric and trait measures for differentiating levels of impact. We also examined the potential influence of regional variation in taxonomy on the ability of metrics and traits to detect the impacts of rural development by combining the Waikato data with Auckland stream data. Finally, we investigated the potential use of traits as a mechanistic tool.

Natural variation in metrics and traits

As a first step, we examined the natural variability of metrics and traits in undisturbed (native forest) sites, focusing on differences between stream types (hard vs. soft bottomed). We found significantly different values between stream types for taxon richness, but not for any other metrics. We also found significant differences between stream types in some trait categories (i.e., the type of trait e.g., reproductive technique) and trait modalities (i.e., groupings of organisms based on characteristics of a trait category e.g., sexual or asexual reproduction)

Response to increasing pastoral development

Landuse (% pastoral) explained a greater proportion of the variation than stream type for all metrics. Landuse was found to explain a greater amount of variation than stream type for 53% of trait categories and 76% of trait modalities.

Landuse explained a greater amount of variation in soft-bottomed (43–63%) than hard-bottomed streams (16–56%) for all metrics. However, the general pattern of response to land use intensity was similar for both stream types (i.e., decreasing values with increasing pastoral development). In contrast to the metrics, landuse explained a comparable amount of variation in both stream types for a number of trait categories. For individual trait modalities, the pattern was similar to that identified for the trait categories. The responses to increasing pastoral development observed in both stream types was an increase in taxa that were smaller, reproduced multiple times per year (plurivoltinism), had greater than one reproductive cycle/individual, lived longer, reproduced asexually, deposited eggs under water (submerged), laid protected eggs, moved by crawling or were attached, were not flexible, were cylindrical in body form, had deposit feeders or algal piercers, were plastron respirers and had both adult and larval stages in aquatic form.

Traits (as categories or as individual modalities) were found to be as powerful at detecting impacts of pastoral development as metrics (as measured by partial eta^2 values).

Effectiveness of measures in differentiating levels of pastoral development

When examined across all streams, all metrics were found to be effective at differentiating between levels of pastoral development (measured as % of catchment area in pasture). The generally higher % dissimilarity for EPT richness and %EPT abundance suggests these metrics may be more effective than MCI or taxon richness. However, for these metrics, the relationship between pastoral development and % dissimilarity was not completely linear, suggesting they may not be as effective at differentiating intermediate levels of impact.

Almost all trait categories were found to be effective in differentiating between high and low levels of impact, but had differing levels of effectiveness for medium impact levels. Particularly effective traits included oviposition site, body flexibility and aquatic stages.

When examined separately for each stream type, there was greater variability in the measures of ability to differentiate levels of pastoral development, but the general pattern was similar for both metrics and traits.

Regional variation

Analysis of combined Auckland and Waikato datasets indicated that landuse explained more of the variation than region or stream type in most metrics. For all metrics, sites subject to rural landuse had lower values than those in native forest. Landuse also explained more of the variation than region or stream type for most trait categories and modalities. Taxa typical of rural streams reproduce more than once a year, reproduce more than once per individual, tend to live longer, reproduce asexually, lay submerged eggs and have both adult and larval forms that were aquatic.

Diagnostic ability of traits

We have identified significant differences in the frequencies of trait modalities that allow us to distinguish native forest sites from urban sites. Further refinement of traits as a diagnostic tool requires more detailed environmental disturbance measures. However, the present data indicate that trait profiles based on mode and frequency of reproduction, oviposition characteristics, movement, respiration and aquatic stages could be employed to detect trends over time in recovery following restoration or degradation following landuse changes.

Trait-based biomonitoring would fit readily into existing biomonitoring frameworks employed by regional authorities, as the basic information (site by species composition matrices) is already collected. Some challenges (for example, due to inconsistencies in the way invertebrate data are collected and enumerated) must be overcome, however, before a New Zealand wide approach could be adopted.

Conclusions and recommendations

For any traits (categories or modalities) to be considered for integration into existing biological monitoring programmes, they would ideally need to satisfy the following criteria:

Display low variation within levels of pastoral development, and significant power to discriminate medium from low or high levels of development

Almost all trait categories were found to be effective in differentiating between high and low levels of impact (especially for hard-bottomed streams), but had differing levels of effectiveness for medium impact levels.

Display greater discriminatory power than that achieved by standard metrics

Traits (as categories or as individual traits) were found to be as powerful at detecting impacts of pastoral development as metrics, especially for hard-bottomed streams.

Possess the ability to diagnose causal factors

Significant differences in the frequencies of trait modalities between native forest and pastoral sites were found, although identification of specific stressors requires finer resolution environmental disturbance measures.

A set of trait categories that consistently met all three of the above criteria included: number of reproductive cycles/individual, reproductive technique, egg mass location, oviposition, flexibility and aquatic stages. These trait categories (and their associated modalities) could be integrated into existing biomonitoring programmes run by regional authorities.

Based on this preliminary assessment of trait and metric responses to increasing pastoral development, recommendations for further development of the trait approach to biomonitoring are detailed below and include:

- Further investigation of diagnostic traits/trait profiles by using more specific measures of disturbance (e.g., contaminant concentrations).
- Investigation and development of stressor-specific traits derived either empirically or through relational analysis of existing datasets.
- Investigation of the development of a trait-based multi-metric using existing datasets.
- Expansion of the regional analysis to encompass a broader range of landuse types (e.g., through integration with other regional councils to provide sufficient numbers of sites with urban and forestry sites).
- Development of a method for integrating a traits approach into standard monitoring protocols.

1 Introduction

Waikato Regional Council (WRC) has been undertaking annual surveys of aquatic macroinvertebrates since 1994 as part of its Regional Ecological Monitoring of Streams (REMS) programme, to document the condition of streams and rivers in the region (Collier & Hamer 2010). From this base data set a number of metrics are derived to describe the relative ecological health of the survey reaches. Associated habitat and water quality metrics are also derived and relationships between invertebrate and physico-chemical metrics examined to investigate possible causal associations. The aims of this programme include determining the current ecological health of the region's rivers and streams, detecting trends over time, reporting on the effects of land use activities, as well as on the effectiveness of restoration activities (Collier 2005). To achieve these aims, measures are required that effectively differentiate between levels of impact. This ability is often hampered by the natural variability of macroinvertebrate communities in time and space (Statzner & Beche 2010). Traditional taxonomic-based invertebrate measures focus on diversity or on the presence or absence of key indicator taxa (Sponseller et al. 2001, Thompson& Townsend 2004). A problem with measures such as abundance, taxon richness and number of EPT (Ephemeroptera, Plecoptera and Trichoptera) is that they are often highly variable over both space and time (Scarsbrook 2002, Pollard & Yuan 2010). Furthermore, such measures generally describe the structural characteristics of a community, but provide little insight into ecosystem functioning.

There is growing interest in the use of macroinvertebrate biological traits as an assessment tool for monitoring human impacts on stream ecosystems (Doledec et al. 2006, Doledec et al. 1999, Stark & Phillips 2009, Statzner et al. 2005). Biological traits describe the biological characteristics of organisms. The use of biological traits offers a fundamentally different way of examining ecosystem responses to human impacts, as traits reflect the functional role that species play within the ecosystem and how disturbance affects this through direct effects on organism performance (McGill et al. 2006). The habitat template model (Southwood 1977, 1988) provides the theoretical basis for this approach. It predicts that where environmental conditions are similar, species trait composition should also be similar, regardless of biogeographical differences in taxonomic composition. Townsend & Hildrew (1994) adapted this model for streams, suggesting that benthic communities should consist of species possessing traits well suited to both the temporal and spatial variability of their local habitats. This model has been used in numerous studies to examine the relationships between traits and environmental drivers (e.g., Scarsbrook & Townsend 1993, Statzner et al. 1997, Townsend & Scarsbrook 1997, Heino 2005, Beche et al. 2006). The approach is simple, intuitive and the effects of individual stressors are often *a priori* predictable. In general, traits have been found as effective, and in some cases, more effective, than traditional biomonitoring methods in differentiating human impacts (Doledec et al. 2006, Magbanua et al. 2010, Rubach et al. 2010), even over large geographic areas (Charvet et al. 2000, Statzner et al. 2001, Lamouroux et al. 2004, Statzner et al. 2005, Doledec et al. 2011).

Biological traits may also be useful for establishing mechanistic linkages between biotic responses and environmental conditions (Baird et al. 2010, Culp et al. 2010, Van den Brink et al. 2010). In contrast, taxonomic-based measures generally only indicate that an ecological change has occurred (Culp et al. 2010). Due to the mechanistic basis of the trait approach, the biological trait approach has recently been proposed for use in ecological risk assessment (Baird et al. 2010, Culp et al. 2010, Van den Brink et al. 2010). A trait-based approach could also provide a framework for mechanistically connecting the occurrence of traits in a community to major environmental drivers. This mechanistic framework may help us better understand and predict response patterns associated with particular stressors, a goal of particular interest for environmental managers.

In this project we aimed to address these specific questions:

- How naturally variable are traits in comparison to metrics?
- How do traits and metrics compare in their ability to differentiate impacted sites?
- Can traits be used to diagnose the mechanisms of impact, by providing linkages between physiological/life history characteristics and potential causal factors?
- Are there specific traits or suites of traits that could be added to the existing suite of invertebrates metrics used in the invertebrate component of monitoring programmes to improve the ability to detect impacts?

Our approach to addressing these questions involved:

- analysis of a 4 year data set of macroinvertebrate abundance for multiple sites within the Waikato region
- derivation of trait-based metrics to identify specific traits or suites of traits that could be added to the existing suite of invertebrate metrics used in the Waikato Regional Council's monitoring programme to improve the ability to detect impacts
- comparison of the relative power of metrics and traits to detect impacts, and
- investigation of the potential of traits to diagnose mechanisms of impact, by examining linkages between landuse and trait frequency.

In addition, a recent analysis of landuse impacts on taxonomic and trait-based measures across a large geographic scale (the whole of New Zealand) (Dolédec et al. 2011), showed ecoregional differences in taxonomic, but not trait, composition. We therefore examined the potential for ecoregional influences on metric and trait responses to rural landuse by undertaking a regional analysis of a combined Waikato Regional Council/ Auckland Council dataset.

2 Methods

2.1 Data preparation

Existing data from Waikato Regional Council's long term Regional Environmental Monitoring of Streams (REMS) programme were used. This dataset includes macroinvertebrate community composition and habitat quality derived from annual assessments during the austral summer (Collier & Hamer 2010). Our dataset was comprised of 90 sites (representing 60 hard bottomed and 30 soft bottomed streams), from which samples had been collected over a period 4 years (2005 to 2008). Land use in the catchments upstream of the sites varied from 100% native forest to 100% pastoral development (median = 40% pastoral). Only one site recorded exotic vegetation (13.3% of catchment), while one site recorded urban development (29% of catchment). The proportion of the various land use types differed between hard and soft bottomed stream types (Fig. 1). Macroinvertebrate sampling is undertaken in accordance with standard New Zealand protocols (Stark et al. 2001), with some modifications specific to the Waikato region (Collier & Kelly 2006a). Samples are preserved in the field with 70% ethanol or isopropynol. A fixed count of at least 100 individuals is used to characterise the macroinvertebrate community at each site. A search for rare taxa is also undertaken and each taxon is allocated a value of 0.5 in the dataset. Invertebrates are identified to the taxonomic level used by Collier & Kelly (2006b). As counts sometimes exceeded 200, data are normalised between 0 and 1 for each site and year and are subsequently referred to as relative frequency. From these counts, a range of standard metrics is derived from the dataset: taxon richness, EPT richness, % EPT abundance (Ephemeroptera, Plecoptera and Trichoptera) and MCI (Macroinvertebrate Community Index (Stark 1985) – a measure of relative sensitivity or tolerance to nutrient pollution based on presence or absence of taxa). As suggested by Maxted et al. (2003), Hydroptilidae caddisflies are excluded from the calculation of %EPT, as they are known to proliferate in filamentous algal blooms and so are not representative of sensitive taxa. Metrics are standardised by the maximum value across all sites to generate values between 0 and 1.0.

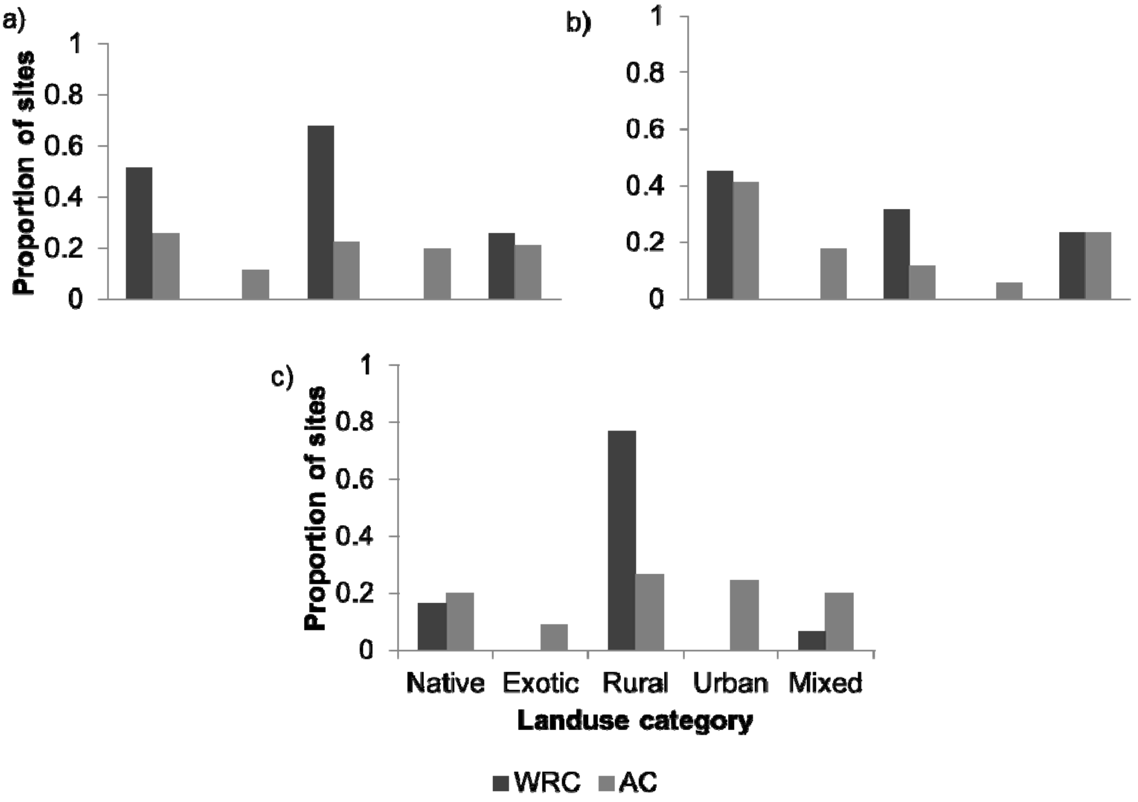

Figure 1: **Distribution of landuse for the Auckland (AC) and Waikato (WRC) regions for a) all sites, b) hard bottomed sites and c) soft bottomed sites.**

For the regional analysis, we combined data from the Waikato Regional Council monitoring programme (2005–2008, 90 sites) and the Auckland Council's Freshwater Ecology Programme (62 sites, 2008–2010) (Moore & Neale 2008). The Auckland Council's programme involves sampling in accordance with standard New Zealand protocols (protocol C1 for hard-bottomed streams, protocol C2 for soft-bottomed streams) (Stark et al. 2001). Samples are processed using Protocol P1 and invertebrates identified to MCI level, with taxa counts being placed into semi-quantitative abundance categories (ranging between rare for 1–4 individuals and very, very abundant for 500+ individuals). A range of taxonomic metrics are derived from this dataset, including those described above for the Waikato Regional Council dataset. Due to differences in macroinvertebrate sampling protocols between the regions, this analysis was necessarily based on presence/absence weighted trait frequencies rather than being weighted by abundance. Landuse varied between the Auckland and Waikato datasets, with substantially more exotic vegetation and urban development in the Auckland region, and more pastoral development in the Waikato region (Fig. 1).

2.2 Biological trait data

For each of the species collected, we employed 15 biological trait categories (e.g., maximum potential body size) divided into 55 trait modalities (e.g., for body size: ≤5 mm, 5–10 mm, 10–20 mm, 20–40 mm, >40 mm). Trait information was generally coded at the generic level, with the exception of some Diptera and non-insect taxa, which were coded at the family or order level. The 15 traits relate to the life history of organisms (e.g., size, number of reproductive cycles) or features that confer resilience or resistance beyond that provided by life history traits (e.g., attachment, body shape), as well as more general biological and physiological features (e.g., feeding habits, respiration; see Appendix A and also the glossary for a definition of the terms used). As a consequence of variation in the source of information used to derive the traits, we used ‘fuzzy coding’ to quantify the affinity of each taxon for each modality that contributed to a trait (Chevenet et al. 1994). Fuzzy coding allows data from a variety of sources (e.g., quantitative, qualitative, observational) to be used and compared statistically. An affinity score of zero indicates no association of the taxon with a trait category, whilst a score of three indicates a high affinity for a given trait category. This approach acknowledges the variability in traits that often occurs at different life stages. For example, a species that is predominantly a predator but feeds by scraping algae in early instars would be given an affinity of three for the feeding category 'predator' and one for the category 'scraper'. We scored traits as zero for any category of a given trait for which information was not available. A description of traits is available at https://secure.niwa.co.nz/fbis/displaycommonsearches.do

Affinity scores were further treated as frequency distributions:

$$q_k = \frac{a_k}{\sum_{k=1}^{h} a_k} \text{ with } q_k \geq 0 \text{ and } \sum_{k=1}^{h} q_k = 1$$

where q_k is the frequency of trait category *k* $(1 \leq k \leq h)$, *h* is the total number of categories of a given trait, and a_k is the assigned affinity. We described the functional composition of communities in terms of trait abundance, by multiplying the frequency of each category per trait by the abundance of species at the site. The resulting trait-by-site array contained the relative frequency of each category per trait in each site. The ade4 library (Thioulouse et al. 1997, Dray & Dufour, 2007) implemented in R freeware (R Development Core Team, 2010) was used to derive the trait frequencies. For the regional analysis encompassing the regional dataset from Auckland and Waikato, trait frequency calculations were based on presence/absence data only, to account for differences in the enumeration methods used by the two agencies.

2.3 Environmental data

The percentage of pastoral land in the catchment upstream of each site was used as our measure of environmental disturbance and was derived from the New Zealand Land Classification database (LCDB2, 2004). Sites were assigned to one of four categories representing degree of disturbance (see Section 5.1 in Collier 2005). Specifically, the categories used were <10%; 10–49%; 50–90%; and >90% pastoral development.

For the regional analysis, the disturbance gradient included the following landuse categories: native forest, exotic forest, rural and urban development, with the assumption being made that sites in urban development represent the most disturbed. The landuse designations were derived from an assessment of dominant landuse defined by the New Zealand Land Classification database (LCDB2, 2004). Only the native forest and rural development categories were used, reflecting the more limited range of landuses represented in the WRC data set.

2.4 *A priori* predictions for trait responses

When exposed to an environmental disturbance, populations conferring traits with resilience (the capacity to return toward the state prior to disturbance) or resistance (the capacity to withstand the disturbance) are predicted to increase (Townsend & Scarsbrook 1997). The predominant stressor in the Waikato dataset is pastoral development. The potential impacts of pastoral development are numerous, comprising multiple stressors including increased temperature, habitat simplification, increased runoff, increased nutrient concentration, modification of food resources and increased sedimentation (see Allan 2004 for review, Quinn and Stroud 2002, Thompson and Townsend 2004). Based on previous studies responses (Doledec et al. 2006, Doledec & Statzner 2008, Thompson et al. 2009, Magbanua et al. 2010, Doledec et al. 2011), we made the following predictions:

- increase in traits associated with population resilience (small size, short generation time, asexual reproduction) responding to increased temperature, habitat simplification, increased runoff and increased nutrient concentration
- increase in autroph feeders (scrapers, algal piercers) and an overall change in the composition of functional feeding groups to reflect the loss of riparian shading and increased nutrient concentrations
- increase in burrowing and detrital-feeding organisms, and decrease in filter feeders and grazing organisms in sediment affected patches, and
- decrease in surface egg laying and an increase in laying of protected eggs associated with increased sedimentation.

We also predicted that differences in functionality and community composition between hard and soft bottomed streams (Stark et al. 2001, Stark & Maxted 2004) would be reflected in variation in some trait responses in these different stream types.

2.5 Statistical analysis

An initial assessment of variability within reference sites (>90% native vegetation) (using One Way ANOVA) was undertaken to determine natural variability of metrics and traits in relation to stream type (hard vs. soft bottomed).

For comparison of the overall effectiveness of taxonomic metrics and trait-based measures for detecting increases in land use impact, one-way Analysis of Variance (ANOVA) was performed on taxonomic metrics and individual trait modalities and Multiple Analysis of Variance (MANOVA) was performed on biological trait composition (groups of trait modalities or trait categories). For each ANOVA/MANOVA, we report effect sizes (partial eta square values, range 0–1; Nakagawa 2004) to compare the magnitude of effects using taxonomic versus trait measures. Eta^2 values are analogous to r^2-values in regressions and are interpreted as the percentage of variance in the dependent variable uniquely attributable to the given effect variable. We used this approach for both the Waikato only analysis and the analysis of the two regions.

Variability of each response measure (metric and trait group) was determined by calculating % similarity within each % pastoral category, with Bray-Curtis as the similarity measure) using SIMPER analysis (PRIMER-E, v6.17, 2007). Although a multivariate measure was not required for the metrics, use of the Bray-Curtis similarity measure allowed direct comparison between metrics and traits (both combine modalities within categories and individual modalities). This measure provides an indication of the overall sensitivity of each measure to a disturbance gradient. We then calculated the % dissimilarity between pairs of % pastoral categories for each measure (metrics and traits), with Bray-Curtis as the dissimilarity measure. One-way ANOVA (for taxonomic measures) or ANOSIM (Analysis of Similarity) (PRIMER-E v6.17, 2007) (for trait measures) were conducted, with Bray-Curtis as the similarity measure and % pastoral development category as the factor for the analyses. Global R and p values are presented for each ANOSIM analysis. The ANOSIM Global R statistic fall between -1 and 1, with R = 0 indicating completely random grouping while R = 1 indicates that all replicates of a site type are more similar to each other than to any replicates of another site type. R>0.75 indicates large differences between groups, R>0.5 indicates overlapping but clearly different groups and R<0.25 (and negative vales) indicates barely separable groups (Clarke & Gorley 2001). A significant Global R (p<0.05) indicates that there are differences between site types somewhere in the analysis. Results of post-hoc pairwise analyses are also presented. This analysis allowed us to compare the effectiveness of the measures in detecting different levels of impact.

For the regional analysis, a Three-Way Analysis of Variance (ANOVA) was performed on the overall dataset to explore the consistency of any differences associated with stream types across the set of metrics/traits, using region (Auckland, Waikato), landuse category (native, exotic, rural, urban) and stream type (hard or soft bottomed) as our factors.

To assess the potential diagnostic ability of the trait approach we calculated Pearson Moment-Product Correlations between trait modalities and % landuse.

3 Variability associated with stream type

As a first step, before examining metric and trait responses to different landuses, we examined the potential influence of stream type (hard versus soft-bottomed). We predicted that differences in functionality and community composition between hard and soft bottomed streams (Stark et al. 2001, Stark & Maxted, 2004) would be reflected in differences in some trait occurrences between these different stream types. Analysis of native forest (reference) sites provides an indication of the natural variability of the metrics and traits, in the absence of human disturbance.

3.1 Reference site variability

Sites with >90% native vegetation were designated as references sites, resulting in 18 hard and 4 soft-bottomed streams being used for the analysis. Analyses indicated that there were significant differences between hard and soft bottomed streams for taxon richness (Table 3-1), with lower values of this metric being recorded for hard-bottomed streams (Fig. 3.1). Given the unbalanced design of this analysis, however, these results should be viewed with caution. Analysis of the subset of 4 soft bottomed and 4 randomly selected hard bottomed sites did not detect any significant differences (Table 3-1).

Table 3-1: Partial eta^2 values for metrics vs stream type (hard or soft bottomed). Significant values are highlighted in bold.

Metric	Stream type (p value)	
	18 hard bottomed, 4 soft bottomed	4 hard bottomed, 4 soft bottomed
Taxon richness	**0.102 (0.003)**	0.059 (0.229)
EPT richness	0.038 (0.077)	0.015 (0.547)
%EPT	0.017 (0.249)	0.095 (0.126)
MCI	0.015 (0.223)	0.002 (0.847)

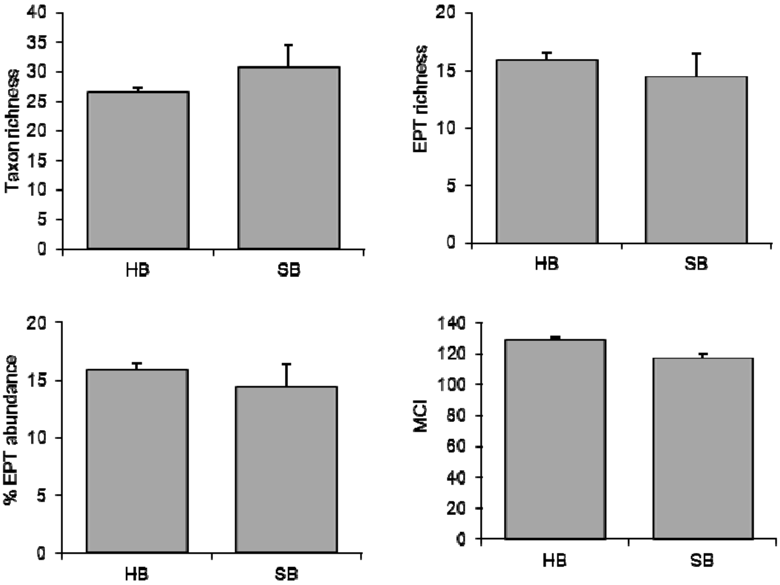

Figure 2: **Plots of metrics recorded from reference sites for each stream type (±S.E.).**

The difference in trait frequencies between hard and soft bottomed streams was also investigated. Significant differences were observed for 9 of the 15 trait categories (Table 3-2). Oviposition site, body form and feeding recorded much higher partial eta^2 values than other traits. Not all trait modalities within a trait category contributed to the observed differences. For example, for the trait category "life duration", the frequency of taxa that lived <1 day varied between hard and soft bottomed streams, while there was no significant difference for any of the other categories (Table 3-2). Similarly, for the trait category "oviposition site", terrestrial and endophytic (inserted in plants) oviposition were significantly different between stream type, but surface or submerged oviposition were not. On the basis of this assessment, reference hard bottomed stream types were characterized by having a greater proportion of taxa that produced eggs that were free or cemented (but not protected), low to medium distance dispersers, of higher flexibility and were less likely to have both adult and larval aquatic stages. In comparison, soft bottomed streams were characterized by having a greater proportion of taxa that were longer lived, had protected eggs, were more highly dispersing, were swimmers, were cylindrical in body form, and had both adult and larval aquatic stages.

This comparison of hard vs. soft bottomed streams indicates that the importance of stream type may need to be taken into account when interpreting both metric and trait analysis in relation to other factors (e.g., landuse impacts).

Table 3-2: Partial eta^2 values for traits (category, modality) vs stream type (hard or soft bottomed). Significant values (P<0.05) for each trait category/modality are in bold.

Trait category	Partial eta^2	Trait modality	Partial eta^2
	Life history traits		
Maximum potential size (mm)	0.066	≤5	0.007
		≥5-10	0.007
		≥10-20	<0.001
		≥20-40	0.039
		>40	0.001
Number of reproductive cycles per year	0.086	semivoltine	0.076
		univoltine	<0.001
		plurivoltine	<0.001
Number of reproductive cycles per individual	0.034	1	0.033
		≥2	0.033
Life duration (days)	**0.253**	≤1	**0.204**
		1–10	0.014
		10–30	0.011
		30–365	0.014
		>365	0.011
Reproductive technique	0.015	asexual	0.003
		hermaphroditism	0.012
		sexual	0.005
Oviposition site	**1.000**	water surface	0.003
		beneath the water surface	0.025
		terrestrial	**0.119**
		eggs laid within plants	**0.099**
Egg/egg mass location	**0.210**	cemented eggs	**0.209**
		eggs protected in/or female	**0.094**
		free eggs	**0.049**
	Resilience/resistance traits		
Dispersal	**0.230**	low (10 m)	**0.181**
		medium (1 km)	0.043
		high (>1 km)	**0.173**
Attachment to substrate	**0.270**	swimmers	**0.215**
		crawlers	**0.234**
		burrowers	0.024
		attached	0.010
Body flexibility	**0.126**	none (<10°)	0.002
		low (>10–45°)	0.041
		high (>45°)	**0.119**

Trait category	Partial eta^2	Trait modality	Partial eta^2
Body form	**1.000**	streamlined	**0.075**
		flattened	**0.204**
		cylindrical	**0.108**
		spherical	**0.078**
General physiological traits			
Feeding habits	**1.000**	shredders	**0.090**
		scrapers	**0.059**
		filter-feeders	**0.054**
		deposit feeder	<0.001
		predators	0.004
		algal piercers	<0.001
Dietary preferences	0.070	strong (specialist)	0.067
		moderate	0.005
		weak (generalist)	0.022
Respiration of aquatic stages	0.069	tegument	0.011
		gills	<0.001
		plastron	**0.054**
		aerial	0.011
Aquatic stages	**0.303**	adult and larva	0.017
		adult or larva	**0.235**
		larva or pupa	**0.265**

4 Comparison of metric and trait responses to pastoral development

4.1 Stream type versus landuse

An initial comparison of the effects of stream type and landuse (different levels of pastoral development) on macroinvertebrate communities was undertaken for metrics and traits (Table 4-1and Table 4-2).

4.1.1 Metrics

For all metrics pastoral development explained more of the observed variation among sites than stream type. Pastoral development explained from 21% (taxon richness) to almost 51% (MCI) (Table 4-1), whereas stream type, explained from 4.5% (taxon richness) to almost 20% (EPT richness).

Table 4-1: Partial eta^2 values for metrics vs stream type (hard or soft bottomed) and pastoral development. Highest value for each metric is in bold. P<0.001 in all cases.

Metric	Stream type (Soft bottomed=30, hard bottomed=60)	Pastoral development (<10%=24, 11-49% =8, 50-90=28, >90=30)
Taxon richness	0.045	**0.210**
EPT richness	0.198	**0.398**
%EPT abundance	0.163	**0.496**
MCI	0.158	**0.509**

4.1.2 Traits

Analysis of the relative importance of landuse and stream type to the variation in trait composition provides an indication of how reliable a trait will be in differentiating landuse impacts. Based on the results presented in Table 4-2, it can be seen that the trait categories maximum potential size, life duration, egg mass/location, dissemination potential, attachment to substrate and respiration were explained more by stream type than by landuse. This suggests these traits may not be suitable as indicators of landuse impacts in Waikato streams. However, individual trait modalities within these trait categories may be more sensitive to landuse than stream type, and thus a multi-indicator approach would be prudent. For example, although a greater proportion of variation was attributed to stream type for the trait category attachment to substrate, individual trait modalities within this category showed stronger relationships with pastoral development. This is also the case for respiration.

Table 4-2: Partial eta^2 values for traits (category, modality) vs stream type (hard or soft bottomed) and pastoral development. Highest value for each trait category/modality is in bold. P<0.001 in all cases, unless otherwise indicated.

Trait category	Pastoral development	Stream type	Trait modality	Pastoral development	Stream type
Life history traits					
Maximum potential size (mm)	0.115	**0.186**	≤5	**0.065**	0.001
			≥5-10	**0.170**	0.009
			≥10–20	**0.249**	0.010
			≥20–40	0.057	**0.147**
			>40	0.019	**0.066**
Number of reproductive cycles per year	**0.102**	0.094	semivoltine	0.008	**0.014**
			univoltine	**0.187**	0.084
			plurivoltine	**0.184**	0.090
Number of reproductive cycles per individual	0.207	**0.217**	1	0.207	**0.217**
			≥2	0.207	**0.217**
Life duration of adults (days)	0.126	**0.170**	≤1	**0.154**	0.095
			1–10	**0.163**	0.080
			10–30	**0.064**	0.036
			30–365	**0.154**	0.057
			>365	0.015	0.**038**
Reproductive technique	**0.119**	0.076	single individual	**0.191**	0.063
			hermaphroditism	**0.058**	0.027
			male and female	**0.187**	0.076
Oviposition site	**1.000**	**1.000**	water surface	**0.508**	0.066
			beneath the water surface	**0.437**	0.169
			terrestrial	0.023	**0.318**
			free eggs	0.049	**0.130**
Egg/egg mass	0.119	**0.234**	cemented eggs	0.036	**0.216**
			female bears eggs in/on body	**0.198**	0.033
			eggs endophytic	**0.175**	**0.175**
Resilience/resistance traits					
Dissemination potential	0.085	**0.126**	low (10 m)	0.027	**0.079**
			medium (1 km)	0.093	**0.125**
			high (>1 km)	**0.044**	0.008
Attachment to substrate	0.171	**0.239**	swimmers	**0.314**	0.236
			crawlers	**0.150**	0.130
			burrowers	**0.052**	0.000
			attached	**0.047**	0.002

Trait category	Pastoral development	Stream type	Trait modality	Pastoral development	Stream type
Body flexibility	**0.237**	0.025	none (<10°)	**0.240**	0.006
			low (>10-45°)	**0.370**	0.003
			high (>45°)	**0.097**	0.025
Body form	**0.158**	0.062	streamlined	**0.265**	0.002
			flattened	**0.129**	0.003
			cylindrical	**0.189**	0.015
			spherical	0.032	**0.061**
General physiological traits					
Feeding habits	**0.184**	0.036	shredders	**0.254**	0.014
			scrapers	**0.110**	0.004
			filter-feeders	**0.055**	0.002
			deposit feeder	**0.125**	0.006
			predators	**0.092**	0.001
			algal piercers	**0.062**	0.011
Dietary preferences	**0.155**	0.066	strong (specialist)	**0.078**	0.039
			moderate	**0.213**	0.021
			weak (generalist)	**0.083**	0.003
Respiration of aquatic stages	0.076	**0.121**	tegument	**0.080**	0.011
			gills	**0.086**	0.002
			plastron	**0.092**	0.087
			aerial	**0.022**	0.020
Aquatic stages	**0.278**	0.173	adult and larva	**0.278**	0.150
			adult or larva	**0.454**	0.104
			Larva or pupa	0.013	**0.033**

4.2 Landuse impacts

4.2.1 All streams – metrics

For all metrics pastoral development explained a significant percentage of the variation, ranging from 26% (taxon richness) to almost 54% (MCI) (Table 4-3). In general there was a decrease in the value of all metrics with increasing level of pastoral development (Figure 3).

Table 4-3: Partial eta^2 values for metrics vs stream type (hard or soft bottomed) and pastoral development.

Metric	Pastoral development
Taxon richness	0.261
EPT richness	0.443
%EPT abundance	0.534
MCI	0.539

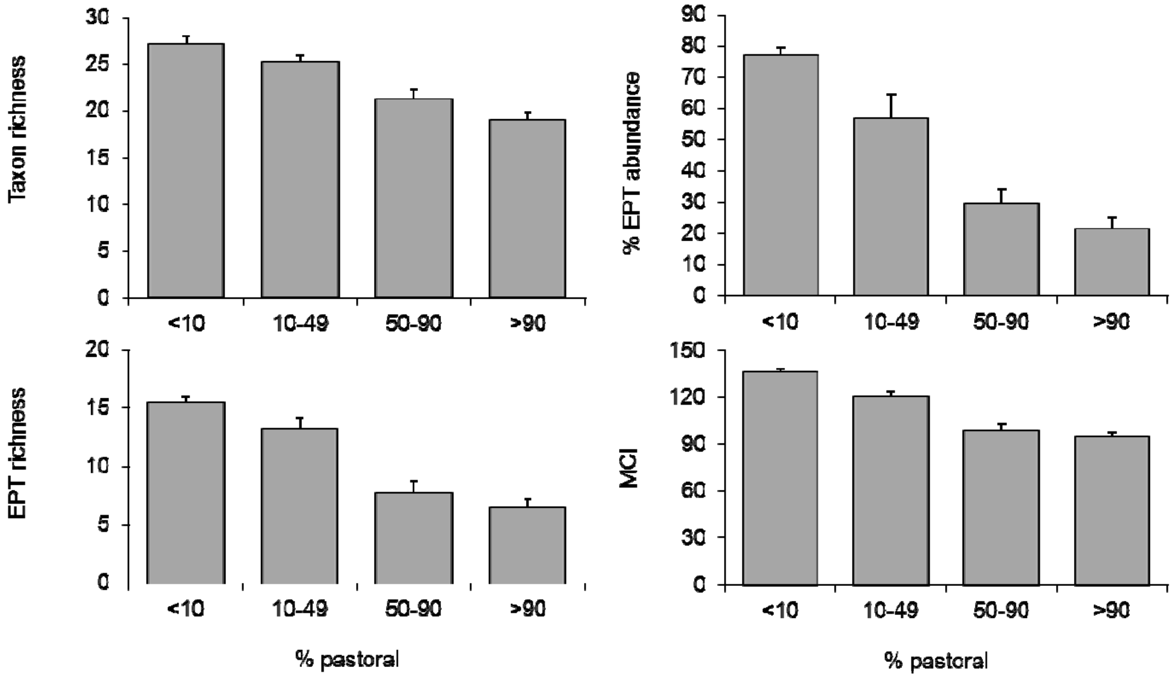

Figure 3: Plots of metrics recorded from all streams versus % pastoral category. Error bars = ±1 S.E.

4.2.2 All streams – traits

When considered at the trait category level, it is evident that many traits were responsive to increasing pastoral development (Table 4-4) with oviposition location recording the highest % variation explained (99.8%) and respiration the lowest value (11.1%). These values were comparable with those recorded for metrics. These differences are reflected in the trait frequency profiles associated with % pastoral development (Figure 4). Increased pastoral development (across all stream types) was associated with an increase in taxa that were smaller sized, plurivoltine (multiple reproductive cycles in one year), had more than one reproductive cycle per individual, were longer lived, reproduced asexually, laid submerged eggs or protected eggs in the body, had low dispersal, low body flexibility and were cylindrical in form and had both adult and larva as aquatic forms.

Table 4-4 shows that not all trait modalities within a category are equally sensitive to stream type or land use. For example, univoltine and plurivoltine taxa decrease and increase respectively in relation to pastoral development, while semivoltine taxa are much less influenced (see also Figure 4). This will reflect to some extent the relative frequency of occurrence of taxa with these traits. Examination of individual trait modalities within trait categories also enables determination of possible mechanisms of response to the multiple stressors associated with pastoral development, by providing insight into exactly which traits are sensitive and the relative impacts on different traits. For example, a greater percentage of variation in small size classes (up to 20 mm) was explained by pastoral development than larger size classes (>20 mm).

Table 4-4: Partial eta^2 values for traits vs pastoral development across all streams.

Trait category	Partial eta^2	Trait modality	Partial eta^2
	Life history traits		
Maximum potential size (mm)	0.126	≤5	0.064
		≥5–10	0.188
		≥10–20	0.241
		≥20–40	0.042
		>40	0.051
Maximum number of reproductive cycles per year	0.140	semivoltine	0.014
		univoltine	0.252
		plurivoltine	0.251
Number of reproductive cycles per individual	0.297	1	0.297
		≥2	0.297
Life duration (days)	0.167	≤1	0.231
		1–10	0.236
		10–30	0.076
		30–365	0.219
		>365	0.038
Reproductive technique	0.157	asexual	0.258
		hermaphroditism	0.077
		sexual	0.254
Oviposition site	0.996	water surface	0.555
		beneath the water surface	0.492
		terrestrial	0.035
		eggs laid within plants	0.072
Egg/egg mass location	0.167	cemented eggs	0.109
		eggs protected in/or female	0.248
		free eggs	0.264

Trait category	Partial eta^2	Trait modality	Partial eta^2
Resilience/resistance traits			
Dispersal	0.123	low (10 m)	0.054
		medium (1 km)	0.162
		high (>1 km)	0.038
Attachment to substrate	0.111	swimmers	0.183
		crawlers	0.092
		burrowers	0.053
		attached	0.045
Body flexibility	0.251	none (<10°)	0.279
		low (>10–45°)	0.379
		high (>45°)	0.097
Body form	0.173	streamlined	0.275
		flattened	0.132
		cylindrical	0.178
		spherical	0.048
General physiological traits			
Feeding habits	0.187	shredders	0.244
		scrapers	0.112
		filter-feeders	0.062
		deposit feeder	0.121
		predators	0.092
		algal piercers	0.080
Dietary preferences	0.184	strong (specialist)	0.093
		moderate	0.256
		weak (generalist)	0.081
Respiration of aquatic stages	0.056	tegument	0.074
		gills	0.087
		plastron	0.048
		aerial	0.023
Aquatic stages	0.318	adult and larva	0.357
		adult or larva	0.511
		larva or pupa	0.016

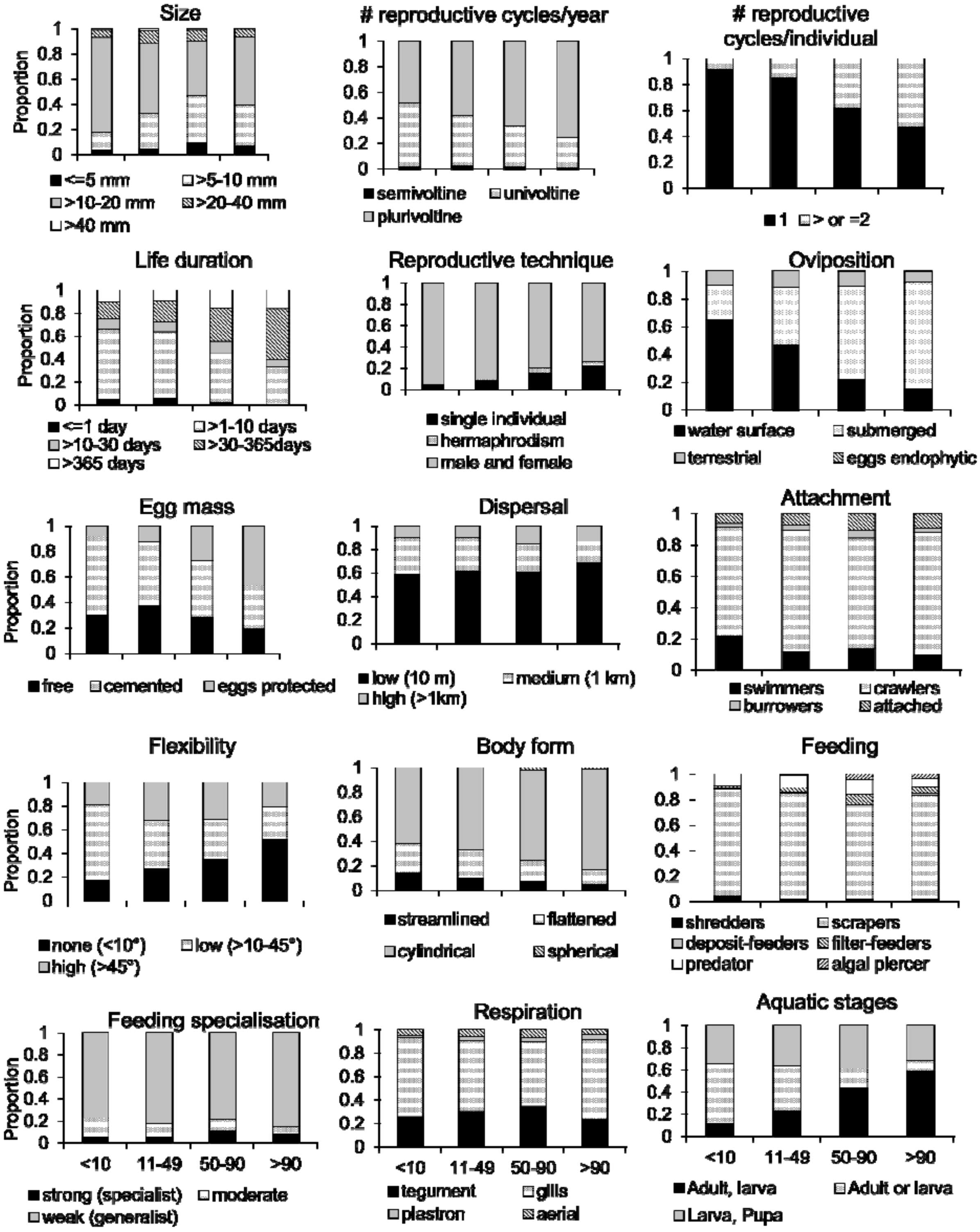

Figure 4: Plots of traits vs % pastoral landuse across all streams.

4.2.3 Separate analyses on hard and soft-bottomed streams – metrics

As stream type was found to be an important factor determining variation in at least some metrics and traits (section 4.1), even in reference sites (section 3), analysis of landuse impacts were conducted on hard and soft bottomed streams separately.

A greater amount of variation in taxonomic metrics was explained by increasing pastoral development for soft bottomed streams than for hard bottomed streams (Table 4-5). For EPT richness and MCI this difference was less than 10%, but for taxon richness the difference was more than 60%. It should be noted that there were no soft bottomed sites in the 10–49% pastoral category. However, re-analysis of the hard bottomed data set without this category (to account for this difference) resulted in only minor changes to the partial eta^2 values (Table 4-5). In general there was a significant decrease in the value of all metrics with increasing level of pastoral development for both stream types (Figure 5).

Table 4-5: Partial eta^2 values for metrics vs pastoral development across hard and soft bottomed streams. Highest values are highlighted in bold.

	% pastoral		% pastoral (excluding 10-49% category)
Metric	Hard bottomed	Soft bottomed	Hard bottomed
Taxon richness	0.159	**0.433**	0.178
EPT richness	0.432	**0.474**	**0.448**
%EPT abundance	0.503	**0.626**	0.527
MCI	0.559	**0.573**	**0.560**

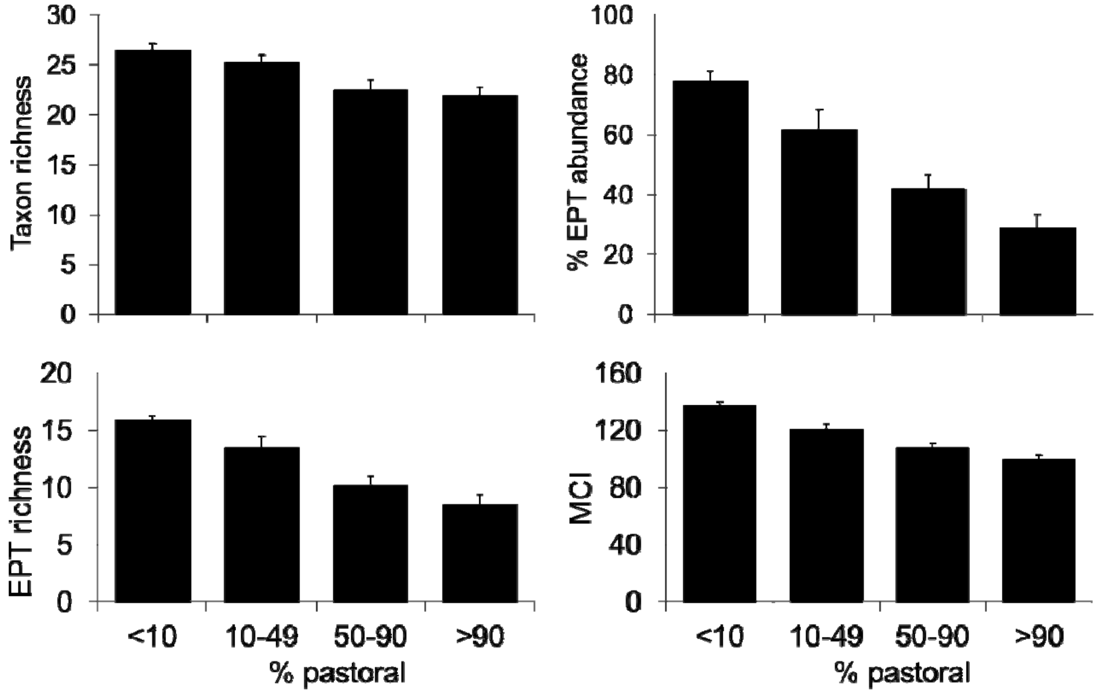

Figure 5: Plots of metrics recorded from hard bottomed streams versus % pastoral category. Error bars = ±1 S.E.

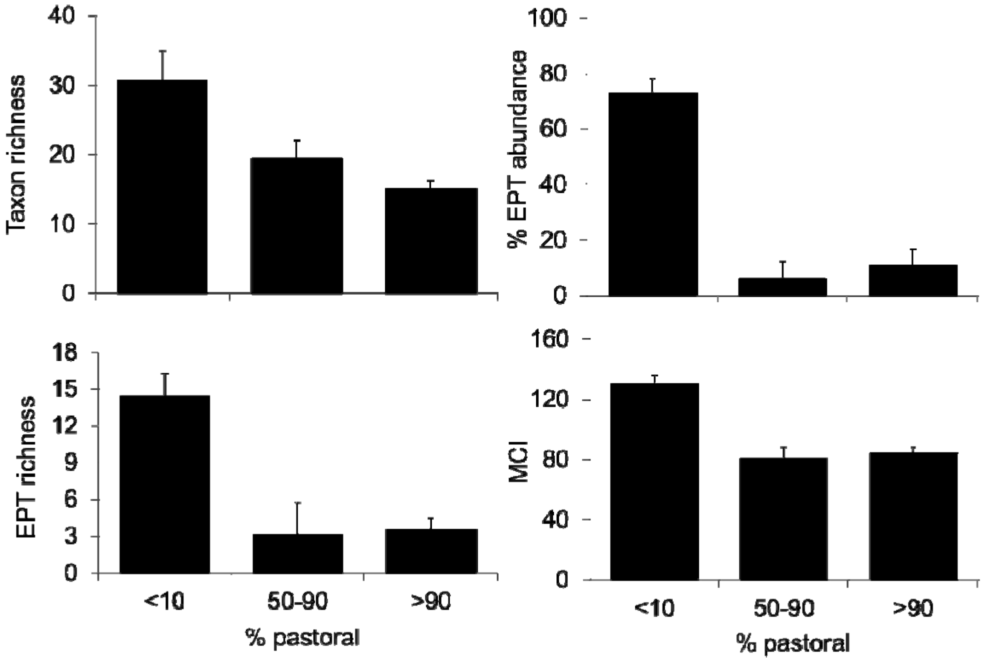

Figure 6: Plots of metrics recorded from soft bottomed streams versus % pastoral category. Error bars = ±1 S.E.

4.2.4 Separate analyses on hard and soft-bottomed streams – Traits

Trait responses were examined for trait categories (combination of trait modalities) and individual trait modalities. When considered at the trait category level, it is evident that many traits are equally responsive to pastoral development in both hard and soft bottomed streams (Table 4-2). Traits more strongly associated with pastoral development in soft bottomed streams include maximum potential size, life duration, body form, and feeding habit and dietary preferences. In contrast, number of reproductive cycles per individual, reproductive technique, egg mass location, body flexibility and aquatic stages were more strongly associated with pastoral development in hard bottomed streams. These differences may in part be due to the lack of soft bottomed sites in the 10–49% pastoral category. However, only one trait category showed a marked change in partial eta^2 values when hard bottomed streams were analysed without this pastoral category (attachment, partial eta^2 increased to 0.320). It should be noted that some of the differences in percentage explained between hard and soft-bottomed streams was quite small.

Table 6-26 shows that individual trait responses to pastoral development may vary between stream types, at least in the strength of the relationships. For example, % pastoral explained more than 10% of variation in two of the three dissemination trait modalities for hard bottomed streams, but only one trait modality for soft bottomed streams. This is reflected in the different trait profiles for this trait in Figure 67 and 8. Similarly, % pastoral explained greater than 10% of variation in four of the feeding habit modalities in soft bottomed streams, but in only one trait modality for hard bottom streams.

Table 4-6: Partial eta^2 values for traits (category, modality) vs pastoral development for hard and soft-bottomed streams. Values in bold indicate highest values for a specific trait category or modality.

Trait category	Hard bottomed	Soft bottomed	Trait modality	Hard bottomed	Soft bottomed
Life history traits					
Maximum potential size (mm)	0.165	**1.000**	≤5	0.077	**0.087**
			≥5–10	**0.248**	0.102
			≥10–20	**0.343**	0.135
			≥20–40	**0.082**	0.008
			>40	0.036	**0.118**
Number of reproductive cycles per year	0.140	**0.178**	semivoltine	**0.043**	0.017
			univoltine	0.144	**0.318**
			plurivoltine	0.143	**0.307**
Number of reproductive cycles per individual	**0.297**	0.256	1	0.248	**0.256**
			≥2	0.248	**0.256**
Life duration of adults (days)	0.167	**0.207**	≤1	**0.190**	0.036
			1–10	**0.219**	0.190
			10–30	0.025	**0.177**
			30–365	**0.189**	0.135
			>365	0.037	**0.040**
Reproductive technique	**0.157**	0.155	single individual	**0.249**	0.162
			hermaphroditism	0.089	**0.152**
			male and female	**0.279**	0.168
Oviposition site	**1.000**	**1.000**	water surface	**0.528**	0.510
			beneath the water surface	0.446	**0.489**
			terrestrial	0.060	**0.081**
			free eggs	0.045	**0.100**
Egg/egg mass	**0.167**	0.147	cemented eggs	**0.041**	0.026
			female bears eggs in/on body	0.194	**0.259**
			eggs endophytic	0.187	**0.196**
Resilience/resistance traits					
Dissemination potential	0.123	**0.156**	low (10 m)	0.039	**0.165**
			medium (1 km)	0.042	**0.264**
			high (>1 km)	**0.133**	0.016

Trait category	Hard bottomed	Soft bottomed	Trait modality	Hard bottomed	Soft bottomed
Attachment to substrate	0.111	**0.165**	swimmers	**0.488**	0.162
			crawlers	**0.200**	0.171
			burrowers	0.024	**0.135**
			attached	**0.107**	0.020
Body flexibility	**0.251**	0.230	none (<10°)	**0.307**	0.154
			low (>10–45°)	**0.462**	0.242
			high (>45°)	**0.116**	0.076
Body form	0.173	**0.350**	streamlined	**0.279**	0.249
			flattened	**0.244**	0.136
			cylindrical	**0.287**	0.164
			spherical	0.040	**0.103**
General physiological traits					
Feeding habits	0.187	**0.256**	shredders	0.224	**0.361**
			scrapers	0.089	**0.198**
			filter-feeders	0.057	**0.091**
			deposit feeder	**0.139**	0.091
			predators	0.039	**0.107**
			algal piercers	0.096	**0.168**
Dietary preferences	0.184	**0.256**	strong (specialist)	0.063	**0.106**
			moderate	0.183	**0.197**
			weak (generalist)	0.035	**0.286**
Respiration of aquatic stages	0.056	**0.093**	tegument	0.042	**0.201**
			gills	0.074	**0.142**
			plastron	0.120	**0.137**
			aerial	0.023	**0.028**
Aquatic stages	**0.318**	0.250	adult and larva	**0.348**	0.268
			adult or larva	**0.478**	0.440
			Larva or pupa	0.070	**0.101**

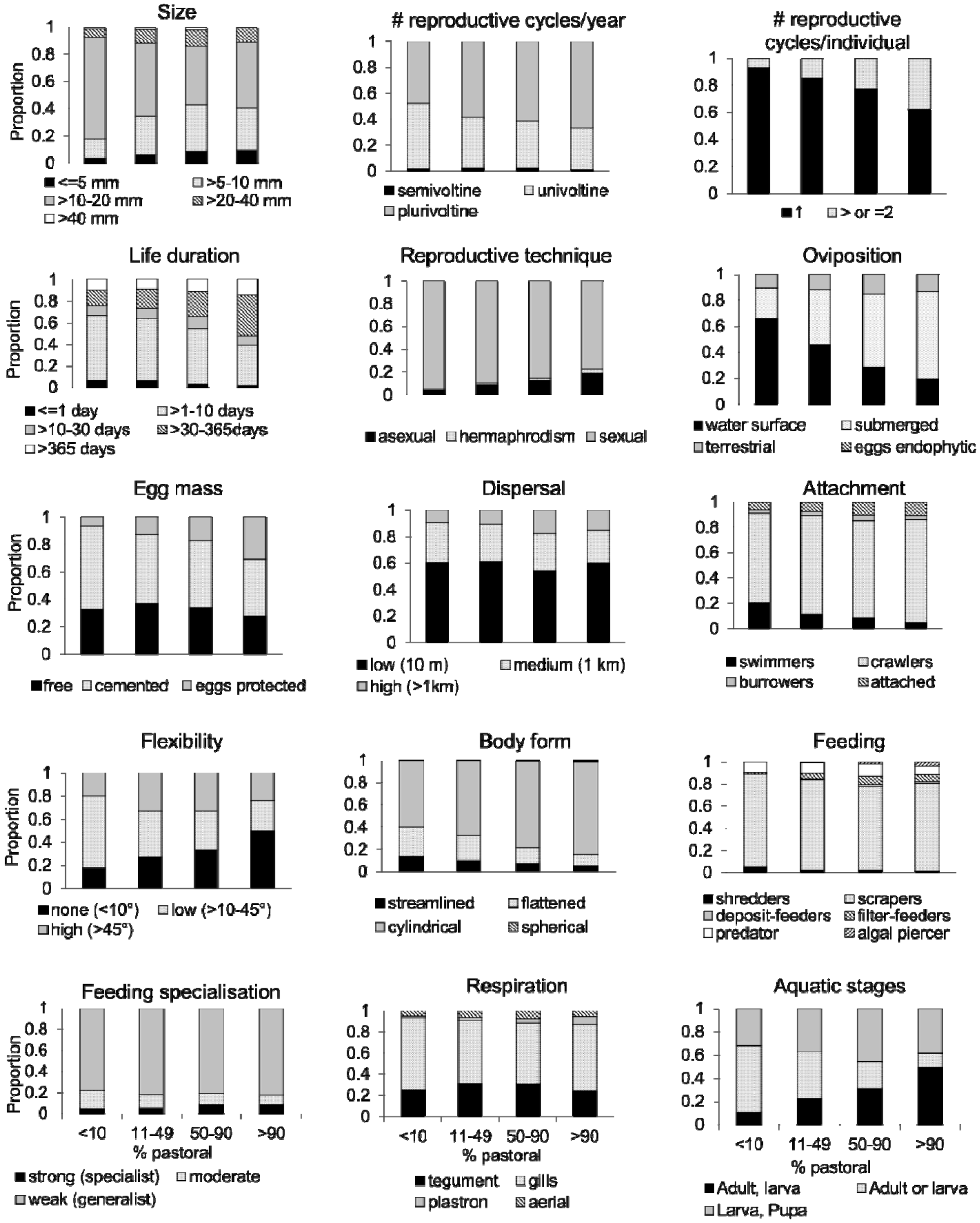

Figure 7: Trait frequencies (relative frequency) (annual average trait frequencies) versus % pastoral for hard bottomed streams.

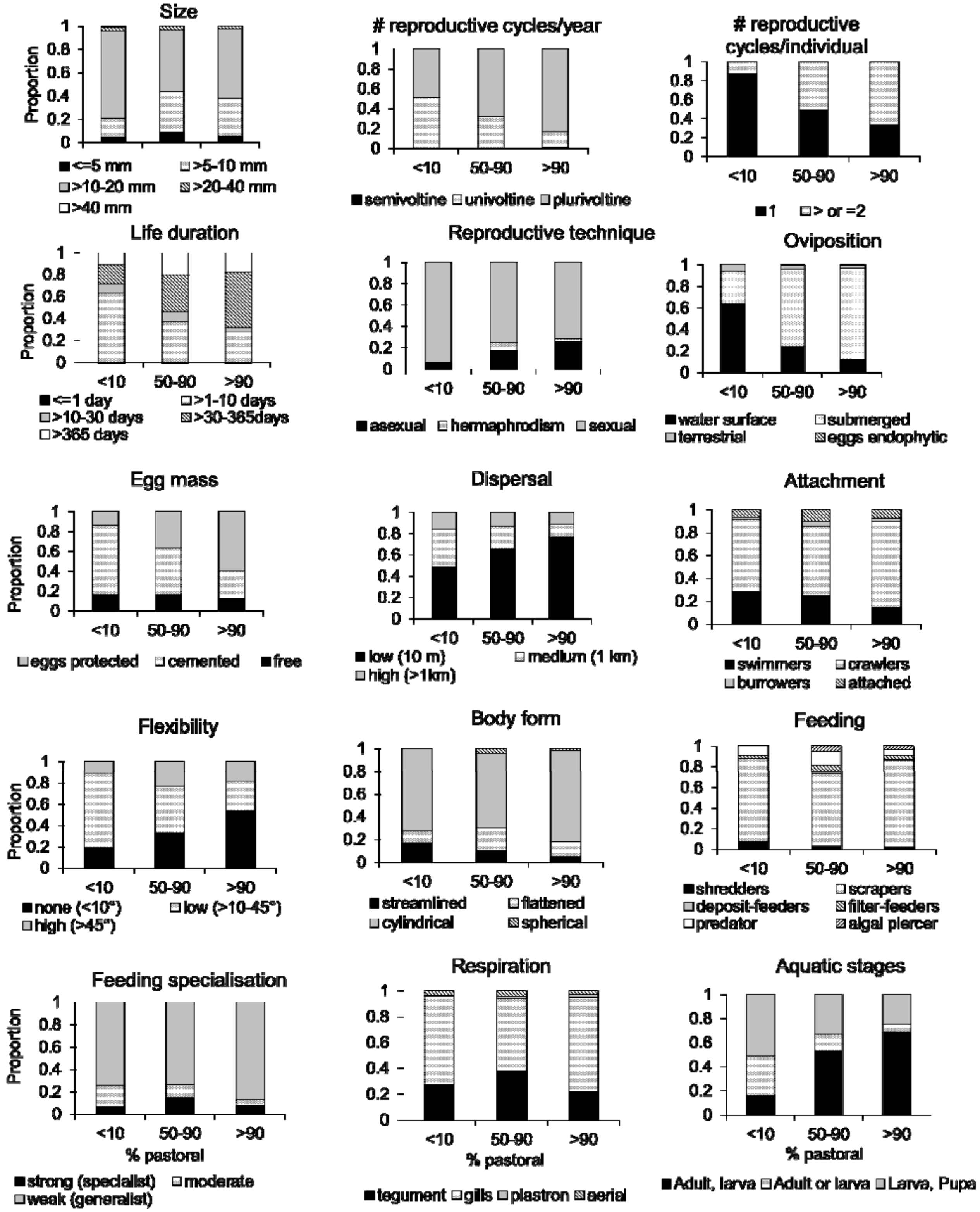

Figure 8: Trait frequencies (relative frequency) (annual average trait frequencies) versus % pastoral for soft bottomed streams.

Despite the statistical differences between stream types, the responses to increasing pastoral development for both stream types observed was an **increase** in taxa that:

- were smaller
- reproduced multiple times per year
- had greater than one reproductive cycle/individual
- lived longer
- reproduced asexually
- deposited eggs under water (submerged)
- laid protected eggs (within the female)
- crawled or were attached
- were not flexible
- were cylindrical in body form
- were deposit feeders or algal piercers
- were plastron respirers
- had both adult and larval stages in aquatic form.

A comparison of the difference in response between hard and soft bottomed streams enables the identification of trait categories/modalities which may be less effective in differentiating land use impacts, due to confounding responses associated with stream type. The only difference observed between stream types was for dispersal, where an increase in low and decrease in highly dispersing taxa was observed. This suggests that, generally, a separate analysis of landuse effects for each stream type may not be required. However, the limitations of the dataset should be taken into consideration i.e., the differences in number of sites within each % pastoral category.

5 Ability to differentiate levels of impact

5.1 Introduction

The effectiveness of each metric and trait was examined by considering a) how much variability there is amongst sites within each pastoral development category (i.e., >10%, 10–49%, 50–90%, >90%) and b) how much variability there is between pastoral development categories for a given measure. To assess these characteristics we calculated Bray Curtis **similarity** values to test for variation **within** each pastoral development category and **dissimilarity** values to test for differences **between** pastoral development categories.

For variation **within** pastoral development categories we would expect the >10% pastoral development) and >90% pastoral development categories to have the lowest variation, while the intermediate categories would be most variable. This is based on the intermediate disturbance hypothesis, which predicts lowest stability in moderately disturbed communities (Connell 1978, Townsend & Scarsbrook 1997). For an effective measure of impact, variation in >10% pastoral development sites would ideally be least, enabling easier detection of deviations from this state (Figure 9a).

For variation **between** landuse categories, an effective measure of impact would show:

- greatest dissimilarity between sites in the >10% and >90% pastoral development categories, and
- least dissimilarity between site groupings with >10% and 10–49% forest and between rural and >90% pastoral development landuse (Figure 9b).

The extent to which each of the taxonomic and trait measures addresses these predictions was explored. ANOSIM analyses were conducted to test the significance of any observed differences. Analyses are presented across all stream types and for hard and soft bottomed streams separately, consistent with previous chapters indicating some differences in response associated with stream type. For visual clarity, results are presented as line graphs rather than bar graphs to enable easier detection of trends related to landuse for multiple metrics/traits on the same graph.

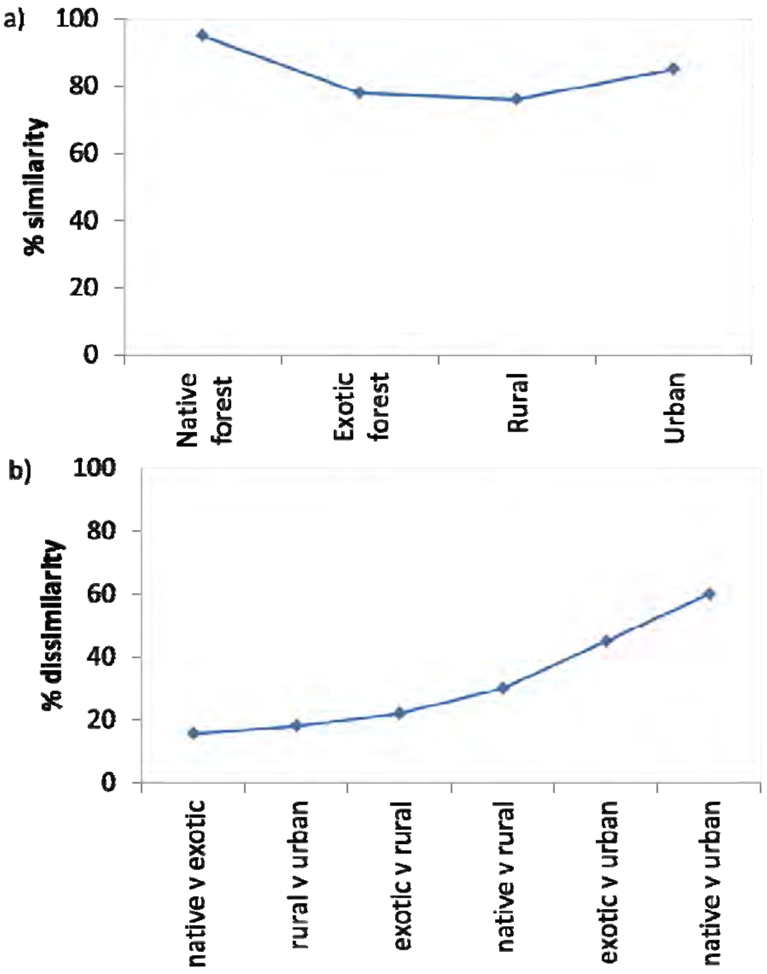

Figure 9: Example of the % similarity within landuse categories and % dissimilarity between landuse categories for an idealized metric.

5.2 Metrics – all stream types

For the analysis across all sites the number of sites within each landuse category was: <10% = 24, 10–49% = 8, 50–90% = 28, >90% = 30.

There was little variation within % pastoral categories for taxon richness and MCI (Figure 10), with % similarity values of greater than 80% for both measures. In contrast, both EPT richness and %EPT abundance showed decreasing % similarity with increasing pastoral development.

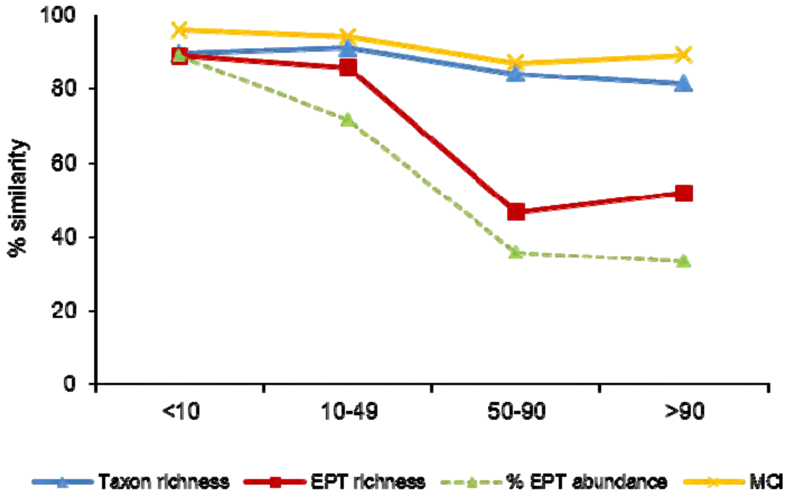

Figure 10: % similarity within % pastoral groups for individual metrics for all streams.

Not surprisingly, % dissimilarity between all landuse categories was low for taxon richness and MCI (Fig. 5.3). Lowest dissimilarity between % pastoral category pairings for EPT richness and %EPT was for <10% v 10–29% pastoral development. Greatest % dissimilarity was between the 50–90 v >90% pairing. Percentage dissimilarity generally increased with increasing difference in % pastoral landuse.

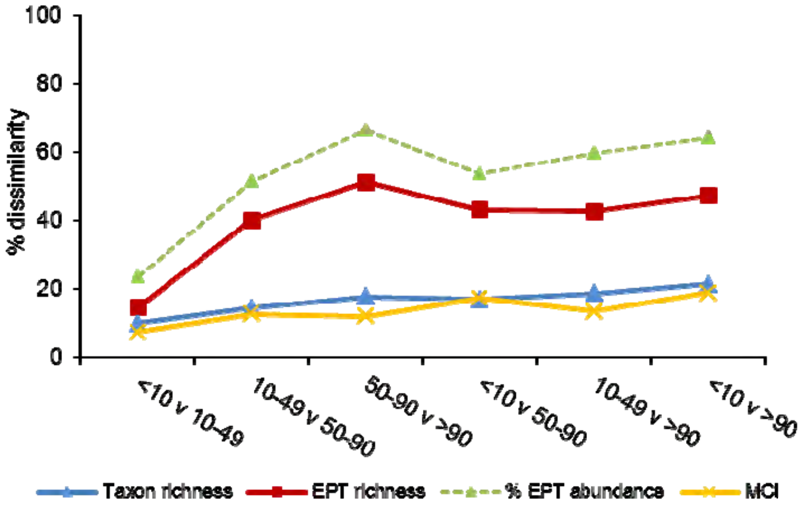

Figure 11: % dissimilarity between % pastoral groups for individual metrics for all streams.

The results of ANOVA analyses indicated significant deviation from random for all metrics (Table 5-1). Tukey's HSD post-hoc pairwise comparisons indicated significant differences between all % pastoral pairings for % EPT abundance, while for EPT richness and MCI there was no significant difference between the 50-90 and >90% categories. For taxon richness there was no significant difference between the <10 and 10–49% categories. These results suggest that all metrics are effective at differentiating between at least some of the categories of pastoral development. The generally higher % dissimilarity for EPT richness and %EPT abundance suggests these metrics may be more effective than MCI or taxon richness. However, for these metrics, the relationship between pastoral development and % dissimilarity is not completely linear, suggesting they may not be as effective at differentiating intermediate levels of impact.

Table 5-1: ANOVA results for metrics versus % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) for all streams.

Factor	% pastoral	
	P value	Pairwise tests (p value)
Taxon richness	<0.001	1v2 (0.233) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.009)
EPT richness	<0.001	1v2 (0.043) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.146)
% EPT abundance	<0.001	1v2 (<0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.032)
MCI	<0.001	1v2 (<0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.305)

5.3 Traits – all streams

Variation within the % pastoral categories across all of the trait categories ranged from 54 to 94% (Figure 12). For most traits, sites in the <10% pastoral land use group showed the greatest similarity, while sites in the >90% pastoral land use group showed the lowest similarity. For the trait categories number of reproductive cycles per year, dietary preference and body form this pattern differed. For some trait categories (e.g., body form and dietary preference) there was very little differentiation between % pastoral categories.

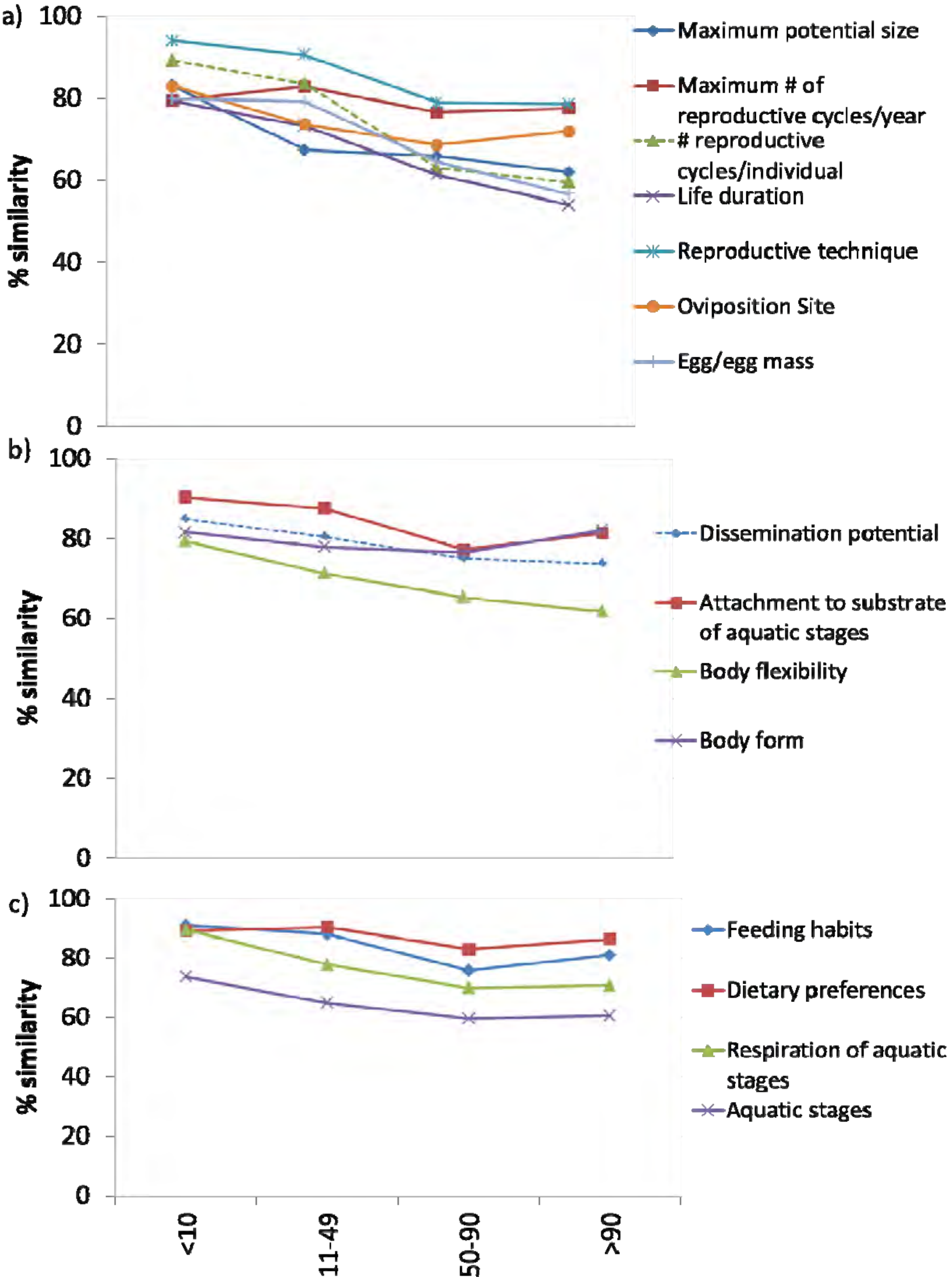

Figure 12: % similarity within % pastoral groups for a) life history, b) resilience resistant and c) general biological trait categories for all stream types.

Per cent dissimilarity between <10 and 11–49% pastoral categories was the lowest of all the % pastoral pairings for all traits, although the absolute % dissimilarity ranged from 8.9 to 32.5 (Figure 13). Greatest % dissimilarity was detected for the <10 v >90% pairing for a number of traits, including number of reproductive cycles per individual, life duration, oviposition site, reproductive technique, egg mass, body flexibility and form and aquatic stages.

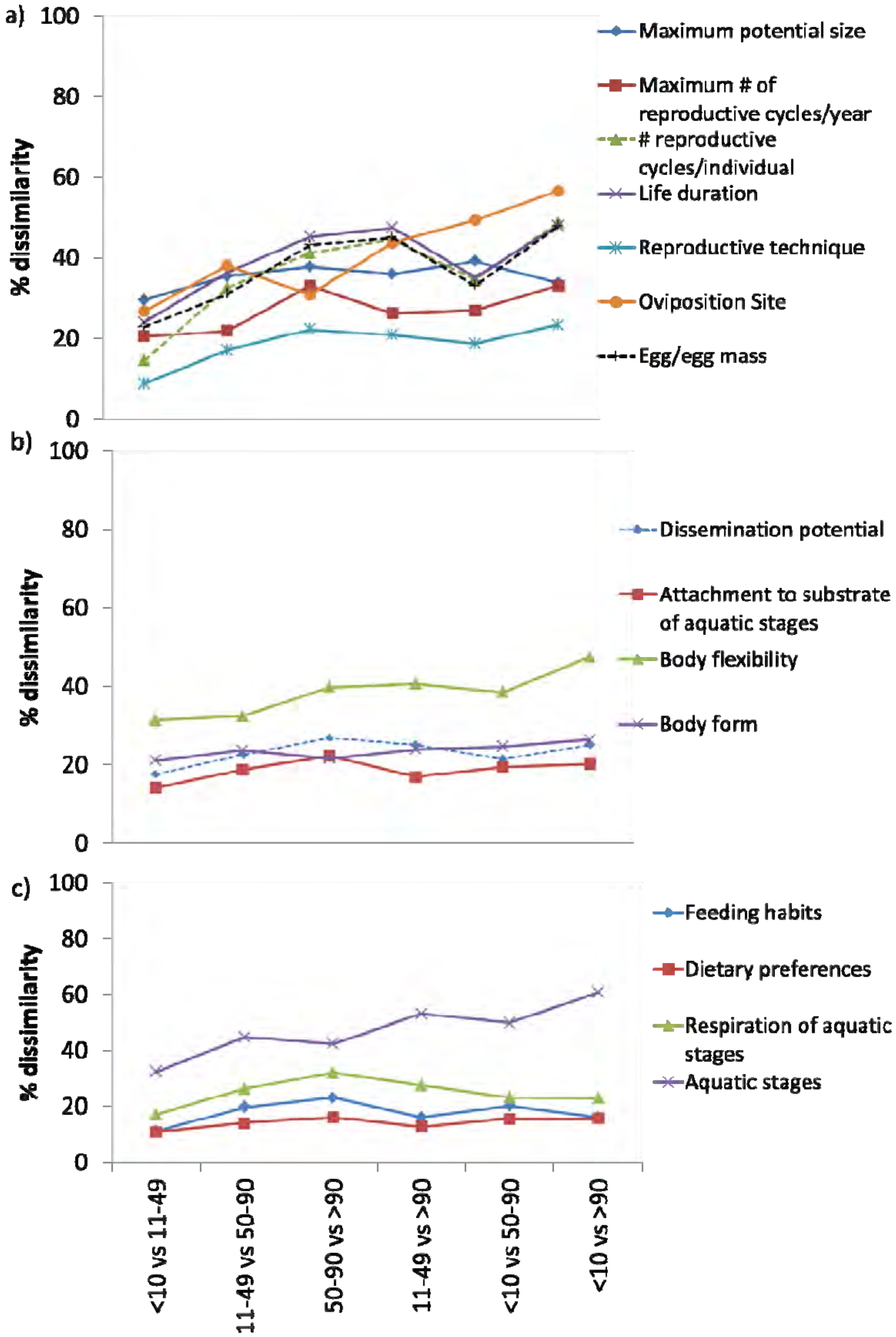

Figure 13: % dissimilarity between % pastoral groups for a) life history, b) resilience resistant and c) general biological trait categories for all stream types.

ANOSIM analyses indicated significant deviation from random similarity for all traits (Table 5-2). R values varied between traits, with the highest value recorded for oviposition (0.380), aquatic stages (0.291) and flexibility (0.221). Pairwise tests for these trait categories indicated highest R values for the <10v>90% comparison, with progressively lower R values for comparisons between % pastoral groups that were predicted to be more similar (Table 5-2). A number of other trait categories displayed the predicted pattern described in section 5.1, including number of reproductive cycles per year, egg mass and body. For some trait categories, this pattern was not observed. For example, the R value was highest for the <10v50–90% comparison. Likewise, the highest R value for respiration was the <10v10–49% comparison. Percentage dissimilarity generally increased with increasing difference in pastoral development, although for some traits this relationship was not completely linear, suggesting they may not be as effective at differentiating intermediate levels of impact. This is particularly evident for egg mass and reproductive technique.

Table 5-2: ANOSIM results - % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) using all data.

Trait	Global R	Significance (p)	Pairwise tests (Global R, p)
Life history traits			
Size	0.135	0.001	1v2 (0.333, 0.001) 1v3 (0.362, 0.001) 1v4 (0.097, 0.001) 3v4 (0.039, 0.008)
Number of reproductive cycles per year	0.142	0.001	1v3 (0.134, 0.001) 1v4 (0.335, 0.001) 2v4 (0.176, 0.001) 3v4 (0.046, 0.003)
Number reproductive cycles/individual	0.149	0.001	1v3 (0.152, 0.010) 1v2 (0.179, 0.006) 1v4 (0.337, 0.001) 2v4 (0.113, 0.003) 3v4 (0.041, 0.022)
Life duration	0.119	0.001	1v2 (0.105, 0.001) 1v3 (0.104, 0.045) 1v4 (0.272, 0.001) 2v4 (0.087, 0.002) 3v4 (0.065, 0.017)
Reproduction	0.126	0.001	1v2 (0.269, 0.001) 1v3 (0.168, 0.001) 1v4 (0.289, 0.001) 3v4 (0.054, 0.001)
Oviposition	0.380	0.001	1v2 (0.296, 0.001) 1v3 (0.555, 0.001) 1v4 (0.696, 0.001) 2v3 (0.165, 0.001) 2v4 (0.401, 0.001) 3v4 (0.023, 0.042)
Egg mass	0.116	0.001	1v2 (0.090, 0.048) 1v3 (0.092, 0.001) 1v4 (0.274, 0.001) 2v4 (0.087, 0.013) 3v4 (0.072, 0.002)
Resilience/resistance traits			
Dissemination	0.062	0.001	1v2 (0.114, 0.012) 1v4 (0.158, 0.001) 1v3 (0.049, 0.005) 3v4 (0.04, 0.017)
Attachment	0.135	0.001	1v2 (0.349, 0.001) 1v3 (0.212, 0.001) 1v4 (0.314, 0.001) 3v4 (0.042, 0.002)

Trait	Global R	Significance (p)	Pairwise tests (Global R, p)
Flexibility	0.221	0.001	1v2 (0.313, 0.001) 1v3 (0.281, 0.001) 1v4 (0.415, 0.001) 2v4 (0.110, 0.009) 3v4 (0.088, 0.001)
Body form	0.149	0.001	1v2 (0.088, 0.019) 1v3 (0.147, 0.001) 2v4 (0.214, 0.001) 3v4 (0.031, 0.015)
General physiological traits			
Feeding	0.067	0.001	1v2 (0.209, 0.001) 1v3 (0.129, 0.001) 1v4 (0.120, 0.001) 3v4 (0.031, 0.015)
Dietary preference	0.105	0.001	1v3 (0.118, 0.001) 1v4 (0.255, 0.001) 3v4 (0.060, 0.001)
Respiration	0.068	0.001	1v2 (0.264, 0.001) 1v3 (0.086 0.001) 1v4 (0.119, 0.004) 3v4 (0.065, 0.001)
Aquatic stages	0.291	0.001	1v2 (0.153, 0.003) 1v3 (0.360, 0.001) 1v4 (0.570, 0.001) 2v3 (0.102, 0.008) 2v4 (0.360, 0.001) 3v4 (0.057, 0.002)

5.4 Metrics – Hard-bottomed stream

For the analysis across hard-bottomed sites, the number of sites within each landuse category was: <10% = 20, 10–49% = 8, 50–90% = 18, >90% = 14.

There was relatively limited variation in % similarity within % pastoral groups based on taxon richness, EPT richness and MCI, with greatest difference between % pastoral groups based on %EPT abundance (Figure 14). Similarity was generally highest for the <10% group and lowest for the >90% group, although the latter was not observed for MCI.

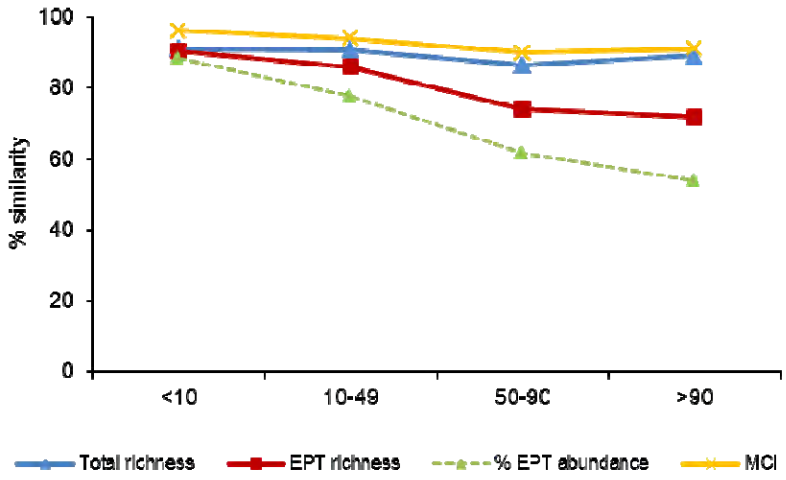

Figure 14: % similarity within % pastoral groups for individual metrics for hard bottomed streams.

For all metrics, greatest dissimilarity was found between <10% and >90% sites (Figure 15). For % EPT abundance and EPT richness, the pattern of % dissimilarity between impact groups was generally as predicted i.e., <10v>90% is most dissimilar and other comparisons showed lower dissimilarities (also see section 5.1).

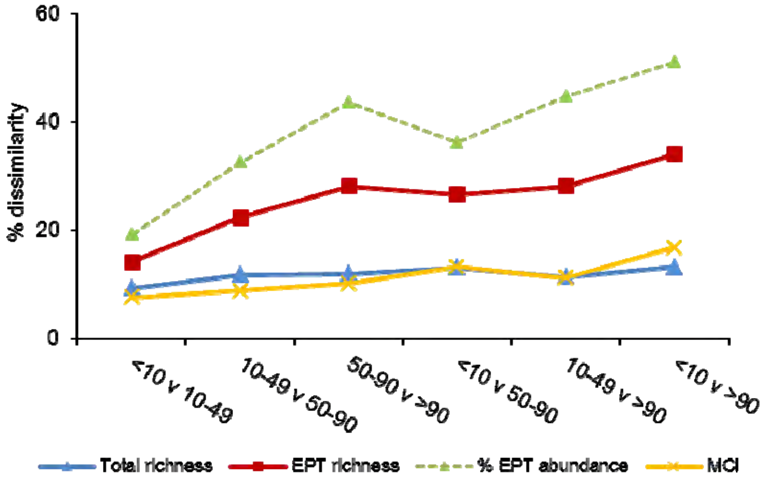

Figure 15: % dissimilarity between % pastoral groups for individual metrics for hard bottomed streams.

The results of ANOVA analyses indicated significant differences between pastoral development classes for all metrics (Table 5-3). Tukey's HSD post-hoc pairwise comparisons indicated significant differences between all % pastoral pairings for % EPT abundance, while for EPT richness and MCI there was no significant difference between the 50–90 and <90% categories. For taxon richness there was no significant difference between the <10% and 10–49%, 50–90% and >90%, and 10–49 and >90% categories. These results suggest that all metrics are effective at differentiating between the different categories of pastoral development. The generally higher % dissimilarity for EPT richness and %EPT abundance suggests these metrics may be more effective than MCI or taxon richness. However, for these metrics, the relationship between pastoral development and % dissimilarity is not completely linear, suggesting they may not be as effective at differentiating intermediate levels of impact.

Table 5-3: ANOVA results for metrics % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) using hard bottomed data only.

Variable	P value	Pairwise tests
Taxon richness	<0.001	1v2 (0.495) 1v3 (<0.001) 1v4 (<0.001) 2v3 (0.021) 2v4 (0.066) 3v4 (0.948)
EPT richness	<0.001	1v2 (0.006) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.055)
% EPT abundance	<0.001	1v2 (0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.002)
MCI	<0.001	1v2 (<0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.102)

5.5 Traits – hard bottomed streams

Percentage similarity within the % pastoral categories across all of the trait categories ranged from 61 and 94% (Figure 16). For number of reproductive cycles per individual, life duration, reproductive technique, respiration and aquatic stages, sites in the <10% pastoral land use group showed the greatest similarity, while sites in the >90% pastoral land use group showed the lowest similarity. For other traits this pattern differed slightly; e.g., for oviposition site similarity in the 50–90% category was lower than the >90% category. For body form, the >90% category showed the greatest similarity within the group, although differentiation of % pastoral group was generally poor for this trait.

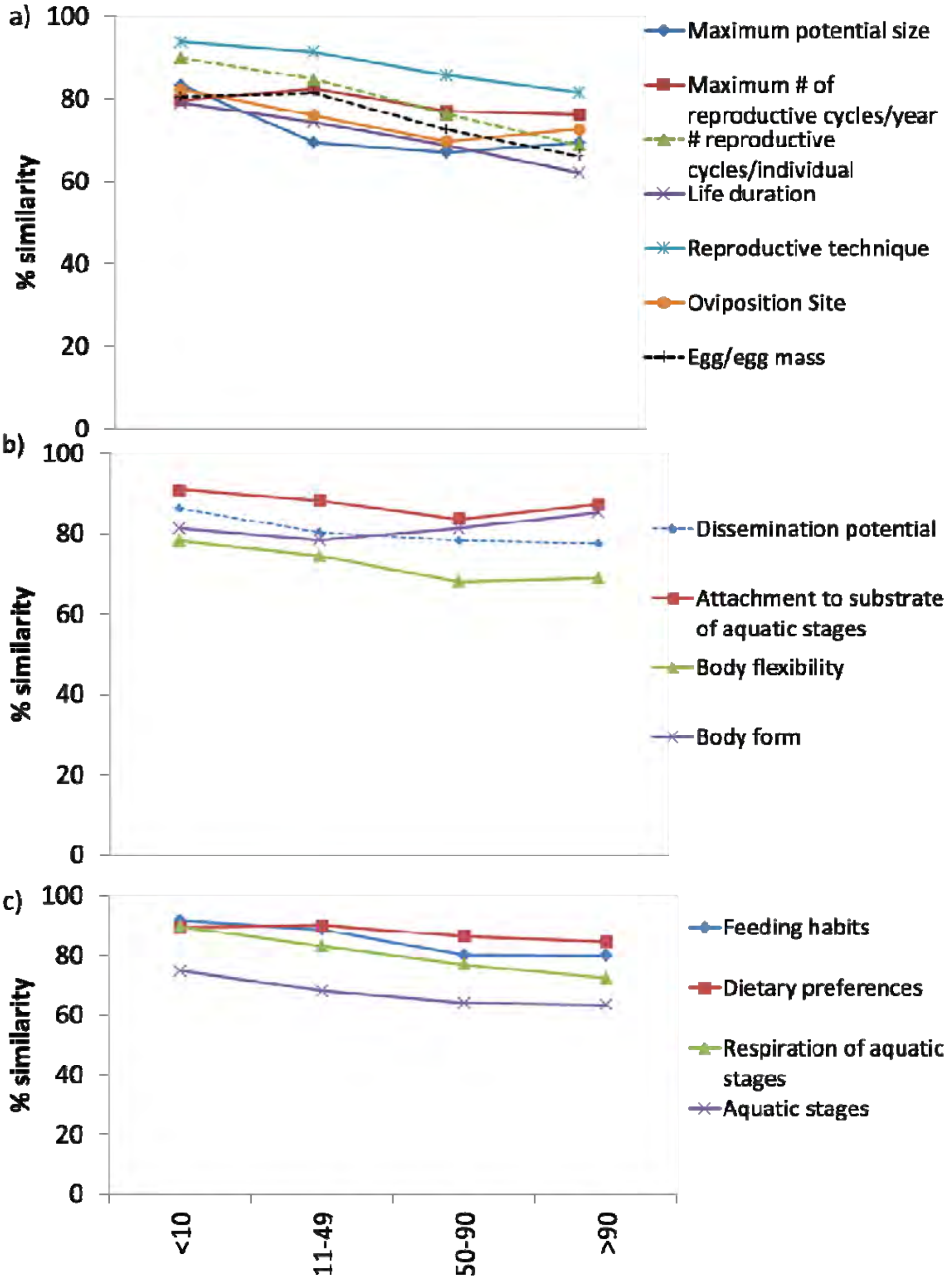

Figure 16: % similarity within % pastoral groups for a) life history, b) resilience or resistance and c) general biological trait categories for hard bottomed streams.

Percent dissimilarity between <10 and 11–49% pastoral categories was the lowest for all the % pastoral pairings for all traits other than number of reproductive cycles per year and body form. The absolute % dissimilarity ranged from 8.7 to 54.2% (Figure 17). Greatest % dissimilarity was detected for the <10 v >90% pairing for a number of traits, including number of reproductive cycles per year and individual, life duration, oviposition site, reproductive technique, oviposition site, egg mass, attachment, body flexibility and form and aquatic stages.

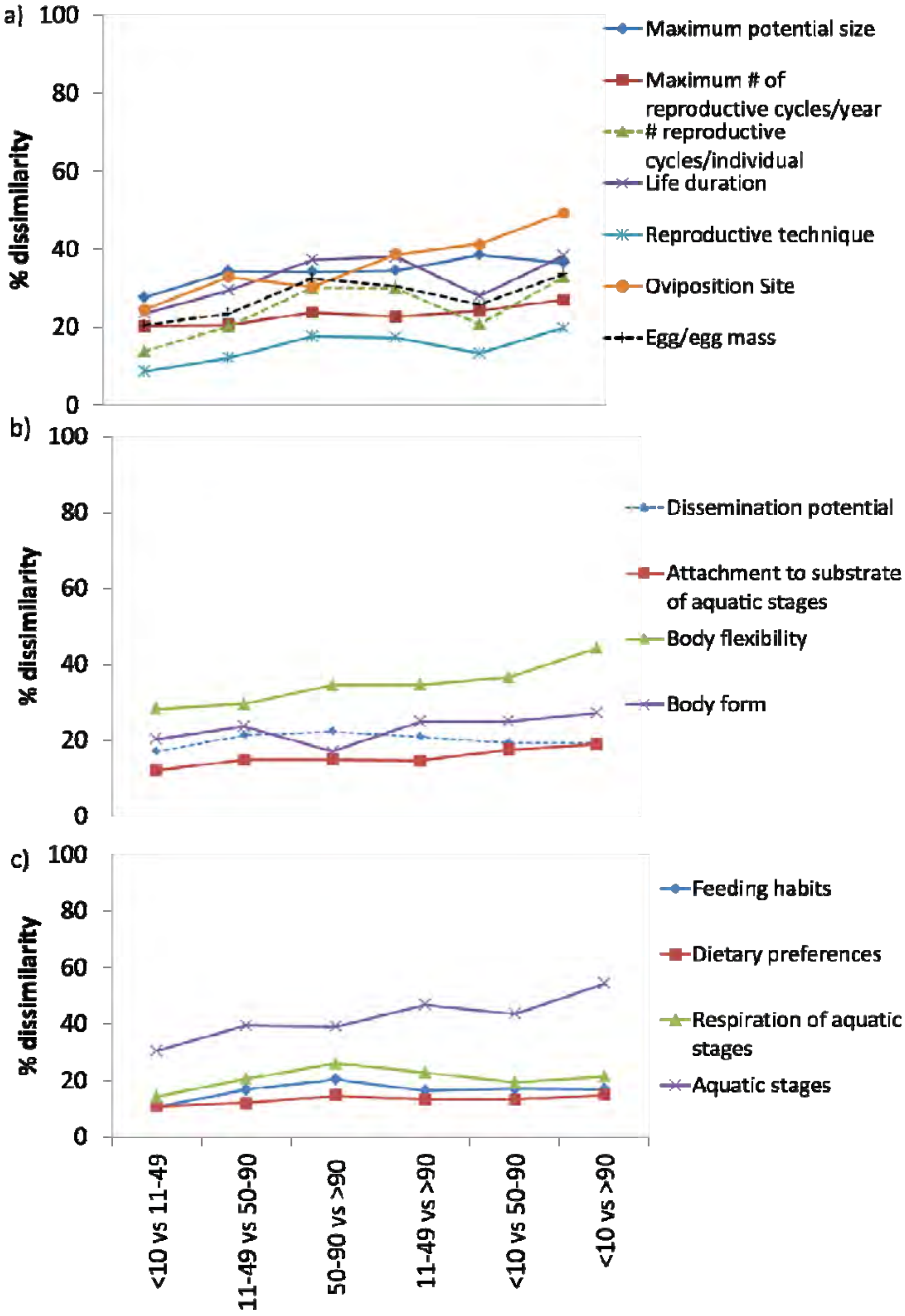

Figure 17: % dissimilarity between % pastoral groups for a) life history, b) resilience resistant and c) general biological trait categories for hard bottomed streams.

ANOSIM analyses indicated significant deviation from random for all traits (Table 5-4). Global R values varied between traits, with the highest value recorded for oviposition (0.346), aquatic stages (0.308) and attachment (0.307). Pairwise tests for attachment indicated highest R values for the <10v>90% comparison, with progressively lower R values for comparisons between % pastoral groups that were predicted to be more similar (Table 5-4). For oviposition and aquatic stages, this pattern wasn't observed, with highest R values for 10-49 v >90% and <10 v 50–90% comparisons. However, R values for the <10 v >90% comparisons were high. Percentage dissimilarity generally increased with increasing difference in pastoral development, although for some traits this relationship was not completely linear, suggesting they may not be as effective at differentiating intermediate levels of impact. This is particularly evident for life duration and reproductive technique.

Table 5-4: ANOSIM results - % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) using hard bottom data only.

Variable	Global R	Significance (p)	Pairwise tests (Global R, p)
Life history traits			
Size	0.025	0.001	1v2 (0.293, 0.001) 1v3 (0.419, 0.001) 1v4 (0.394, 0.001)
Number of reproductive cycles per year	0.044	0.019	1v3 (0.064, 0.006) 1v4 (0.159, 0.001)
Number reproductive cycles/individual	0.173	0.001	1v2 (0.184, 0.001) 1v3 (0.162, 0.006) 1v4 (0.398, 0.001) 3v4 (0.115, 0.004)
Life duration	0.134	0.001	1v3 (0.055, 0.009) 1v4 (0.369, 0.001) 2v4 (0.147, 0.006) 3v4 (0.130, 0.001)
Reproduction	0.200	0.001	1v2 (0.289, 0.001) 1v3 (0.209, 0.001) 1v4 (0.447, 0.001) 3v4 (0.101, 0.005)
Oviposition	0.346	0.001	1v2 (0.341, 0.003) 1v3 (0.441, 0.001) 1v4 (0.214, 0.001) 2v4 (0.699, 0.001)
Egg mass	0.097	0.001	1v3 (0.069, 0.003) 1v4 (0.277, 0.001) 3v4 (0.107, 0.001)
Resilience/resistance traits			
Dissemination	0.075	0.001	1v2 (0.146, 0.004) 1v3 (0.102, 0.001) 1v4 (0.162, 0.001)
Attachment	0.307	0.001	1v2 (0.278, 0.004) 1v3 (0.411, 0.001) 1v4 (0.689, 0.001) 2v4 (0.205, 0.002)
Flexibility	0.257	0.001	1v2 (0.218, 0.001) 1v3 (0.288, 0.001) 1v4 (0.575, 0.001) 2v4 (0.086, 0.002) 3v4 (0.125, 0.005)

Variable	Global R	Significance (p)	Pairwise tests (Global R, p)
Body form	0.186	0.001	1v3 (0.242, 0.001) 1v4 (0.379, 0.001) 2v3 (0.173, 0.003) 2v4 (0.330, 0.001)
General physiological traits			
Feeding	0.102	0.001	1v2 (0.184, 0.001) 1v3 (0.166, 0.001) 1v4 (0.283, 0.001)
Dietary preference	0.066	0.002	1v3 (0.108, 0.001) 1v4 (0.202, 0.001)
Respiration	0.114	0.001	1v2 (0.276, 0.004) 1v3 (0.140, 0.001) 1v4 (0.208, 0.001)
Aquatic stages	0.308	0.001	1v2 (0.124, 0.002) 1v3 (0.349, 0.001) 1v4 (0.124, 0.001) 2v3 (0.091, 0.033) 2v4 (0.323, 0.001) 3v4 (0.084, 0.008)

5.6 Metrics – Soft-bottomed stream

For the analysis across soft-bottomed sites, the number of sites within each landuse category was: <10% = 4, 10–49% = 0, 50–90% = 10, >90% = 16.

There was relatively limited variation in % similarity within % pastoral groups based on total richness and MCI, with greatest difference between impact groups based on EPT richness and %EPT abundance (Figure 18). Similarity was generally highest for the <10% group and lowest for the >90% group.

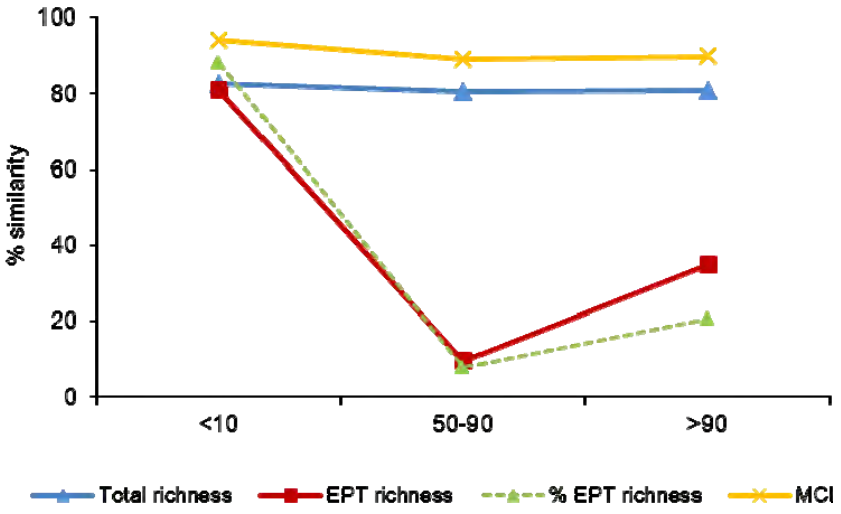

Figure 18: % similarity within % pastoral groups for individual metrics for soft bottomed streams.

The greatest dissimilarity for MCI and total richness was found between <10% and >90% sites (Figure 15). This was not the case for EPT richness or %EPt abundance, where the greatest difference was for the 50–90 versus >90% comparison.

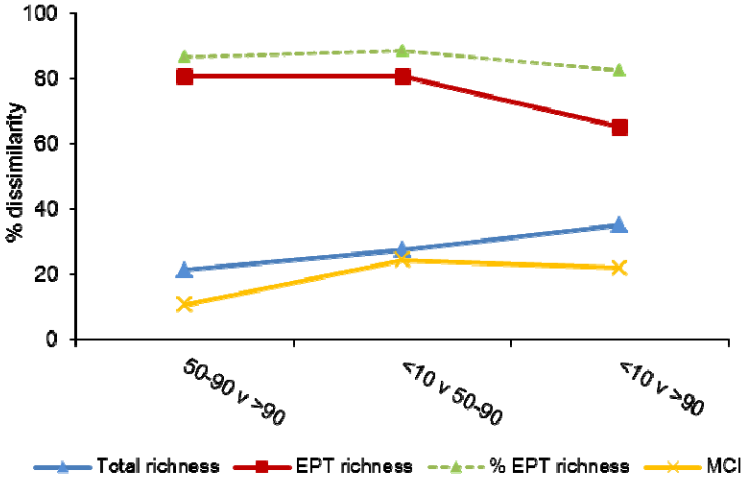

Figure 19: **% dissimilarity between % pastoral groups for individual metrics for soft bottomed streams.**

The results of ANOVA analyses indicated significant deviation from random for all metrics (Table 5-5). Post-hoc pairwise comparisons indicated significant differences between all % pastoral pairings for % EPT abundance, while for EPT richness and MCI there was no significant difference between the 50–90 and <90% categories. For taxon richness there was no significant difference between the <10 and 10–49%, 50–90% and >90%, and 10–49% and >90% categories.

Table 5-5: ANOVA results for metrics % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) using soft bottomed data only.

Variable	P value	Pairwise tests
Taxon richness	<0.001	1v2 (0.495) 1v3 (<0.001) 1v4 (<0.001) 2v3 (0.021) 2v4 (0.066) 3v4 (0.948)
EPT richness	<0.001	1v2 (0.006) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.055)
% EPT abundance	<0.001	1v2 (0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.002)
MCI	<0.001	1v2 (<0.001) 1v3 (<0.001) 1v4 (<0.001) 2v3 (<0.001) 2v4 (<0.001) 3v4 (0.102)

5.7 Traits – Soft-bottomed stream

Variation within the % pastoral categories across all of the trait categories ranged from 52 and 95% (

Figure 20). For size, number of reproductive cycles per individual, life duration, oviposition, egg mass, body flexibility and aquatic stages, sites in the <10% pastoral land use group showed the greatest similarity, while sites in the >90% pastoral land use group showed the lowest similarity. For other traits this pattern differed e.g., for number of reproductive cycles per year similarity in the <90% category was highest.

Per cent dissimilarity between <10 and 11–49% pastoral categories was lowest of all the % pastoral pairings for all traits other than number of reproductive cycles per year and body form (Figure 21). The absolute % dissimilarity ranged from 17.8 to 62.3 (Figure 21). Greatest

% dissimilarity was detected for the <10 v >90% pairing for a number of traits, including number of reproductive cycles per year and individual, life duration, oviposition site, egg mass, dissemination potential, body flexibility and aquatic stages.

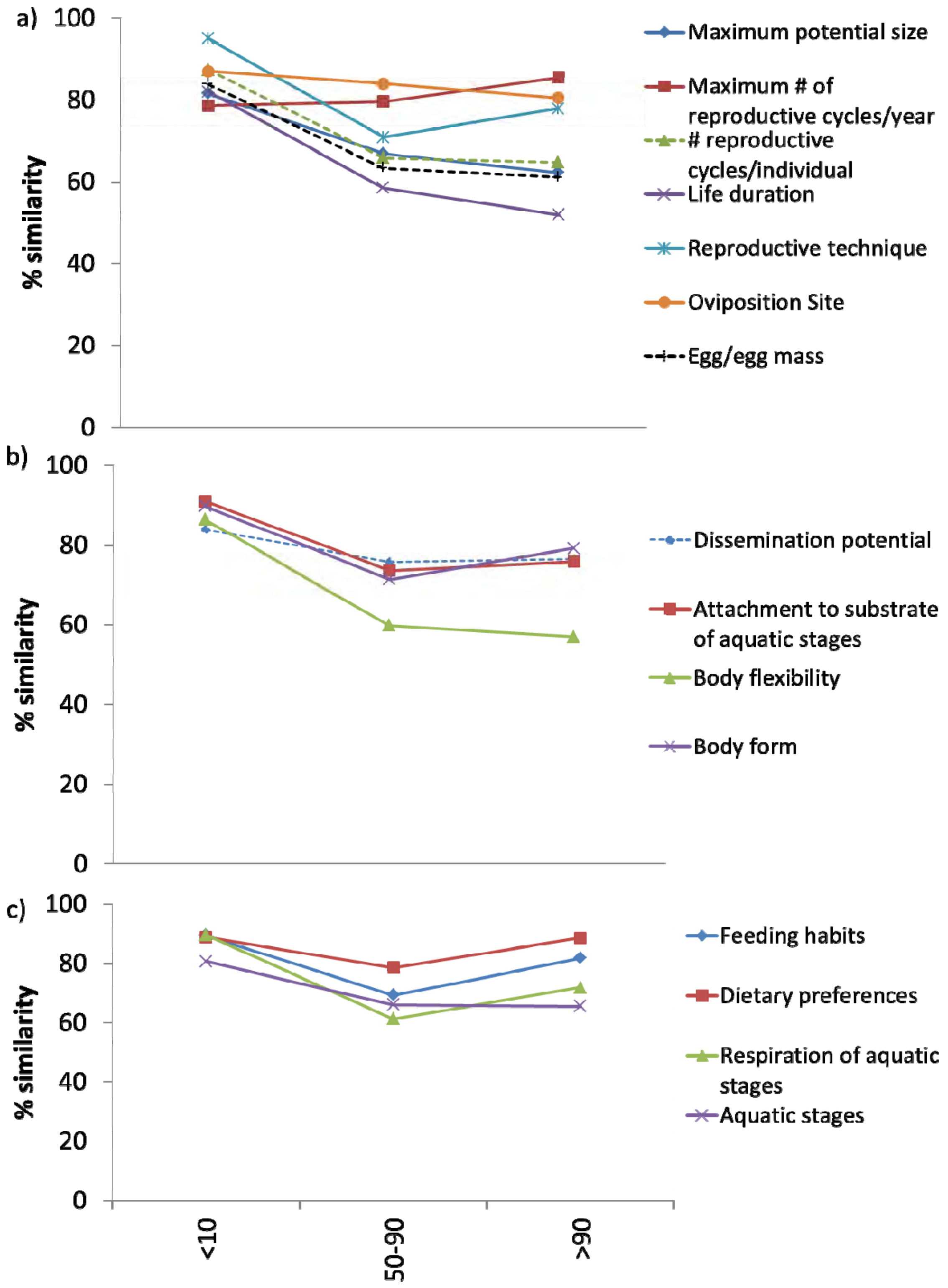

Figure 20: % similarity within % pastoral groups for a) life history, b) resilience or resistance and c) general biological trait categories for soft bottomed streams.

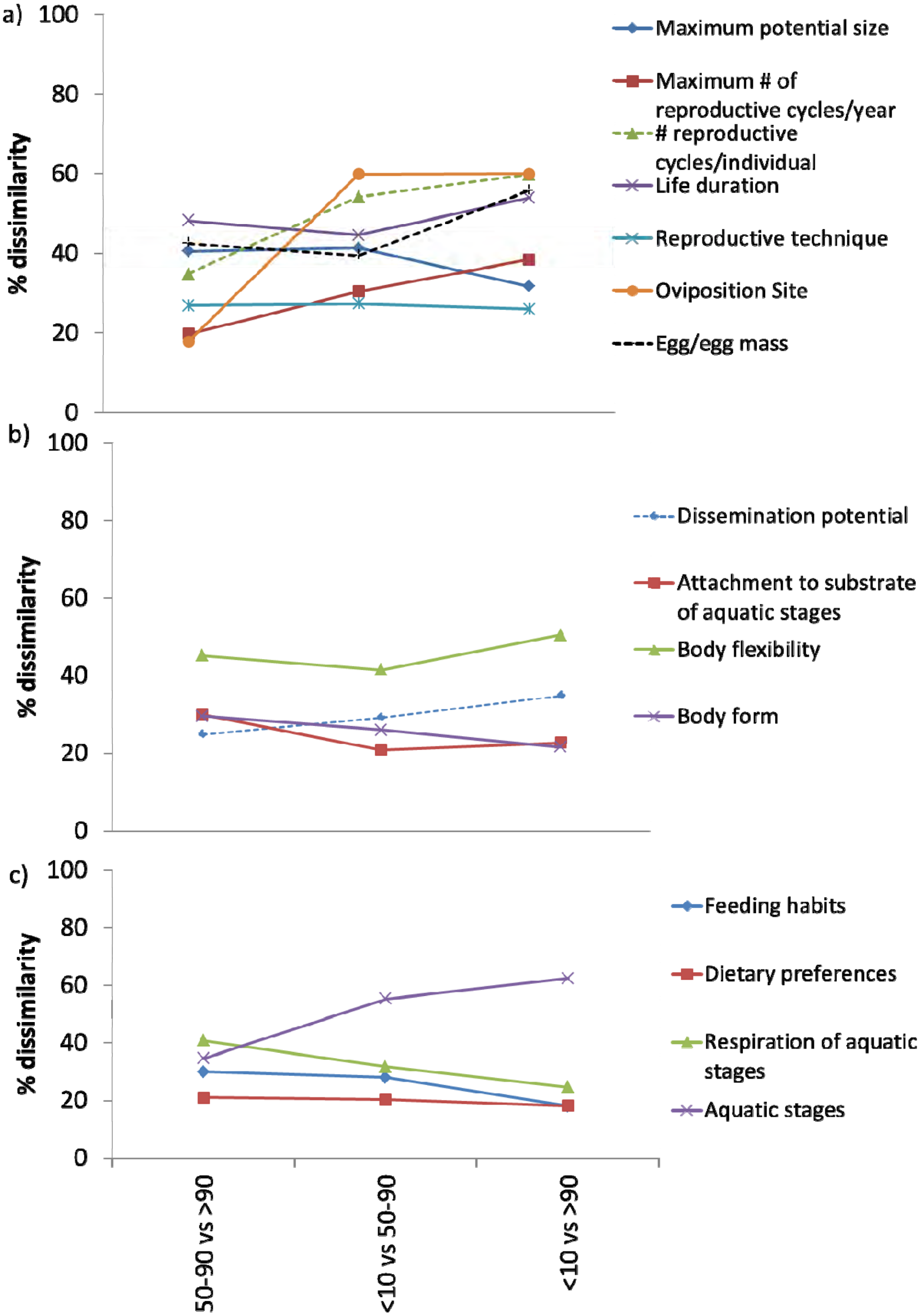

Figure 21: % similarity between % pastoral groups for a) life history, b) resilience resistant and c) general biological trait categories for soft bottomed streams.

ANOSIM analyses indicated significant deviation from random for all traits (Table 5-6). R values varied between traits, with the highest value recorded for oviposition (0.346), Number of reproductive cycles per year (0.311) and dietary preference (0.316). Pairwise tests for Number of reproductive cycles per year, life duration, egg mass, dissemination, flexibility, dietary preference and aquatic stages indicated highest R values for the <10v>90% comparison (Table 5-6). Using the criteria from section 5.1 as the basis for determining the effectiveness of a measure in detecting differences between impact state, many traits would satisfy this criterion, with oviposition, aquatic stages, volitinism and dissemination providing the greatest ability.

Table 5-6: ANOSIM results - % pastoral (1=<10%, 2=11–49%, 3=50–90%, 4=>90%) using soft bottom data only.

Trait	Global R	Significance (p)	Pairwise tests (Global R, p)
Life history traits			
Size	0.071	0.044	1v3 (0.319, 0.001) 3v4 (0.009, 0.021)
Number of reproductive cycles per year	0.311	0.001	1v3 (0.262, 0.001) 1v4 (0.659, 0.001) 3v4 (0.145, 0.005)
Number reproductive cycles/individual	0.177	0.001	1v3 (0.441, 0.001) 1v4 (0.403, 0.001)
Life duration	0.082	0.018	1v3 (0.150, 0.019) 1v4 (0.143, 0.008)
Reproduction	0.101	0.012	1v4 (0.164, 0.005) 3v4 (0.087, 0.021)
Oviposition	0.337	0.001	1v3 (0.922, 0.001) 1v4 (0.750, 0.001)
Egg mass	0.172	0.001	1v3 (0.129, 0.054) 1v4 (0.331, 0.001) 3v4 (0.081, 0.033)
Resilience or Resistance traits			
Dissemination	0.198	0.001	1v3 (0.220, 0.005) 1v4 (0.395, 0.001)
Attachment	0.100	0.009	3v4 (0.190, 0.002)
Flexibility	0.133	0.001	1v3 (0.124, 0.006) 1v4 (0.215, 0.003)
Body form	0.142	0.002	3v4 (0.183, 0.001)
General biological traits			
Feeding	0.195	0.002	3v4 (0.277, 0.001)
Dietary preference	0.307	0.001	1v4 (0.431, 0.001) 3v4 (0.296, 0.001)
Respiration	0.128	0.007	3v4 (0.201, 0.001)
Aquatic stages	0.231	0.001	1v3 (0.483, 0.001) 1v4 (0.513, 0.001)

6 Combined analysis – Auckland Council and Waikato Regional Council

6.1 Introduction

Stream macroinvertebrates have a range of environmental preferences and represent a diverse group that integrates ecosystem changes over time. Therefore, they are widely used as indicators of environmental disturbance (Wright et al. 1993, Stark et al. 2001, Metzeling et al. 2003). However, an important limitation in their use is that taxonomic composition and abundance vary considerably as a consequence of biogeography (e.g., Poff 1997, Heino 2001, Bonada et al. 2007) and the observed patterns are, in fact, the product of natural stochastic variation and independent deterministic changes associated with disturbance from human activities. Thus, the use of taxonomic composition alone may be insufficient to unambiguously distinguish local landuse effects from natural biogeographic variation of populations. Ecoregional differences in macrofaunal communities have been observed in New Zealand rivers (Harding and Winterbourn, 1997).

We investigated the influence of regional differences on the potential to differentiate impacts on streams affected by differing landuses in the Auckland (AC) and Waikato (WRC) regions. Due to differences in the number of sites in each landuse within each region, this analysis was only undertaken using sites in predominantly native forest or rural landuses. In addition, as different enumeration methods were used to derive abundance data, presence/absence data were used. Because of differences in stream type amongst reference sites, we also included stream type as a factor in our analysis.

6.2 Variation in Metrics

Landuse explained a greater proportion of variation than region or stream type for all metrics (Table 6-1), with percentage variation explained by landuse ranging from 15.2% (taxon richness) to 45% (EPT richness). However, variation in taxon richness was almost equally explained by landuse (15.2%), region (12.2%) and stream type (10%).

Table 6-1: Results (partial eta^2) of a three-way ANOVA to test for differences between regions, landuse and stream type. Values in bold indicate highest values.

Metric	All sites (103)		
	Landuse (Native=56, Rural=47)	Region (WRC=74, AC=29)	Stream type (HB=69, SB=44)
Taxon richness	0.152	0.122	0.100
EPT richness	0.450	0.001	0.190
MCI	0.410	0.020	0.030

Figure 22, Figure 23 and Figure 24 illustrate the differences in metric values based on region, landuse and stream type, respectively. As described above, there was little difference between any of the metrics based on region. In contrast, native stream sites recorded significantly higher values than rural streams for all metrics. Differences in MCI and EPT richness were also noted based on stream type. However, in general land use is the strongest driver of invertebrate metric scores.

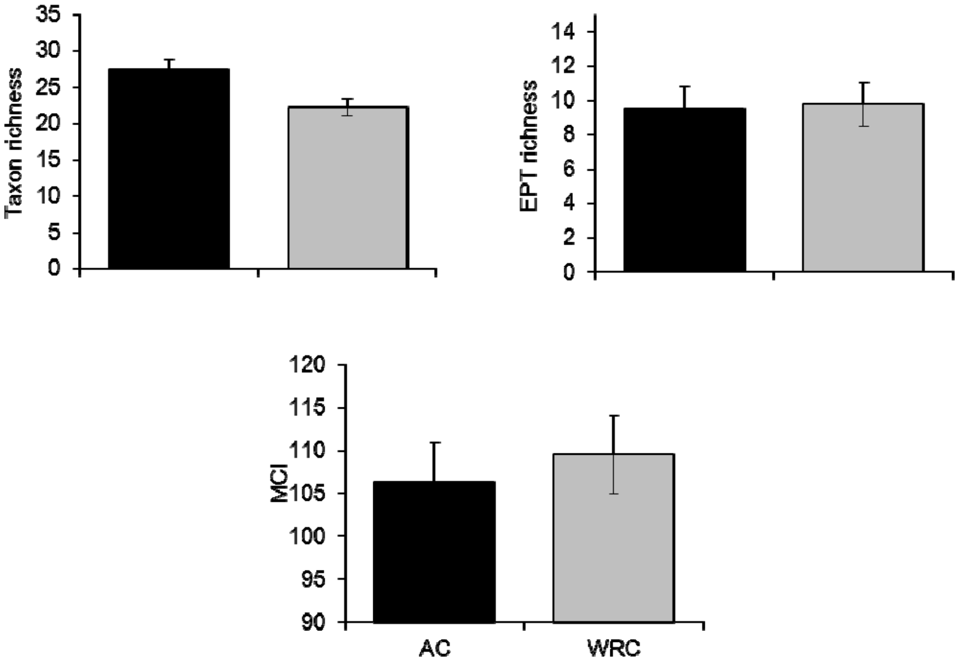

Figure 22: Metrics based on region (Auckland Council – AC, Waikato Regional Council – WRC) (±1 S.E.)

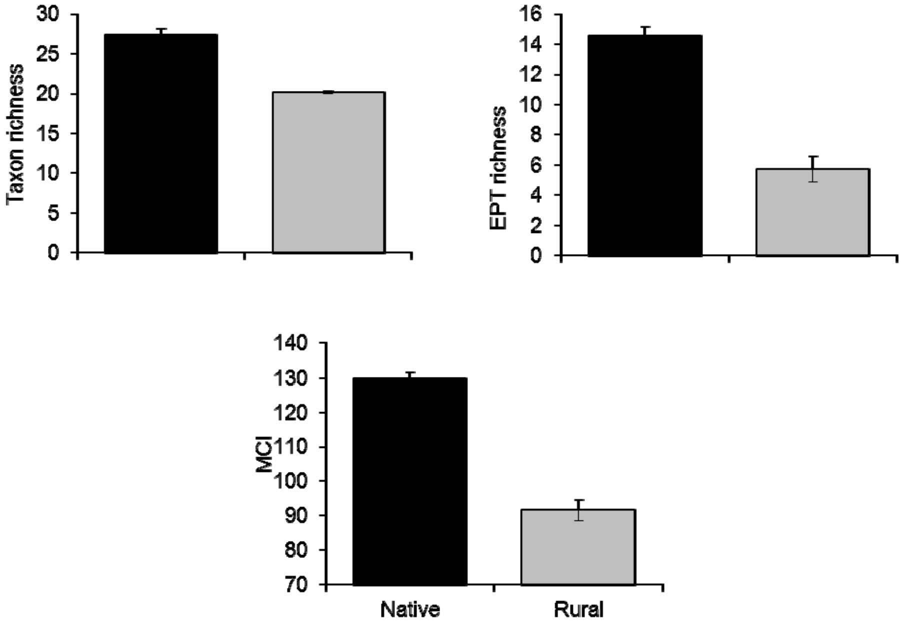

Figure 23: Metrics based on landuse (native and rural) across Auckland and Waikato regions (±1 S.E).

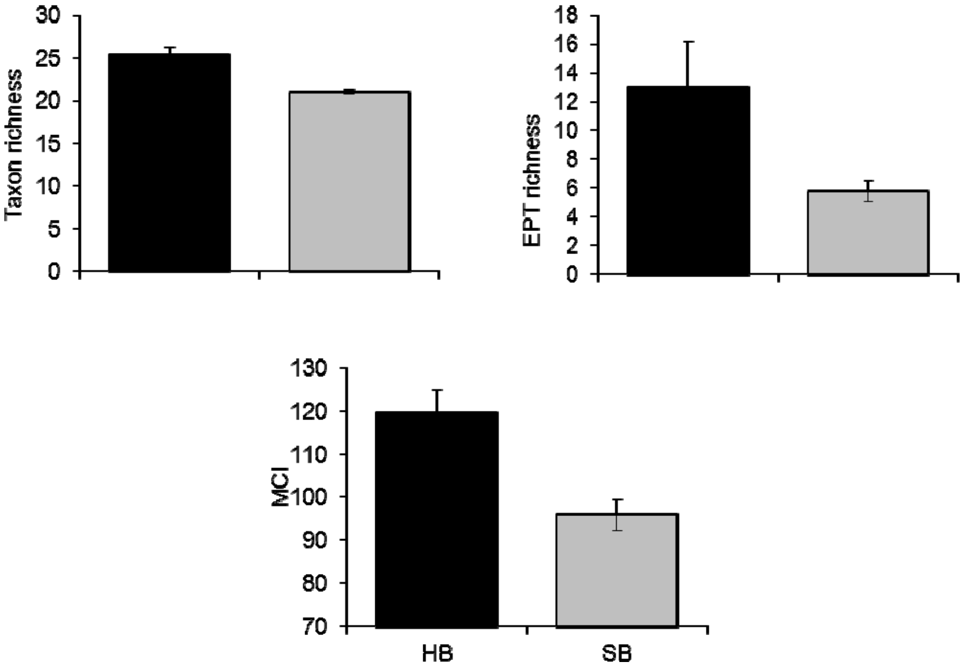

Figure 24: Metrics based on stream type (soft and hard bottomed) across Auckland and Waikato regions (±1 S.E.).

6.3 Variation in Traits

When examined across all sites (regardless of stream type), landuse explained more variation than region or stream type for almost all trait categories (Table 6-2), with percentage variation explained ranging from 17% (dispersal, body form) to 37% (oviposition). Region explained more of the variation in size (16%) and attachment to substrate (16%), while stream type explained more of the variation in egg mass location (23%).

Examination of individual trait modalities provides insight into specific mechanisms of response, as well as variability in sensitivity of specific traits/trait modalities to specific stressors. A greater proportion of variation was explained by landuse than regional differences in 36 of the 55 trait modalities examined. This result indicates that, in general, landuse is a much more significant driver of macroinvertebrate trait profiles than any differences associated with regionality.

Table 6-2: Partial eta^2 values for traits (category, modality) vs region for the combined data set for all streams. Values in bold indicate highest values for a specific trait category or modality.

Trait category	Landuse	Region	Stream type	Trait modality	Landuse	Region	Stream type
				Life history traits			
Maximum potential size (mm)	0.09	**0.16**	0.12	≤5	0.03	**0.08**	0.07
				≥5–10	0.01	<0.01	**0.03**
				≥10–20	<0.01	**0.02**	**0.02**
				≥20–40	0.02	0.06	**0.10**
				>40	**0.06**	0.01	0.01
Number of reproductive cycles per year	**0.18**	<0.01	0.04	semivoltine	<0.01	<0.01	<0.01
				univoltine	**0.17**	<0.01	0.03
				plurivoltine	**0.18**	<0.01	0.04
Number of reproductive cycles per individual	**0.24**	0.07	0.12	1	**0.24**	0.07	0.12
				≥2	**0.24**	0.07	0.12
Life duration (days)	**0.25**	0.18	0.15	≤1	0.10	**0.11**	0.06
				1–10	**0.09**	0.03	0.07
				10–30	**0.18**	0.09	0.07
				30–365	**0.18**	0.09	0.12
				>365	**0.04**	0.01	0.02
Reproductive technique	**0.25**	0.03	0.05	asexual	**0.17**	<0.01	0.04
				hermaphroditism	**0.23**	0.02	<0.01
				sexual	**0.25**	0.01	<0.01

Trait category	Landuse	Region	Stream type	Trait modality	Landuse	Region	Stream type
Oviposition site	**0.37**	0.03	0.14	water surface	**0.36**	0.02	0.06
				beneath the water surface	**0.32**	0.01	0.10
				terrestrial	0.05	<0.01	**0.11**
				eggs endophytic	**0.07**	0.03	**0.07**
Egg/egg mass location	0.14	0.18	**0.23**	cemented eggs	0.10	**0.11**	0.03
				female bears eggs in/on body	0.12	0.17	**0.22**
				free eggs	<0.01	0.01	**0.13**
Resilience/resistance traits							
Dispersal	**0.17**	0.02	0.02	low (10 m)	**0.02**	0.01	**0.02**
				medium (1 km)	**0.17**	0.01	<0.01
				high (>1km)	**0.06**	<0.01	0.01
Attachment to substrate	0.07	**0.21**	0.16	swimmers	<0.01	**0.19**	0.15
				crawlers	0.05	**0.14**	0.06
				burrowers	0.01	**0.02**	<0.01
				attached	0.02	**0.06**	**0.06**
Body flexibility	**0.31**	0.02	<0.01	none (<10°)	**0.14**	<0.01	<0.01
				low (>10–45°)	**0.31**	0.02	<0.01
				high (>45°)	**0.13**	<0.01	<0.01
Body form	**0.17**	0.10	0.03	streamlined	<0.01	0.01	<0.01
				flattened	0.02	<0.01	<0.01
				cylindrical	**0.06**	0.02	<0.01
				spherical	**0.16**	0.08	0.01
General physiological traits							
Feeding habits	**0.21**	0.09	0.09	shredders	**0.13**	<0.01	0.03
				scrapers	**0.06**	0.02	0.03
				filter-feeders	**0.09**	<0.01	<0.01
				deposit feeder	**0.06**	**0.06**	0.03
				predators	0.03	0.03	<0.01
				algal piercers	**0.07**	0.01	<0.01
Dietary preferences	**0.18**	0.05	0.12	strong (specialist)	**0.09**	0.02	0.12
				moderate	**0.13**	0.03	0.09
				weak (generalist)	<0.01	<0.01	**0.05**

Trait category	Landuse	Region	Stream type	Trait modality	Landuse	Region	Stream type
Respiration of aquatic stages	**0.21**	0.03	<0.01	tegument	**0.03**	0.01	<0.01
				gills	**0.13**	<0.01	<0.01
				plastron	**0.15**	<0.01	<0.01
				aerial	0.02	**0.11**	<0.01
Aquatic stages	**0.24**	0.10	0.11	adult and larva	**0.24**	0.10	0.11
				adult or larva	**0.36**	0.03	0.05
				Larva or pupa	<0.01	0.05	0.05

These results are illustrated graphically in Figure 25, Figure 26 and Figure 27. There is little difference between regions for most trait categories. In contrast, there were clear differences in the trait profiles of streams based on native and rural landuses (Fig. 6.4). The traits of taxa typical of rural streams include those that:

- reproduce more than once per year (plurivoltine)
- reproduce more than once per individual
- tend to live longer
- reproduce asexually (or through hermaphrodism)
- lay submerged eggs, and
- have both adult and larval aquatic forms.

The similarity in plots between landuse and stream type reflects the predominance of hard bottomed streams in native landuse (61%) and soft bottomed streams in rural landuse (also 61%). These results from Table 6-2 indicate that landuse, rather than stream type, was the dominant driver for most traits. These results also suggest that trait analysis can be reliably undertaken across regions (other than for size and attachment, where region explained more of the variation).

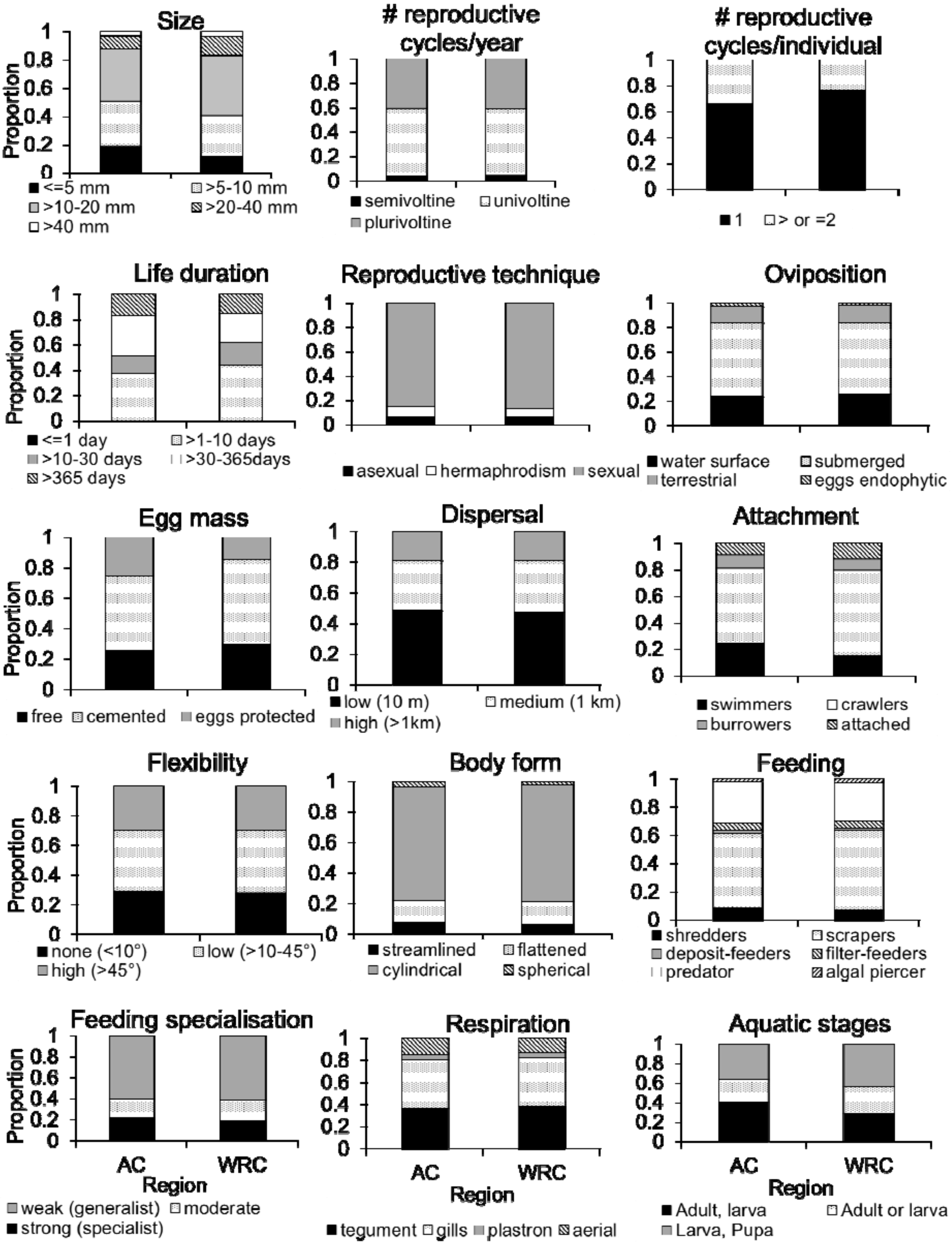

Figure 25: Trait profiles based on region (across all stream types and landuses).

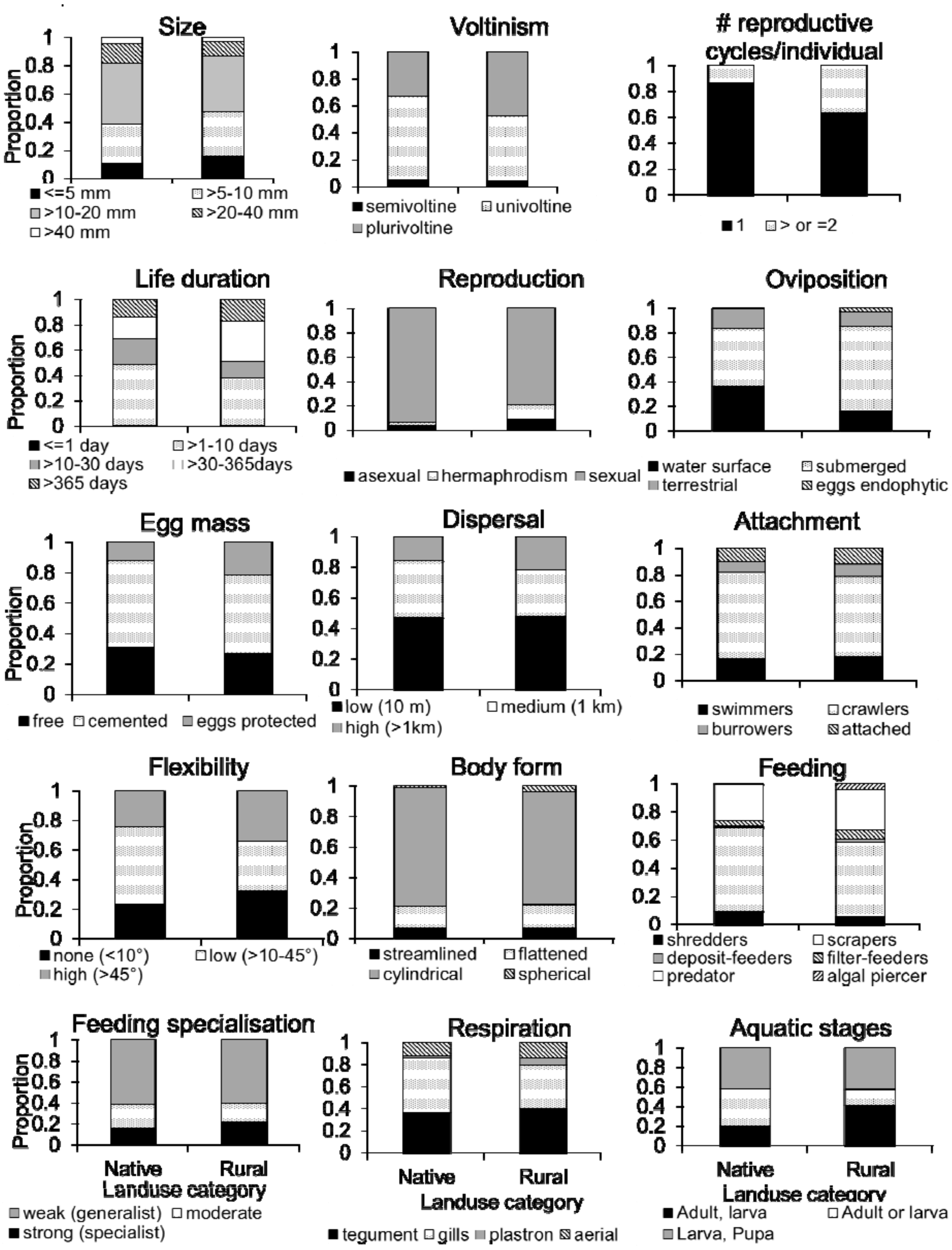

Figure 26: Trait profiles based on landuse (across all regions and stream types).

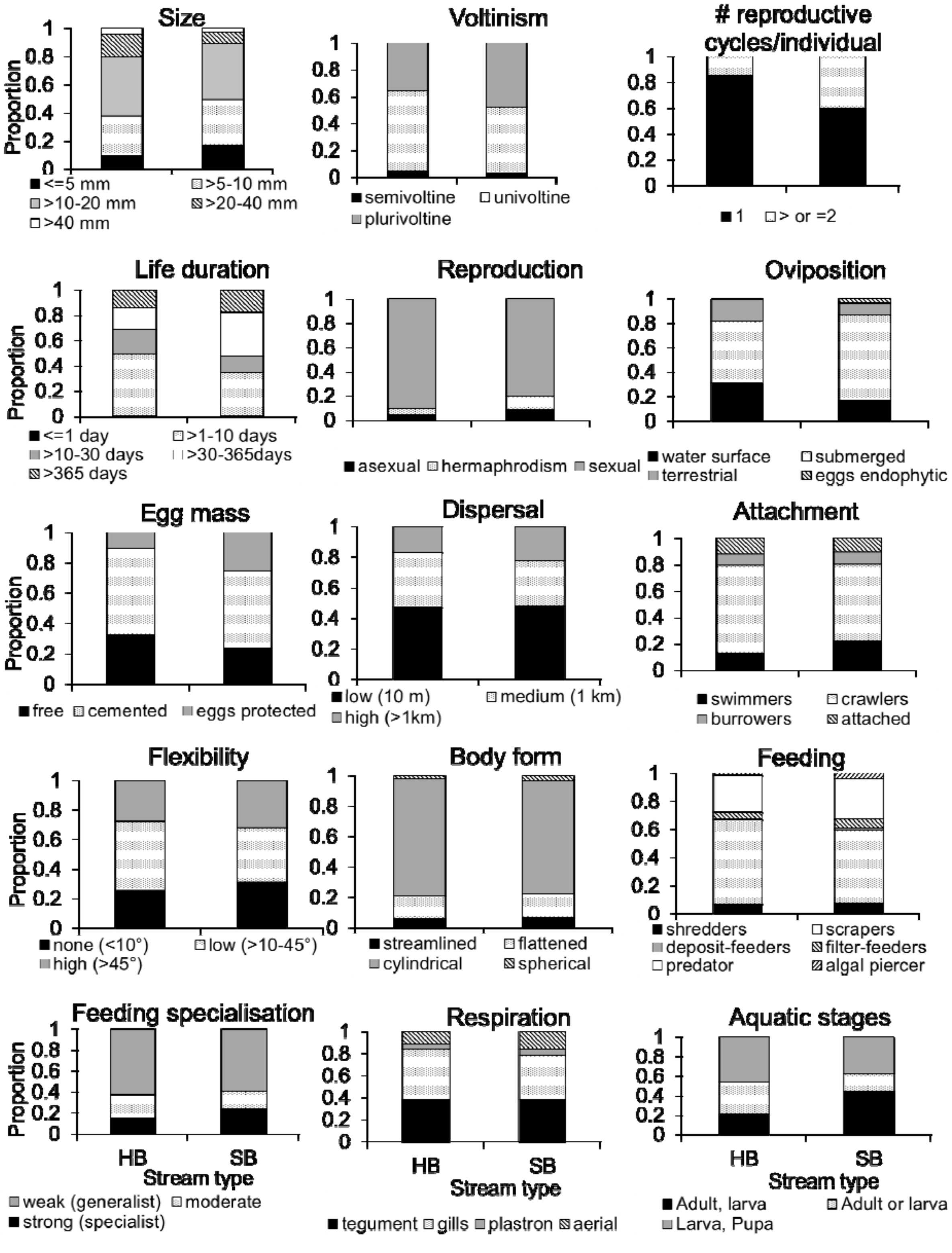

Figure 27: Trait profiles based on stream type (across all regions and landuses).

7 Diagnostic value of traits

One of the potentially valuable applications of the trait approach to biomonitoring is its ability to diagnose different landuse impacts. In previous chapters we have identified significant changes in the frequencies of trait modalities in association with increasing pastoral development. In general, the complete absence of a trait modality is not observed; rather there is a change in frequency. By identifying the dominant trait modality indicative of a particular landuse it should be possible to track changes in the functional profile of sites associated with changes in landuse. As an initial step in this process, we examined the relationship between % pastoral development and individual trait modalities by calculating Pearsons Product-Moment Correlations.

Table 7-1 presents those trait modalities for which correlations ≥0.50 or ≤-0.50 were detected. These cut-off values were chosen as the aim was to determine the predominant trait modalities. Eight of the traits recorded correlation coefficients ≥0.50 or ≤-0.50. When a cut-off of R value of ≥0.40 or ≤-0.40 was employed, the number of significant correlations increased to 20.

Table 7-1: Pearsons Product-Moment Correlation coefficients for % pastoral/trait modality correlations for all sites. The yellow cells are those with R values ≥0.50 or ≤-0.50). Green shading indicates R values ≥0.40 or ≤-0.40).

Trait category	Trait modality	% Pastoral
Number reproductive cycles/year	Plurivoltine (2)	0.48
	Univoltine (1)	-0.48
Number reproductive cycles/individual	1	-0.53
	> or =2	0.53
Life duration	<1 day	-0.45
	>1–10 days	-0.47
	>30–365days	0.43
Reproductive technique	asexual	0.49
	hermaphrodism	0.26
	sexual	-0.50
Oviposition site	water surface	-0.72
	submerged	0.69
	terrestrial	-0.14
Egg mass	free	-0.26
	cemented	-0.46
Dispersal	medium	-0.40
Body flexibility	none	0.46
	low	-0.56
	high	0.09
Body form	streamlined	-0.47
	cylindrical	0.37

Trait category	Trait modality	% Pastoral
Feeding habits	shredders	-0.45
	filter feeders	-0.12
Dietary specialisation	moderate	-0.48
	strong (specialist)	0.21
Aquatic stages	Adult, larva	0.58
	Adult or larva	-0.69

The relative frequency of each dominant trait modality identified in Table 7-1 is plotted to illustrate the changes associated with increasing pastoral development Figure 28. On this basis it can be concluded that, in comparison to sites in native forest, sites subject to pastoral development were more likely to have a greater proportion of taxa that:

- reproduce more than once per year
- have more than one reproductive cycle per individual
- live longer
- reproduce asexually
- lay submerged eggs
- have protected eggs
- have inflexible body form and tend to be cylindrical
- have both adult and larval aquatic forms.

The ability to identify trait modalities that are responding to specific landuses requires analysis of the environmental measures specifically associated with these landuses, as at least some measures will be common to more than one landuse. For example, rural streams are often characterized as having high temperatures, though loss of riparian vegetation (Quinn et al. 1997). This is also a characteristic of urban streams (Walsh et al. 2001). Identifying key drivers will be an important next step in developing traits as a diagnostic tool.

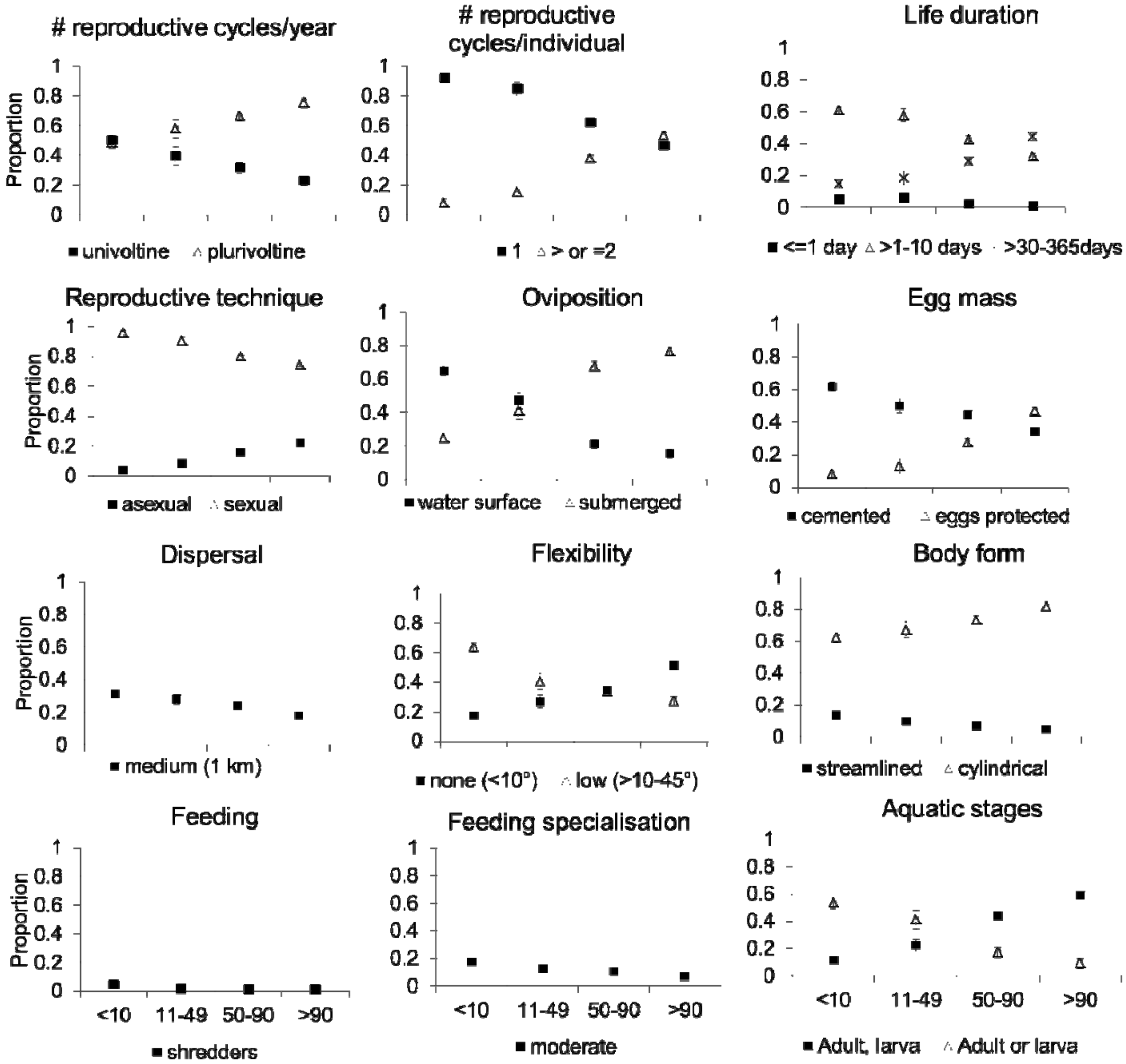

Figure 28: Plots of frequencies of trait modalities for each category with correlation coefficients ≥0.50 with % pastoral.

8 Discussion

In this project we determined the response of both metrics and traits to a gradient of pastoral development (as measured by % pastoral landuse in the catchment) for Waikato streams. Based on existing information, we developed *a priori* hypotheses of likely responses of traits to these stressors. As part of this analysis we also compared the effectiveness of metric and trait measures for differentiating levels of impact. We examined the potential influence of regional differences in invertebrate communities on the ability of metrics and traits to detect the impacts of rural development. Finally, we investigated the potential use of traits as a mechanistic tool.

The aim of this study was not simply to determine whether traits were “better” or “worse” than taxonomic-based metrics at differentiating between impacted sites. Rather it was aimed at determining how traits could “add value” to existing, well established monitoring tools.

Analysis of metrics and traits in reference sites (native vegetation in >90% of the catchment) identified significant differences associated with stream type (hard vs. soft bottomed streams) for taxon richness. Similarly stream type explained a significant amount of variation in some trait categories and trait modalities. Taken in isolation, the findings of differences associated with stream type may be of concern for biomonitoring purposes for both metrics and traits, requiring the factoring out of stream type analyses where sampling includes both stream types. However, the importance of this result needs to be considered in the context of multiple environmental drivers i.e., what is the relative contribution of stream type compared to other drivers?

When the relative contribution of stream type and % pastoral to metric and trait values was investigated, landuse was found to be the predominant factor in all metrics. Similarly landuse explained more of the variation in 53% of trait categories and 76% trait modalities. Dolédec et al. (2006) found 26% of trait categories differed significantly amongst landuse practices (from ungrazed tussock land to highly intensive deer and dairy farming), so our result is significant.

Many traits were found to be responsive to pastoral development. Traits more strongly associated with pastoral development included number of reproductive cycles per year and per individual, reproductive technique, oviposition, body flexibility and form, feeding habits and specialization and aquatic stages. Dolédec et al. (2006) found traits relating to life history (number of reproductive cycles/individual and year, life duration, egg laying modes and parental care behaviour) provided the strongest separation along a landuse gradient. Weaker but significant relationships were found with feeding ecology, body shape and respiration. Previous studies have demonstrated that traits related to body size shape and feeding strategies were more weakly related to perturbations (Dolédec at al 1999, 2006). However, Townsend & Thompson (2007) suggested that average invertebrate body size would increase with agricultural intensity because of the relationship between growth and nutrient-induced stream productivity.

A comparison of the ranges of partial eta ^{2} values derived for metrics and traits suggests that traits (as categories or as individual traits) were as powerful at detecting impacts of pastoral development as metrics (Table 8-1). Traits displayed a much greater range of partial eta ^{2} values than metrics, indicating that not all trait categories/modalities were equally effective.

Table 8-1: Ranges of partial eta^2 values derived from tests of metric and trait responses to increasing pastoral development for Waikato streams.

	Metrics		Trait category		Individual trait	
Stream type	**Range**	**Best**	**Range**	**Best**	**Range**	**Best**
All	0.26–0.54	MCI	0.06–1.00	Oviposition	0.05–0.56	Surface laying
Hard bottomed	0.16–0.56	MCI	0.06–1.00	Oviposition	0.03–0.53	Surface laying
Soft bottomed	0.43–0.63	%EPT abundance	0.09–1.00	Oviposition, Size	0.01–0.51	Surface laying

In our study we predicted a number of trait responses based on the potential influences of multiple environmental stressors. Table 8-2 summarises the trait responses to increasing pastoral development observed for Waikato streams and assesses these responses against our *a priori* predictions. For many traits, our prediction held true, although short generation time did not increase with pastoral development, as would have been predicted from previous studies (and from ecological theory). For some traits, a more complex response was evident e.g., while algal piercers increased (a response to increasing algal biomass typical of rural streams), filter feeders also increased (an unexpected result given the anticipated increase in sedimentation with increasing rural development). Dolédec et al. (2010) found no relationship between the frequency of filter feeders and increasing landuse intensification. The increase in the laying of free eggs was inconsistent with the predicted increase in bed sediment cover associated with landuse intensification and was contrary to previous findings (Dolédec et al. 2010). Tomanova et al. (2008) have suggested that inconsistencies in functional responses to anthropogenic impacts could be due to the simultaneous operation of several stressors.

Table 8-2: Summary of trait responses to pastoral development compared to a priori predictions.

Factor Variable	Predictions (section 2.4)	Ecological basis[1]	Change in relation to increasing land use development			Prediction supported?
			All stream types	Hard bottomed	Soft bottomed	
Size	↑small sizes	Increased resilience[1]	↑ >5–10mm, ↓ >10–20mm	↑ >5mm, >5–10mm, ↓ >10–20mm	↑ >5–10mm, ↓ >10–20mm	Yes
Number of reproductive cycles per year	↑plurivoltinism (multiple of reproduction cycles/year)	Increased resilience[1]	↑plurivoltinism	↑plurivoltinism	↑plurivoltinism	Yes
Number reproductive cycles/individual	↑in rapidly reproducing taxa	Rapid population turnover[1]	↑ >1, ↓1	↑ >1, ↓1	↑ >1, ↓1	Yes
Life duration	↑short generation time	Rapid population turnover[1]	↑ >30–365 days, ↓ >1–10, >10–30 days	↑ >30-365 days, ↓ >1-10, >10–30 days	↑ >30–365 days, ↓ >1–10, >11–30 days	No
Reproduction	↑asexual	Rapid recolonisation in variable environments[1]	↑asexual, hermaphroditism, ↓sexual	↑asexual, hermaphroditism, ↓sexual	↑asexual, hermaphroditism, ↓sexual	Yes
Oviposition	↓surface egg laying	Increased sediment cover smothers eggs[2]	↑submerged, endophytic, ↓water surface, terrestrial	↑submerged, endophytic, ↓water surface, terrestrial	↑submerged, endophytic, ↓water surface, terrestrial	Yes
Egg mass	↑in protected eggs	Increased sediment cover smothers eggs[2]	↑free, protected eggs, ↓cemented	↑free, protected eggs, ↓cemented	↑free, protected eggs, ↓cemented	Yes
Dissemination	↑highly dispersive taxa	Promotes refuge use and recolonisatons[1]	↑low, ↓medium	↑low, ↓medium	↑low, ↓medium	No
Attachment	↑ burrowers	Increased sediment[2]	↑ crawlers, ↓ swimmers	↑ crawlers, ↓ swimmers	↑ crawlers, ↓ swimmers	Somewhat
Flexibility	↑ flexibility[1]	Response to high flows[1]	↑none, ↓low	↑none, ↓low	↑none, ↓low	No

Factor Variable	Predictions (section 2.4)	Ecological basis[1]	Change in relation to increasing land use development			Prediction supported?
			All stream types	Hard bottomed	Soft bottomed	
Body form	↑ Flattened[1]	Response to high flows[1]	↑ cylindrical, ↓ streamlined	↑ cylindrical, ↓ streamlined	↑ cylindrical, ↓ streamlined	No
Feeding	↑algal piercers, detritivores, ↓scraper, filter feeders	increase in autrophs[2]	↑ filter feeder, algal piercer	↑ filter feeder, algal piercer	↑ filter feeder, algal piercer	Somewhat
Dietary preference	↑specialist		↑specialist, ↓ generalist, moderate	↑specialist, ↓ generalist	↑specialist, ↓ moderate	Somewhat
Respiration	↑plastron, ↓gills	Increased sediment[2]	↑ gills, ↓ tegument	↑ gills, ↓ tegument	↑ gills, ↓ tegument	No
Aquatic stages	↓adults aquatic	Aerial adults for increased dispersal[1]	↑adult and larvae, ↓adult or larvae	↑adult and larvae, ↓adult or larvae	↑adult and larvae, ↓adult or larvae, larvae or pupae	No

[1] Based on (Townsend & Hildrew 1994), [2] (Doledec et al. 2006).

Both metrics and traits were found to be effective in differentiating between levels of impact. Particularly effective traits included number of reproductive cycles/individual, reproductive technique, oviposition site, body flexibility and aquatic stages.

Dolédec et al. (2011) examined variation in taxonomic metrics and biological traits over a broad regional scale (the whole of New Zealand) in relation to landuse intensification. They found a greater proportion of taxonomic metrics were influenced by ecoregional differences than biological traits. In addition, they found landuse explained twice as much variation in traits as in taxonomic metrics. In our study we found that landuse explained more of the variation in metrics than regional differences, with between 26 and 59% of the total variation explained. A similar result was recorded for most trait categories (with 22–59% of variation explained) and many individual traits (with 1–59% of variation explained). Collectively this suggests that both metrics and traits can be used to differentiate landuse effects across Auckland and Waikato regions. Inclusion of additional regional datasets would provide a more robust analysis of this issue, as the dataset available was limited due to the need to match pastoral development.

There is growing interest in the use of traits for diagnosing causal mechanisms of response in benthic invertebrate communities (Culp et al. 2010). In our study, we have identified significant differences in the frequencies of trait modalities that allow us to distinguish native forest sites from pastoral sites. However, the measures of landuse employed (% pastoral landuse) were insufficient to identify specific causal mechanisms such as reduced oxygen, increased temperature, increased nutrient concentrations, which would commonly be associated with a change from native to pastoral landuse. Further investigation is required to refine these results. Existing data sets are likely to be available to undertake such analyses. Notwithstanding this, trait profiles based on mode and frequency of reproduction, oviposition characteristics, movement, feeding and aquatic stages could be employed to detect trends over time in recovery following restoration or degradation following landuse changes.

Trait-based biomonitoring would fit readily into existing biomonitoring frameworks, as the basic information (site by species composition matrices) is already collected. Challenges exist for the general application at a national level, for example, due to inconsistencies in the way invertebrate data are collected and enumerated (coded abundance versus fixed count data). The use of presence/absence data may address this challenge. Other challenges related to consistency, availability, applicability and understanding of the trait data (Culp et al. 2010).

9 Conclusions and recommendations

For traits to be considered for integration into existing biological monitoring programmes, they would ideally need to satisfy the following criteria:

- display low levels of variation at high and low levels of pastoral development, and significant discriminatory power at intermediate levels
- display greater discriminatory power over and above that achieved by standard metrics
- possess the ability to diagnose causal factors.

Almost all trait categories were found to be effective in differentiating between high and low levels of impact, with differing levels of effectiveness for medium impact levels. Particularly effective traits included number of reproductive cycles/individual, life duration, reproductive technique, egg mass location, oviposition, flexibility and aquatic stages.

Traits (as categories or as individual traits) were found to be as powerful at detecting impacts of pastoral development as metrics. The trait categories number of reproductive cycles/individual, reproductive technique, oviposition, dispersal, flexibility and aquatic stages were especially effective.

We have identified significant differences in the frequencies of trait modalities that allow us to identify native forest sites from pastoral sites. Further refinement of traits as a diagnostic tool requires further analysis using specific environmental variables associated with disturbance. However, trait profiles based on mode and frequency of reproduction, oviposition characteristics, movement, feeding and aquatic stages could be employed to detect trends over time in recovery following restoration or degradation following landuse changes.

A set of trait categories consistently meeting all three of the above criteria includes: the number of reproductive cycles/year and individual, reproductive technique, egg mass location, oviposition, flexibility and aquatic stages. These traits categories (and their associated modalities) could be integrated into existing biomonitoring programmes.

The effectiveness of a trait-based measure in detecting a stressor will depend on the specificity of the stressor under investigation. The development of *a priori* predictions based on an understanding of likely ecological and physiological responses of individual taxa significantly enhances the value of this approach. A number of studies derived stressor-specific traits or suites of traits (e.g., salinity - Schafer et al. 2011, metals and cargo-ship traffic – Dolédec and Statzner 2008, toxic substances – Archaimbault et al. 2010). In reality, few stressors operate in isolation and one of the advantages of the trait approach is the ability to detect multiple stressor responses. Statzner & Beche (2011) have suggested that resolving the effects of multiple human-caused stressors on ecosystems requires a high diversity of response variables that react mechanistically to specific stressors so that their responses can be *a priori* predicted. They further suggest that using multiple biological traits is the only feasible way of addressing this challenge.

Based on the results of this preliminary assessment of trait and metric responses to increasing pastoral development, recommendations for further development of this approach for use in biomonitoring are detailed below and include:

- Further investigation of diagnostic traits/trait profiles by using more specific measures of disturbance (e.g., nutrient concentrations).
- Investigation and development of stressor-specific traits, derived either empirically (e.g., Rubach et al. 2010, Schäfer et al. 2011) or through relational analysis of existing datasets.
- Investigation of the development of a trait-based multi-metric (Archaimbault et al. 2010) using existing datasets.
- Expansion of the regional analysis to encompass a broader range of landuse types (e.g., through integration with other regional councils).
- Development of a method for integrating a traits approach into standard monitoring protocols.

10 Glossary of abbreviations and terms

Aquatic stages - Are all stages of the life cycle of the animal aquatic? E.g., All stages of Dytiscidae are aquatic, whereas only larvae of Megaloptera are aquatic.

Attachment to substrate of aquatic stages (excluding eggs) - How does the animal move within its' habitat? Does it swim, crawl, burrow or is it attached?

Body flexibility - How flexible is the animal? Not flexible (<10°), low (>10–45°) or high (>45°)? E.g., snails are not flexible, worms have high flexibility

Body form - What is the shape of the animal? Is it streamlined, flattened (dorso-ventral or lateral), cylindrical or spherical?

Dietary preferences - Does the animal specialise in a particular species or type of food (e.g., wood feeder) or is it more generalised?

Dispersal (all stages) - How far can the larvae, pupae and adults move? Upto10 m (low dispersion potential), 1 km (medium) or >1 km (high dispersion potential).

Egg/egg mass - Do the eggs float freely on the water surface or stream bed or are they cemented to rocks and other debris or to plant material? Are they retained within the body (protected)?

Feeding habits - How does the animal feed? Is it a shredder, scraper, deposit-feeder, filter-feeder, predator or algal piercer?

Habitat - Where are the animals found?

Life duration of adults (including subimago of Ephemeroptera) - How long do adults live? Short (e.g., 1 day) to long (e.g., greater than 1 year)?

MCI - A measure of the relative sensitivity or tolerance of an organism to pollution (Stark et al. 2001). Ranges from 1 (pollution tolerant) to 10 (pollution sensitive)

Number of reproductive cycles per individual - How many times does an animal reproduce before it dies?

Oviposition site - Where are the eggs deposited? On or under water, on land or are they inserted into plants (endophytic)?

Potential number of descendants per reproductive cycle - Generally measured as the number of eggs (or number of individuals if live-bearing) produced per reproductive cycle.

Potential size - Refers to the maximum recorded size of the animal

Reproductive technique - May be sexual, asexual (through budding or cloning) or may be hermaphroditic (so male and female sexual organs are both present on the animal)

Respiration of aquatic stages (not including eggs) - How does the animal obtain its' oxygen? If in dissolved form, then respiration will be by gills or over the general body surface (tegument and spiracles). If in atmospheric form, then the animal may have a respiratory siphon or may be able to take bubbles of air under its wings (plastron; temporary air storage) or other structures to use while under water.

Trait category – the type of trait e.g., reproductive technique, size

Trait modality – groupings of organisms based on characteristics of a trait category e.g., sexual or asexual reproduction

Voltinism (Potential number of reproductive cycles per year) - May be less than once a year (semi-voltine), once a year (univoltine) or greater than once a year (plurivoltine). This measure is known to vary with temperature and hence latitude.

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Appendix A Biological traits and trait categories and their codes

Biological trait	Code	Trait category
	Life history	
Maximum potential size (mm)	SIZE1	≤5
	SIZE2	≥5–10
	SIZE3	≥10–20
	SIZE4	≥20–40
	SIZE5	>40
Number of reproductive cycles per year	SEMI	semivoltine
	UNIV	univoltine
	PLURIV	plurivoltine
Number of reproductive cycles per individual	CPI1	1
	CPI2	≥2
Life duration of adults (days)	LDA1	≤1
	LDA2	1–10
	LDA3	10–30
	LDA4	30–365
	LDA5	>365
Reproductive technique	SINGLE	single individual
	HERMA	hermaphroditism
	TWO	male and female
Oviposition site	SURFACE	water surface
	SUBMERGED	beneath the water surface
	TERRESTRIAL	terrestrial
Egg/egg mass	EGGFREE	free eggs
	EGGCEMENT	cemented eggs
	EGGPROTECTED	female bears eggs in/on body
	EGGENDO	eggs endophytic
	Resistance and resilience	
Dissemination potential (all stages)	DISSLOW	low (10 m)
	DISSMEDIUM	medium (1 km)
	DISSHIGH	high (>1 km)
Attachment to substrate of aquatic stages (excluding eggs)	SWIMMER	swimmers (water column)
	CRAWLER	crawlers (epibenthic)
	BURROWER	burrowers (infauna)
	ATTACHED	attached

Biological trait	Code	Trait category
Body flexibility	NOFLEX	none (<10°)
	LOWFLEX	low (>10–45°)
	HIGHFLEX	high (>45°)
Body form	STREAMLINED	streamlined
	FLATTENED	flattened (dorso-ventral or lateral)
	CYLINDRICAL	cylindrical
	SPHERICAL	spherical
General biological characteristics		
Feeding habits	SHREDDER	shredders
	SCRAPER	scrapers
	FILTERFEED	filter-feeders
	DEPOSIT	deposit feeder
	PREDATOR	predators
	ALGALP	algal piercers
Dietary preferences	SPECIALIST	strong (specialist)
	MODERATESPE	moderate
	GENERALIST	weak (generalist)
Respiration of aquatic stages (excluding eggs)	TEGUMENT	tegument
	GILL	gills
	PLASTRON	plastron
	AERIAL	aerial
Aquatic stages	ADUANDLAR	adult and larva
	ADUORLAR	adult or larva
	LARANDPUP	larva and pupa