TITLE: The development of a novel macroinvertebrate indexing tool for the determination of salinity effects in freshwater habitats

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AUTHORS AND DETAILS

Alex Pickwell[1], Drew Constable[2], Richard Chadd[3], Chris Extence[3], Sally Little[4]

[1] AECOM, 12 Regan Way, Chetwynd Business Park, Chilwell, Nottingham, NG9 6RZ, UK.

[2] Environment Agency of England, Bromholme Lane, Brampton, Huntingdon, Cambridgeshire, PE28 4NE, UK.

[3] Environment Agency of England, Stepping Stone Walk, Winfrey Avenue, Spalding, Lincolnshire, PE11 1DA, UK.

[4] School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, NG25 0QF, UK.

CORRESPONDING AUTHOR

Alex Pickwell – mrapickwell@gmail.com

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CRediT AUTHOR CONTRIBUTIONS

Alex Pickwell: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Drew Constable:** Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization. **Richard Chadd:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Chris Extence:** Writing - Original Draft, Writing - Review & Editing, Supervision. **Sally Little:** Investigation, Writing - Review & Editing.

ABSTRACT

Salinisation is a global threat to freshwater habitats and is predicted to worsen with climate change. Increases in salinity can result in substantial modification of freshwater biotic communities through both direct toxic effects and indirect effects such as altering prey resources, competitive interactions, predator abundances, and facilitating the spread of invasive species. Traditional techniques to determine salinity, such as point sampling chemical assessment, are typically periodic and may not reveal intermittent changes in salinity concentration. Halo-stratification and a lack of standardised depth to collect chemical data further complicates using these methods. More importantly, such methods do not show the ecological impacts of salinity increases in freshwater habitats. Complementing traditional techniques with biological assessments may resolve these issues. Pressure-specific biotic indices using aquatic macroinvertebrate community data have long been used to assess aspects of aquatic habitats, although relatively few have focussed on salinity. This paper presents the Salinity Association Group Index (SAGI), a novel aquatic macroinvertebrate index to assess salinity in freshwater habitats. SAGI is compatible with data derived from established survey techniques employed by regulatory bodies and for Water Framework Directive assessments, amongst others. The method integrates taxonomic resolution beyond family level with taxon abundance weighting in a scoring matrix to increase the efficacy of the tool and make best use of publicly available data. Application of SAGI in case studies demonstrates a positive, moderate to strong correlation with conductivity used as a measure of salinity. The range of correlations (R^2 = 0.57-0.91) compares favourably with pressure-specific, macroinvertebrate-based monitoring tools used in Europe for WFD monitoring. Furthermore, SAGI is shown to be highly effective in comparison to alternative salinity-specific biotic indices.

KEYWORDS

Index, macroinvertebrate, salinity, Water Framework Directive

1. INTRODUCTION

The salinisation of freshwater systems (both inland and coastal) is an important global issue (Williams, 2001; Kefford *et al*., 2016) that in recent decades has been occurring at an unprecedented rate (Nielsen *et al.*, 2003), threatening the biodiversity of freshwater habitats and the ecosystem goods and services they provide (Cañedo-Argüelles *et al*., 2013; Herbert *et al*., 2015). Freshwater salinisation is now considered a major global environmental issue (MEA, 2005; Cañedo-Argüelles *et al.*, 2016) and is predicted to intensify due to the impacts of climate change (Le *et al*., 2019; Reid *et al*., 2019) and increased water demand (Cañedo-Argüelles *et al*., 2013). The monitoring and management of freshwater habitats at risk is essential to avoid the significant economic, social and environmental costs that can result from the impact of salinisation on freshwater ecosystems (Williams, 2001).

Salinisation due to natural factors and occurring at rates uninfluenced by anthropogenic activities is termed primary salinisation (Williams, 2001; Cañedo-Argüelles *et*

al., 2013). In contrast, anthropogenic activities resulting in increases in salinity, either directly or indirectly, are referred to as secondary, or anthropogenic salinisation (Cañedo-Argüelles *et al*., 2013). These activities include the application of road salt (Williams *et al*., 1999; Hintz & Relyea, 2019) and the disposal of waste water from industry (Williams, 1987; Williams, 2001; Piscart *et al.*, 2005; Wolf *et al.*, 2009; Vengosh *et al*., 2014). Furthermore, disturbance of natural hydrological cycles mobilising naturally accumulated salts held in groundwater (Williams *et al.*, 1991; Kay *et al.*, 2001; Hart *et al*., 2003), soil (Kay *et al*., 2001) and rocks (Pillsbury, 1981) substantially contribute to the salinisation of inland waters (Williams, 1987). Such activities include the abstraction and diversion of water for irrigation benefit (Pillsbury, 1981; Williams, 1987; Cañedo-Argüelles *et al*., 2013) and the removal of native deep-rooted plants for agricultural purposes (Williams, 1987; Kay *et al.*, 2001). Whilst seawater inundation and incursion (e.g. Williams *et al.*, 1991; Wolf *et al.*, 2009) are natural processes associated with the transition from the freshwater to the marine environment, the effect on the resident aquatic community is important. Sea level rise resulting from climate change leads to increased seawater inundation and incursion of freshwater habitats (Little *et al.*, 2017; Little *et al*., in press). This will be exacerbated by ongoing reductions in freshwater flow resulting from impoundments, droughts and increased abstraction, which have been shown to increase seawater inundation and saline intrusion in surface (Attrill *et al.*, 1996; Reid *et al*., 2019) and ground waters (e.g. through natural deposits of prehistoric marine sediments underlying drained land). In the last few decades, secondary salinisation has become one of the primary drivers influencing freshwater biotic communities (Bäthe & Coring, 2011; Kefford *et al*., 2016).

Whilst salinity can be inexpensively and effectively determined by measuring electrical conductivity as a proxy, such chemical assessments reveal little about the effect of increased salinity on freshwater biota (Wright, 1994; Clarke *et al.*, 2003). Even small increases in salinity (*ca.* 2 $mScm^{-1}$) have been shown to result in the loss of halo-sensitive taxa (Hart *et al*., 1991; Chapman *et al.*, 2000; James *et al*., 2003; Böhme, 2011) and the gain of halo-tolerant taxa (Nielsen *et al*., 2003; Piscart *et al.*, 2005; Schröder *et al*., 2015). In a European context, the Water Framework Directive (WFD) (European Commission, 2000) requires the assessment of a water body to be based on the determination of its ecological condition (Chave, 2001; Teixeira *et al*., 2009) relative to the expected undisturbed condition (European Commission, 2000). Furthermore, the WFD requires salinity, alongside other physico-chemical elements, to be monitored (European Commission, 2000).

Globally, a range of biotic indices using macroinvertebrate community data have been developed to assess the impact of many pressures on freshwater biological communities, such as the Walley, Hawkes Paisley and Trigg (WHPT) index (Walley & Hawkes, 1996; Walley & Hawkes, 1997; Paisley *et al*., 2014) for assessing organic pollution, the Lotic-invertebrate Index for Flow Evaluation (LIFE) score (Extence *et al.*, 1999) for assessing river flow variability, the Drought Effect of Habitat Loss on Invertebrates (DEHLI) index for drought-mediated habitat change (Chadd *et al*., 2017), and the Proportion of Sediment-sensitive Invertebrates (PSI) score (Extence *et al*., 2011; Extence *et al*., 2017; Turley *et al*., 2014) for assessing fine sediment deposition. Relatively few indices have been proposed for the detection and determination of the impact of salinity on the benthic macroinvertebrate community, although such indices have been developed by Williams *et al*. (1999), Horrigan *et al*. (2005),

Wolf *et al*. (2009), Schäfer *et al*. (2011) and Palmer *et al.* (2013). Three of these indices have been designed for specific habitat types. Williams *et al*. (1999) developed an index to detect chloride contamination of freshwater springs in the Greater Toronto Area, whilst Palmer *et al*. (2013) proposed a salinity index for the assessment of coastal grazing marsh ditches. Wolf *et al*. (2009) designed their metric to indicate the longitudinal salinity zonation of tidal marshland streams. In contrast, both Horrigan *et al*. (2005) and Schäfer *et al*. (2011) proposed non-habitat specific salinity indices to measure the change in freshwater macroinvertebrate communities caused by salinity increases.

This paper proposes a novel salinity-specific index (SAGI – the Salinity Association Group Index) for the assessment of salinity increases on freshwater macroinvertebrates communities in nominally freshwater habitats, and presents the results obtained from three study areas following its application. The indices developed by Horrigan *et al*. (2005) and Schäfer *et al*. (2011) are locally adapted for application with the same dataset and the results of the two indices are compared with those of SAGI. We predict that SAGI will provide an effective tool for application in future monitoring programmes and investigations, enabling and facilitating the appropriate management and conservation of freshwater habitats.

2. METHODS

Electrical conductivity ($mScm^{-1}$) has been used to express salinity throughout this study.

2.1. Index structure

SAGI is based on ecological niche theory (Hutchinson, 1957) wherein the optimal conditions for species are found towards the centroid of their multidimensional ecological niche resulting in minimised death rate and maximised birth rate. Macroinvertebrate taxa (1103 species, genera and families) were assigned to one of five bandings, termed Salinity Association Groups (SAG; see Table 1 for definitions), based upon their association with salinity. Five bandings were chosen to match the number of salinity zones classified according to the Venice System (Battaglia, 1959) used in the WFD (European Commission, 2000). SAG-I represents typically freshwater taxa tolerant of only slight increases in salinity. Groups II-IV represent taxa associated with increasing salinities along the freshwater-seawater continuum, whilst SAG-V represents coastal seawater taxa rarely found at more dilute salinities. Taxa with wide salinity tolerances were attributed to the SAG where the main population of the taxon are typically found, indicating the primary salinity affiliation of that taxon. An extensive literature review of 144 published sources of data comprising macroinvertebrate species salinity preferences, species field distributions in relation to salinity and along salinity gradients, single species laboratory salinity tolerance tests, and multiple species mesocosm salinity tolerance experiments (references appended as Supplementary Material 1) was undertaken. Horrigan *et al.* (2007) reported that macroinvertebrate species salinity sensitivity derived from laboratory experiments reflects that derived from field distributions, allowing evaluation of these types of studies alongside

each other. The data sources were assessed with expert judgement and used to inform the allocation of taxa to the SAGs, which are shown in Supplementary Material 2. This methodology has been used previously in the development of the LIFE (Extence *et al*., 1999) and PSI (Extence *et al*., 2011) metrics, and provides SAGI with a sound biological basis which results in the tool having a mechanistic link between salinity and the biotic response rather than a purely correlative link (Friberg, 2014). Consequently, SAGI is not susceptible to statistical artefacts that can occur in purely statistically-derived biomonitoring tools (Turley *et al.*, 2016).

++ Table 1 ++

It is recognised that species level identification gives the most detailed ecological data (Armitage *et al*., 1990) and can result in the most accurate metric outputs (e.g. Extence *et al*. 1999; Monk *et al*., 2012; Vilmi *et al*., 2015). However, several studies have reported similar results when comparing family-level data with species- or genus-level data (e.g. Kefford, 1998; Clements *et al*., 2000; Chessman *et al*., 2007) and as such lower levels of data resolution can be adequate for the detection of community disturbances. SAGI employs a largely species level of identification, although several taxonomic groups have been retained at genus level (e.g. *Elodes, Dryops, Hydroptila*) where species resolution of taxa at different life stages has not yet been fully determined and the index employs family level identification for Diptera due to a lack of information on species' salinity association. Species-level identification is recommended where possible and practical to allow the accurate calculation of complementary indexing tools such as the Community Conservation Index (CCI – Chadd & Extence, 2004), LIFE (Extence *et al*., 1999), Monitoring Intermittent Streams index (MIS-index – England *et al*., 2019), PSI (Extence *et al*., 2011; Turley *et al*., 2014; Extence *et al*., 2017), and mixed level Empirically-weighted PSI (E-PSI, Turley *et al*., 2016). Where species data are unavailable, it is possible to use SAGI with family level data.

The SAGI methodology uses a scoring matrix (Table 2) incorporating the Salinity Association Groups and logarithmic abundance categories (1-9, 10-99, 100-999 and 1000+ individuals present) to calculate a final score for a macroinvertebrate sample. The abundance categories are derived from the UKTAG methodology for macro-invertebrate sampling and analysis (Murray-Bligh *et al*., 1997; Chadd, 2010). The matrix design in Table 2 is inspired by the structure of the biotic scoring system proposed by Chandler (1970) for assessing water quality and used in the LIFE metric (Extence *et al*., 1999). Calculation of SAGI is undertaken by determining the individual Salinity Association Score (SAS) for each scoring taxon present in a sample by referring to the scoring matrix in Table 2 and defined SAG allocations (Supplementary material 2).

++ Table 2 ++

To calculate the SAGI score for a sample, the following formula is applied;

$$SAGI = \frac{\Sigma SAS}{n}$$

where ΣSAS = the sum of individual taxon salinity association scores in the sample, and n = the number of SAGI scoring taxa in the sample. Higher SAGI scores reflect higher environmental salinities.

SAGI can be calculated for samples collected using any sampling methodology and therefore can be adapted for use in any country or eco-region. However, the same sampling protocol must be employed when comparing outputs of different studies. As such, it is recommended that samples are collected in accordance with the procedure defined within the UK Technical Advisory Group methodology for macro-invertebrate sampling and analysis in nominally freshwater habitats (Murray-Bligh *et al*., 1997) and employed by the UK regulatory bodies (Environment Agency, Natural Resources Wales, Scottish Environment Protection Agency and Northern Ireland Environment Agency). The procedure consists of using a Freshwater Biological Association pattern pond-net (square aperture 0.5m^2, 1-mm net mesh) to undertake a 3-min kick/sweep net sampling of all habitats present at a site, with the different habitats sampled in proportion to their occurrence, supplemented with a 1-min active hand search.

2.2. Study areas

SAGI has been developed in Great Britain and Ireland for wide application across a range of temporal scales and waterbody types. It is, however, expected that the index will also function effectively in mainland Europe. Furthermore, the methodology for assigning taxa can be followed to adapt the index to local conditions in bioregions beyond Europe. In this context the design of SAGI mirrored the conceptualisation and testing process used in developing the LIFE index (Extence *et al*., 1999), a metric that has now been adapted for use across several continents. Below we illustrate the application of SAGI using three case studies from England at different temporal resolutions. Sample sites were selected where it was considered that salinity was the most significant stressor on the ecological communities present and the freshwater survey locations are known to be subject to saline ingress or substratum chloride concentration issues.

2.2.1. Sussex rivers (Adur and Ouse)

The River Adur in southern England has a catchment area of approximately 540km^2 (Environment Agency, 2008). The estuary of the river extends from Shoreham-by-Sea upstream to the tidal limits at 21km on the eastern branch, which rises on Ditchling common, and 18.9km on the western branch which rises near the village of Slinfold.

The Sussex River Ouse, also in southern England, rises near Lower Beeding and has a catchment area of over 650km^2 (Environment Agency, 2009a). The normal tidal limit of the River Ouse occurs at Barcombe Mills, with the estuary of the river extending from this point

for 21.8km to Newhaven where it discharges into the English Channel (Environment Agency, 2009b; Environment Agency, 2010).

The estuary channels of both the River Ouse and River Adur have been narrowed, deepened and constrained for navigation and flood defence, exacerbating saline incursion by decreasing frictional drag and increasing tidal flow velocity which results in funnelling and propagation of the tidal wave upstream into nominally freshwater habitats (Savenije & Veling, 2005). Consequently, the upper Adur and Ouse estuaries have extensive tidal freshwater zones (Little *et al.*, 2017). Short- and long-term projections for southeast England indicate climate change and other anthropogenic impacts in the catchment are likely to exacerbate saline incursion within these estuaries (Robins *et al*., 2016). Furthermore, in a study on both the Adur and Ouse estuaries Little *et al.* (2017) found that whilst substratum type was locally important at two sampling locations, salinity was the primary and dominant variable driving benthic macroinvertebrate species distribution and community composition along both riverine to estuarine transitions.

2.2.2. South Holland Main Drain and South Forty Foot Drain

The South Holland Main Drain (SHMD) and the South Forty Foot Drain (SFFD) are man-made channels constructed to drain fenland areas of South Lincolnshire, eastern England. The SHMD was constructed as a result of the South Holland Drainage Act 1793 (Mossop & Elms, 1984) and with its subsidiaries currently drains a catchment of 169km^2. The drain starts 2km south of Cowbit and flows 23km in an easterly direction before discharging into the Wash and subsequently the North Sea via the tidal River Nene (Mossop & Elms, 1984). The SFFD is 34.7km long and, with its upstream tributaries included, drains a total area of 651km^2. The first section of the drain was constructed in the 1630s (Taylor, 1999), with further extension work undertaken in the late eighteenth century resulting in the current course (Barnwell, 1998). The SFFD starts near Guthram Gowt and flows north to Swineshead, where it turns east, ultimately discharging into the tidal section of the River Witham, known as The Haven, at Boston (Faulkner, 2009).

The underlying geology and leakage of seawater through the tidal sluice gates of both drains contribute to the saline ingress known to occur in the SHMD and SFFD. Examination of Environment Agency data collected monthly during the period 2000-2020 from the SFFD and the SHMD has shown that salinity can vary between 0.21-14.34mScm^{-1} in the SFFD and 0.42-22.12mScm^{-1} in the SHMD (EA water quality data archive online, examined 29 March 2020).

The SHMD, the SFFD, the Sussex River Ouse and River Adur are ideal water bodies to investigate the ecological effect of an increasing salinity concentration on aquatic macroinvertebrate fauna along salinity gradients at geographically distinct locations (Figure 1). Furthermore, the rivers Adur and Ouse are ideal case studies of riverine-estuary continuums where it is known salinity is the primary variable controlling macroinvertebrate community composition (Little *et al*., 2017), whilst the SHMD and the SFFD provide examples of large drainage channels with limited estuarine connectivity with which to examine the relationship between SAGI and salinity.

++ Figure 1 ++

2.3. Macroinvertebrate sample and chemistry data collection

All macroinvertebrate samples were collected using a Freshwater Biological Association pattern pond-net (square aperture of 0.5m^2, 1-mm net mesh) following the standard three-minute protocol, wherein habitats are sampled in proportion to their occurrence, and a one minute additional hand search is undertaken, as described by Murray-Bligh *et al*. (1997). The protocol is recognised as an international standard method (ISO, 2012).

2.3.1. Sussex rivers (Adur and Ouse)

Macroinvertebrate sampling was undertaken at 12 and 15 sites respectively along the riverine to estuarine transition of the River Adur and Sussex River Ouse (Figure 1A) during August 2008 and February 2009 to coincide with low summer and high winter river flows, indicative of high and low salinities and degree of tidal saline incursion, respectively. The survey sites along the rivers covered the complete transition from marine to freshwater conditions.

Tide and salinity profiles for the River Adur and River Ouse were recorded using SEBA Dipper-TEC sondes (recording water level (m), total dissolved solids (gL^{-1}), temperature (°C), conductivity (mScm^{-1}) and salinity (PSU) at 2-minute intervals). The sondes were positioned at five stations along each river over four consecutive days (during the macroinvertebrate sampling period) for 12-hour periods in August 2008 and February 2009. Furthermore, spot samples of physico-chemical parameters (water temperature (°C), pH, dissolved oxygen concentration (DO %), conductivity (mScm^{-1}) and salinity (PSU)) were recorded at each macroinvertebrate sample site using a hand-held WinLab® Data-Line Conductivity Meter. Surface sediment samples were collected at each sample site and analysed for grain size, particle roundness, sphericity, organic carbon, water content, calcium carbonate and minerogenic content.

The tide and salinity profiles enabled the interpolation of the maximum, minimum, average and range of salinity experienced at each macroinvertebrate sampling site along the two rivers under low freshwater discharge conditions and high freshwater discharge conditions. Full details on the sampling undertaken in the River Adur and River Ouse can be found in Little *et al.* (2017).

2.3.2. South Holland Main Drain

Three sites located along the length of the SHMD (Figure 1B) from typically freshwater (SHMD1) and increasing in salinity (SHMD2, SHMD3) were surveyed for macroinvertebrate and chemistry data during March 2010, June 2010 and October 2011. Chemistry data (conductivity (mScm^{-1}), redox potential (mV), dissolved oxygen (mgL^{-1}, DO %), and water temperature (°C)) was collected using a YSI-556 multiprobe field meter.

2.3.3. Swineshead Bridge, South Forty Foot Drain

Swineshead Bridge (where the A17 road passes over the SFFD) is the approximate location of the freshwater/saline-influence interface on the SFFD (Figure 1B). The site is a routine monitoring point for the Environment Agency of England (EA). Water samples and chemistry data have been collected using standard EA protocols on a generally monthly basis throughout February 1990 to September 2019, except for a gap from December 2002 to February 2007. Point sample chemistry data (ammonia (mgL^{-1}), total oxidised nitrogen (mgL^{-1}), orthophosphate (mgL^{-1}), dissolved oxygen (DO %, mgL^{-1}), pH, water temperature (°C) and conductivity ($mScm^{-1}$)) were used to investigate the selectiveness of SAGI. Dissolved oxygen, pH, water temperature and conductivity were collected using a YSI multimeter. Ammonia, total oxidised nitrogen and orthophosphate were determined by laboratory analysis of water samples.

Given the nature of the EA sample collection programmes, the collection of chemistry and macroinvertebrate samples are not synchronised. To pair the data a 180-day mean for all physico-chemical parameters preceding the macroinvertebrate sample dates was calculated. This was chosen to provide an appropriate measure of the antecedent environmental conditions acting on the macroinvertebrate community. A combined total of 30 macroinvertebrate samples, collected and analysed by the EA using standard protocols (described in Murray-Bligh *et al*., 1997) throughout July 1990 to September 2019, were matched with the EA chemistry data.

2.4. Data analysis

Each of the three case studies presented used the same data analysis steps that follow. Pearson correlation analysis was used to screen independent environmental variables for collinearity ($|r| > 0.7$) to select a set of uncorrelated variables for subsequent analysis. The distribution of selected variables was checked for normality and appropriately transformed if necessary. Conductivity was $\log(x + 1)$ transformed and percentage data were normalised using arcsin square-root. Stepwise multiple linear regression analysis was then undertaken to examine the ability of salinity and other retained environmental variables to account for the variation in the SAGI score. Models were then compared using Akaike's information criterion (AICc). Statistical analyses were performed with R version 4.0.2 and R Studio version 1.3.1073 (R Core Team, 2020; R-Studio Team, 2020). A full list of variables used in the data analysis is provided in Table S1 (Supplementary Material 3).

2.5. Adaptation and comparison of Salinity Index and SPEARsalinity

Salinity Index (Horrigan *et al*., 2005) and $SPEAR_{salinity}$ (Schäfer *et al*., 2011) were both adapted for use with the case studies datasets to examine and compare the efficacy of these indices with SAGI using common datasets. Both metrics were originally developed to function with

family level data. To ensure parity with their respective original work, both Salinity Index and $SPEAR_{salinity}$ were adapted and calculated at family level.

For full details of Salinity Index calculation, reference should be made to Horrigan *et al*. (2005). In brief, Salinity Index assigns taxa at family level one of three salinity sensitivity scores (SSS): '10' for sensitive, '5' for generally tolerant, and '1' for very tolerant. The average score of all taxa found present in a sample is calculated to form the final index score representative of salinity sensitivity of the sampled macroinvertebrate community. The index was adapted using SAG taxa classification to assign taxa to SSS; SAG-I taxa were assigned to the 'sensitive' SSS, SAG-II taxa were assigned to the 'generally tolerant' SSS, and taxa from SAG-III, SAG-IV and SAG-V were all assigned to the 'very tolerant' SSS.

For full details of $SPEAR_{salinity}$ calculation, reference should be made to Schäfer *et al*. (2011). Briefly, $SPEAR_{salinity}$ assigns families to one of either 'sensitive' or 'tolerant' and calculates the fraction of the abundance of sensitive individuals in a community for salinity. The index was adapted using SAG taxa classification to assign taxa to either 'sensitive' or 'tolerant'. Taxa classified to SAG-I and SAG-II were assigned as 'sensitive', whilst taxa classified to SAG-III, SAG-IV and SAG-V were assigned to 'tolerant'.

The adapted Salinity Index and $SPEAR_{salinity}$ were applied to the three case studies and the resulting correlation values (R^2) between the indices and conductivity were compared with those attained using SAGI. The salinity indices proposed by Williams *et al.* (1999) and Palmer *et al.* (2013) were not considered for adaptation as these indices were developed for application in specific habitat types which are not present in the analysed dataset (freshwater springs for Williams *et al*. (1999) and coastal grazing marsh ditches in England and Wales for Palmer *et al*. (2013)). Additionally, Wolf *et al*. (2009) was not considered as the metric does not apply a scoring system to result in a numerical output, but instead results in a graphical representation of the salinity preference of the macroinvertebrate community.

3. RESULTS

3.1. Sussex rivers (Adur and Ouse)

A total of 11207 individuals were recorded from 98 taxa (identified to five Orders, 11 families, five genera, 77 species) from all samples collected from the River Adur. 4872 individuals were recorded from 68 taxa (identified to five Orders, seven families, four genera, and 52 species) from the samples collected in August 2008, whereas 6335 individuals were recorded from 68 taxa (identified to four Orders, six families, two genera, and 56 species) from the samples collected in February 2009. Relative abundance and number of taxa was lower in August 2008 (mean 406 individuals; 11.8 taxa) compared to February 2009 (mean 528 individuals; 12.4 taxa). A total of 14757 individuals were recorded from 90 taxa (identified to seven Orders, 11 families, four genera, and 68 species) from all samples collected from the River Ouse. 9239 individuals were recorded from 72 taxa (identified to seven Orders, 11 families, three genera, and 51 species) from the samples collected in August 2008, whereas 5518 individuals were recorded from 60 taxa (identified to five Orders, five families, three genera, and 47 species)

from the samples collected in February 2009. Relative abundance and number of taxa was slightly higher in August 2008 (mean 660 individuals; 10.4 taxa) compared to February 2009 (mean 345 individuals; 10.0 taxa).

SAGI scores increased along the salinity gradients present in the River Adur and River Ouse in both the August 2008 (Figure 2) and the February 2009 (Figure 3) surveys. The change in SAGI score resulted from modification of the community composition due to salinity conditions. Figure 2 and Figure 3 demonstrates the general trend of decreasing presence of SAG-I and SAG-II taxa (reflective of lower salinities) and the increasing presence and eventual dominance of SAG-III and SAG-IV taxa (reflective of higher salinities) along the River Adur and River Ouse salinity continuums.

++ Figure 2 ++

++ Figure 3 ++

SAGI scores ranged from 4.76 to 13.29 and conductivity 0.21 to 52.19mScm^{-1} for the River Adur during the August survey period. During the February survey period SAGI scores ranged from 4.55 to 13.11 and conductivity from 0.21 to 52.19mScm^{-1}. The salinity profile along the River Adur is similar in the August and February survey periods, although conductivity starts increasing rapidly at Site A9 in the February survey compared to Site A8 during the August surveys; one site further downstream. Conductivity consistently increased between consecutive sites moving upstream to downstream along the River Adur during both survey periods. SAGI also resulted in a similar profile along the River Adur for the August and February survey periods. In the August surveys, SAGI scores increased between consecutive sites moving upstream to downstream along the River Adur. The sole exception was between sites A3 and A4 where a minor decrease in SAGI score from 5.00 to 4.71 was determined. Conductivity between the same sites increased by 0.06mScm^{-1} in the same survey period. The February surveys found four occasions where the downstream site attained a slightly lower SAGI score than the adjacent upstream site (A2 to A3, decrease of 0.57; A4 to A5, decrease of 0.22; A5 to A6, decrease of 0.28; A5 to A6, decrease of 0.67), contrasting with the consistent increases in conductivity between adjacent sites moving downstream.

During the August survey of the River Ouse, SAGI scores ranged from 4.15 to 13.00 and conductivity from 0.21 to 46.87mScm^{-1}. SAGI scores ranged from 4.12 to 12.44 and conductivity 0.21 to 46.8mScm^{-1} during the February survey period. The salinity profile along the River Ouse is similar in the both survey periods, although conductivity increased more between Sites O7 - O9 in the August survey compared to the February survey. The SAGI profile is also similar for both survey periods; decreases in SAGI score were found between sites O2 and O3 (August, 0.56 decrease; February, 0.20), and sites O6 and O7 (August, 1.00 decrease; February, 0.37) in both the August and February survey periods. The August survey period also determined a decrease of 0.87 in SAGI score between sites O8 and O9. The February surveys found two further occasions where the downstream site attained a slightly lower SAGI score than the adjacent upstream site (O12 to O13, decrease of 0.77; O15 to O16). This contrasts with conductivity consistently increasing between adjacent sites moving

downstream along the River Ouse during both survey periods. Nonetheless, SAGI appears to reflect the salinity continuum of both rivers in both survey periods due to the salinity-mediated change in community composition of the resident aquatic macroinvertebrate fauna.

SAGI scores showed a strong positive correlation with conductivity (R^2 = 0.912, $p < 0.001$; see Figure 4 and Table 3). A summary of the results of the stepwise multiple regression analysis are shown in Table 3, with the top three performing models being shown and ranked using AICc values. Also presented is the parameter output of Model 4, which features conductivity only. The model summaries for Models 1 and 4 are shown in Table S2 (Supplementary Material 3). Table 3 shows that Models 1 to 3 are very similar in terms of goodness-of-fit displaying very similar R^2 and AICc values. Given Models 2 and 3 have Δi values very close to 2 they can be considered to be essentially as good as Model 1 - the 'best' model. Similarly, there is only a small improvement in the amount of explained variation (R^2) between Model 4, which only features the predictor conductivity, and those models with additional predictor variables.

++ Figure 4 ++

++ Table 3 ++

3.2. South Holland Main Drain

A total of 6968 individuals were recorded from 54 taxa (identified to four families, five genera, and 45 species) from all samples. The relative abundance was highest at SHMD3 (mean 1274 individuals), the most saline site, whilst number of taxa was highest at SHMD1 (mean 26.3 taxa), the most freshwater site. Relative abundance and number of taxa were lowest at SHMD2 (mean 321 individuals; 7.6 taxa). Seasonally, relative abundance and number of taxa were both lowest in spring (mean 178 individuals; 13.0 taxa). Abundance was highest in autumn (mean 1568 individuals), whereas number of taxa was highest in summer (mean 15.7 taxa). The most abundant taxon at SHMD1 was *Potamopyrgus antipodarum*, followed by *Ampullaceana balthica* and *Asellus aquaticus*. In comparison, the most abundant taxon at SHMD2 was *Mytilopsis leucophaeta*, followed by *Dreissena polymorpha* and *Corophium multisetosum*. Finally, the most abundant taxon at SHMD3 was *Gammarus zaddachi*, followed by *Neomysis integer* and *Gammarus tigrinus*, demonstrating a clear shift from mollusc-dominated communities at SHMD1 and SHMD2 to an amphipod dominated community at SHMD3.

In the SHMD the SAGI score increased as conductivity increased (Figure 5; Figure 6). In both spring and summer there was a consistent increase in conductivity along the SHMD continuum, although the increase occurred at a greater rate in summer than in spring. The increase in SAGI along the SHMD continuum in the same two seasons generally responded appropriately to the rate of increase in conductivity and was also larger in summer than in spring. Autumn showed the largest increase in conductivity between any two adjacent sites; from 0.91 to 22.75mScm^{-1} between SHMD1 and SHMD2. This was also reflected by SAGI,

which similarly recorded the largest SAGI score increase (from 5.12 to 8.25) between the same two sites. A small decrease in conductivity of 0.53mScm^{-1} was recorded between SHMD2 and SHMD3 in autumn. In contrast SAGI continued to increase up to 9.00 at SHMD3, signifying higher salinity conditions prior to conductivity readings being collected and the signal persisting in the composition of the macroinvertebrate community. The increase in salinity between SHMD1 and SHDM2 resulted in the exclusion of all SAG-I assigned taxa from the latter, resulting in only SAG-II and SAG-III assigned taxa contributing to SAGI scores for SHMD2 (Figure 5). A co-occurring decrease in taxon richness from 28 to six was also recorded between these two sites. From SHMD2 the macroinvertebrate community further shifted to a greater percentage of SAG-III and SAG-IV taxa present at SHMD3 concomitant with an increase in salinity. Across the sites there was a positive correlation between log-transformed conductivity concentration and SAGI (Figure 6; $R^2 = 0.767$, $p < 0.01$).

++ Figure 5 ++

++ Figure 6 ++

A summary of the top three performing models from the stepwise regression analysis is shown in Table 4 and the models are ranked by AICc values. The model summary for Model 1 is shown in Table S3 (Supplementary Material 3). Salinity alone was the best model in terms of explaining the variation in SAGI scores with an AICc value of 30.0. Although Models 2 and 3 had similar R^2 values to Model 1, the models have been penalised for being less parsimonious and not significantly improving goodness-of-fit by including uninformative additional parameters.

++ Table 4 ++

3.3. Swineshead Bridge, SFFD

A total of 14518 individuals were recorded from 171 taxa (identified to five Orders, 31 families, 14 genera, and 121 species) from all samples. Relative abundance and number of taxa were highest in autumn (mean 490 individuals; 23.0 taxa) and lowest in winter (mean 78 individuals; 13.0 taxa). Summer (mean 296 individuals; 22.6 taxa) and spring (mean 311 individuals; 18.8 taxa) also had substantially higher relative abundance and taxon richness than winter. The most abundant taxon was Oligochaeta, followed by Chironomidae and *Gammarus tigrinus*. The most frequently recorded taxon was Chironomidae followed by *Potamopyrgus antipodarum* and *Ampullaceana balthica.*

Conductivity at Swineshead Bridge showed a seasonal pattern, with peak values generally occurring in autumn months (Figure 7) and spring conductivity values remaining consistently below 6mScm^{-1} and exceeding 4mScm^{-1} on only three occasions. SAGI scores and the community composition appeared to loosely follow the general conductivity pattern through the time series. When spring (March, April, May) and autumn (September, October, November) macroinvertebrate data were combined, the relationship between SAGI and

conductivity was weak (R^2 = 0.20, p = 0.013; Figure 8). When considered separately, the results showed a seasonal effect with the autumn data (R^2 = 0.566, p < 0.001; Figure 9) having a much stronger and significant relationship than spring (R^2 = 0.152, p > 0.05). The data was subsequently analysed separately for each season. The results of the multiple regression for spring and autumn are shown in Table 5. Model summaries for Model Spr1 and Model Aut1 are shown in Table S4 (Supplementary Material 3). The best ranked model for spring showed that SAGI was being influenced by salinity and orthophosphate (R^2 = 0.511, p = 0.028, AICc = 11.2). In contrast, the best ranked model for autumn featured salinity only (R^2 = 0.566, p < 0.001, AICc = -0.6).

++ Figure 7 ++

++ Figure 8 ++

++ Figure 9 ++

++ Table 5 ++

3.4. Comparison of SAGI with adapted Salinity Index and SPEARsalinity

In order to compare the efficacy of SAGI with alternative salinity-specific macroinvertebrate indices, the amount of variance in SAGI, adapted Salinity Index and adapted $SPEAR_{salinity}$ explained by conductivity in the three case studies is presented in Table 6.

++ Table 6 ++

SAGI performed favourably when compared to the two alternative salinity indices adapted for use with the datasets. Conductivity was shown to have statistically significant relationships (p <0.05) with all salinity indices, albeit with varying relationship strengths. SAGI had the highest correlation coefficients for both the Sussex rivers (Adur and Ouse) and the SFFD Autumn datasets, whereas the Salinity Index correlation was highest for the South Holland Main Drain. $SPEAR_{salinity}$ index had a higher correlation coefficient than SAGI for the South Holland Main Drain only.

4. DISCUSSION

4.1. Performance of SAGI to determine the effects of salinity

The results of this study demonstrate that SAGI is a very effective and robust metric in reflecting changes in the macroinvertebrate community structure in response to changing salinity conditions. The efficacy of SAGI as an evidence-gathering tool was shown to be positive under different scenarios; acting across multiple sites along a river length and salinity gradient, and for a single site subject to saline ingress over multiple decades. Greater than 75% variation in SAGI scores is accounted for by conductivity in both the South Holland Main Drain and the Sussex rivers case studies, demonstrating a strong relationship between SAGI and salinity. Furthermore, the South Holland Main Drain and the Sussex rivers case studies

illustrate that salinity-mediated changes in macroinvertebrate community structure in response to salinity conditions is driving the change in SAGI score.

In the SFFD case study, however, conductivity explained less of the variation in the SAGI scores. Of the additional water quality variables available for inclusion in the analysis, only orthophosphate improved the model for the spring. Orthophosphate is an oxoanion of phosphorus and is one of the most common contributors to nutrient enrichment of surface waters. Nutrient enrichment results in complex macroinvertebrate community responses, although a decrease in the diversity of aquatic insect orders has been reported (Friberg *et al*., 2010; Yuan, 2010). The data in the SFFD example is heavily focused at the lower end of the salinity scale where the macroinvertebrate community is likely to be strongly affected by other environmental variables, such as orthophosphate, which become increasingly dominant in comparison with the effect of salinity at this scale. It is at the lower end of the salinity range (*ca*. 0-3mScm^{-1}) and at such fine scale where the salinity and SAGI relationship begins to degrade. One possible reason for this breakdown in relationship is the lack of resolution of certain taxonomic groups which are frequent in the SFFD dataset (e.g. Diptera families and the genera *Lymnaea, Aeshna, Corixa, Haliplus, Hydroporus, Laccobius* and *Enochrus*) which, if identified to species could increase the accuracy of SAGI at such fine scales. For example, *Enochrus bicolor* has a strong association with brackish conditions, whilst *E. fuscipennis* shows no such association. Similarly for *Aeshna juncea* and *A. mixta*, with *A. juncea* rarely occurring in brackish conditions whilst *A. mixta* is a typical inhabitant of brackish conditions and is tolerant of increased salinities. Furthermore, studies to more accurately define salinity tolerances for freshwater taxa and other taxa without SAG assignments would also benefit the accuracy and precision of SAGI. In addition to the lack of taxonomic resolution for SAGI in certain groups, the salinity tolerances of the majority of freshwater species are not as well defined as brackish water and estuarine taxa. Further research focussing on identifying and excluding those taxa with wide salinity tolerances would also enhance the precision and accuracy of SAGI. A breakdown in relationship between the index and the variable of interest at fine scales has also been found between the LIFE index and flow when examining extremely low flow conditions (Monk *et al*., 2006), resulting in the development of DEHLI which better reflects community changes through extreme low flow, drought-mediated conditions and the subsequent community recovery (Chadd *et al*., 2017).

A further important factor which is expected to contribute to reducing the unexplained variation between conductivity and SAGI in the SFFD case study is water level. This factor was not accounted for in the analyses due to the lack of available data for the parameter. The SFFD is a heavily managed system with water levels being controlled and operating over varying regimes over the datasets 20-year period (1990-2009). It is primarily managed for flood risk and drainage of the arable land which dominates the landscape of the catchment. Water levels are substantially decreased in winter months, stranding marginal habitats (e.g. mature reed margins, boulder rip-rap bank reinforcements) exploited by aquatic biotic communities during the summer months. Comparing the average number of taxa recorded in autumn (23.0) with to spring (18.8) demonstrates a reduction in taxon richness potentially resulting from the water level management and thus possibly affecting SAGI. This highlights a major difficulty when using biological metrics in isolation where results can be

affected, and interpretations skewed, by the strong influence of stressors other than that for which the metric was designed.

The SFFD example also revealed a seasonal influence affecting SAGI, with different relationships evident when spring and autumn data were analysed separately. Seasonal dependence, related to the life history of macro-invertebrate taxa (Johnson *et al*., 2012), is a well-known issue affecting many biological metrics (Rosenberg & Resh, 1993; Zamora-Muñoz *et al*., 1995). For example, Šporka *et al*. (2006) examined 76 biotic indices and found that 31 of the metrics exhibited statistically significant seasonal variations. It has long been recognised that many insect taxa have seasonal life cycles which influence aquatic macro-invertebrate community composition throughout the year (Hynes, 1970; Wright *et al*., 1984). Non-insect macro-invertebrate taxa are also known to present well-defined seasonal variations in abundance and distribution (Rosenberg & Resh, 1993). Predictive models such as the River InVertebrate Prediction And Classification System (RIVPACS; Wright *et al*., 1984; Moss *et al*., 1987; Wright, 2000) can be used to account for seasonal dependence in biological metrics, and as such linking SAGI with a biological modelling technique would resolve the seasonal influence observed in the SFFD case study and enhance the metric.

The case study examples presented in this study are from England, however the development of SAGI and the conclusions of this study are internationally applicable. Future work should look to test SAGI in continental Europe and further afield. Additionally, the current case studies examine SAGI in largely riverine systems with connectivity to an estuary and exhibiting a salinity gradient. SAGI should also be examined using data collected from isolated inland systems, such as in the scenario of localised saline pollution events, where high salinity sites may be located upstream of decreasing salinities.

4.2. Comparison with alternative salinity-specific macroinvertebrate-based indices

The results demonstrate that SAGI had a positive, moderate to strong correlation with conductivity in each case study. The range of correlations (R^2 = 0.57-0.91) compares favourably with those for similar tools. For example, Birk *et al*. (2012) reported the median correlation coefficient for pressure-specific, macroinvertebrate-based monitoring tools used in the EU is 0.64 (R^2 = 0.41, calculated for comparability). Furthermore, the amount of variance in SAGI explained by conductivity in the case studies is comparable and generally better than the amount of explained variance reported by alternative salinity indices (Table 6). It is also worth noting that the lowest correlation, resulting from the SFFD case study, focussed on a single site over multiple decades subject to short and long-term salinity variation amongst other long-term pressures.

SAGI was only slightly outperformed by Salinity Index and SPEARsalinity in the South Holland Main Drain case study. SAGI marginally outperformed both alternative salinity indices in the Sussex rivers (Adur and Ouse) dataset and performed substantially better in the SFFD dataset. Both the Sussex Rivers and SFFD datasets were comprised of multiple data points with the SFFD data also occurring over a 20 year time period. In contrast the dataset of the South Holland Main Drain (SHMD) was relatively small (n = 9) and collected over a much

shorter period of time. SAGI performed best when there was a greater availability of data which subsequently gives more confidence in the results and its ability to detect changes in salinity levels.

Several factors may explain the generally stronger correlation of SAGI with conductivity compared with other salinity indices. Firstly, SAGI is predominantly based on species-level taxonomic resolution. Both $SPEAR_{salinity}$ (Schäfer *et al*., 2011) and Salinity Index (Horrigan *et al*., 2005) are based on family-level taxonomic resolution. There is a growing body of evidence demonstrating that increased taxonomic resolution results in the most accurate index outputs. For example, Pond *et al*. (2008) found that correlations between a genus-level index and water-quality variables were stronger than correlations using the family-level index in an investigation of mining disturbance on West Virginia streams, whilst Hawkins *et al*. (2000) found that predictive models based on species-level data gave better predictions of watershed alterations resulting from logging than models based on family-level data. Furthermore, Extence *et al*. (1999) found that Lotic-invertebrate Index for Flow Evaluation (LIFE) scores obtained from family level data had a weaker correlation with flow rate than scores obtained using species level data. This result has since been further verified by Monk *et al.* (2012) in an assessment using a long-term dataset from 14 river sites in eastern England. Increased taxonomic resolution was a proposal made by Horrigan *et al*. (2005) to improve the accuracy and precision of the Salinity Index. Secondly, the Salinity Index (Horrigan *et al*., 2005) uses presence/absence data. In comparison, SAGI incorporates an abundance-weighted scoring system, and as such will reflect more subtle saline-induced changes in taxa abundances that may not affect their presence at a location. The importance of abundance in ecological assessments has long been acknowledged (Hynes, 1960), and studies continue to reaffirm this assertion. For example, Melo (2005) concluded from a five-year study of five streams in southeast Brazil that using presence/absence data in place of abundance data results in a significant loss of information. Furthermore, integration of abundance in ecological indices has been shown to improve metric accuracy. Extence *et al*. (1999) asserted that the use of relative abundance data in the calculation of LIFE scores rather than presence/absence data resulted in LIFE scores exhibiting stronger correlations with flow. Horrigan *et al*. (2005) also suggested the integration of abundance data into the Salinity Index to improve the accuracy of the tool.

4.3. Application of SAGI

The Water Framework Directive (WFD) requires the recognition of biological abundance in the assessment of water quality (European Commission, 2000). Further desirable features for biomonitoring tools include a biological basis and testing over the full range of water bodies to which the tool is intended to be applied (Turley *et al*., 2016), compatibility with the sampling protocol used by other biomonitoring tools and surveys (Bonada *et al*., 2006), and reliable indication of change in the targeted pressure (Dolédec *et al*., 1999; Birk *et al*., 2012). SAGI exhibits many of these features, having a biological basis and incorporating relative abundance data whilst also being compatible with the UKTAG methodology for macro-invertebrate sampling and analysis (Murray-Bligh *et al*., 1997; Chadd, 2010) employed by the

675 UK regulatory authorities in the assessment of water bodies for the WFD (WFD-UKTAG, 2008).
676 The efficacy of SAGI in quantifying salinity-induced change in the aquatic macroinvertebrate
677 community has been demonstrated in this study, showing that SAGI is an effective tool for
678 investigating change which can be related to a range of actions (e.g. river habitat
679 modifications resulting in alterations to riverine salinity profiles, increased saline intrusion
680 due to decreased freshwater flow following drought or increased tidal pressure).

681 A study of over 9000 sites throughout 14 European countries found that 47% of rivers
682 (90% of lowland rivers) were subject to multiple pressures (Schinegger *et al*., 2012),
683 demonstrating the potential for pressure-specific biomonitoring tools to be confounded by
684 multiple factors when applied in isolation. The diagnostic capabilities of pressure-specific
685 biomonitoring tools can be much improved when used collectively, as demonstrated by Clews
686 & Ormerod (2009) using the acidification metric AWIC with BMWP-ASPT and LIFE in a study
687 of the River Wye catchment in the United Kingdom. Chadd *et al.* (2017) applied the very low
688 flow effect metric DEHLI in tandem with LIFE to study drought effects in several UK water
689 bodies. Furthermore, a graphical multi-metric tool incorporating LIFE, PSI, BMWP-ASPT and
690 BMWP-NTAXA is routinely used by UK regulatory authorities for diagnostic purposes. Thus,
691 when used in combination and informed by alternative pressure-specific biomonitoring tools,
692 SAGI can improve our understanding of how aquatic macroinvertebrate communities respond
693 to salinity changes, informing more targeted monitoring and mitigation measures.
694 Furthermore, SAGI provides a means of measuring the effect of salinity on aquatic biotic
695 communities, resulting in better informed decision-making during conservation and
696 management of freshwater habitats.

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975 dependent? Factors explaining their variations. *Water Research*, 29 (1), 285-290.
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977

978 **DATA AVAILABILITY STATEMENT**

979 The data that support the findings of this study are available from the corresponding author,
980 AP, for the South Forty Foot Drain and South Holland Main Drain, and from co-author SL for
981 the River Adur and Sussex River Ouse, upon reasonable request.

982

983 **TABLES**

984 **Table 1: Definitions of the Salinity Association groups**

Salinity Association Group (SAG)	Group definition
I	Taxa which tolerate only salinities below 3.35mScm^{-1}. *Typically freshwater taxa; may be tolerant of slightly brackish conditions, or completely intolerant.*
II	Taxa which can tolerate salinities over 3.4mScm^{-1} up to a salinity of 13.1mScm^{-1}. Taxa may be present at slightly higher salinities, but only in small numbers. *Freshwater taxa tolerant of mild brackish conditions.*
III	Taxa which are characterised by the largest abundance occurring in the salinity range 10.6-26.2mScm^{-1}. Taxa are tolerant of the salinity range 5.6-33.5mScm^{-1} but may also be recorded in very low numbers at greater or lower salinities. *Characteristic brackish water taxa, tolerant of a wide range of salinity conditions from long term brackish to near freshwater.*
IV	Taxa which typically occur at salinities greater than 24.7mScm^{-1}. Taxa may be present at slightly lower salinities, but only in small numbers. *Long-term brackish taxa tolerant of lower salinities, i.e. transition zones.*
V	Taxa which tolerate only salinities greater than 27.7mScm^{-1}. *Full coastal seawater taxa rarely moving into nominally freshwater habitats.*

985

986 **Table 2: Scoring matrix for determining Salinity Association Scores (SASs)**

Salinity Association Group (SAG)	Abundance category (estimated number of individuals)			
	A (1-9)	**B (10-99)**	**C (100-999)**	**D/E (1000+)**
I	4	3	2	1
II	5	6	7	8
III	9	10	11	12
IV	13	14	15	16
V	17	18	19	20

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Table 3: Summary of models of stepwise multiple linear regression for the Sussex rivers

Model	Model Parameters	df	R^2	AICc	Δi	Weight
1	Intercept + log(conductivity + 1) + arcsin sqrt(calcium carbonate) + arcsin sqrt(sediment water content)	5	0.934	139.5	0.00	0.580
2	Intercept + log(conductivity + 1) + arcsin sqrt(calcium carbonate)	4	0.928	141.5	2.03	0.210
3	Intercept + log(conductivity + 1)+ arcsin sqrt(calcium carbonate) + sqrt(sediment water content) + factor(river)	6	0.934	141.5	2.07	0.206
4	Intercept + log(conductivity + 1)	3	0.912	149.7	10.2	0.004

Table 4: Summary of models of stepwise multiple linear regression for the South Holland Main Drain

Model	Model Parameters	df	R^2	AICc	Δi	Weight
1	Intercept + log(conductivity + 1)	3	0.767	30.0	0.00	0.949
2	Intercept + log(conductivity + 1) + redox potential	4	0.800	34.1	5.84	0.051
3	Intercept + log(conductivity + 1) + redox potential + log(dissolved oxygen + 1)	5	0.841	44.2	15.8	0.000

995 **Table 5: Summary of spring and autumn models of the stepwise multiple linear regression**
996 **for Swineshead, SFFD**

Model	Model Parameters	df	R^2	AICc	Δi	Weight
Spr1	Intercept + log(conductivity + 1) + log(orthophosphate + 1)	4	0.511	11.2	0.00	0.791
Spr2	Intercept + log(conductivity + 1) + log(orthophosphate + 1) + temperature	5	0.602	14.1	2.88	0.187
Spr3	Intercept + log(conductivity + 1) + log(orthophosphate + 1) + temperature + log(dissolved oxygen + 1)	6	0.686	18.4	7.23	0.021
Aut1	Intercept + log(conductivity + 1)	3	0.566	-0.6	0.00	0.591
Aut2	Intercept + log(conductivity + 1) + log(dissolved oxygen + 1)	4	0.615	1.1	1.71	0.251
Aut3	Intercept + log(conductivity + 1) + log(dissolved oxygen + 1) + log(ammonia + 1)	5	0.687	2.2	2.79	0.146

997

998 **Table 6: R^2 matrix between SAGI and adapted alternative salinity indices against**
999 **log(conductivity + 1)**

Index	SAGI	Salinity Index (Horrigan *et al.*, 2005)	$SPEAR_{salinity}$ index (Schäfer *et al.*, 2011)
Sussex rivers (Adur and Ouse)	0.912***	0.847***	0.881***
South Holland Main Drain (SHMD)	0.767**	0.872***	0.782**
South Forty Foot Drain (SFFD) - Autumn	0.566***	0.427**	0.344*

(* p <0.05, ** p <0.01, *** p <0.001)

1000

FIGURE LEGENDS

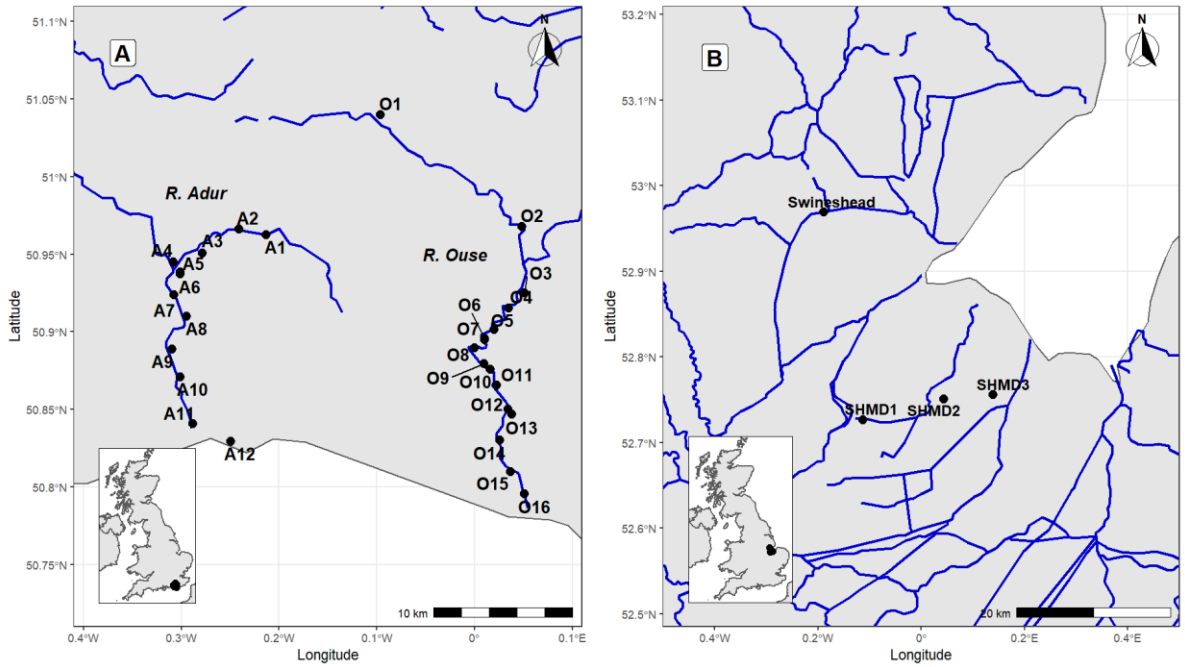


Figure 1: Location of sampling points on (A) the River Adur and River Ouse, Sussex, and (B) the South Forty Foot Drain and South Holland Main Drain, Lincolnshire

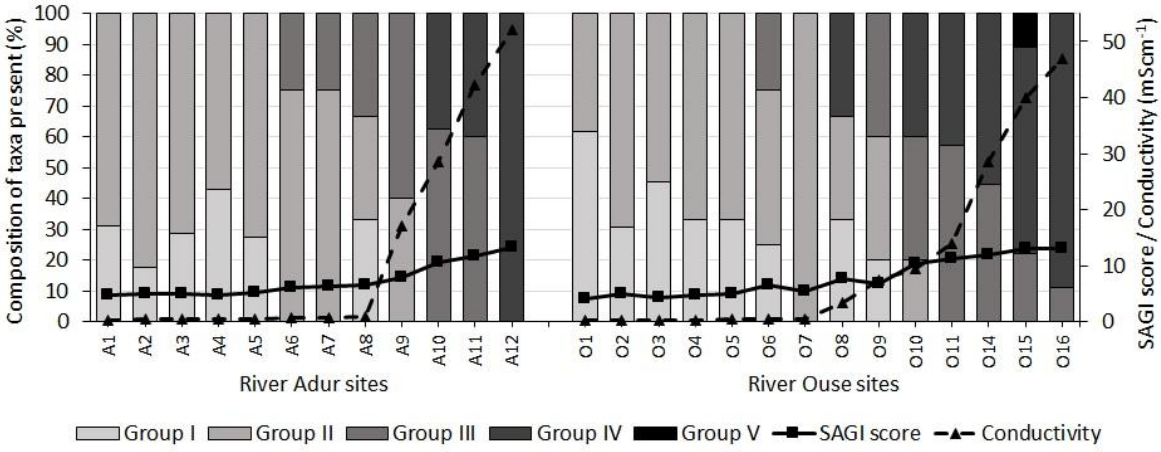
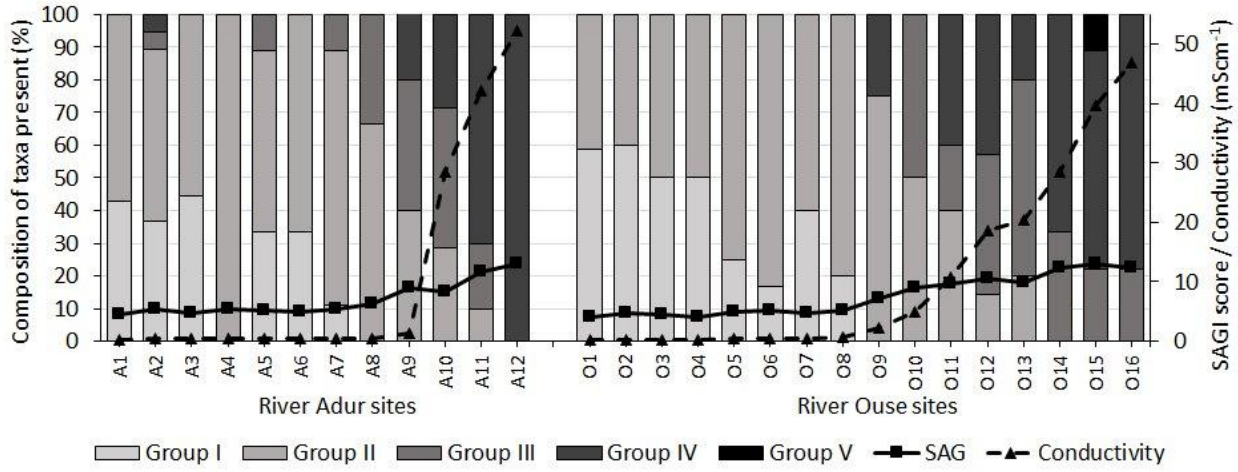
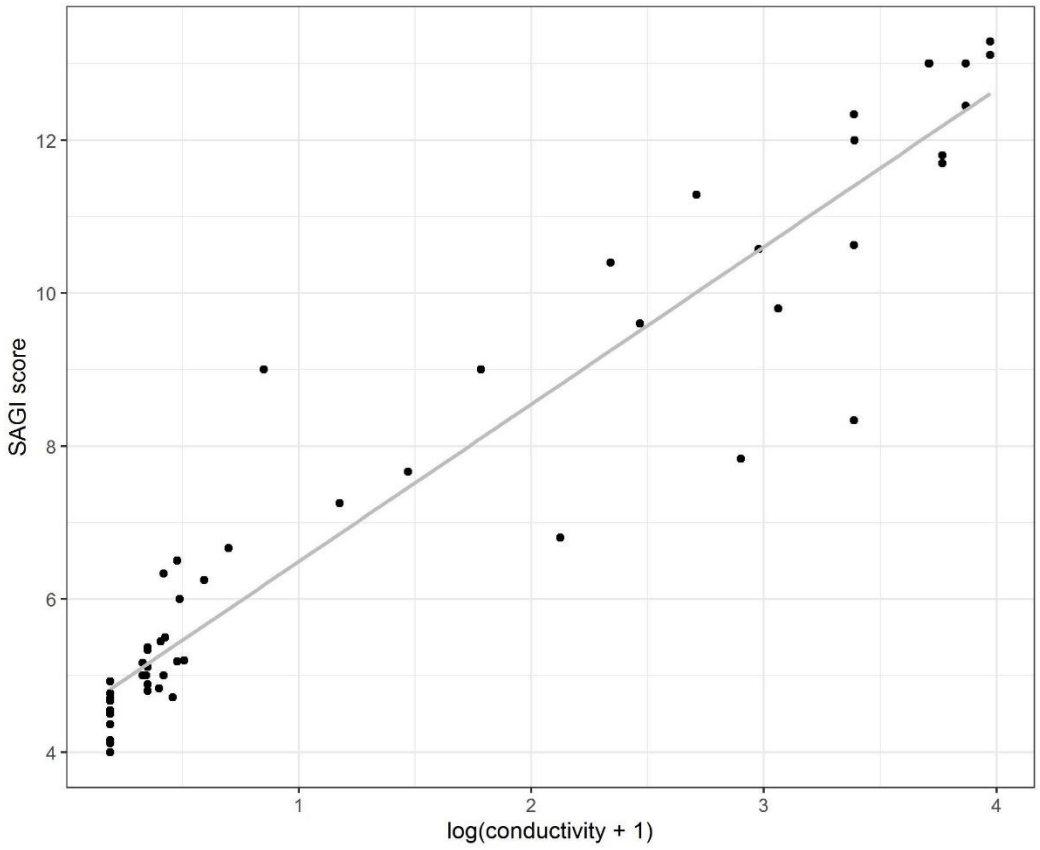


Figure 2: SAGI score, conductivity and SAG-assigned invertebrate community composition in the River Adur and Sussex River Ouse sites in August 2008 surveys



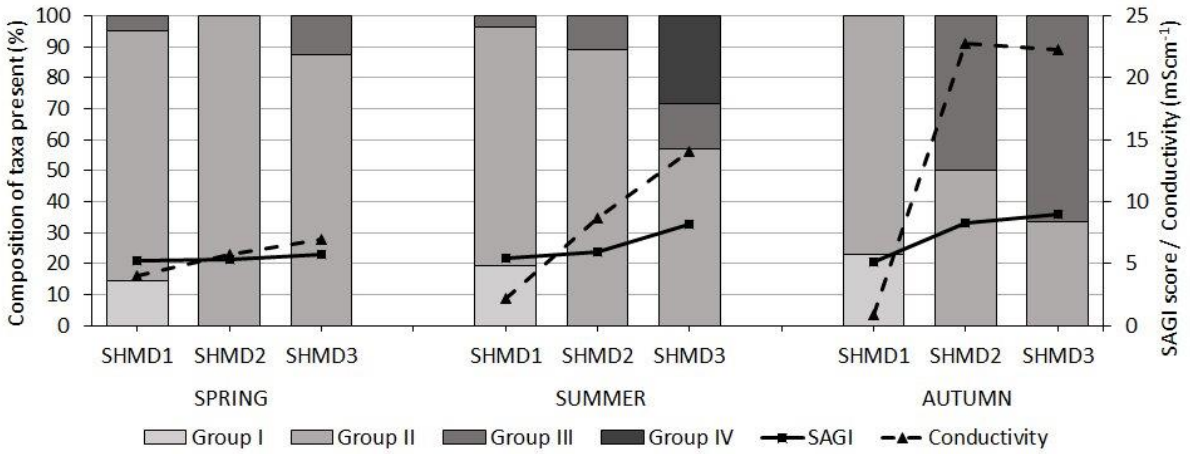
1008

1009 Figure 3: SAGI score, conductivity and SAG-assigned invertebrate community composition in
1010 the River Adur and Sussex River Ouse sites in February 2009 surveys



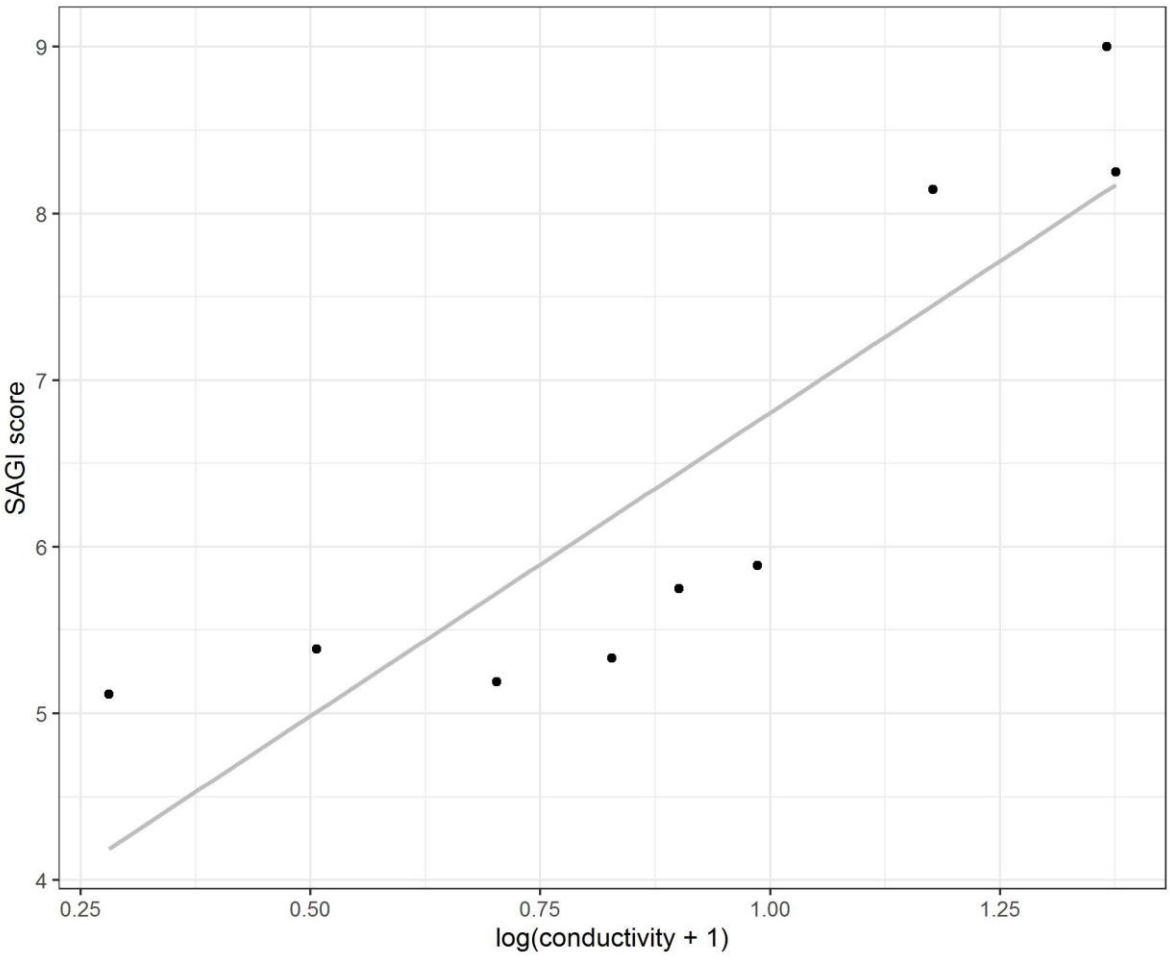
1011

1012 Figure 4: Linear regression between conductivity and SAGI index score for the rivers Adur
1013 and Ouse



1014

1015 Figure 5: SAGI score, conductivity and SAG-assigned invertebrate community composition at
1016 South Holland Main Drain sites



1017

1018 Figure 6: Linear regression between conductivity and SAGI for the South Holland Main Drain

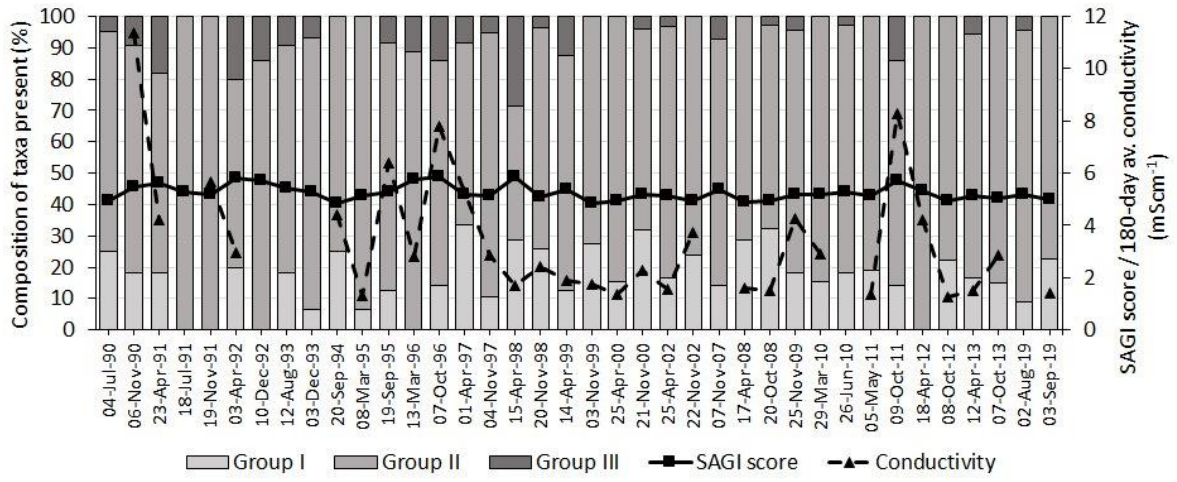


Figure 7: SAGI score, conductivity and SAG-assigned invertebrate community composition at South Forty Foot Drain sites

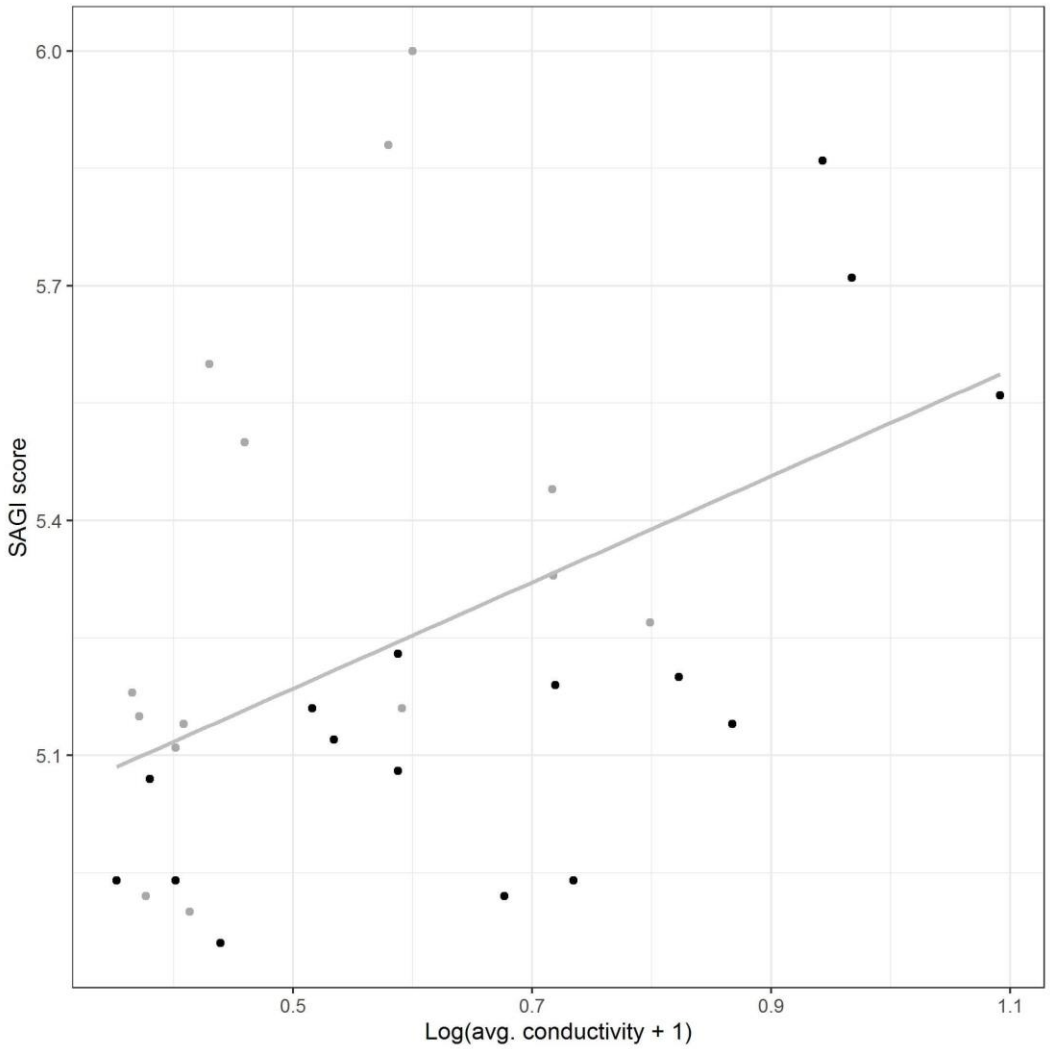


Figure 8: Linear regression between conductivity and SAGI index score for Swineshead Bridge, SFFD (grey dots = spring data; black dots = autumn data)

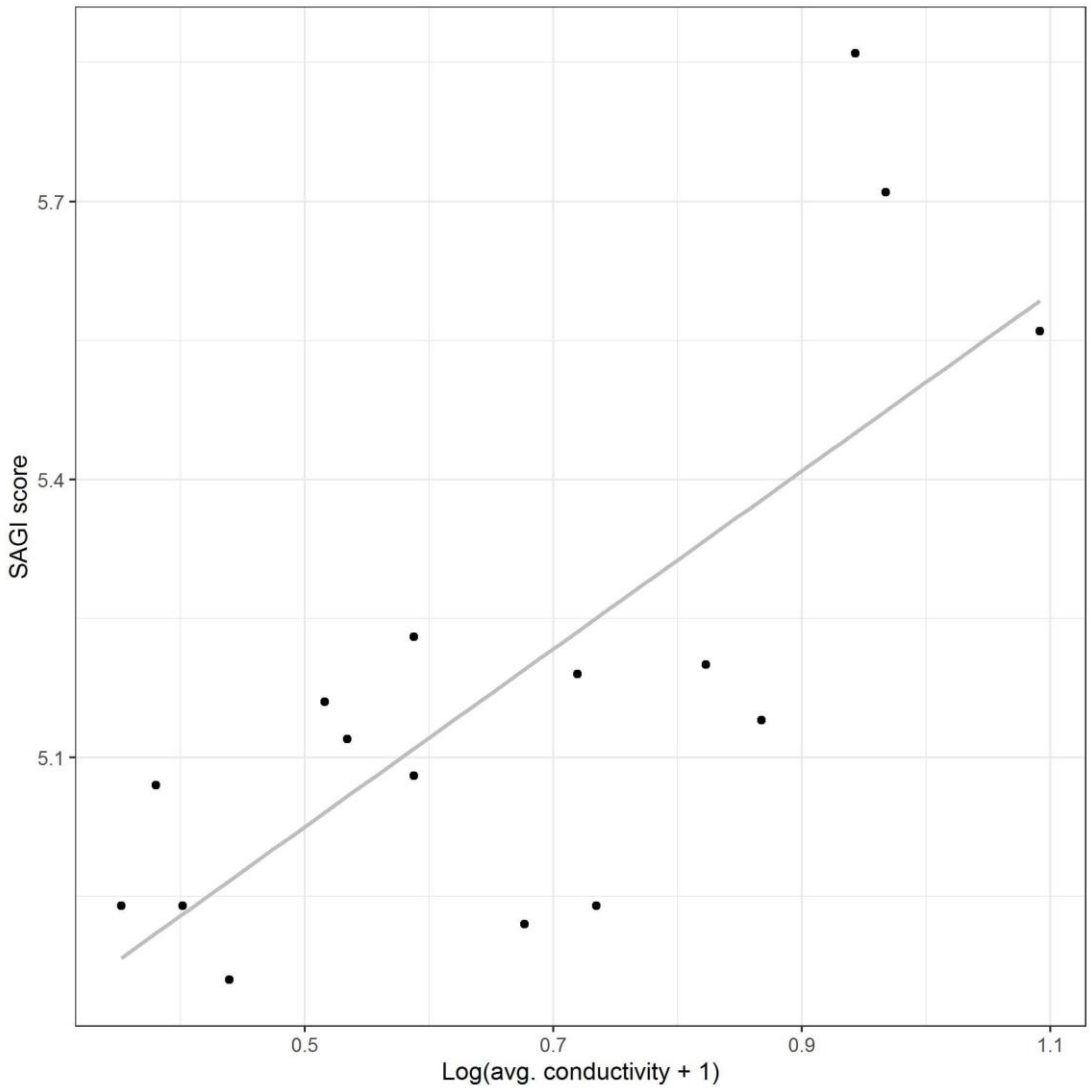


Figure 9: Autumn Linear regression between conductivity and SAGI index score for Swineshead Bridge, SFFD