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

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Key Words: fine sediment; macroinvertebrates; streams; biological traits; bioassessment.

Page header: TRAITS AS INDICATORS OF FINE SEDIMENT STRESS

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ABSTRACT

1
2 Excessive inputs of fine-grained sediment can damage aquatic ecosystems both by degrading
3 habitat condition and by directly impairing biota. Recent research has improved our understanding
4 of how benthic macroinvertebrates respond to fine-grained sediment stress, leading to the
5 development of a variety of bioassessment indices based on changes in taxonomic composition and
6 biological trait composition. Use of biological traits as indicators of stress has been advocated on
7 the basis of a better mechanistic understanding of the biotic and abiotic factors acting on benthic
8 communities. We quantified changes in the macroinvertebrate biological trait assemblage from a
9 large number of river reaches spanning a national-scale gradient of increasing agricultural fine
10 sediment delivery and retention, having first factored out variation associated with the natural
11 environmental gradient, with the aim of robustly testing predictions of trait response. We found
12 strong support for two of 18 predictions of how macroinvertebrate traits would respond to fine
13 sediment stress. Furthermore, using an independent dataset, we were able to confirm the response
14 of five of six trait-classes which partial RLQ-Fourth corner analysis found to be significantly
15 associated with the fine sediment gradient. Prevalence of eggs as a resistant form, in combination
16 with either an adult aquatic life stage or crawling, provided the best indication of fine sediment
17 conditions in streams, approaching the performance of taxonomic composition-based sediment
18 indices; $CoFSI_{sp}$ and $EPSI_{mtl}$. This study has robustly confirmed the potential of macroinvertebrate
19 biological traits as indicators of fine sediment impacts.

20

21

INTRODUCTION

22

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

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

55 composition-based indices that can be limited to the biogeographic region used for development
56 (Zuellig and Schmidt, 2012).

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

73 Descloux *et al.* (2014) and Mondy and Usseglio-Polatera (2013) made predictions of how trait-
74 classes would respond to increasing colmation (clogging of stream bed interstices) through a
75 number of different driving processes, e.g. decreasing interstitial space. Descloux *et al.* (2014)
76 found strong support for three of 17 predictions, whereas six of the seven predictions made by
77 Mondy and Usseglio-Polatera (2013) were supported. Building on these studies, the current work
78 will quantify changes in the lotic macroinvertebrate biological trait assemblage from a large number
79 of river reaches and across a wide gradient of agricultural fine sediment delivery and retention in
80 the stream bed. Our objectives will be to (i) test 18 of the predictions made by Descloux *et al.*
81 (2014) and Mondy and Usseglio-Polatera (2013) for which we have data (Table I) and (ii) identify
82 suites of trait-classes that are favoured under high-levels of deposited fine sediment in the stream
83 bed and, conversely, those associated with low deposited fine sediment conditions.

85 *Study sites*

86 We identified 205 independent replicate catchments across England and Wales, representative of a
87 range of river types over a gradient of pressure from fine sediment sources (Figure 1). We focussed
88 on rural catchments, as agriculture is the dominant anthropogenic source of fine sediment being
89 delivered to watercourses (Zhang *et al.*, 2014). Furthermore, including urban catchments in the
90 analysis would have introduced many other confounding stressors. In the absence of extensive
91 empirical data on fine sediment delivery to watercourses, we used available national GIS layers and
92 outputs (Collins and Anthony, 2008) in combination with a process-based model of sediment
93 mobilisation and transport to watercourses via surface runoff and drain flow (Davison *et al.*, 2008)
94 to derive estimates of fine sediment inputs from agriculture (incorporating bare soil, winter cereals,
95 spring cereals, potatoes, managed grass, rough grazing and woodland), urban areas, eroding channel
96 banks and sewage treatment works. We then selected stream sites where (i) modelled delivery of
97 fine sediment was predominantly (>75%) from agricultural sources, (ii) modelled sewage inputs
98 were < 0.5 kg ha⁻¹ year⁻¹, (iii) modelled diffuse urban inputs were < 2.0 kg ha⁻¹ year⁻¹ and (iv) there
99 were no lakes or reservoirs in the catchment. Modelled fine sediment delivery rates ranged from 14
100 – 1900 kg ha⁻¹ year⁻¹ across the 205 streams.

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

118 experiencing drought stress, e.g. restricted wetted width and depth: in practice, this was only an
119 issue in autumn 2011.

120 *Biological sampling*

121 Macroinvertebrates were sampled with a pond net (1 mm mesh-size) using a standard three-minute
122 kick/sweep, sampling all in-stream habitats in proportion to their areal coverage over the reach (10
123 – 20 m long depending on stream width), followed by a one-minute hand-search of rare or difficult
124 to sample habitats e.g. large stones, tree roots. This is the standard sampling method used by UK
125 regulatory authorities to monitor river water quality (Murray-Bligh *et al.*, 1997). Environmental
126 variables were recorded either on-site (stream width and depth, velocity, substrate composition) or
127 from map-based data (mean discharge category, elevation, distance from source and slope).
128 Macroinvertebrate community samples were immediately fixed in 10% formalin, returned to the
129 laboratory for subsequent identification and quantification (semi-quantitative numbers per sample)
130 to the lowest practicable taxonomic level; usually species or genus but family for more difficult
131 groups, e.g. Oligochaeta and some Diptera. Prior to data analysis, taxonomic resolution of the
132 complete macroinvertebrate dataset was standardised to ensure that it only contained discrete taxa
133 (as described in Appendix 3 of Chinnayakanahalli *et al.*, 2011).

134 Two existing trait resources were used to gather available biological trait information: the French
135 Genus Trait Database (Tachet *et al.*, 2003) and the on-line database www.freshwaterecology.info,
136 version 5.0, accessed on 30th January 2013 (Schmidt-Kloiber and Hering, 2015). Since October
137 2016 both sources are now available from www.freshwaterecology.info. The French data was the
138 primary source of information and was supplemented with information from the on-line resource
139 for those taxa or traits that were not included in the French database. Each biological trait, e.g.
140 maximal potential body size, was described by several trait-classes, e.g. ≤ 0.25 cm, $> 0.25-0.5$ cm.
141 The trait characteristics of each taxon were scored by assigning a value to each trait-class reflecting
142 the affinity of the taxon to the trait-class. Scores ranged from 0 to 5 indicating no to high affinity
143 respectively (Chevenet *et al.*, 1994). We compiled information on 11 biological traits across 62
144 trait-classes (Table II) for 192 distinct taxa identified across the 205 sites.

145 *Fine sediment sampling*

146 At each site a reach-scale estimate of the amount of fine sediment deposited on the stream bed was
147 made immediately upstream of the macroinvertebrate sampling area using the disturbance technique
148 (Duerdoth *et al.*, 2015). A steel cylinder (height 75 cm, diameter 48.5 cm) was inserted into an
149 undisturbed section of the stream bed and the water column agitated vigorously for one minute,

150 without touching the stream bed, to raise fine sediment deposited on the surface of the stream bed.
151 A pair of water samples was then collected quickly from within the cylinder. Then one minute was
152 spent disturbing the stream bed to a depth of approximately 10 cm, and vigorously agitating the
153 water and bed to raise any sub-surface fine sediment in addition to re-suspended surface deposits.
154 A second pair of water samples was then collected from within the cylinder. Four such sets of
155 water samples (surface, and combined surface and subsurface) were collected from each site, two
156 from erosional patches and two from depositional patches. Samples were refrigerated and returned
157 to the laboratory within five days, where they were processed for dry mass and organic content (i.e.
158 volatile solids following combustion at 550°C). Particle size distributions of material <1mm
159 diameter was also measured using a Malvern Mastersizer 2000. Reach-averaged values for surface
160 and total (combined surface and subsurface) deposited fine sediment were derived subsequently
161 (Table III).

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

166 *Data Analysis*

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

183 used as row weights. RLQ-analysis combined these three separate ordinations by defining a linear
184 combination of traits (taxon scores in Q-FCA) and a linear combination of environmental variables
185 (sample scores in R_r-PCA) that maximised covariance between taxon and site scores, measured
186 through the L_r-table (Dolédec *et al.*, 1996; Wesuls *et al.*, 2012).

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

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

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

203 *Independent testing*

204 Trait-classes confirmed by RLQ_p-Fourth-corner analysis to have significant associations with the
205 fine sediment gradient were applied to an independent dataset consisting of simultaneously
206 collected macroinvertebrate assemblage and deposited fine sediment data from 57 stream sites in
207 Wales sampled as part of a study investigating environmental impacts of agri-environment schemes
208 (Jones *et al.*, 2017). Field sampling and laboratory processing protocols were identical to those
209 used in the 205-site dataset. The dataset included multiple streams from each of the four stream
210 types. Measured deposited fine sediment mass in the stream bed ranged from 0.05 – 31.2 kg m⁻²
211 across the 57 sites. Relative prevalence of each trait-class (within a trait) at each site was calculated
212 by log-transformed abundance weighting trait-class affinity scores for each taxon for a given site.
213 Sums of weighted scores (one per trait-class) were expressed as the relative abundance distribution
214 (within a trait), giving the site trait profile. Measures of trait-class prevalence at a site were

215 correlated against reach-scale geometric mean mass of deposited fine sediment and organic fine
216 sediment in the stream bed. The strength of their association with deposited fine sediment gradients
217 was also compared to that for established fine sediment indices CoFSI_{sp} (Murphy *et al.*, 2015) and
218 E-PSI_{mtl} (Turley *et al.*, 2016). We corrected for family-wise error rate using the Holm-Bonferroni
219 method (Holm, 1979) to reduce the chance of Type I errors.

220

221

RESULTS

222 RLQ_p axis 1 was the dominant axis defining the ordination space (accounting for 84% of
223 explanatory power of the RLQ_p) and was significantly negatively correlated with all six measures of
224 deposited fine sediment mass (Table III, Figure 2a). RLQ_p axis 1 was to a lesser extent also
225 positively correlated with the modelled delivery of fine sediment from agriculture (Table III, Figure
226 2a). This negative association between modelled delivery and retained sediment is due to different
227 factors affecting load and retention (Naden *et al.* 2016). RLQ_p axis 2 (accounting for 10% of
228 explanatory power of the RLQ_p) was correlated with variables describing composition of the fine
229 sediment; coarser fine sediments tended to have relatively less organic content (Table III, Figure
230 2a).

231 Of the 18 predictions of biological trait-class response to fine sediment stress (Table I) only two
232 were confirmed by our data: prevalence of ovoviviparity was negatively correlated with RLQ_p axis
233 1, describing decreasing stress from fine sediment, while that of crawling was positively correlated
234 (Table II). Outside of predicted trait-class responses to increasing fine sediment, there was also a
235 significant negative correlation between RLQ_p axis 1 and prevalence of an aquatic adult stage. In
236 addition, we found significant positive correlations between RLQ_p axis 1 and prevalence of aerial
237 active and aquatic active dispersal, and eggs or statoblasts as resistance forms (Table II, Figure 2b).
238 There were no significant correlations between RLQ_p axis 2 and trait-classes.

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

254 cemented eggs (Figure 3, Figure S1). This syndrome included *Baetis*, *Rhithrogena*, *Elmis* and
255 *Leuctra*.

256 Analysis of the independent dataset confirmed that prevalence of an aquatic adult life stage and
257 ovoviviparity increased significantly with increasing mass of deposited fine sediment and fine
258 organic sediment in the stream bed (Table IV). In addition, prevalence of crawling, eggs or
259 statoblasts as resistance forms and aerial active dispersal decreased significantly with increasing
260 mass of deposited fine sediment and fine organic sediment in the stream bed (Table IV). Both
261 CoFSI_{sp} and E-PSI_{mtl} had significant negative correlations with deposited fine sediment gradients,
262 the strengths of which were greater than that for individual trait-classes correlations (Table IV).
263 Mass of deposited fine sediment (*logSed*) was most parsimoniously predicted (stepwise selection
264 from the suite of six biological trait-classes) by a combination of prevalence of eggs or statoblasts
265 as resistance forms (*Rest_egg*) and crawling (*Loco_crw*) ($\log Sed = 5541 - 2.99Rest_egg - 3.44$
266 $Loco_crw$, $R^2 = 0.358$, $P < 0.001$). Similarly, mass of deposited fine organic sediment (*logVs*) was
267 most parsimoniously predicted by a combination of prevalence of an aquatic adult life stage
268 (*AqSt_ad*) and *Rest_egg* ($\log Vs = 2.236 + 329AqSt_ad - 3.06 Rest_egg$, $R^2 = 0.379$, $P < 0.001$).
269 These models incorporate diagnostic aspects of both trait syndromes A and B to best distinguish
270 sites, and do so with similar power to community composition indices (Table IV).

271

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

303 In a comparison between results of the current study and other published work we found little
304 concordance in the identity or direction of significant associations (Table S2). Of 31 trait-classes
305 across 11 traits that had significant associations with increasing mass of fine sediment in at least one

306 of the four studies, none showed a consistent trend. Prevalence of ovoviviparity increased with
307 increasing deposited fine sediment mass in the current study and in Mondy and Usseglio-Polatera
308 (2013), decreased in Descloux *et al.* (2014) and showed no trend in Buendia *et al.* (2013).
309 Prevalence of scrapers decreased with increasing deposited fine sediment mass in Mondy and
310 Usseglio-Polatera (2013), but increased in Descloux *et al.* (2014) and Buendia *et al.* (2013) and
311 showed no relationship in the current study. For the remaining 26 trait-classes compared, there was
312 a consistent result across studies of no significant correlation with increasing deposited fine
313 sediment mass. It is possible that cross-study inconsistencies are a function of differing sampling
314 methodologies, sample sizes, and extent of stressor gradient encompassed. Mesh size of the
315 sampling nets used varied across studies from 300 to 1000 μm , which may have influenced relative
316 prevalence of traits in assemblages, e.g. maximal potential size. In addition, it may well be that the
317 complexity of the habitat template, where different biotic and abiotic filters act over varying
318 temporal and spatial scales to define the assemblage of traits present in a given reach, makes
319 formulating predictions very difficult (Menezes *et al.*, 2010). Furthermore, not all impacts of fine
320 sediment are direct: macroinvertebrate assemblages will respond to changes in other parts of the
321 biological community which may, in turn, select for traits other than those presumed to be impacted
322 directly by fine sediment.

323 However, it is likely that inconsistencies between different studies arise, in part, from the fact that
324 previous studies did not explicitly consider that trait responses to environmental gradients cannot be
325 regarded as independent discrete entities. Traits do not respond in isolation to the environment,
326 rather the environment acts on combinations of trait-classes, as mediated through species (Verberk
327 *et al.*, 2013). It should be recognised that they interact through trade-offs in energy investment, and
328 efforts should be made to identify colinearities. Clusters of traits with similar patterns of variation
329 across the extensive dataset are linked for ecological or evolutionary reasons, and together offer
330 some form of adaptation to life in fine sediment-rich or coarse substrate habitats. Rather than
331 testing hypotheses on a trait-class by trait-class basis we should be formulating hypotheses
332 regarding combinations of compatible trait-classes that together form a life history strategy for
333 dealing with the challenges posed by the environment. In the present study we directly addressed
334 this concern using multivariate RLQ_p-Fourth corner and cluster analysis to identify three distinct
335 trait syndromes along the deposited fine sediment gradients. These represent life-history strategies
336 that enable taxa to persist at both ends of the gradient. The combination of trait-classes we found to
337 be significantly associated with the fine sediment stress gradient incorporated all four of the major
338 trait domains proposed by Verberk *et al.* (2008) to be critical in dealing with environmental
339 constraints, namely reproduction, development, dispersal and synchronisation. Streams with little

340 deposited fine sediment tended to favour a strategy characterised by eggs as resistant forms, aerial
341 active dispersal and crawling, which together with a tendency for the laying of fixed eggs and
342 univoltine or semivoltine life cycles offer a fitness advantage under such minimally impacted
343 conditions. These are all trait characteristics of non-Dipteran insect orders, and indeed 75 of the 82
344 taxa assigned to this cluster were mayflies, stoneflies, beetles and caddis flies. We found that
345 streams with high amounts of deposited fine sediment selected for a strategy defined primarily by
346 an aquatic adult life stage and ovoviviparity. It is not difficult to see how greater investment in egg
347 protection and parental care provided by ovoviviparity can be advantageous in an environment
348 where un-attended eggs deposited in the stream bed or adhered to hard substrates would be
349 smothered or abraded by excessive quantities of fine sediment. Dolédec *et al.* (2006) and Lange *et*
350 *al.* (2014) also found that the prevalence of ovoviviparity increased in more intensively farmed
351 catchments with a greater mass of fines in the stream bed. They attributed this to the increased
352 probability of smothering of eggs by fines and algal mats in such streams. When combined with an
353 extended adult aquatic stage and multiple life cycles per year, as for amphipod and isopod
354 crustaceans, this strategy allows for a strong and sustained recruitment at the more stressed end of
355 the gradient.

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

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

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

389

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390

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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 <i>et al.</i> (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
Resistance forms	7	Decreased prevalence of isolated cemented eggs	Decreasing availability of clean coarse substrates	-	Yes
	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

	flier	Loco_fli	0.015	0.903
	surface swimmer	Loco_ssw	-0.046	0.422
	full water swimmer	Loco_swi	-0.019	0.855
Locomotion and substrate relation	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
	microorganisms	Food_mio	-0.007	0.921
	detritus (< 1mm)	Food_det	-0.001	0.986
	dead plant (>= 1mm)	Food_dep	-0.047	0.422
Food	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
	absorber	Feed_abs	-0.025	0.744
	deposit feeder	Feed_dep	-0.034	0.657
	shredder	Feed_shr	-0.033	0.674
Feeding habit	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}, E-PSI_{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}	E-PSI _{mtl}
	CoFSI _{sp}	-0.625	-0.616		
	E-PSI _{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	<i>0.268</i>	<i>0.218</i>	<i>-0.280</i>	<i>-0.390</i>
	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).

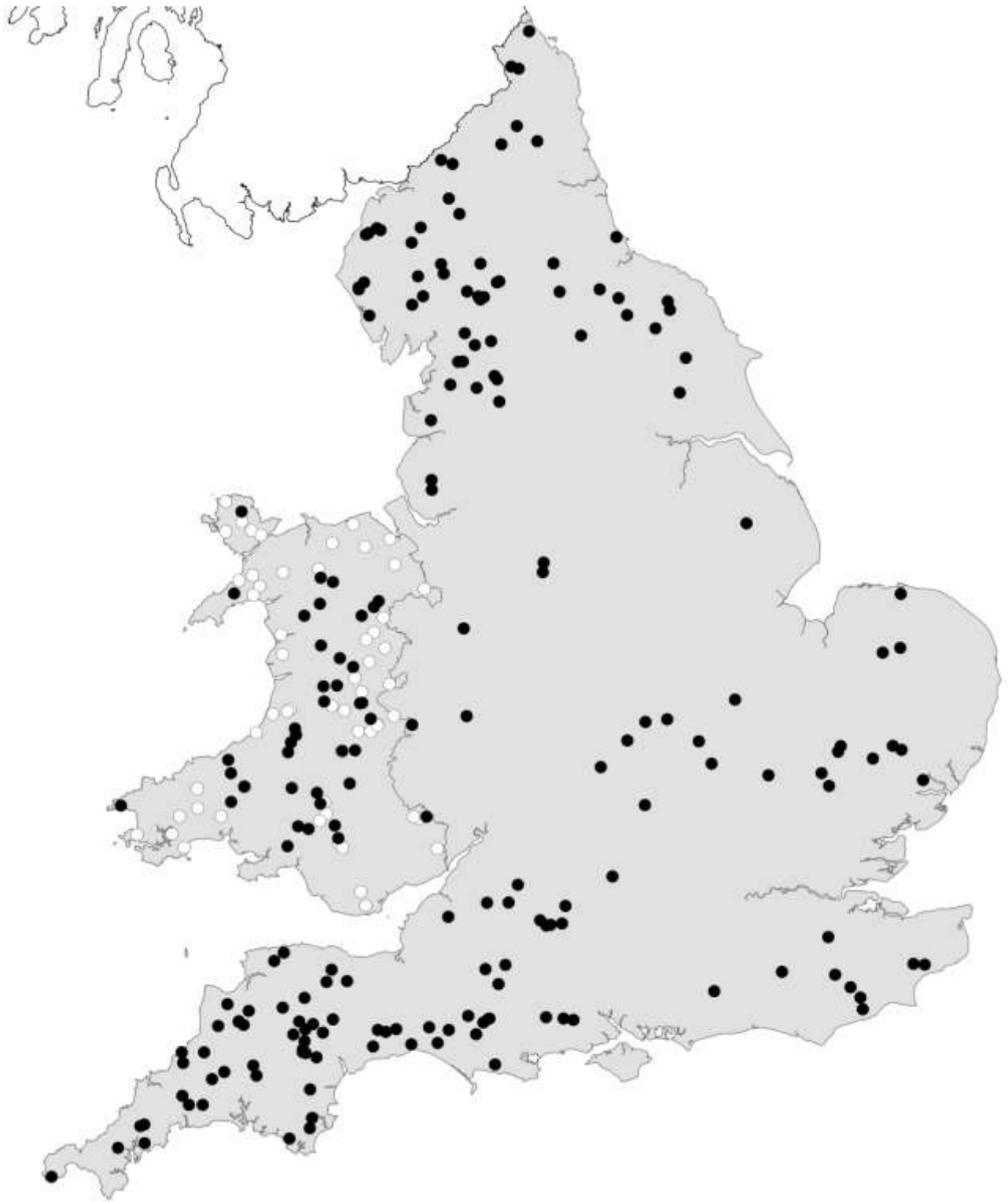


Figure 1.

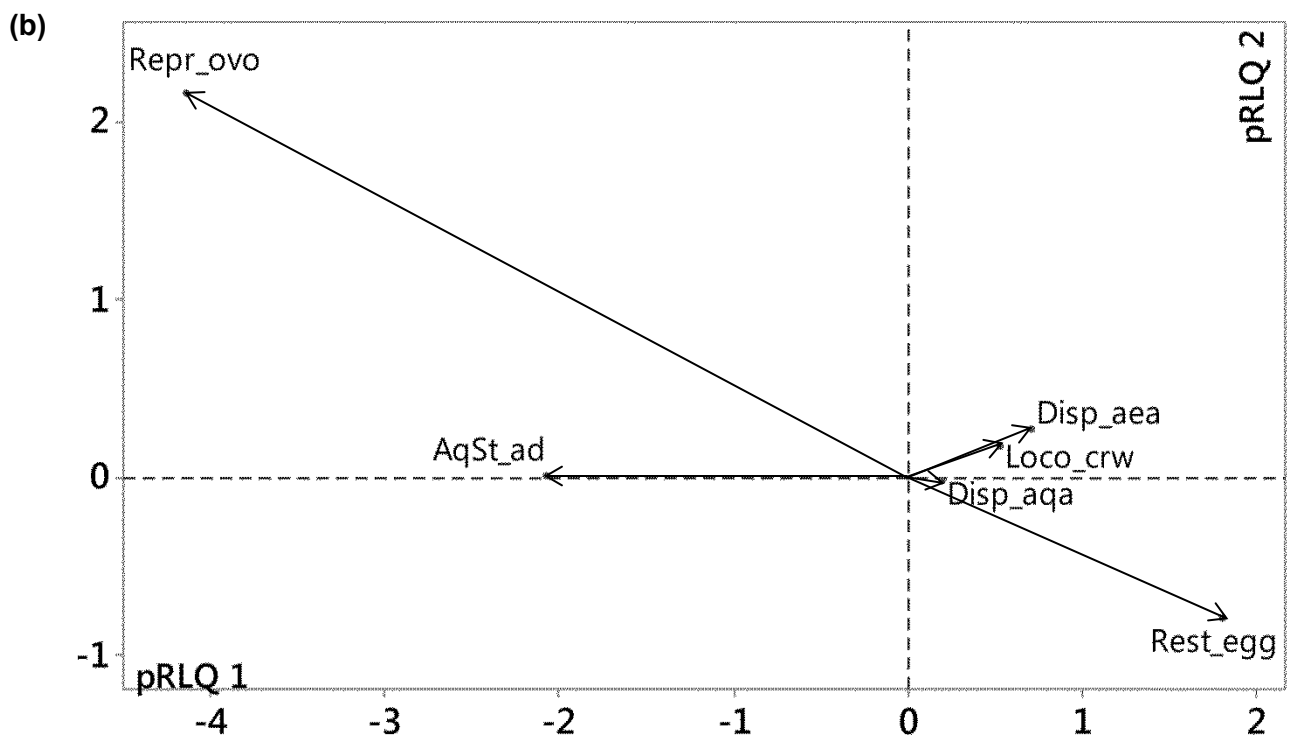
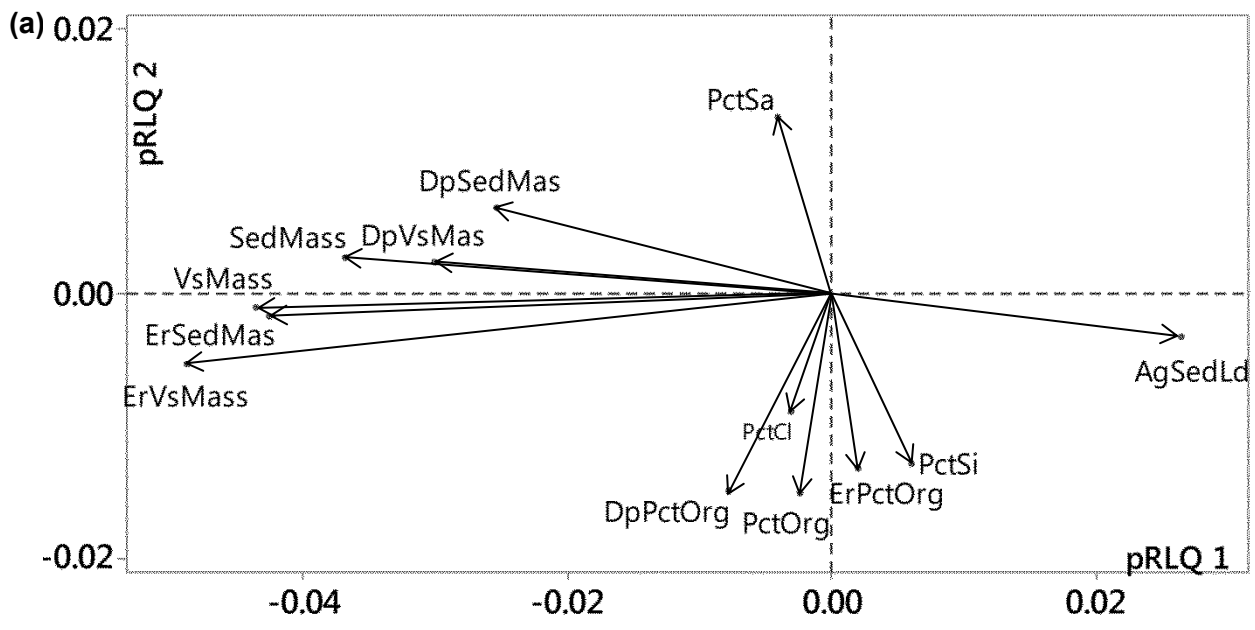


Figure 2.

(c)

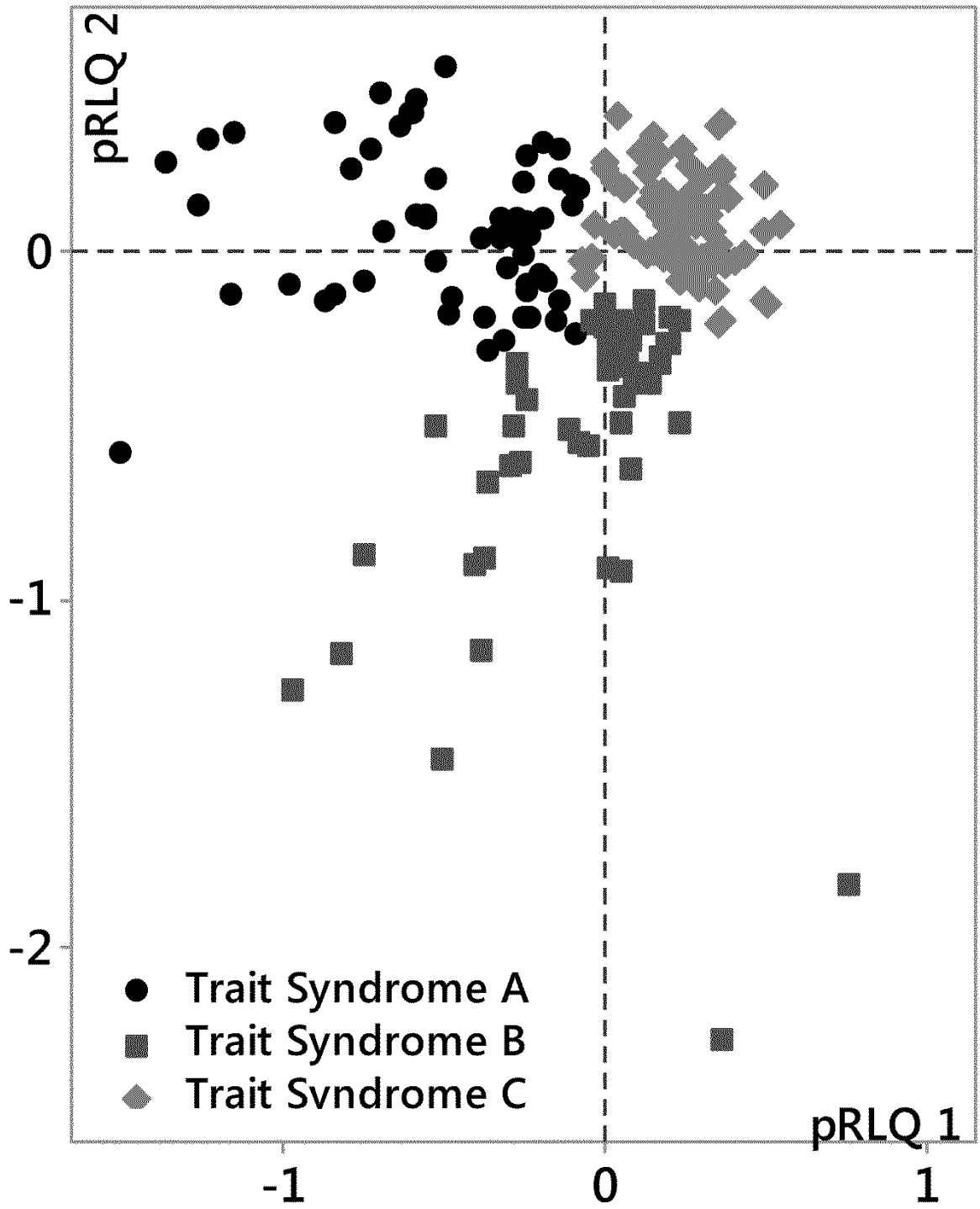


Figure 2. (cont'd.)

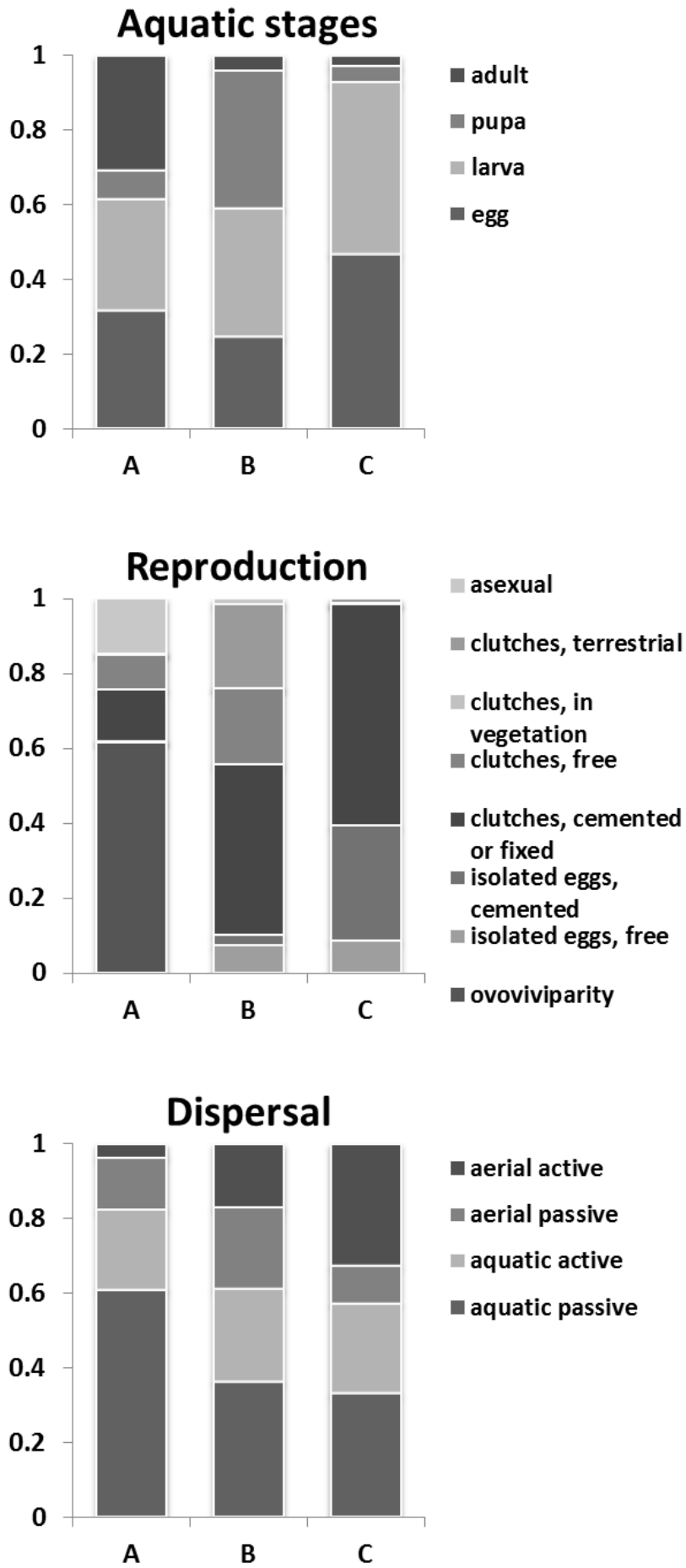


Figure 3.

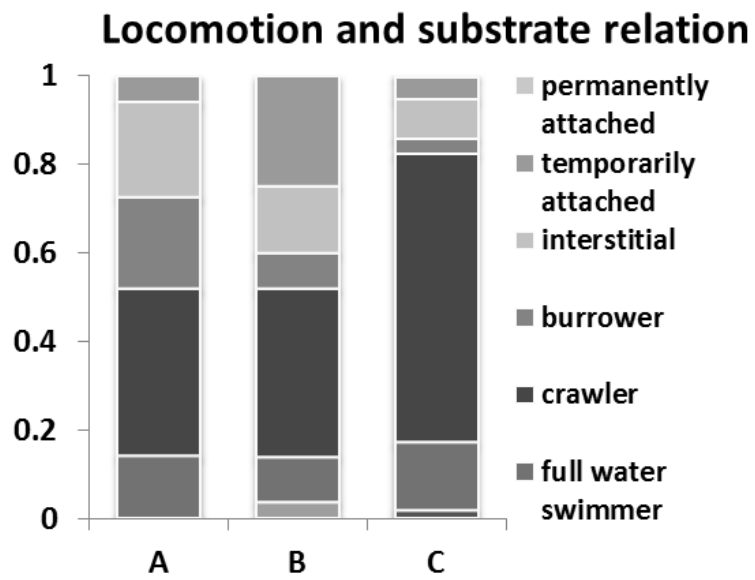
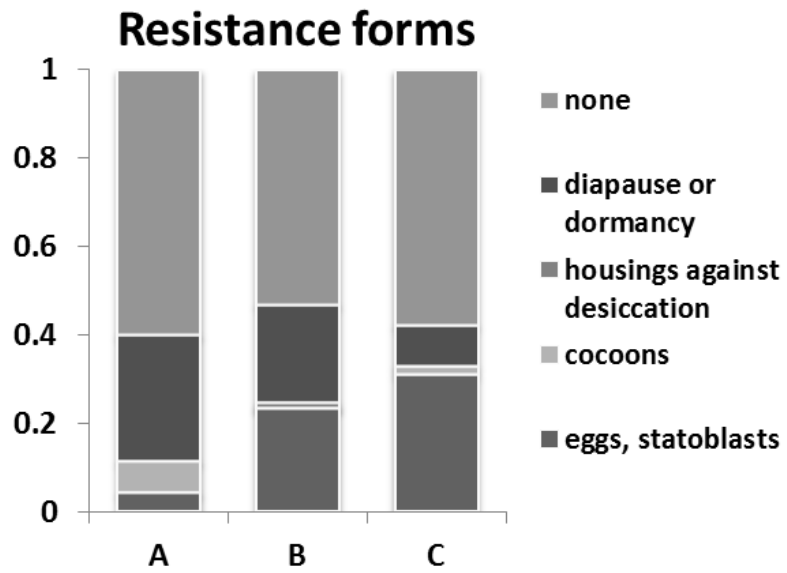


Figure 3. (cont'd.)