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The implications of an invasive species on the reliability of macroinvertebrate biomonitoring tools used in freshwater ecological assessments.

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1 The implications of an invasive species on the reliability of macroinvertebrate biomonitoring tools
2 used in freshwater ecological assessments.

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51 **The implications of an invasive species on the reliability of macroinvertebrate**
52 **biomonitoring tools used in freshwater ecological assessments.**

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54 Mathers, K.L., Chadd, R.P, Extence, C.A, Rice, S.P, and Wood, P.J.

55
56 **Abstract**

57 Invasive species represent one of greatest threats to aquatic biodiversity globally and are widely
58 acknowledged to be instrumental in modifying native community structure. Despite this, little is
59 known about how the increasing range expansion of invasive taxa may affect routine
60 biomonitoring tools widely employed to measure or quantify environmental quality in lotic
61 systems. This study examined the impact of an invasive freshwater crayfish on commonly
62 employed riverine macroinvertebrate biomonitoring tools (scores and indices) designed to
63 respond to a range of stressors. Data from long term monitoring sites on both 'control' and
64 invaded rivers in England were examined to assess changes to biomonitoring scores following
65 invasion by signal crayfish (*Pacifastacus leniusculus*). Results indicate that routine biomonitoring
66 tools used to quantify potential ecological stressors which are weighted by abundance, such as
67 the Lotic-invertebrate Index for Flow Evaluation (LIFE) score and Proportion of Sediment-
68 sensitive Invertebrates (PSI), were subject to significant inflation following invasion. In contrast,
69 indices based simply on the presence of taxa, such as the Average Score Per-Taxon (ASPT - a
70 derivative of BMWP), displayed no changes compared to control rivers; or in the case of the
71 Biological Monitoring Working Party Score (BMWP), NTAXA and EPT richness, no consistent
72 pattern following invasion. Season had a significant effect on the interaction of crayfish and LIFE
73 and PSI scores. Autumn samples were subject to statistical inflation following crayfish invasion
74 whilst Spring samples exhibited no significant change. The results suggest that care should be
75 taken when interpreting routine macroinvertebrate biomonitoring data where non-native crayfish
76 are present, or in instances where their presence is suspected.

77
78 **Keywords.** non-native taxa, crayfish, macroinvertebrate, seasonal sampling, WFD, biological
79 monitoring.

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90 **1. Introduction**

91 Invasive species are considered to be one of the greatest threats to global biodiversity (Simberloff
92 et al., 2013). The extent of biological invasions has increased rapidly over the last century and it
93 is likely that this rate will continue in the future (Pysek and Richardson, 2010). The translocation
94 of non-native taxa can have significant and far reaching implications for the functioning of invaded
95 ecosystems including habitat modifications, acting as vectors in the transmission of disease, and
96 altering assemblage composition through predation and resource competition (Manchester and
97 Bullock, 2000). The spatial and biological implications of invasions are driven and influenced by
98 natural and anthropogenic global environmental change (Lapointe et al., 2012). Anthropogenic
99 modifications are altering the structure of many aquatic ecosystems (Friberg, 2014) and
100 biomonitoring programmes that assess the status of freshwater water bodies have become an
101 essential means of monitoring and evaluating such pressures (Buss, 2015).

102

103 Benthic macroinvertebrates are one of the most commonly employed freshwater groups globally
104 (Carter et al., 2006) and in Europe are designated as one of the biological quality elements
105 employed in the implementation of the EU Water Framework Directive (WFD; EU, 2000). The
106 occurrence of indicative aquatic invertebrate taxa and assemblages based upon functional traits
107 and life histories have enabled the development of a multitude of biomonitoring tools used for the
108 identification and quantification of a range of anthropogenic disturbances and stressors (Bonada
109 et al., 2006). However, species expansion, in particular invasive species, may significantly
110 compromise the use of aquatic macroinvertebrates as bioindicators (MacNeil et al., 2013).
111 Selective predation by many invasive taxa such as crayfish (e.g *Procambarus clarkii*, *Orconectes*
112 *limosus*; *Pacifastacus leniusculus*) is likely to modify communities (Gheradi and Acquistapace,
113 2007; Ercoli et al., 2015) and may thereby reduce the effectiveness of commonly employed
114 biomonitoring indices to accurately characterise the pressures they were designed to assess.

115 Crayfish are one of the largest freshwater invertebrates, typically dominating the biomass of
116 benthic communities where they occur (Momot, 1995, Holdich, 2002). They are widely considered
117 to be keystone species in both lotic and lentic habitats due to their size, population density and
118 functional role in the ecosystem (Stenroth and Nyström, 2003). Invasive crayfish have been
119 widely documented to reduce the biomass and richness of other aquatic macroinvertebrates and
120 macrophytes (Twardocleb et al., 2013; Ercoli et al., 2015). Consequently, their introduction may
121 have substantial effects on aquatic environments and communities. Although it is widely
122 acknowledged that invasive crayfish species may be instrumental in modifying freshwater
123 invertebrate community structure, little attention has been given to whether these potential
124 community modifications influence the effectiveness of widely utilised biomonitoring tools. The
125 aim of this study was to determine whether the performance of six commonly employed
126 macroinvertebrate biomonitoring tools used in the routine ecological assessment and

127 management of freshwaters have been affected following invasion of the signal crayfish
128 (*Pacifastacus leniusculus*) in English rivers.

129

130 **2. Methods**

131 *2.1. Data*

132 The main dataset employed in this study was derived from the Environment Agency of England
133 and Wales 'BIOSYS' database. The data-set comprised 846 samples (380 and 467 from invaded
134 and control samples respectively) with the majority of samples collected between 1990 and 2013
135 (three sites had data extending back to the 1970's and an additional four sites had data from the
136 mid 1980's). Nine invaded sites and eight reaches where signal crayfish were absent throughout
137 the record (control sites) from three English regions (East, North West and South East England)
138 formed the basis of the analysis. The sites reflected different geological, hydrological and
139 biogeographical characteristics regionally and were subject to no other significant anthropogenic
140 stressors. Both native white-clawed crayfish (*Austropotamobius pallipes*) and other invasive
141 invertebrate species were absent from the selected sites (with the exception of the long-
142 established gastropod *Potamopyrgus antipodarum*). *P. antipodarum* is widely distributed across
143 most regions in England since its introduction over a century ago (Ponder, 1988) and is not
144 considered to have any significant effects on freshwater invertebrate communities in most
145 European streams (Murria, et al. 2008).

146

147 All benthic invertebrate samples were collected using the Environment Agency's standard
148 sampling protocol for routine biomonitoring, comprising a 3-minute 'kick-sample', which
149 encompasses all available habitats, and an additional 1-minute, detailed hand search using a
150 standard FBA pattern pond net (Murray-Bligh, 1999). Each site has a season specific record of
151 community composition; Spring (March –May, df 300), Summer (June-August, df 119), Autumn
152 (September – November, df 352) and Winter, (December – February, df 75). Macroinvertebrates
153 were recorded to either species or genus level. Diptera larvae were only resolved to family level
154 and Hydracarina to order throughout the series. In total, 596 taxa were recorded.

155

156 Six standard biomonitoring indices of ecological and hydrological quality were derived for each
157 sample. Three of these indicators are routinely used to assess water quality by the Environment
158 Agency: the Biological Monitoring Working Party Score (BMWP; Chesters, 1980), the Number of
159 BMWP-scoring families present (NTAXA) and the Average Score Per-Taxon (a derivative of
160 BMWP). The Lotic Invertebrate index for Flow Evaluation (LIFE; Extence et al., 1999) which
161 quantifies river flow pressures (e.g. low flows during drought, abstraction or impoundment
162 pressures), and the Proportion of Sediment-sensitive Invertebrates (PSI; Extence et al., 2013) ,
163 which provides a measure of community sensitivity to fine sediment were also calculated for each
164 sample. For some samples PSI scores were unclassified reducing the sample number from 827

165 to 745. All five of the above indices are widely employed by the Environment Agency to provide a
166 measure of ecosystem health within lotic ecosystems. The final index employed was the richness
167 of aquatic insect larvae within the orders Ephemeroptera, Plecoptera and Trichoptera (EPT
168 richness) and is widely used internationally (e.g. Ligeiro et al., 2013; Tonkin et al., 2015). All
169 indices were standardized by site (Z-scores) to control for natural variability associated with
170 individual rivers.

171 2.2 .Data analysis

172 Data were categorised into four groups: i) Control-before invasion, ii) Control-after invasion, iii)
173 Invaded-before invasion, iv) Invaded-after invasion). For sites invaded by *P. leniusculus* the
174 approximate date of invasion was determined based on the first occurrence in the historical faunal
175 series. Detecting signal crayfish is difficult due to their high mobility (Gladman et al., 2010) and
176 there are currently no methods of determining crayfish populations below a density of 0.2m^{-2}
177 (Peay, 2003). It is likely that the true detection limit is higher, probably approaching a density of
178 1.0m^{-2} for the kick-net samples utilised in this study. As a result, it is important to acknowledge
179 that signal crayfish may have been present at the study sites for a number of years prior to formal
180 detection in biomonitoring samples. Also, routine sampling of crayfish populations is not a
181 standard practice following invasion, and it is likely that variations in population densities between
182 sites over time will be present in the dataset.

183 Control sites were divided into two periods (before invasion and after invasion) based on the
184 mean date of invasion for the respective region (1999 for East; 1997 for North West; and 2003 for
185 South East). This provided a means of assessing whether there were temporal shifts in
186 invertebrate community composition and bioindicators not associated with crayfish invasion. This
187 factor was included in the analysis as previous long-term analyses of UK data sets have revealed
188 changes in community composition associated with drought (Monk et al., 2008), channel
189 morphology modifications (Dunbar et al., 2010) and improvements in water quality (Durance and
190 Ormerod, 2009).

191

192 To assess the potential influence of crayfish invasion on the biomonitoring indices, Generalised
193 Linear Models (GLMs) were fitted to each metric. To enable a GLM to be fitted to the data, Z-
194 scores were normalised to positive values prior to analysis. This standardised the indices, making
195 them comparable with each other, without modifying the variance or trends within the series.
196 Models were fitted using the glm function in R Version 3.1.2 (R development Core Team, 2014).
197 Inspection of the Akaike's Information Criteria (AIC) indicated that a Gaussian error distribution
198 and identity link was the most suitable structure. Only significant terms were included in the final
199 model and were examined using the drop function. For each index, a GLM was fitted which
200 encompassed all available data. To assess the effect of crayfish on indices, the significance of
201 the interaction term (time x treatment) was examined. To determine any seasonal effects GLMs

202 were fitted to indices based on Spring and Autumn samples (df 300, and 352 respectively – 265
203 and 321 for PSI). Temporal changes in index scores were visualised and inspected via error plots
204 in IBM SPSS Statistics (version 21, IBM Corporation, New York). Index scores were visualised on
205 a river, region and global basis to identify and confirm the consistency of the trends.

206

207 **3. Results**

208 ASPT scores (derived from BMWP) demonstrated no significant changes following crayfish
209 invasion, with both 'control' and invaded rivers demonstrating an increase in scores over time
210 ($T_{3,827} = -0.183$, $P > 0.05$; Fig. 1a). BMWP, NTAXA and EPT richness displayed inconsistent
211 responses following crayfish invasion when individual rivers and regions were considered; some
212 rivers and regions displayed a decrease comparative to the 'control' rivers whilst others displayed
213 increases (Figs. 1b, c & d). Both LIFE and PSI displayed a significant elevation of scores
214 following crayfish invasion compared to control sites. For both indices, the overall temporal trend
215 of increasing scores was present in both 'control' and invaded streams, however the increases in
216 invaded streams following invasion were determined to be statistically inflated ($T_{=3,827} = 3.905$, P
217 < 0.001 and $T_{=3,745} = 3.905$, $P < 0.001$ respectively; Figs. 1e & f). When season was considered,
218 LIFE and PSI scores displayed significantly inflated scores within invaded rivers for the autumn
219 season ($T_{3,350} = 2.906$, $P < 0.005$ and $T_{=3,321} = 4.529$, $P < 0.001$ respectively; Figs. 2a and b). In
220 contrast no significant differences in the temporal trends between invaded and 'control' sites were
221 identified for any of the biomonitoring scores for the spring sampling period ($P > 0.05$). All
222 statistical significance values and measures of standard error for the Before-After-Control-
223 Invaded interaction effect for each index and season (Spring and Autumn) are presented in Table
224 1.

225

226 **4. Discussion**

227 Results from this study indicate that the presence of signal crayfish has not significantly changed
228 the effectiveness of the commonly utilized water quality indices, ASPT, NTAXA or BMWP,
229 employed for EU WFD ecological assessment (Furse et al., 2006). ASPT displayed no significant
230 differences among control or invaded streams, with a similar magnitude of increase in the score
231 over time. BMWP, NTAXA and the biometric of EPT richness all demonstrated no consistent
232 pattern in either control or invaded streams, between regions or between rivers in the same
233 region. All four of these scores are based on records of presence only and do not incorporate any
234 weighting for the abundance of the taxa contributing to the score (recorded at family level).
235 Furthermore, BMWP, NTAXA and EPT richness are additive measures which may be influenced
236 by habitat richness and sampling effort, and are inherently more variable than their numerical
237 average typically suggests (Clarke et al., 2003). It is likely that should these metrics include
238 abundance weightings in their future derivations (as in the case of the proposed BMWP / NTAXA
239 replacement – the Whalley, Hawkes, Paisley & Trigg metric; WHPT, WFD-UKTAG, 2014), then

240 alterations to scores following invasion may occur and the resulting scores would need to be
241 interpreted with this in mind. Moreover, the taxonomic resolution used in scoring may play a key
242 role in determining crayfish invasion effects, with greater taxonomic level (genus or species level
243 data), making identification of invasion effects more likely. Reduced taxa richness (number of
244 taxa) has been observed in other studies associated with crayfish invasion (Crawford et al., 2006;
245 Ruokonen et al., 2014), although the family level data used to derive the metric NTAXA did not
246 identify any assemblage changes in the current investigation.

247

248 LIFE and PSI indices, which incorporate abundance weightings of the taxa contributing to their
249 score, both displayed significantly inflated scores following crayfish invasion compared to control
250 rivers. The application of the LIFE scores enables flow regime variability to be quantified based
251 upon the flow requirements of invertebrate species (Extence et al., 1999). Aquatic invertebrate
252 community composition following crayfish invasion has been reported to shift towards more
253 mobile taxa adapted to faster flow velocities at the expense of slower, less mobile taxa (Parkyn et
254 al., 1997). Studies have reported increasing or unaltered abundances or dominance of highly
255 mobile and flow velocity sensitive Ephemeroptera larvae at sites where invasive crayfish are
256 present (Usio and Townsend, 2004; Grandjean et al., 2011). The inflated LIFE scores recorded
257 within invaded streams most likely reflects the greater mobility of the remaining flow sensitive
258 taxa, characteristics which are likely to enhance their ability to evade crayfish predation
259 (Peckarsky, 1996). Predator avoidance strategies, including enhanced locomotion and vertical
260 migration to the waterline or into the river bed (Crowl and Covich, 1990; Haddaway et al., 2014),
261 by some taxa could potentially lead to the inflation or depression of biomonitoring scores. The
262 application of biological indicators typically assumes that the impacts of predation and competition
263 within macroinvertebrate assemblages are minor relative to the environmental changes that the
264 index was designed (and employed) to detect. It is likely that native predatory species have little
265 effect on the performance of biomonitoring tools because the community is familiar with them.
266 However, the invasion of a non-native 'alien species' into a waterbody may disrupt this natural
267 equilibrium leading to changes in the performance of biomonitoring tools.

268

269 The PSI score was designed to identify the effect of fine sediment pressure (primarily deposition)
270 based upon tolerance ranges of individual taxa (Extence et al., 2013). It appears that the inflated
271 PSI and LIFE scores recorded in this study were influenced by the markedly reduced abundance
272 of Gastropoda, Bivalvia and Hirudinea taxa. These are among the most widely documented prey
273 items of crayfish and may be selectively or preferentially predated by crayfish in many lotic
274 ecosystems (Dorn, 2013). Although the prevalence of some prey taxa are likely to decrease in the
275 presence of invasive crayfish, there is limited evidence to suggest that they become locally
276 extinct. Consequently, the inflated PSI and LIFE scores may represent a shift to a community
277 dominated, by fine sediment and flow sensitive taxa through predation rather than a shift in flow

278 regime or fine sediment present at a site. Future application and potential modifications to these
279 indices should consider the potential effect of invasive species upon them. The use of these
280 indices in their current form could be used to help identify sites subject to invasive taxa but may
281 also lead to the misinterpretation of the stressors affecting water bodies if not identified. Given the
282 variety of invertebrate biomonitoring tools available we recommend that, where feasible, a multi-
283 metric approach is employed in the ecological assessment of freshwater bodies. The application
284 of individual metrics may not indicate pressures associated with the stressor it was designed to
285 quantify, but when used in combination with other metrics derived in different ways (e.g. presence
286 / absence data, total abundance or abundance weighted), may provide evidence to indicate the
287 presence of an ecological stressor(s). Together with knowledge regarding the wider
288 environmental and ecological context, this approach may help inform water resource and river
289 managers of potential threats to the ecological status of freshwater bodies associated with the
290 spread of invasive species.

291
292 When individual seasons were considered, no significant differences were recorded between
293 control and invaded sites / rivers for the spring sampling period. Crayfish movement and growth is
294 strongly regulated by water temperatures, with activity increasing with rising temperatures
295 (Johnson et al., 2014). Spring samples typically occur when crayfish activity is at its minimum and
296 consequently it is unsurprising that none of the indices were significantly affected at this time of
297 year. In contrast, Autumn samples are usually collected at the height or toward the end of crayfish
298 activity (notably directly after the breeding season); with inflated elevation of both the LIFE and
299 PSI scores evident at invaded sites. It is therefore recommended, that routine biomonitoring
300 samples collected in autumn need to be interpreted with caution if invasive crayfish are present or
301 if their presence is suspected. Samples collected in spring were not determined to be significantly
302 affected but should still be considered with caution. We also advise that those applying
303 macroinvertebrate biomonitoring indices to identify environmental stressors or those developing
304 new indices should be conscious of the potential influence that invasive species may have on the
305 effectiveness of such tools, especially if abundance weightings are incorporated in their
306 derivation.

307
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314
315

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416
417 **List of Figures**

418 **Fig. 1.** Macroinvertebrate biomonitoring indices (mean ± 95% CI) recorded for each before, after,
419 invaded and control factor in East, South East and North West England; a) ASPT; b) BMWP; c)
420 NTAXA ; d) EPT richness; e) PSI and; f) LIFE; Black solid = Before Invaded; Grey solid = After
421 Invaded; Black dashed = Before Control and; Grey dashed = After Control. Metrics standardised
422 to Z-scores.

423
424 **Fig. 2.** Macroinvertebrate biomonitoring indices (mean ± 95% CI) recorded for each before, after,
425 invaded and control factor for spring and Autumn samples from all three regions; a) LIFE and; b)
426 PSI. Black solid = Before Invaded; Grey solid = After Invaded; Black dashed = Before Control
427 and; Grey dashed = After Control. Metrics standardised to Z-scores.

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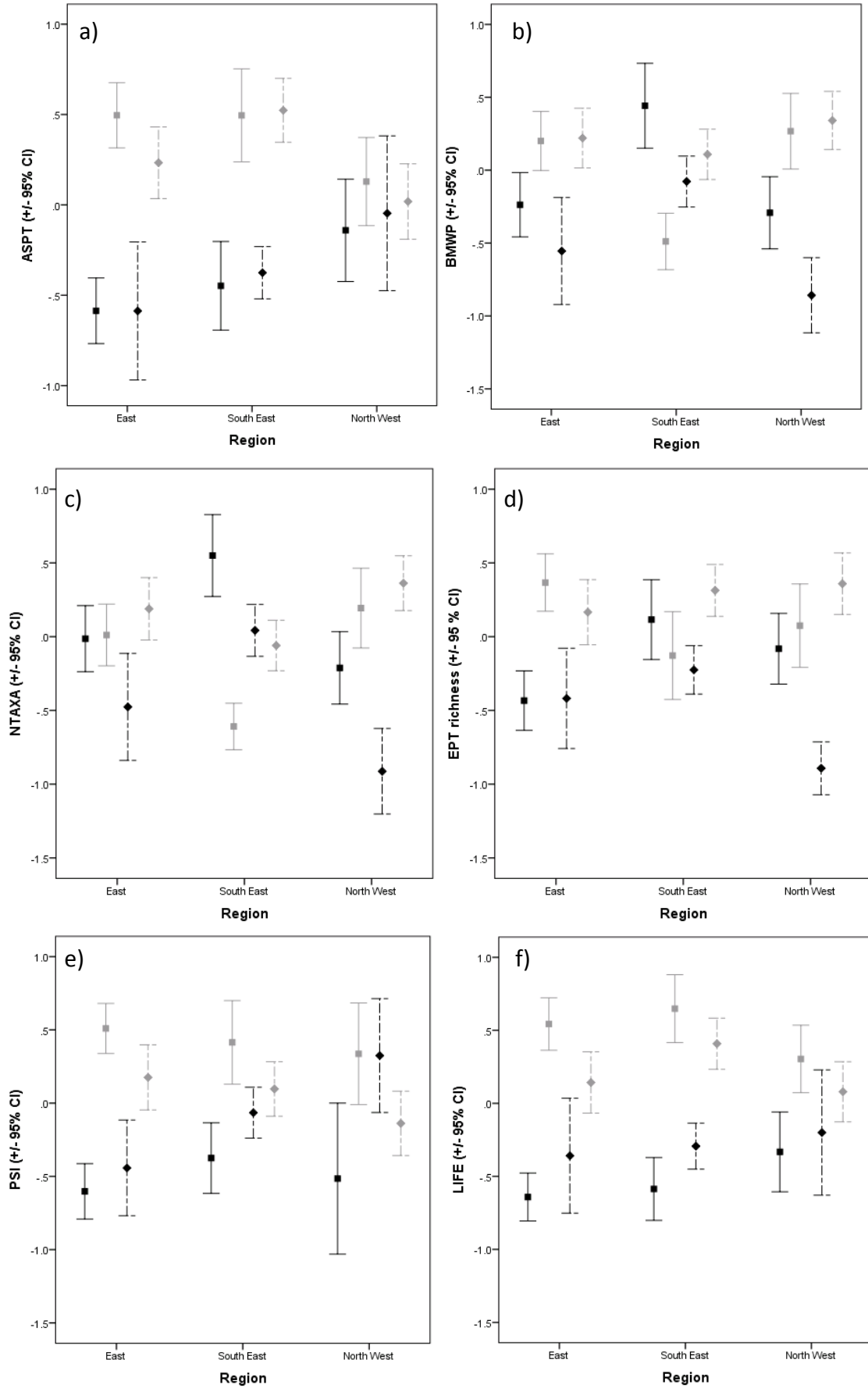


Figure 1

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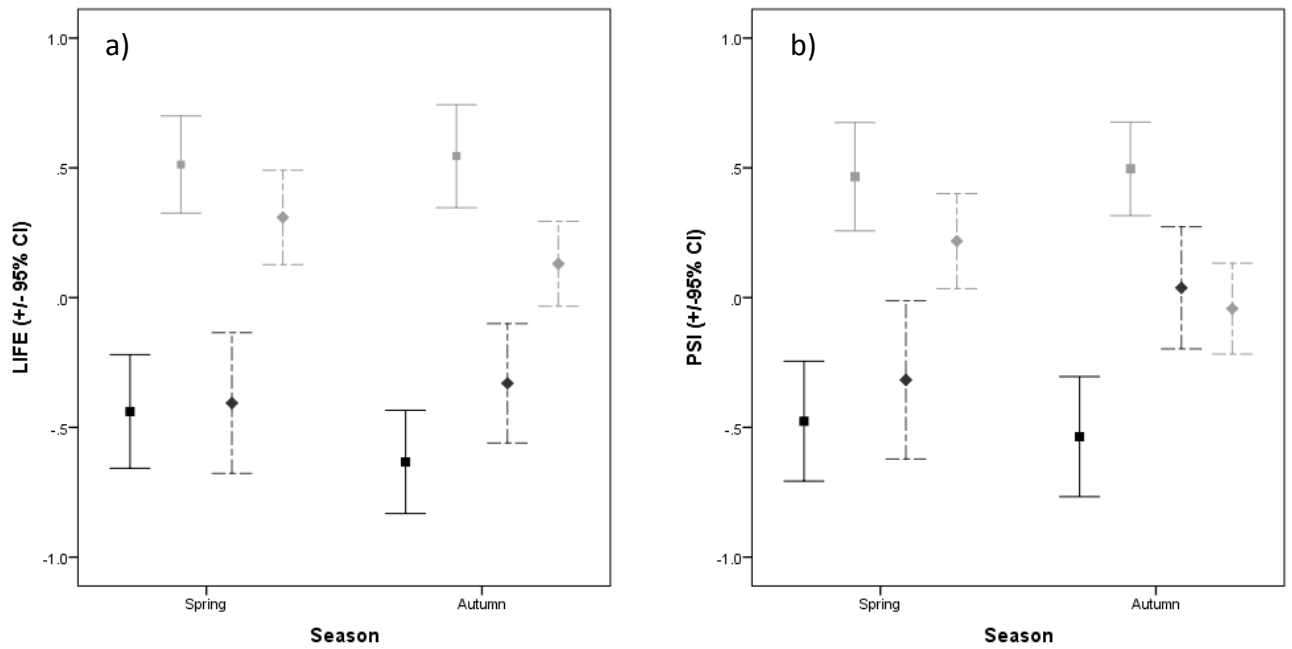


Figure 2

Table 1. Summary values for the Before-After-Invaded -Control interaction effects from the GLM for each bio-monitoring index

	SE	T-Value	P-Value	Degree of sig
All seasons				
BMWP	0.141	-3.374	<0.001	***
ASPT	0.135	-0.183	0.855	
NTAXA	0.148	-4.188	<0.001	***
EPT richness	0.139	-3.121	0.002	**
LIFE	0.134	3.905	<0.001	***
PSI	0.150	5.239	<0.001	***
Spring				
BMWP	0.253	-0.786	0.433	
ASPT	0.244	-0.008	0.994	
NTAXA	0.248	-0.692	0.489	
EPT richness	0.266	-1.513	0.131	
LIFE	0.233	1.576	0.116	
PSI	0.265	1.626	0.105	
Autumn				
BMWP	0.217	-2.605	0.010	*
ASPT	0.200	0.569	0.570	
NTAXA	0.224	-3.280	0.001	**
EPT richness	0.219	-2.429	0.015	*
LIFE	0.212	2.906	0.004	**
PSI	0.232	4.529	<0.001	***

N.B *** = $P \leq 0.001$, ** = $P \leq 0.005$, * = $P \leq 0.05$