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

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Abstract

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

Keywords. non-native taxa, crayfish, macroinvertebrate, seasonal sampling, WFD, biological monitoring.

1. Introduction

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

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

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

2. Methods

2.1. Data

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

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

Six standard biomonitoring indices of ecological and hydrological quality were derived for each sample. Three of these indicators are routinely used to assess water quality by the Environment Agency: the Biological Monitoring Working Party Score (BMWP; Chesters, 1980), the Number of BMWP-scoring families present (NTAXA) and the Average Score Per-Taxon (a derivative of BMWP). The Lotic Invertebrate index for Flow Evaluation (LIFE; Extence et al., 1999) which quantifies river flow pressures (e.g. low flows during drought, abstraction or impoundment pressures), and the Proportion of Sediment-sensitive Invertebrates (PSI; Extence et al., 2013) , which provides a measure of community sensitivity to fine sediment were also calculated for each sample. For some samples PSI scores were unclassified reducing the sample number from 827 to 745. All five of the above indices are widely employed by the Environment Agency to provide a measure of ecosystem health within lotic ecosystems. The final index employed was the richness of aquatic insect larvae within the orders Ephemeroptera, Plecoptera and Trichoptera (EPT richness) and is widely used internationally (e.g. Ligeiro et al., 2013; Tonkin et al., 2015). All indices were standardized by site (Z-scores) to control for natural variability associated with individual rivers.

2.2 .Data analysis

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

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

To assess the potential influence of crayfish invasion on the biomonitoring indices, Generalised Linear Models (GLMs) were fitted to each metric. To enable a GLM to be fitted to the data, Z-scores were normalised to positive values prior to analysis. This standardised the indices, making them comparable with each other, without modifying the variance or trends within the series. Models were fitted using the glm function in R Version 3.1.2 (R development Core Team, 2014). Inspection of the Akaike's Information Criteria (AIC) indicated that a Gaussian error distribution and identity link was the most suitable structure. Only significant terms were included in the final model and were examined using the drop function. For each index, a GLM was fitted which encompassed all available data. To assess the effect of crayfish on indices, the significance of the interaction term (time x treatment) was examined. To determine any seasonal effects GLMs were fitted to indices based on Spring and Autumn samples (df 300, and 352 respectively – 265 and 321 for PSI). Temporal changes in index scores were visualised and inspected via error plots in IBM SPSS Statistics (version 21, IBM Corporation, New York). Index scores were visualised on a river, region and global basis to identify and confirm the consistency of the trends.

3. Results

ASPT scores (derived from BMWP) demonstrated no significant changes following crayfish invasion, with both 'control' and invaded rivers demonstrating an increase in scores over time ($T_{3, 827} = -0.183$, $P > 0.05$; Fig. 1a). BMWP, NTAXA and EPT richness displayed inconsistent responses following crayfish invasion when individual rivers and regions were considered; some rivers and regions displayed a decrease comparative to the 'control' rivers whilst others displayed increases (Figs. 1b, c & d). Both LIFE and PSI displayed a significant elevation of scores following crayfish invasion compared to control sites. For both indices, the overall temporal trend of increasing scores was present in both 'control' and invaded streams, however the increases in invaded streams following invasion were determined to be statistically inflated ($T =_{3, 827} = 3.905$, $P < 0.001$ and $T =_{3, 745} = 3.905$, $P < 0.001$ respectively; Figs. 1e & f). When season was considered, LIFE and PSI scores displayed significantly inflated scores within invaded rivers for the autumn season ($T_{3, 350} = 2.906$, $P <0.005$ and $T = _{3, 321} = 4.529$, $P<0.001$ respectively; Figs. 2a and b). In contrast no significant differences in the temporal trends between invaded and 'control' sites were identified for any of the biomonitoring scores for the spring sampling period ($P > 0.05$). All statistical significance values and measures of standard error for the Before-After-Control-Invaded interaction effect for each index and season (Spring and Autumn) are presented in Table 1.

4. Discussion

Results from this study indicate that the presence of signal crayfish has not significantly changed the effectiveness of the commonly utilized water quality indices, ASPT, NTAXA or BMWP, employed for EU WFD ecological assessment (Furse et al., 2006). ASPT displayed no significant differences among control or invaded streams, with a similar magnitude of increase in the score over time. BMWP, NTAXA and the biometric of EPT richness all demonstrated no consistent pattern in either control or invaded streams, between regions or between rivers in the same region. All four of these scores are based on records of presence only and do not incorporate any weighting for the abundance of the taxa contributing to the score (recorded at family level). Furthermore, BMWP, NTAXA and EPT richness are additive measures which may be influenced by habitat richness and sampling effort, and are inherently more variable than their numerical average typically suggests (Clarke et al., 2003). It is likely that should these metrics include abundance weightings in their future derivations (as in the case of the proposed BMWP / NTAXA replacement – the Whalley, Hawkes, Paisley & Trigg metric; WHPT, WFD-UKTAG, 2014), then alterations to scores following invasion may occur and the resulting scores would need to be interpreted with this in mind. Moreover, the taxonomic resolution used in scoring may play a key role in determining crayfish invasion effects, with greater taxonomic level (genus or species level data), making identification of invasion effects more likely. Reduced taxa richness (number of taxa) has been observed in other studies associated with crayfish invasion (Crawford et al., 2006; Ruokonen et al., 2014), although the family level data used to derive the metric NTAXA did not identify any assemblage changes in the current investigation.

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

The PSI score was designed to identify the effect of fine sediment pressure (primarily deposition) based upon tolerance ranges of individual taxa (Extence et al., 2013). It appears that the inflated PSI and LIFE scores recorded in this study were influenced by the markedly reduced abundance of Gastropoda, Bivalvia and Hirudinea taxa. These are among the most widely documented prey items of crayfish and may be selectively or preferentially predated by crayfish in many lotic ecosystems (Dorn, 2013). Although the prevalence of some prey taxa are likely to decrease in the presence of invasive crayfish, there is limited evidence to suggest that they become locally extinct. Consequently, the inflated PSI and LIFE scores may represent a shift to a community dominated, by fine sediment and flow sensitive taxa through predation rather than a shift in flow regime or fine sediment present at a site. Future application and potential modifications to these indices should consider the potential effect of invasive species upon them. The use of these indices in their current form could be used to help identify sites subject to invasive taxa but may also lead to the misinterpretation of the stressors affecting water bodies if not identified. Given the variety of invertebrate biomonitoring tools available we recommend that, where feasible, a multi-metric approach is employed in the ecological assessment of freshwater bodies. The application of individual metrics may not indicate pressures associated with the stressor it was designed to quantify, but when used in combination with other metrics derived in different ways (e.g. presence / absence data, total abundance or abundance weighted), may provide evidence to indicate the presence of an ecological stressor(s). Together with knowledge regarding the wider environmental and ecological context, this approach may help inform water resource and river managers of potential threats to the ecological status of freshwater bodies associated with the spread of invasive species.

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

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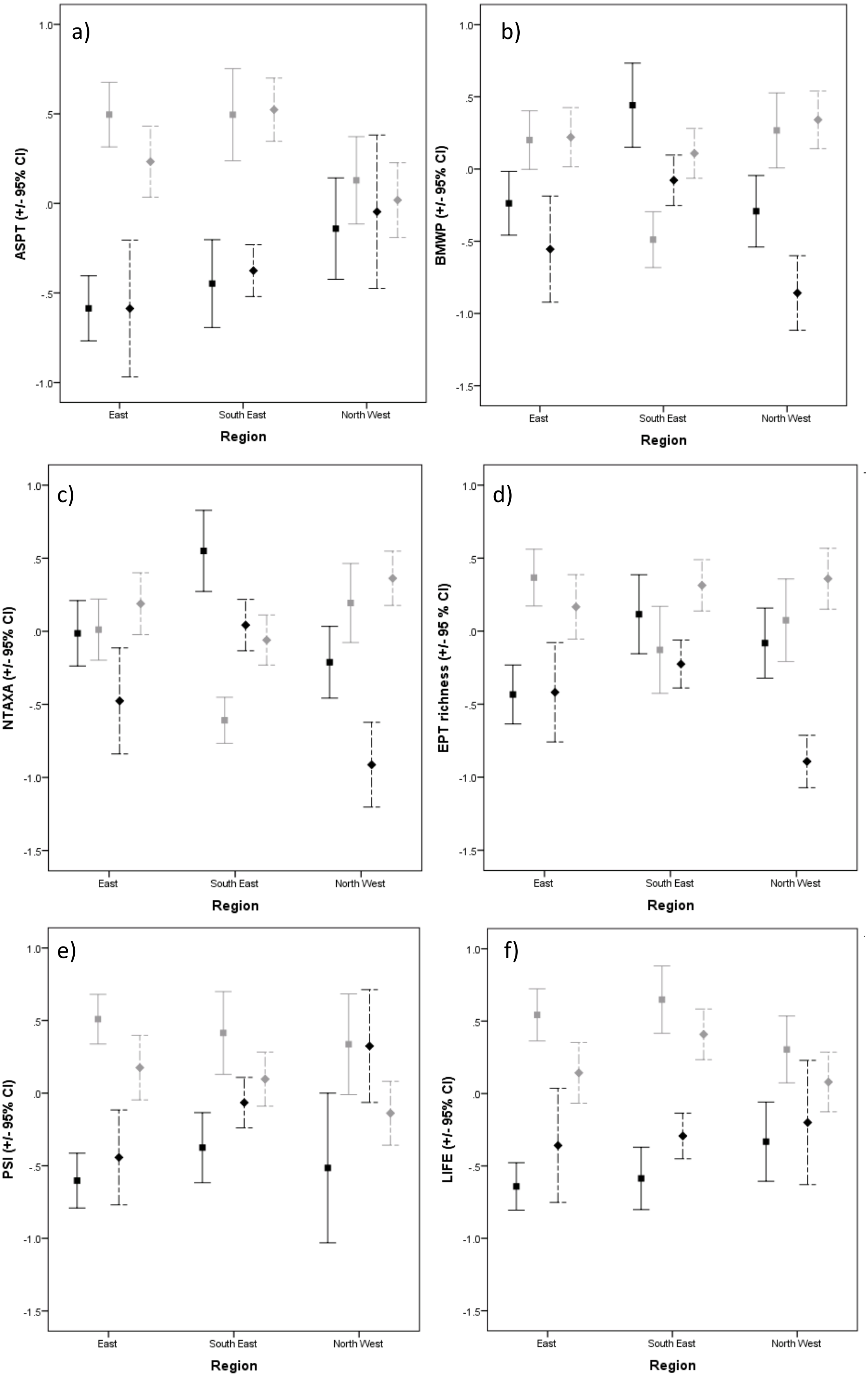
List of Figures

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

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


a)
ASPT (+/- 95% CI)
1.0
.5
.0
-.5
-1.0
East
South East
North West
Region
b)
BMWP (+/- 95% CI)
1.0
.5
.0
-.5
-1.0
-1.5
East
South East
North West
Region
c)
NTAXA (+/-95% CI)
1.0
.5
.0
-.5
-1.0
-1.5
East
South East
North West
Region
d)
EPT richness (+/-95 % CI)
1.0
.5
.0
-.5
-1.0
-1.5
East
South East
North West
Region
e)
PSI (+/-95% CI)
1.0
.5
.0
-.5
-1.0
-1.5
East
South East
North West
Region
f)
LIFE (+/-95% CI)
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East
South East
North West
Region

Figure 1

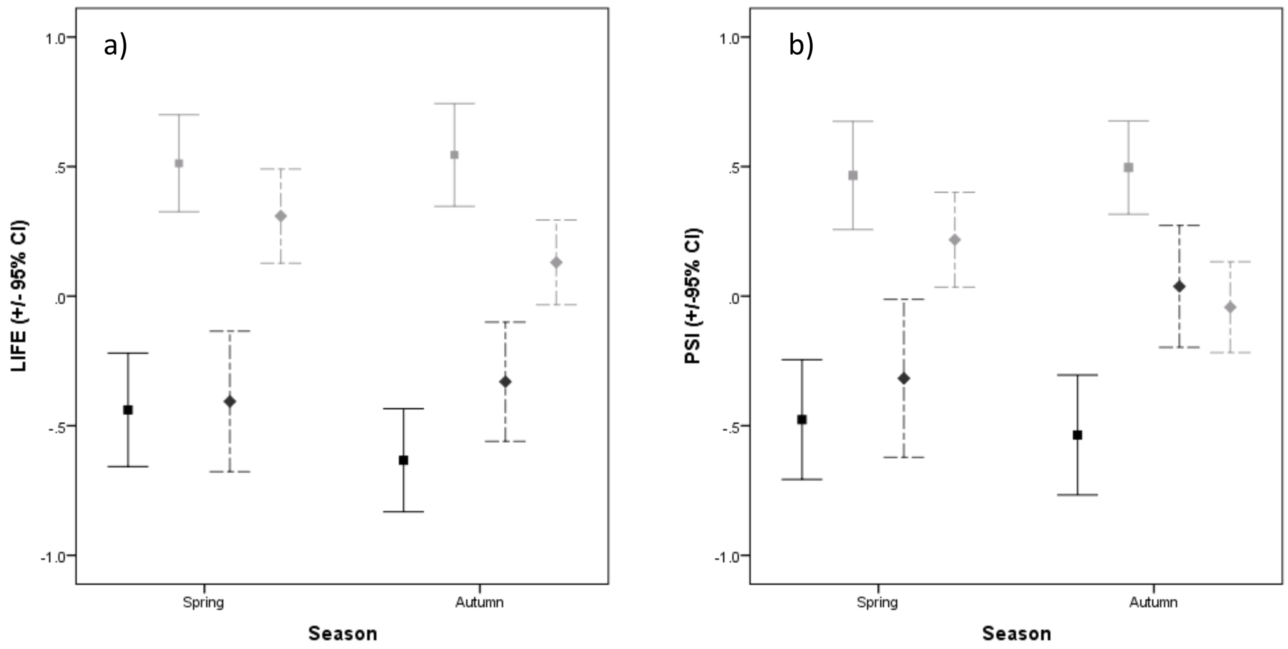

Figure 2

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 ≤ 0.001 , ** = P ≤ 0.005, * = P ≤ 0.05