CAN MACROINVERTEBRATE BIOLOGICAL TRAITS INDICATE FINE-GRAINED SEDIMENT CONDITIONS IN STREAMS?

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ABSTRACT

Excessive inputs of fine-grained sediment can damage aquatic ecosystems both by degrading habitat condition and by directly impairing biota. Recent research has improved our understanding of how benthic macroinvertebrates respond to fine-grained sediment stress, leading to the development of a variety of bioassessment indices based on changes in taxonomic composition and biological trait composition. Use of biological traits as indicators of stress has been advocated on the basis of a better mechanistic understanding of the biotic and abiotic factors acting on benthic communities. We quantified changes in the macroinvertebrate biological trait assemblage from a large number of river reaches spanning a national-scale gradient of increasing agricultural fine sediment delivery and retention, having first factored out variation associated with the natural environmental gradient, with the aim of robustly testing predictions of trait response. We found strong support for two of 18 predictions of how macroinvertebrate traits would respond to fine sediment stress. Furthermore, using an independent dataset, we were able to confirm the response of five of six trait-classes which partial RLQ-Fourth corner analysis found to be significantly associated with the fine sediment gradient. Prevalence of eggs as a resistant form, in combination with either an adult aquatic life stage or crawling, provided the best indication of fine sediment conditions in streams, approaching the performance of taxonomic composition-based sediment indices; CoFSI$_{sp}$ and EPSI$_{mtl}$. This study has robustly confirmed the potential of macroinvertebrate biological traits as indicators of fine sediment impacts.

INTRODUCTION

While fine sediment plays an important role in the natural functioning of rivers, the detrimental impacts of excessive quantities of sediment on aquatic ecosystems is well established (Wood and Armitage, 1997). Fine sediments (inorganic and organic particles of less than 2 mm diameter) are delivered to watercourses both from natural sources such as channel bank erosion, and as a result of anthropogenic activities in the catchment, e.g. intensive agriculture. Excessive delivery from the catchment and in-stream retention of fine sediments can impact (both directly and indirectly) freshwater biological communities (Collins *et al.*, 2011). Previous studies have shown how abrasion from suspended particles, clogging of gills and filtering structures, and burial by deposited fine sediment can directly harm individuals (Jones *et al.*, 2012). Excess deposition of fine sediment can indirectly affect freshwater communities by altering benthic habitat, e.g. filling interstitial spaces in bed substrate, and food availability, e.g. smothering of periphyton (Jones *et al.*, 2012). Recent research has improved our understanding of how benthic macroinvertebrate communities respond to increasing fine-grained sediment stress (Murphy *et al.*, 2015; Hubler *et al.*, 2016). Based on quantified associations between taxa abundance and benthic substrate conditions, we can infer the extent of fine sediment stress on a stream from the assemblage of in-stream taxa found at a site using biotic indices such as $\mathrm{CoFSI_{sp}}$ (Murphy *et al.*, 2015) or $\mathrm{E\text{-}PSI_{mtl}}$ (Turley *et al.*, 2016). Taxa such as *Heptagenia* have been consistently associated with low fine sediment conditions while *Ptychoptera* and Prodiamesinae can be indicative of high levels of entrained fine sediment (Murphy *et al.*, 2015; Hubler *et al.*, 2016). Such an approach to biological monitoring, focussing on compositional changes along stress gradients, is well-established (Rosenberg and Resh, 1993).

Alternative approaches to biomonitoring have also been considered that may offer additional benefits by complementing or replacing conventional community structural indices (Friberg, 2014). Among the most promising methods is the use of multiple biological traits (Dolédec *et al.*, 1999; Gayraud *et al.*, 2003). Biological traits are intrinsic characteristics of species that influence their fitness, e.g. mode of reproduction and locomotion, body size and food preferences. Within each trait a number of different states or classes may exist, e.g. within the respiration trait there are four trait-classes; gills, tegument, aerial spiracle or plastron. The prevalence of trait-class combinations under particular environmental conditions should reflect the selection pressure of the habitat template (Townsend and Hildrew, 1994) and, thus, provide insight into the underlying causal mechanisms. Indeed the approach allows for predictions to be made regarding the prevalence of certain trait-classes along specific gradients of increasing stress. The multiple biological trait approach could also lead to more widely applicable diagnostic indices of impact, as opposed to the composition-based indices that can be limited to the biogeographic region used for development (Zuellig and Schmidt, 2012).

Macroinvertebrate taxa respond to different aspects of fine sediment pressure, dependent on their intrinsic biological traits. For example, certain taxa may be susceptible to the chemical changes associated with the amount of organic matter deposited on the river bed, whereas others may be more susceptible to the physical impacts of inorganic fine sediments (Culp *et al*., 1986). There is a need to better understand how the prevalence of biological trait-classes in the macroinvertebrate community changes along a gradient of increasing fine sediment stress. Recent studies have provided some information on the macroinvertebrate trait-classes associated with greater amounts of entrained fine sediment (Buendia *et al*., 2013; Mondy and Usseglio-Polatera 2013; Descloux *et al*., 2014). However, across these studies only one trait-class, gill respiration, out of the 48 assessed, was consistently found to be more prevalent with increasing mass of fine sediment. The Buendia *et al*. (2013) and Descloux *et al*. (2014) studies were undertaken over relatively confined spatial scales, sampling only 3-5 discrete watercourses. While Mondy and Usseglio-Polatera (2013) analysed data from 1293 river reaches across 55 stream types in France, their analysis focussed on the response of a select sub-set of traits to fine sediment pressure. Resolving these inconsistencies in observed responses would be best achieved by incorporating a wide range of stream types and a large number of sampling sites from across as wide a fine sediment stress gradient as possible.

Descloux *et al*. (2014) and Mondy and Usseglio-Polatera (2013) made predictions of how trait-classes would respond to increasing colmation (clogging of stream bed interstices) through a number of different driving processes, e.g. decreasing interstitial space. Descloux *et al*. (2014) found strong support for three of 17 predictions, whereas six of the seven predictions made by Mondy and Usseglio-Polatera (2013) were supported. Building on these studies, the current work will quantify changes in the lotic macroinvertebrate biological trait assemblage from a large number of river reaches and across a wide gradient of agricultural fine sediment delivery and retention in the stream bed. Our objectives will be to (i) test 18 of the predictions made by Descloux *et al*. (2014) and Mondy and Usseglio-Polatera (2013) for which we have data (Table I) and (ii) identify suites of trait-classes that are favoured under high-levels of deposited fine sediment in the stream bed and, conversely, those associated with low deposited fine sediment conditions.

METHODS

Study sites

We identified 205 independent replicate catchments across England and Wales, representative of a range of river types over a gradient of pressure from fine sediment sources (Figure 1). We focussed on rural catchments, as agriculture is the dominant anthropogenic source of fine sediment being delivered to watercourses (Zhang *et al.*, 2014). Furthermore, including urban catchments in the analysis would have introduced many other confounding stressors. In the absence of extensive empirical data on fine sediment delivery to watercourses, we used available national GIS layers and outputs (Collins and Anthony, 2008) in combination with a process-based model of sediment mobilisation and transport to watercourses via surface runoff and drain flow (Davison *et al.,* 2008) to derive estimates of fine sediment inputs from agriculture (incorporating bare soil, winter cereals, spring cereals, potatoes, managed grass, rough grazing and woodland), urban areas, eroding channel banks and sewage treatment works. We then selected stream sites where (i) modelled delivery of fine sediment was predominantly (>75%) from agricultural sources, (ii) modelled sewage inputs were < 0.5 kg ha^{-1} year^{-1}, (iii) modelled diffuse urban inputs were < 2.0 kg ha^{-1} year^{-1} and (iv) there were no lakes or reservoirs in the catchment. Modelled fine sediment delivery rates ranged from 14 – 1900 kg ha^{-1} year^{-1} across the 205 streams.

To ensure that sampled macroinvertebrate communities came from as wide a range of natural river types as possible, within limits set by the above site selection criteria, the 205 sites were selected as equally as possible from each of four broad stream types based on catchment geology, distance from source, elevation and slope (Table S1 in Supporting Information). This structured sampling design allowed us to factor out the influence of natural environmental differences between sites and to focus our analysis on the response of macroinvertebrate communities to an un-confounded fine sediment gradient. The spatial distribution of sites across England and Wales revealed a greater density of sites to the north and west relative to central areas and the south east (Figure 1). This pattern was a result of site selection criteria whereby areas of greater population density were avoided. Each stream was sampled once, in either spring (March-May) or autumn (September-November) of 2010 or 2011, with both the macroinvertebrate community and deposited fine sediment being assessed. In each of the four periods sampling effort was spread as equally as possible across England and Wales, across the four stream types and, within each stream type, across the modelled fine sediment input gradient. Sampling coincided with a period of below-average rainfall, and consequently river flows, for much of England and Wales, though the greatest river flow deficiencies were generally subsequent to our sampling period, during winter 2011 and spring 2012 (Marsh *et al*., 2013). As a precaution, we did not sample streams that were evidently experiencing drought stress, e.g. restricted wetted width and depth: in practice, this was only an issue in autumn 2011.

Biological sampling

Macroinvertebrates were sampled with a pond net (1 mm mesh-size) using a standard three-minute kick/sweep, sampling all in-stream habitats in proportion to their areal coverage over the reach (10 – 20 m long depending on stream width), followed by a one-minute hand-search of rare or difficult to sample habitats e.g. large stones, tree roots. This is the standard sampling method used by UK regulatory authorities to monitor river water quality (Murray-Bligh *et al.*, 1997). Environmental variables were recorded either on-site (stream width and depth, velocity, substrate composition) or from map-based data (mean discharge category, elevation, distance from source and slope). Macroinvertebrate community samples were immediately fixed in 10% formalin, returned to the laboratory for subsequent identification and quantification (semi-quantitative numbers per sample) to the lowest practicable taxonomic level; usually species or genus but family for more difficult groups, e.g. Oligochaeta and some Diptera. Prior to data analysis, taxonomic resolution of the complete macroinvertebrate dataset was standardised to ensure that it only contained discrete taxa (as described in Appendix 3 of Chinnayakanahalli *et al.*, 2011).

Two existing trait resources were used to gather available biological trait information: the French Genus Trait Database (Tachet *et al.*, 2003) and the on-line database www.freshwaterecology.info, version 5.0, accessed on 30th January 2013 (Schmidt-Kloiber and Hering, 2015). Since October 2016 both sources are now available from www.freshwaterecology.info. The French data was the primary source of information and was supplemented with information from the on-line resource for those taxa or traits that were not included in the French database. Each biological trait, e.g. maximal potential body size, was described by several trait-classes, e.g. ≤ 0.25 cm, > 0.25-0.5 cm. The trait characteristics of each taxon were scored by assigning a value to each trait-class reflecting the affinity of the taxon to the trait-class. Scores ranged from 0 to 5 indicating no to high affinity respectively (Chevenet *et al.*, 1994). We compiled information on 11 biological traits across 62 trait-classes (Table II) for 192 distinct taxa identified across the 205 sites.

Fine sediment sampling

At each site a reach-scale estimate of the amount of fine sediment deposited on the stream bed was made immediately upstream of the macroinvertebrate sampling area using the disturbance technique (Duerdoth *et al.*, 2015). A steel cylinder (height 75 cm, diameter 48.5 cm) was inserted into an undisturbed section of the stream bed and the water column agitated vigorously for one minute, without touching the stream bed, to raise fine sediment deposited on the surface of the stream bed. A pair of water samples was then collected quickly from within the cylinder. Then one minute was spent disturbing the stream bed to a depth of approximately 10 cm, and vigorously agitating the water and bed to raise any sub-surface fine sediment in addition to re-suspended surface deposits. A second pair of water samples was then collected from within the cylinder. Four such sets of water samples (surface, and combined surface and subsurface) were collected from each site, two from erosional patches and two from depositional patches. Samples were refrigerated and returned to the laboratory within five days, where they were processed for dry mass and organic content (i.e. volatile solids following combustion at 550°C). Particle size distributions of material <1mm diameter was also measured using a Malvern Mastersizer 2000. Reach-averaged values for surface and total (combined surface and subsurface) deposited fine sediment were derived subsequently (Table III).

In summary, for each site, there was an estimate of the quantity of fine sediment being delivered from the catchment (kg ha^{-1} year^{-1}), derived from the process-based model, as well as actual measurements of deposited fine sediment mass and composition (Table III), and a description of the in-stream macroinvertebrate community.

Data Analysis

We applied partial RLQ (RLQ$_p$) analysis (Wesuls *et al.*, 2012; Dray *et al.*, 2014) to statistically test the significance of associations between the prevalence of trait-classes and fine sediment variables, having first factored out variation associated with underlying natural environmental gradients. This approach provided the means to confirm or refute predictions of biological trait response to fine sediment stress set out in Table I. Assignment of sites to one of four broad stream types, as described in the site selection process, provided a categorical description of natural differences between sites. RLQ$_p$ analysis first undertakes two multivariate regressions using stream-type assignment (W-table: 205 sites x 1 site type factor) as an explanatory variable and log-transformed taxon abundance data (L-table: 205 sites x 192 taxa) and fine sediment variable data (R-table: 205 sites x 13 environmental variables) as response tables. Residuals from both these regressions are then used as L$_r$ and R$_r$-tables, along with the original trait data (Q-table: 192 taxa x 62 trait-class data), in a modified RLQ analysis (Wesuls *et al.*, 2012). This involved initially carrying out a correspondence analysis on the L$_r$-table to derive scores for sites and taxa that had maximal covariance. Principal component analysis (PCA) was carried out on the R$_r$-table with sample scores from the L$_r$-table correspondence analysis used as row weights. Fuzzy correspondence analysis (FCA) was carried out on the Q-table with taxon scores from the L$_r$-table correspondence analysis used as row weights. RLQ-analysis combined these three separate ordinations by defining a linear combination of traits (taxon scores in Q-FCA) and a linear combination of environmental variables (sample scores in R_r-PCA) that maximised covariance between taxon and site scores, measured through the L_r-table (Dolédec *et al.*, 1996; Wesuls *et al.*, 2012).

We applied the Fourth-corner approach (Dray and Legendre, 2008) directly to RLQ_p outputs to test (i) correlations between each trait-class and the first two RLQ_p axes for environmental gradients (sample scores from R_r-PCA) and (ii) correlations between each fine sediment variable and the first two RLQ_p axes for trait gradients (taxon scores from Q-FCA: Dray *et al.*, 2014). Significance of correlations was tested using the combined results of 4999 permutations of sites and 4999 permutations of taxa as described in Dray *et al.* (2014), with *P*-values adjusted for multiple comparisons using the false discovery rate method (Benjamini and Hochberg, 1995).

In addition, we identified groups of taxa with similar combinations of trait-class affinities (trait syndromes) by applying hierarchical cluster analysis (based on Euclidian distances and using Ward's minimum variance method) to the first two RLQ_p axes taxon scores. We determined the optimal number of clusters using a combination of 30 clustering indices whereby the optimal cluster number most frequently recommended was chosen. Characteristics of each cluster (trait syndrome) were summarized as the average (across taxa) relative abundance-weighted affinity for each trait-class within a trait for each cluster.

RLQ_p, Fourth-corner and cluster analyses were undertaken using R 3.2.5 (R Core Team, 2016) with the additional ade4 (Dray and Dufour, 2007) and NbClust packages (Charrad *et al.*, 2014).

Independent testing

Trait-classes confirmed by RLQ_p-Fourth-corner analysis to have significant associations with the fine sediment gradient were applied to an independent dataset consisting of simultaneously collected macroinvertebrate assemblage and deposited fine sediment data from 57 stream sites in Wales sampled as part of a study investigating environmental impacts of agri-environment schemes (Jones *et al.*, 2017). Field sampling and laboratory processing protocols were identical to those used in the 205-site dataset. The dataset included multiple streams from each of the four stream types. Measured deposited fine sediment mass in the stream bed ranged from 0.05 – 31.2 kg m^{-2} across the 57 sites. Relative prevalence of each trait-class (within a trait) at each site was calculated by log-transformed abundance weighting trait-class affinity scores for each taxon for a given site. Sums of weighted scores (one per trait-class) were expressed as the relative abundance distribution (within a trait), giving the site trait profile. Measures of trait-class prevalence at a site were correlated against reach-scale geometric mean mass of deposited fine sediment and organic fine sediment in the stream bed. The strength of their association with deposited fine sediment gradients was also compared to that for established fine sediment indices CoFSI_{sp} (Murphy *et al.*, 2015) and E-PSI_{mtl} (Turley *et al.*, 2016). We corrected for family-wise error rate using the Holm-Bonferroni method (Holm, 1979) to reduce the chance of Type I errors.

RESULTS

RLQ$_p$ axis 1 was the dominant axis defining the ordination space (accounting for 84% of explanatory power of the RLQ$_p$) and was significantly negatively correlated with all six measures of deposited fine sediment mass (Table III, Figure 2a). RLQ$_p$ axis 1 was to a lesser extent also positively correlated with the modelled delivery of fine sediment from agriculture (Table III, Figure 2a). This negative association between modelled delivery and retained sediment is due to different factors affecting load and retention (Naden *et al*. 2016). RLQ$_p$ axis 2 (accounting for 10% of explanatory power of the RLQ$_p$) was correlated with variables describing composition of the fine sediment; coarser fine sediments tended to have relatively less organic content (Table III, Figure 2a).

Of the 18 predictions of biological trait-class response to fine sediment stress (Table I) only two were confirmed by our data: prevalence of ovoviviparity was negatively correlated with RLQ$_p$ axis 1, describing decreasing stress from fine sediment, while that of crawling was positively correlated (Table II). Outside of predicted trait-class responses to increasing fine sediment, there was also a significant negative correlation between RLQ$_p$ axis 1 and prevalence of an aquatic adult stage. In addition, we found significant positive correlations between RLQ$_p$ axis 1 and prevalence of aerial active and aquatic active dispersal, and eggs or statoblasts as resistance forms (Table II, Figure 2b). There were no significant correlations between RLQ$_p$ axis 2 and trait-classes.

Taxa were clustered into three distinct groups based on their trait-class affinities. Trait syndrome A (n = 60) was associated with relatively high levels of deposited fine sediment, while trait syndrome B (n = 50) was associated with a moderate to high mass of deposited fine sediment with a relatively high organic content and with the inorganic fraction dominated by silt and clay. Trait syndrome C (n = 82) was associated with relatively low levels of deposited fine sediment (Figure 2). Taxa in trait syndrome A had a greater tendency towards an aquatic adult stage, ovoviviparity, aquatic passive dispersal, larger body size, burrowing and more than one life cycle per year, and included *Gammarus*, *Potamopyrgus antipodarum* and Tubificidae, among others (Figure 3, Figure S1 in supporting information). Trait syndrome B was dominated by taxa with pronounced aquatic larval and pupal stages and that lay clutches of eggs. Trait syndrome B also featured a greater tendency than other syndromes for taxa being attached to the substrate and for predation and filter feeding (Figure 3, Figure S1). Among taxa assigned to this syndrome were *Simulium*, Orthocladiinae, Tanypodinae, *Polycelis* and Pediciidae. In trait syndrome C crawling and swimming were the dominant modes of locomotion, with a greater tendency towards aerial active dispersal, pronounced aquatic egg and larval stages, scraping and shredding as the main feeding habits and the laying of cemented eggs (Figure 3, Figure S1). This syndrome included *Baetis*, *Rhithrogena*, *Elmis* and *Leuctra*.

Analysis of the independent dataset confirmed that prevalence of an aquatic adult life stage and ovoviviparity increased significantly with increasing mass of deposited fine sediment and fine organic sediment in the stream bed (Table IV). In addition, prevalence of crawling, eggs or statoblasts as resistance forms and aerial active dispersal decreased significantly with increasing mass of deposited fine sediment and fine organic sediment in the stream bed (Table IV). Both CoFSI_{sp} and $\text{E-PSI}_{\text{mtl}}$ had significant negative correlations with deposited fine sediment gradients, the strengths of which were greater than that for individual trait-classes correlations (Table IV). Mass of deposited fine sediment (*logSed*) was most parsimoniously predicted (stepwise selection from the suite of six biological trait-classes) by a combination of prevalence of eggs or statoblasts as resistance forms (*Rest_egg*) and crawling (*Loco_crw*) ($logSed = 5541 - 2.99 Rest\_egg - 3.44\ Loco\_crw$, $R^2 = 0.358$, $P < 0.001$). Similarly, mass of deposited fine organic sediment (*logVs*) was most parsimoniously predicted by a combination of prevalence of an aquatic adult life stage (*AqSt_ad*) and *Rest_egg* ($logVs = 2.236 + 329 AqSt\_ad - 3.06\ Rest\_egg$, $R^2 = 0.379$, $P < 0.001$). These models incorporate diagnostic aspects of both trait syndromes A and B to best distinguish sites, and do so with similar power to community composition indices (Table IV).

DISCUSSION

We found strong support for just two of 18 predictions of how macroinvertebrate traits would respond to fine sediment stress: an increasing prevalence of ovoviviparity and a decreased prevalence of crawlers. The large sample size, spatial extent and structure of our dataset mean that we can be confident that our results have more general applicability than smaller-scale studies such as Descloux *et al*. (2014). When compared with another large-scale study of trait responses to fine sediment stress (Mondy and Usseglio-Polatera, 2013), we found agreement for three of the seven trait-classes assessed. However, unlike in our study, Mondy and Usseglio-Polatera (2013) did not factor out the confounding effect of natural differences between the 55 stream-types included in their study prior to assessing trait responses to sedimentation. The degree to which the associations they found were driven by the underlying typology rather than sediment stress *per se* is not known. It is well understood that streams will naturally vary in the amount of entrained fine sediment in their bed as a function of physical aspects of the catchment, watercourse and reach, e.g. geology, elevation, channel slope, stream power (Naden *et al*., 2015). In the present study we were particularly interested in quantifying the response of the macroinvertebrate trait assemblage to variation in fine sediment conditions over and above that expected naturally, i.e. variation due to anthropogenic activity in the catchment. We have successfully applied a partialling-out approach previously when developing the CoFSI_{sp} and AWIC indices (Davy-Bowker *et al*., 2005) but this is the first study of trait responses to fine sediment stress to have addressed the confounding effect of natural environmental factors. We found relatively fewer significant relationships than other studies but this is to be expected as we have factored out a substantial gradient in natural stream typology and then focussed on the residual gradient in fine sediment stress. It is likely that many previously reported associations between traits and fine sediment stress were in fact driven by the stream typology gradient. Furthermore, using an independent dataset, we were able to confirm the response of five of six trait-classes which RLQ_p-Fourth corner analysis found to be significantly associated with the fine sediment gradient. While prevalence of these selected trait-classes was individually not as strongly related to the deposited fine sediment gradient as taxonomic composition-based sediment indices, when combined they did approach the performance of CoFSI_{sp} and EPSI_{mtl}. Lange et al. (2014) also found that indices of community composition, e.g. richness, evenness, were similarly or slightly better correlated with stressor gradients than functional trait-based indices.

In a comparison between results of the current study and other published work we found little concordance in the identity or direction of significant associations (Table S2). Of 31 trait-classes across 11 traits that had significant associations with increasing mass of fine sediment in at least one of the four studies, none showed a consistent trend. Prevalence of ovoviviparity increased with increasing deposited fine sediment mass in the current study and in Mondy and Usseglio-Polatera (2013), decreased in Descloux *et al.* (2014) and showed no trend in Buendia *et al.* (2013). Prevalence of scrapers decreased with increasing deposited fine sediment mass in Mondy and Usseglio-Polatera (2013), but increased in Descloux *et al.* (2014) and Buendia *et al.* (2013) and showed no relationship in the current study. For the remaining 26 trait-classes compared, there was a consistent result across studies of no significant correlation with increasing deposited fine sediment mass. It is possible that cross-study inconsistencies are a function of differing sampling methodologies, sample sizes, and extent of stressor gradient encompassed. Mesh size of the sampling nets used varied across studies from 300 to 1000 µm, which may have influenced relative prevalence of traits in assemblages, e.g. maximal potential size. In addition, it may well be that the complexity of the habitat template, where different biotic and abiotic filters act over varying temporal and spatial scales to define the assemblage of traits present in a given reach, makes formulating predictions very difficult (Menezes *et al.*, 2010). Furthermore, not all impacts of fine sediment are direct: macroinvertebrate assemblages will respond to changes in other parts of the biological community which may, in turn, select for traits other than those presumed to be impacted directly by fine sediment.

However, it is likely that inconsistencies between different studies arise, in part, from the fact that previous studies did not explicitly consider that trait responses to environmental gradients cannot be regarded as independent discrete entities. Traits do not respond in isolation to the environment, rather the environment acts on combinations of trait-classes, as mediated through species (Verberk *et al.*, 2013). It should be recognised that they interact through trade-offs in energy investment, and efforts should be made to identify colinearities. Clusters of traits with similar patterns of variation across the extensive dataset are linked for ecological or evolutionary reasons, and together offer some form of adaptation to life in fine sediment-rich or coarse substrate habitats. Rather than testing hypotheses on a trait-class by trait-class basis we should be formulating hypotheses regarding combinations of compatible trait-classes that together form a life history strategy for dealing with the challenges posed by the environment. In the present study we directly addressed this concern using multivariate RLQ$_p$-Fourth corner and cluster analysis to identify three distinct trait syndromes along the deposited fine sediment gradients. These represent life-history strategies that enable taxa to persist at both ends of the gradient. The combination of trait-classes we found to be significantly associated with the fine sediment stress gradient incorporated all four of the major trait domains proposed by Verberk *et al.* (2008) to be critical in dealing with environmental constraints, namely reproduction, development, dispersal and synchronisation. Streams with little deposited fine sediment tended to favour a strategy characterised by eggs as resistant forms, aerial active dispersal and crawling, which together with a tendency for the laying of fixed eggs and univoltine or semivoltine life cycles offer a fitness advantage under such minimally impacted conditions. These are all trait characteristics of non-Dipteran insect orders, and indeed 75 of the 82 taxa assigned to this cluster were mayflies, stoneflies, beetles and caddis flies. We found that streams with high amounts of deposited fine sediment selected for a strategy defined primarily by an aquatic adult life stage and ovoviviparity. It is not difficult to see how greater investment in egg protection and parental care provided by ovoviviparity can be advantageous in an environment where un-attended eggs deposited in the stream bed or adhered to hard substrates would be smothered or abraded by excessive quantities of fine sediment. Dolédec *et al*. (2006) and Lange *et al*. (2014) also found that the prevalence of ovoviviparity increased in more intensively farmed catchments with a greater mass of fines in the stream bed. They attributed this to the increased probability of smothering of eggs by fines and algal mats in such streams. When combined with an extended adult aquatic stage and multiple life cycles per year, as for amphipod and isopod crustaceans, this strategy allows for a strong and sustained recruitment at the more stressed end of the gradient.

If a trait-based approach is to be used to predict fine-sediment conditions at new test sites, it would be better to have a good mechanistic understanding of observed patterns, or at the very least to have confidence that associations found in one study will hold true elsewhere. Testing of associations identified in the 205 site dataset, using an independent set of data from 57 Welsh streams, provided assurance that our findings were robust. In addition our study agreed with findings of Mondy and Usseglio-Polatera (2013) regarding the response of reproduction, and one of two locomotion traits. Both studies found the prevalence of crawling to decrease with increasing fine sediment stress but our study did not find any association with burrowing. Burrowing would allow an individual to travel through and exploit fine sediment beds or interstitial deposits, and also to maintain their favoured positions within such habitats by avoiding, for example, areas of low oxygen or burial by accreting sediments (Jones et al., 2012). Therefore it is surprising that we did not find a significant association. However, while burrowing animals such as Tubificidae, *Pisidium* and Tipulidae, were markedly associated with a greater mass of deposited fines there were as many other burrowing taxa e.g. *Ephemera*, *Cordulegaster*, and *Leuctra geniculata* that were more associated with intermediate or low levels of deposited fines. These taxa are perhaps limited by some other aspect of the fine sediment gradient, e.g. interstitial oxygen levels: currently there is limited objective information on physiological traits available within macroinvertebrate trait databases.

Independent testing has provided strong support for the prevalence of eggs or statoblasts as a resistant form, in combination with either an aquatic adult life stage or crawling, to be considered as a bioindicator of fine sediment conditions in streams. The trait metrics had similar correlations with the mass of deposited organic fine sediment in the stream bed to CoFSI_{sp} or E-PSI_{mtl}. Taxa with an affinity for eggs as a resistant form include all flatworms, most mayfly and many stonefly taxa. Those taxa with a strong affinity for crawling include all flatworms, stoneflies and Odonata, and some mayfly, caddis fly and beetle taxa. The inclusion of multiple biological trait variables to the bio-indicator model can capture more complex changes in life history strategies along the stressor gradient.

In conclusion, this study has confirmed significant association between the condition of streams, in terms of the quantity and quality of deposited fine sediment, and biological trait characteristics of the benthic macroinvertebrate community. Correlative analysis of a spatially extensive dataset, designed to investigate benthic fine sediment impacts, has identified consistent patterns in the trait assemblage that could in the future be applied to manipulative experimental situations or broad-scale bioassessment surveys. This knowledge will help lead to better protection of lotic communities from excessive inputs of inorganic and organic fine sediment.

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Table I. Eighteen hypotheses of which adaptations (trait-classes) within a trait would become more or less prevalent under high stress conditions, following Descloux *et al*. (2014) and Mondy and Usseglio-Polatera (2013). The two final columns record whether Descloux *et al*. (2014) and Mondy and Usseglio-Polatera (2013) found support for each hypothesis (- indicates that the hypothesis was not tested in that study).

Trait		Prediction under high benthic fine sediment stress	Mechanism of impact	Supported by Descloux *et al*. (2014)	Supported by Mondy and Usseglio-Polatera (2013)
Maximal potential size	1	Increased prevalence of < 0.5 cm	Reduced interstitial space with increasing colmation	No	-
Number of reproductive cycles per year	2	Increased prevalence of > 1 life cycle per year	Increased temporal stability of harsh conditions	No	-
Reproduction technique	3	Increased prevalence of asexual	Increased temporal stability of harsh conditions	No	-
	4	Increased prevalence of free eggs	Decreasing availability of clean coarse substrates	No	-
	5	Increased prevalence of free clutches	Decreasing availability of clean coarse substrates	No	-
	6	Increased prevalence of ovoviviparity	Increased temporal stability of harsh conditions	No	Yes
	7	Decreased prevalence of isolated cemented eggs	Decreasing availability of clean coarse substrates	-	Yes
Resistance forms	8	Increased prevalence of no resistant forms	Increased temporal stability of harsh conditions	No	-
Respiration	9	Increased prevalence of tegumental	Internal respiratory surfaces protected from abrasion	No	-
	10	Decreased prevalence of gill	Abrasion of exposed gill surfaces by fine particles	No	-
Locomotion and substrate relation	11	Decreased prevalence of crawlers	Reduction in mean substrate particle size and area of clean hard substrates	-	Yes
	12	Increased prevalence of burrowers	Reduction in mean substrate particle size and increased cover of fine sediment beds	No	Yes
	13	Increased prevalence of attached	Attached taxa with cases or shells protected from abrasion	Yes	-
Food	14	Increased prevalence of microorganisms in fine sediment	Increased availability of fine particulate matter	No	-

	15	Increased prevalence of fine detritus	Increased availability of fine particulate matter	No	-
Feeding habit	16	Increased prevalence of deposit-feeders	Increased availability of fine particulate matter	No	No
	17	Increased prevalence of filter-feeders	Increased availability of fine particulate matter	No	Yes
	18	Decreased prevalence of scrapers	Deposited fine particulate matter decreases quantity and quality of biofilm	No	Yes

Table II. Correlation coefficients (*r*) from Fourth-corner tests between the first partial RLQ axis for environmental variables (pRLQ-R1) and trait-classes. Significant correlations (P_{adj}<0.05) are in bold. *P*-values were adjusted for multiple comparisons using the false discovery rate procedure.

TRAIT	TRAIT-CLASS	Abbreviation	*r*	P_{adj}
Maximal potential size	≤ .25 cm	MaxS_25cm	0.023	0.674
	> .25-.5 cm	MaxS_5cm	-0.013	0.921
	> .5-1 cm	MaxS_1cm	0.010	0.921
	> 1-2 cm	MaxS_2cm	0.020	0.855
	> 2-4 cm	MaxS_4cm	-0.030	0.674
	> 4-8 cm	MaxS_8cm	-0.029	0.674
	> 8 cm	MaxSm8cm	-0.006	0.939
Life cycle duration	≤ 1 year	Lcyc_m1	0.038	0.557
	> 1 year	Lcyc_l1	-0.017	0.855
Potential number of cycles per year	< 1	Pcyc_lt1	0.041	0.434
	1	Pcyc_1	0.044	0.442
	> 1	Pcyc_gt1	-0.054	0.297
Aquatic stages	egg	AqSt_eg	0.053	0.297
	larva	AqSt_la	0.041	0.457
	pupa	AqSt_pu	-0.003	0.962
	adult	**AqSt_ad**	**-0.086**	**0.025**
Reproduction	**ovoviviparity**	**Repr_ovo**	**-0.097**	**0.017**
	isolated eggs, free	Repr_ief	0.041	0.424
	isolated eggs, cemented	Repr_iec	0.063	0.149
	clutches, cemented or fixed	Repr_ccf	0.060	0.214
	clutches, free	Repr_cfr	-0.053	0.286
	clutches, in vegetation	Repr_cvg	-0.064	0.149
	clutches, terrestrial	Repr_ctr	0.010	0.921
	asexual reproduction	Repr_asr	-0.025	0.744
Dispersal	aquatic passive	Disp_aqp	0.066	0.149
	aquatic active	**Disp_aqa**	**0.087**	**0.017**
	aerial passive	Disp_aep	-0.007	0.921
	aerial active	**Disp_aea**	**0.098**	**0.017**
Resistance forms	**eggs, statoblasts**	**Rest_egg**	**0.081**	**0.029**
	cocoons	Rest_coc	-0.016	0.903
	housings against desiccation	Rest_hou	-0.052	0.161
	diapause or dormancy	Rest_dia	-0.055	0.297
	none	Rest_non	0.033	0.674
Respiration	tegument	Resp_teg	0.015	0.904
	gill	Resp_gil	0.022	0.805
	plastron	Resp_pla	0.036	0.621
	spiracle	Resp_spi	-0.019	0.855

Locomotion and substrate relation	flier	Loco_fli	0.015	0.903
	surface swimmer	Loco_ssw	-0.046	0.422
	full water swimmer	Loco_swi	-0.019	0.855
	crawler	**Loco_crw**	**0.085**	**0.029**
	burrower	Loco_bur	-0.065	0.149
	interstitial	Loco_int	-0.004	0.939
	temporarily attached	Loco_tpa	0.014	0.909
	permanently attached	Loco_pat	0.005	0.921
Food	microorganisms	Food_mio	-0.007	0.921
	detritus (< 1mm)	Food_det	-0.001	0.986
	dead plant (>= 1mm)	Food_dep	-0.047	0.422
	living microphytes	Food_mip	0.025	0.744
	living macrophytes	Food_map	-0.027	0.722
	dead animal (>= 1mm)	Food_dea	-0.036	0.634
	living microinvertebrates	Food_mii	-0.046	0.422
	living macroinvertebrates	Food_mai	0.000	0.998
	vertebrates	Food_vrt	-0.047	0.297
Feeding habit	absorber	Feed_abs	-0.025	0.744
	deposit feeder	Feed_dep	-0.034	0.657
	shredder	Feed_shr	-0.033	0.674
	scraper	Feed_scr	0.067	0.149
	filter-feeder	Feed_fil	-0.007	0.921
	piercer	Feed_prc	-0.042	0.469
	predator	Feed_pre	-0.013	0.921
	parasite	Feed_par	-0.029	0.674

Table III. Correlation coefficients (*r*) from Fourth-corner tests between the first and second partial RLQ axis for traits (pRLQ-Q) and each fine sediment variable. Significant correlations ($P_{adj}<0.05$) are in bold. *P*-values were adjusted for multiple comparisons using the false discovery rate procedure.

Variable type	Variable	Abbreviation	pRLQ axis 1		pRLQ axis 2	
			r	P_{adj}	*r*	P_{adj}
Measured deposited fine sediment variables	Reach mean total sediment mass (log-transformed g m^{-2})	SedMass	**-0.140**	**0.001**	0.013	0.674
	Reach mean Depositional area sediment mass (log-transformed g m^{-2})	DpSedMas	**-0.090**	**0.004**	0.029	0.203
	Reach mean Erosional area sediment mass (log-transformed g m^{-2})	ErSedMas	**-0.162**	**0.001**	-0.005	0.884
	Reach mean total organic mass (log-transformed g m^{-2})	VsMass	**-0.157**	**0.001**	-0.009	0.799
	Reach mean Depositional area organic mass (log-transformed g m^{-2})	DpVsMas	**-0.106**	**0.001**	0.010	0.674
	Reach mean Erosional area organic mass (log-transformed g m^{-2})	ErVsMass	**-0.171**	**0.001**	-0.024	0.587
	Mean % organic (log-transformed)	PctOrg	-0.007	0.810	**-0.058**	**0.001**
	Mean Depositional area % organic (log-transformed)	DpPctOrg	-0.022	0.593	**-0.054**	**0.001**
	Mean Erosional area % organic (log-transformed)	ErPctOrg	0.006	0.810	**-0.053**	**0.001**
	% by volume of particles in sand size category	PctSa	-0.012	0.786	**0.048**	**0.001**
	% by volume of particles in silt size category	PctSi	0.018	0.671	**-0.048**	**0.001**
	% by volume of particles in clay size category	PctCl	-0.009	0.810	**-0.031**	**0.050**
Modelled fine sediment delivery	PSYCHIC model estimate of agricultural fine sediment load to site from catchment (log x+1-transformed kg ha^{-1} yr^{-1})	AgSedLd	**0.089**	**0.002**	-0.014	0.639

Table IV. Correlation coefficients (*r*) between log-transformed deposited bed sediment mass, CoFSI_{sp}, $\text{E-PSI}_{\text{mtl}}$, and the prevalence of selected biological trait-classes. Non-significant correlations (Holm-Bonferroni corrected $P < 0.05$) are in italics.

	Sediment Index	Deposited fine sediment mass (log g m^{-2})	Deposited organic fine sediment mass (log g m^{-2})	CoFSI_{sp}	$\text{E-PSI}_{\text{mtl}}$
	CoFSI_{sp}	-0.625	-0.616		
	$\text{E-PSI}_{\text{mtl}}$	-0.640	-0.644	0.887	
TRAIT	TRAIT-CLASS				
Aquatic Stages	adult	0.561	0.571	-0.853	-0.871
Reproduction technique	ovoviviparity	0.496	0.495	-0.862	-0.872
Dispersal	aquatic active	*0.268*	*0.218*	*-0.280*	-0.390
	aerial active	-0.385	-0.411	0.720	0.797
Resistance forms	eggs, statoblasts	-0.552	-0.556	0.759	0.609
Locomotion and substrate relation	crawler	-0.569	-0.571	0.870	0.801

Figure Headings:

Figure 1. Location of 205 sampled stream sites (black circles) across England and Wales and the 57 independent stream sites (white circles) in Wales.

Figure 2. Results from the first two axes of partial RLQ analysis illustrating, in the same ordination space, the direction of greatest variability for (a) significant environmental variables, (b) significant trait-classes and (c) the centre of distribution for each of the 192 taxa, each of which is assigned to one of three trait syndromes based on their trait-class affinities. Axis 1 and axis 2 have eigenvalues of 0.0099 and 0.0012 respectively; axis 1 accounting for 84% of explanatory power of the partial RLQ. See Tables II and III for explanation of abbreviated labels.

Figure 3. Average prevalence of each trait-class, within each of the five traits which were significantly associated with partial RLQ axes, for trait syndromes A, B and C (see Figure 2c).

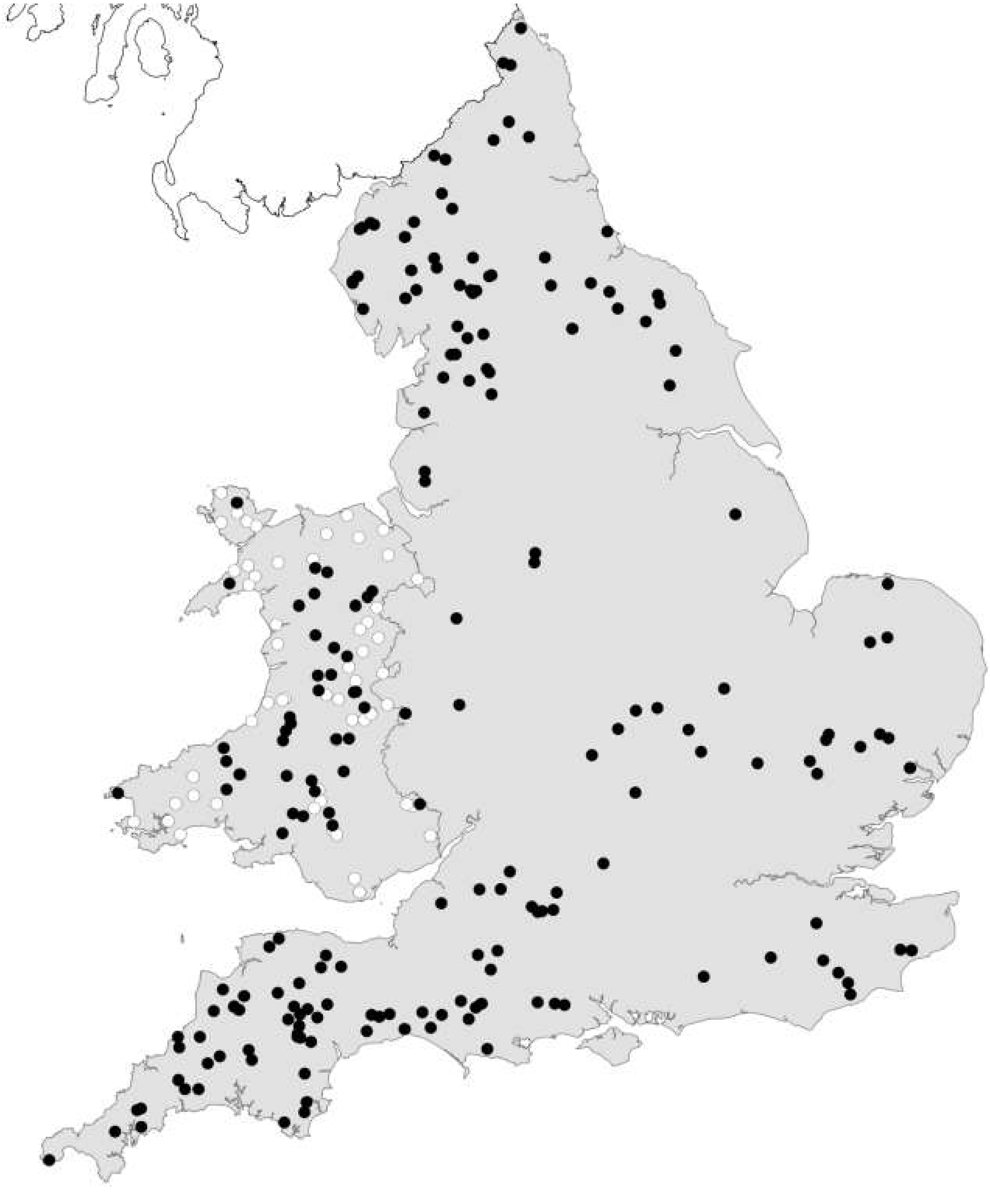


Figure 1.

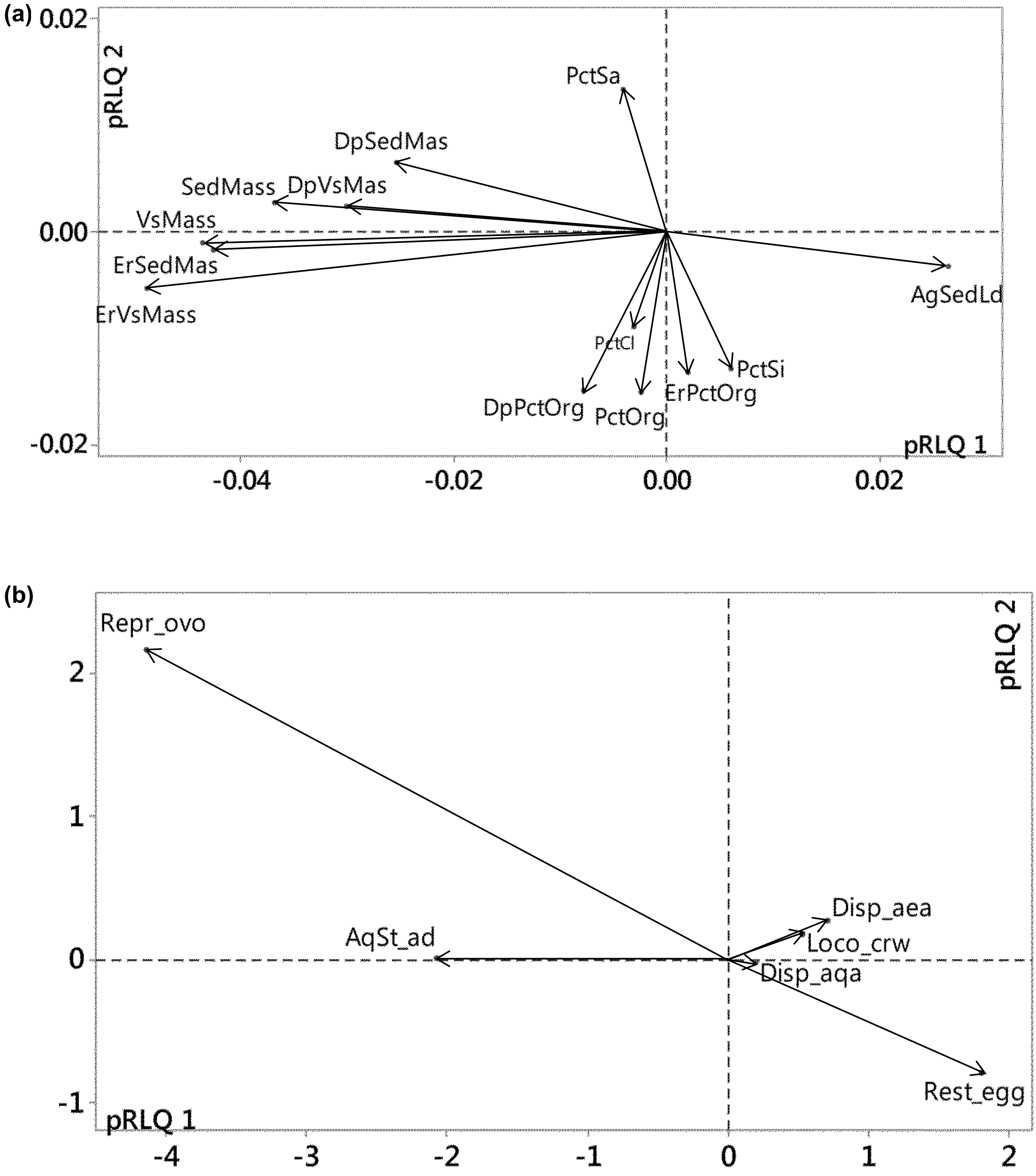

(a)
pRLQ 2
pRLQ 1
0.02
0.00
-0.02
-0.04
-0.02
0.00
0.02
PctSa
DpSedMas
SedMass
DpVsMas
VsMass
ErSedMas
ErVsMass
AgSedLd
PctCl
PctSi
ErPctOrg
DpPctOrg
PctOrg
(b)
pRLQ 2
pRLQ 1
2
1
0
-1
-4
-3
-2
-1
0
1
2
Repr_ovo
AqSt_ad
Disp_aea
Loco_crw
Disp_aqa
Rest_egg

Figure 2.

(c)

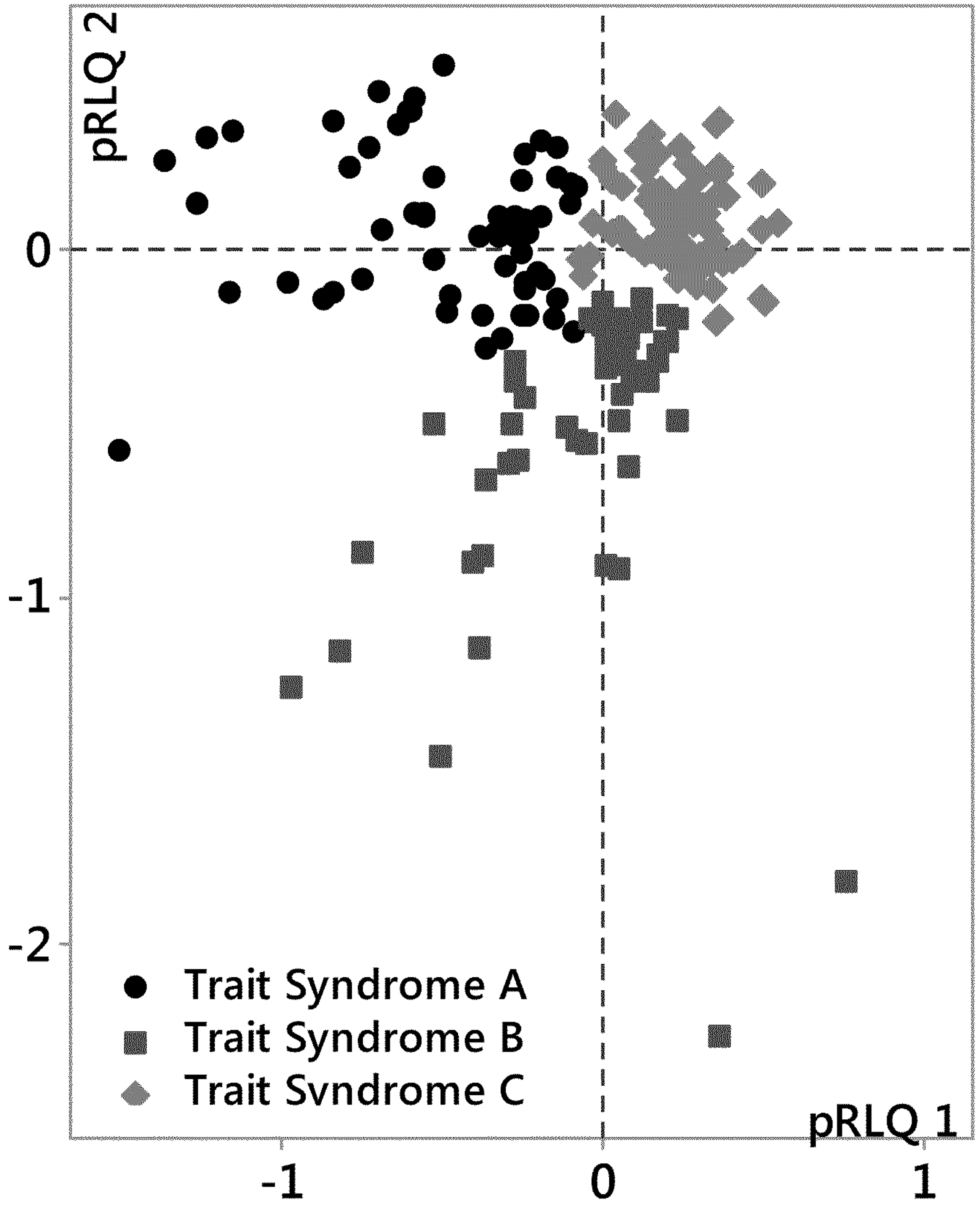


Figure 2. (cont'd,)

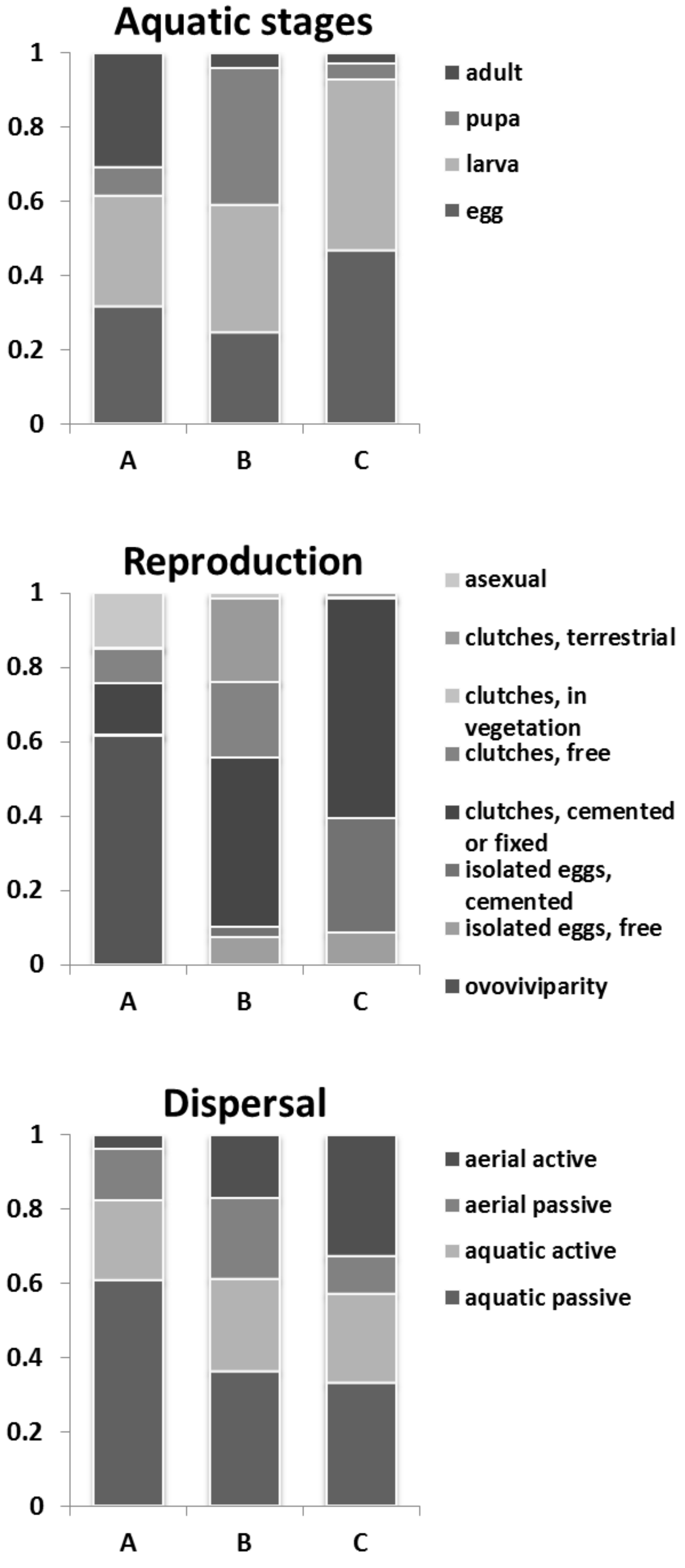
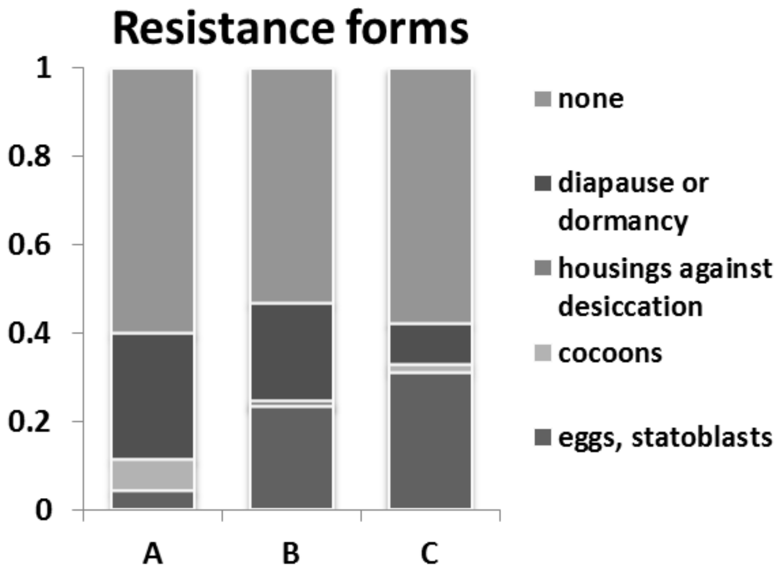

Aquatic stages
1
0.8
0.6
0.4
0.2
0
A
B
C
adult
pupa
larva
egg
Reproduction
1
0.8
0.6
0.4
0.2
0
A
B
C
asexual
clutches, terrestrial
clutches, in vegetation
clutches, free
clutches, cemented or fixed
isolated eggs, cemented
isolated eggs, free
ovoviviparity
Dispersal
1
0.8
0.6
0.4
0.2
0
A
B
C
aerial active
aerial passive
aquatic active
aquatic passive

Figure 3.



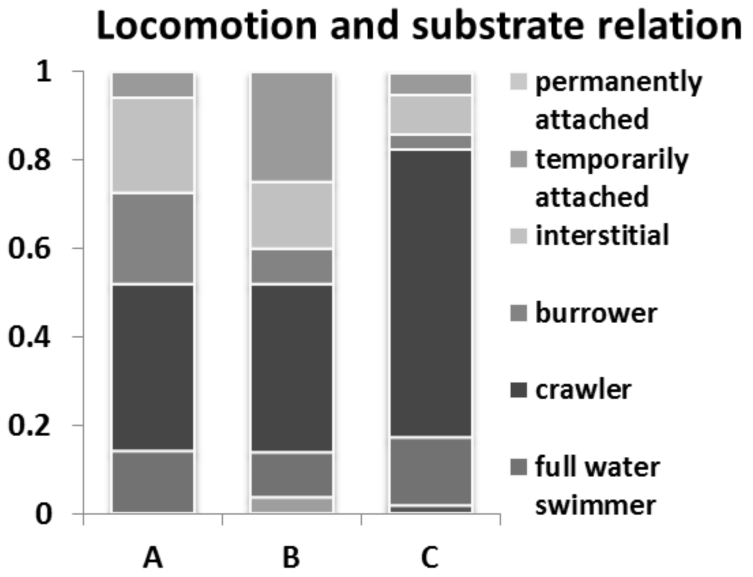


Figure 3. (cont'd.)