Aquascience Consultancy Limited

A BENCHMARK OF ECOLOGICAL CONDITION IN THE BOSSINGTON ESTATE WATERS IN OCTOBER 2017 PRIOR TO RIVER WORKS

Completed on the 2nd March 2018

Dr. Nick Everall MIFM C Env

☎ **(01246) 239344**

✉ nick@aquascienceconsultancy.co.uk

Website: www.aquascienceconsultancy.co.uk

Executive Summary

Firstly, it is important to note that this is just an interim report and a report of the full findings summarising both the Autumn 2017 plus the Spring and Autumn 2018 survey results will be produced in the winter of 2018. The Autumn 2017 survey findings from the Bossington Estate Waters will provide the benchmark, prior to the in-stream habitat works undertaken in the winter or 2017, of:

i. the aquatic faunal plus associated river fly population status through the River Test Home Beat of the Bossington Estate Waters

ii. the spatial watercourse condition in terms of the flow, sediment, organic and nutrient enrichment fingerprints through the River Test Home Beat of the Bossington Estate Waters

From the survey by Aquascience Consultancy Limited in the Autumn of 2017, of the River Test Home Beat in the Bossington Estate Waters appeared potentially lacking in riverfly and overall aquatic faunal species richness and abundance, but the Spring and Autumn 2018 survey work will be required to get a better handle on this (see requisite mapped data on page 12, 13 and 14 respectively).

The Bossington Estate Waters exhibited good conditions with respect to organic pollution, when referenced against all current UK and EC Regulatory Biological Standards, plus their inferred chemical standards associated with e.g. ammonia and dissolved oxygen sediment, organic and nutrient pollution (map on page 15). This means that there were no signs of marked organic pollution typically associated with e.g. sewage works discharges or agricultural run-off in the Autumn of 2017.

There were, however, some fingerprints of moderate sediment and nutrient impact in the Home Beat waters of the River Test at Sites 1 and 3 in the Autumn of 2017 (maps on page 16 and 17 respectively). The nutrient signatures were probably closely associated with the sediment signatures in that this was likely to be sediment-P impacts rather than water column. Although a likely legacy of historic or existing intermittent water column levels of P dissociating in to the sediment.

Flow velocity appeared most impeded in the Home Beat waters of the River Test at Sites 1 and 3 in the Autumn of 2017 from the map on page 18. These were sites immediately upstream of the existing weir impoundment and further downstream from the weir where prior to river works the natural energy of the river had been tempered.

There were traces of a pesticide-herbicide biosignature in the aquatic faunal communities at all 3 survey sites in the Home Beat waters of the River Test in the Autumn of 2017 as shown on page 19. As they were trace signatures they may (or may not) be a legacy of historic upstream incursions of these compounds but worthy of some thought as to whether or not any upstream agricultural practices such as plant spray drift could be continuing to impact the receiving river invertebrates. If the river works bring about major improvements in the ecological condition of the river then it is unlikely that the issue is a current and continuing one. That said there were similar fingerprints further downstream from the 2015-2017 S & TC study in the River Test and we have included some details of those findings for further spatial and temporal context on page 20 of this report.

The overview in the Autumn of 2017 was of a reach of the River Test with varied benchmark environmental pressures and consequent ecological condition, which in-stream remediation work e.g. weir removal and habitat creation were likely to produce large improvements upon.

From all of the aquatic fauna surveyed through the Bossington Estate Home Beat waters of the River Test in the Autumn of 2017, there were no conservation and regulatory important species found. Of importance to fly angling and a sustainable food bed for the trout and grayling fishery was the presence in the Autumn of 2017 of representative up-winged mayflies like The Mayfly (*Ephemera danica*), Blue Winged Olive (*Serratella ignita*), Large - Small Dark Olives (*Baetis rhodani* - *Baetis scambus*) and Small Spurwing (*Centroptilum luteolum*) pre-river habitat work. A few stoneflies were found through the Bossington waters in the form of Willow or Needle Fly (*Leuctra* sp.) and Large Brown Stonefly (*Perla bipunctata*). Amongst the sedges important to fly fishers there were Grannom (*Brachycentrus subnubilis*), Black Sedge (*Silo nigricornis*), Welshman's Button (*Sericostoma personatum*), Cinnamon Sedge (*Limnephilus lunatus*), Black Silverhorn (*Mystacides azurea*), Brown Silverhorn (*Athripsodes aterrimus*), Sand fly (*Rhyacophila dorsalis*), Dark Spotted Sedge (*Polycentropus flavomaculatus*) and Grey Flag (*Hydropsyche pellucidula*). Damselfly (*Calopteryx splendens*), Alder fly (*Sialis lutaria*), Reed Smuts (*Simulium* spp.) and Midges (Orthocladiinae and Tanytarsinii families) were also present throughout the Bossington Estate Home Beat waters. The freshwater shrimp (*Gammarus pulex*) is a stable taxa of healthy chalk rivers with trout and grayling historically seen 'tailing' in the Autumn to fatten themselves up for the winter months. Prior to the river works the Home Beat waters of the River Test at the study sites was relatively depauperate in freshwater shrimp (*Gammarus pulex*) numbers. Aside to the importance of the aquatic fauna as a stable base for the fishery food chain, the gnats and other riverflies found through the Home Beat waters also provide a vital food source for local birds and bat populations which hunt over the water like Daubenton's (*Myotis daubentonii*) and Pipistrelle (*Pipistrellus pipistrellus*) bats.

Albeit potentially impoverished at present, the benchmark diversity of aquatic invertebrates found through the Bossington study site waters in the Autumn of 2017 highlighted the potential for fly fishing connoisseurs to have good 'matching the hatch' sport once the full watercourse and linked water quality work had been implemented. This project was particularly exciting because the meagre baseline condition of the watercourse held vast potential for improvement and delivery of a sustainable salmon spawning plus trout and grayling fishery. Indeed, improvements in ecological condition were apparent from in-stream and bank work carried out in a carrier of the Bossington Estate waters from the 2015-2017 S & TC studies but have not yet been written up (Everall *pers. obs*., 2015-2017). It will therefore be very interesting to see what impacts the river works have upon the ecological condition of the receiving River Test in the Bossington Estate Home Beat Waters at the 3 study sites over the next and future years.

Contents

Study Limitations

This short report was prepared by Dr. Everall of Aquascience Consultancy Limited at the request of the Bossington Estate to determine the preliminary biological quality in a selected study reach of the Home Beat of the River Test as a pre-river works improvement benchmark. The findings in this report are a seasonal snapshot of the biological status of these watercourses in October 2017 which will be complimented by further seasonal aquatic ecological condition assessment in the Spring and Autumn of 2018.

This report has been produced by Aquascience Consultancy Limited within the terms of the contract with the client and taking account of the resources devoted to it by agreement with the client. We disclaim any responsibility to the client and others in respect of any matters outside of the specific scoping of this report. This report is confidential to the client, and we accept no responsibilities to third parties to whom this report, or any part thereof, is made known. Any such party relies on the contents of the report at their own risk. Any changes to any current UK and international ecotoxicological standards outlined in this report may cause the opinion, advice and conclusions set out in this report to become inappropriate or incorrect.

1. Survey methodologies

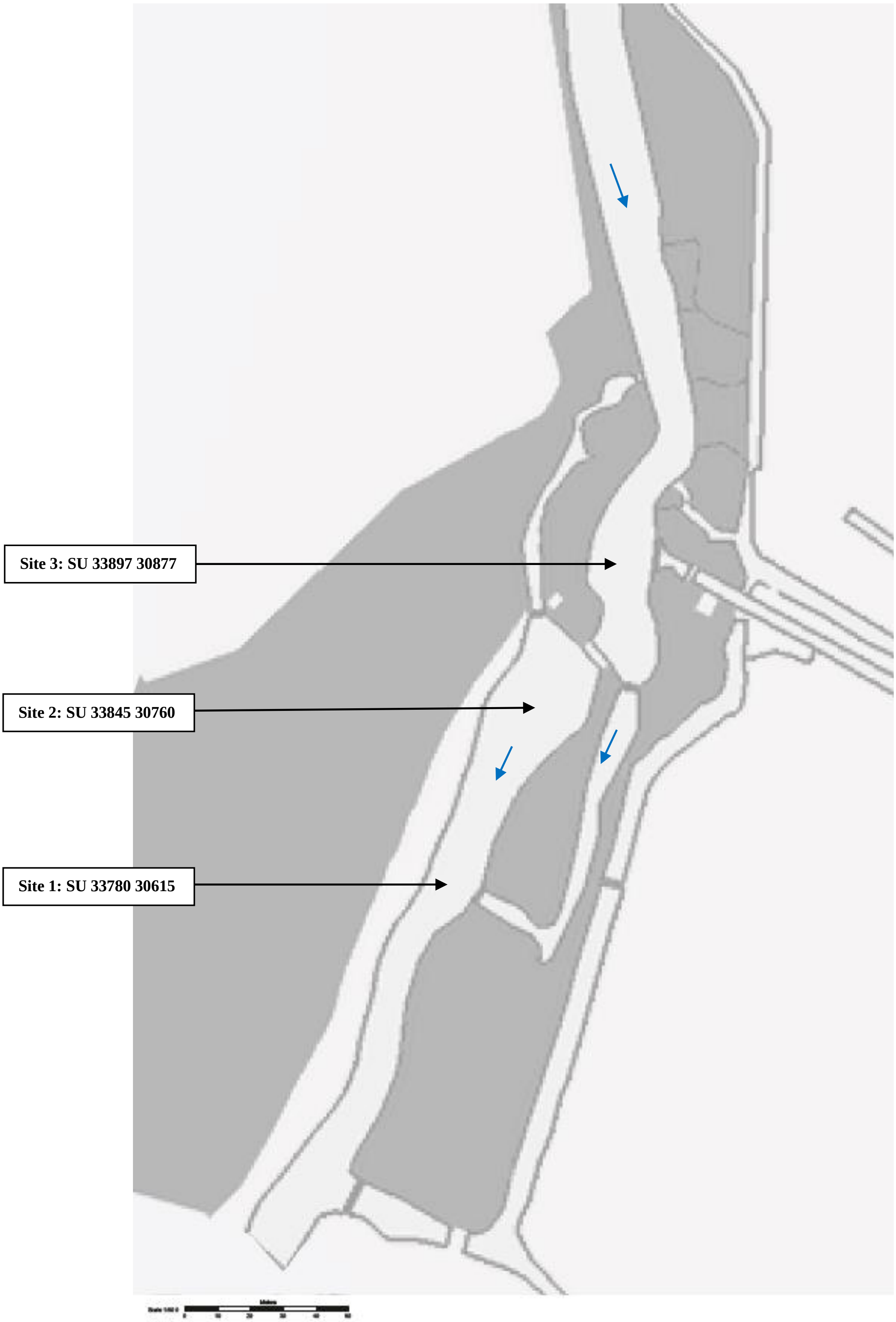
1.1 Watercourse sampling

Following a general watercourse inspection 3-minute kick-sweep net sampling and 1-minute hand search sampling of macro-invertebrates was undertaken at the 3 chosen River Test sample sites through the Home beat of the Bossington Estate waters in the Autumn of 2017. The watercourse sampling was in accord with standard Environmental Impact Assessment (EIA) protocols (HMSO, 1985) and later ISO 7828 (1994) methods adopted by the Environment Agency (Environment Agency, 2009a).

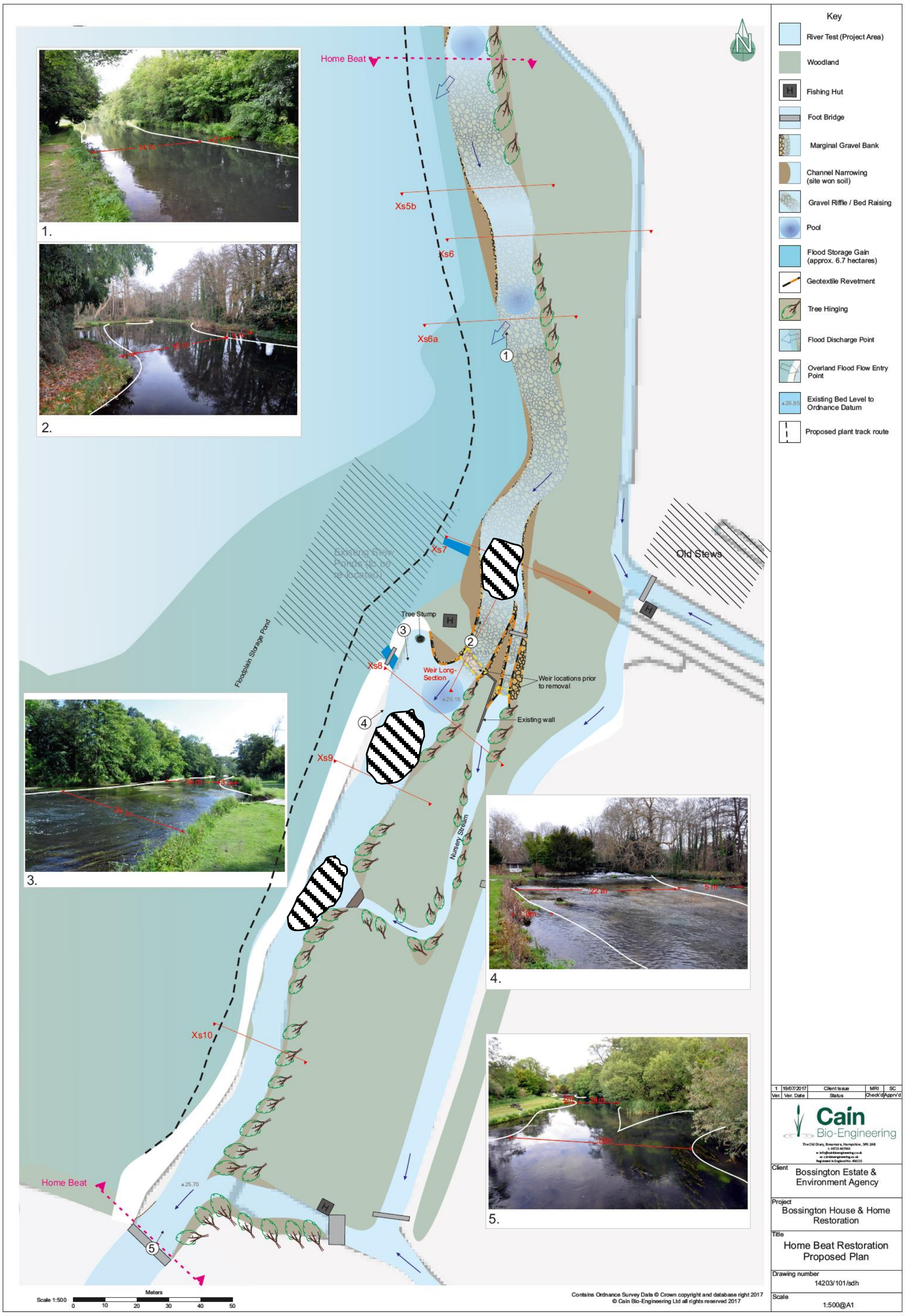
The Environment Agency protocol for 3 minute kick-sweep sampling was strictly adhered to at each of the sample sites chosen in the River Test at Bossington using a stop watch (Environment Agency, 2009a). An additional 1 minute timed hand search of larger substrate e.g. bricks and/or rocks were also undertaken. All representative flow habitats were sampled at each site. All samples contained >95% live animals at the time of sampling and this was recorded on the sealed field sample buckets (Everall *pers. obs.*, 2017). The samples were kept cool in transit, processed and preserved in 70% Industrial Denatured Alcohol (IDA) within 3 months of collection.

1.2 Bio monitoring site locations

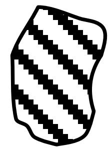
The macroinvertebrate biological sample sites through the Bossington Estate water are shown in the map overleaf along with the direction of watercourse flow →.



The sample sites integrate in to the proposed works shown in the map overleaf with all maps in this report kind courtesy of Cain Bio-Engineering.



Faunal sample areas



The macroinvertebrate sampling was undertaken in the spatial sequence Site 1 → 3 in the autumn of 2017. Each of the chosen homogeneous sample reaches at Sites 1-3 consisted of a ~20m stretch where 3 minute kick-sweep net samples were taken in an upstream chronology. The assessment sample site details for the Bossington Estate River Test study areas are listed in the table below.

Table 1 - Key details for Bossington Estate survey sites on 2nd October 2017.

Site no.	Grid ref	Descriptor
3	SU 33897 30877	River Test upstream weir removal site
2	SU 33845 30760	River Test 1st downstream weir removal site
1	SU 33780 30615	River Test 2nd downstream weir removal site

Specific site photographs and details from Autumn 2017.

Site 1: River Test upstream weir removal site

Bed: <1% Boulders, ~20% cobbles/pebbles, ~10% gravel and 70% sand/silt. Macrophyte cover: minor emergent and submerged. Immersed tree root: <1%. Surrounding land use: deciduous woodland (some river tree canopy cover-shading), rough pasture and estate. Altitude 36m.

Sample site view

River bed view

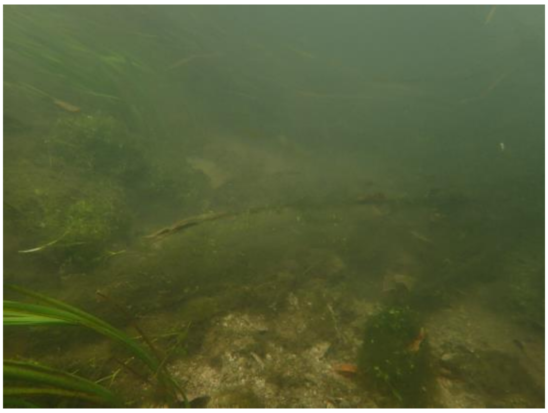
Site 2: River Test 1st downstream weir removal site

Bed: <1% Boulders, ~30-40% cobbles/pebbles, ~30-40% gravel and 10% sand/silt. Macrophyte cover: minor emergent and submerged (*Ranunculus* ~30% cover). Immersed tree root: <1%. Surrounding land use: deciduous woodland (little tree canopy cover-shading), rough pasture and estate. Altitude 34m.


Sample site view River bed view

Site 3: River Test 2nd downstream weir removal site

Bed: <1% Boulders, ~30-40% cobbles/pebbles, ~30-40% gravel and 10% sand/silt. Macrophyte cover: minor emergent and submerged (*Ranunculus* ~30% cover). Immersed tree root: <1%. Surrounding land use: deciduous woodland (little tree canopy cover-shading), rough pasture and estate. Altitude 34m.

Sample site view River bed view

1.3 Macroinvertebrate sample sorting and analysis

At the Aquascience Consultancy Limited laboratory in Chesterfield the preserved macro-invertebrate river samples were washed and sorted using large stainless-steel sieves down to a final retaining sieve of 500um in size within 8 hours of sample collection. The 3 x 1-minute sub-samples were separately analysed but results for this Autumn preliminary report have been presented as a pooled 3-minute sample.

The primary washed, preserved and sieved samples were then carefully decanted and very gently rinsed with tap water into large (sub-divided) white trays for sorting, counting and identification of the sample macro-invertebrates. Each of the 3 x 1-minute kick-sweep net samples were carefully sorted by hand using 8 sub-divisional areas of a tray into the respective groups of macro-invertebrates e.g. cased caddis, caseless caddis, mayflies, stoneflies, etc. These organisms were then placed into compartmental petri-dishes for identification and counting under a low power binocular microscope using FBA level keys. Previously, sorted/preserved organisms like small flatworms were also identified and counted at this stage.

All macro-invertebrate samples were identified to the lowest taxonomic resolution possible and apart from a few gnat larvae and worms this was mainly to species and very occasionally genus level. The sorting and analysis of all macroinvertebrate samples was in accordance with Environment Agency Best Practices (Environment Agency, 2009b) but to a higher species and actual organism count level than that routinely employed by the Environment Agency. It is now widely accepted expert opinion that aquatic faunal data often needs to be resolved to species level to adequately fulfil operational and legislative obligations for river management and conservation purposes (Arscott *et al*., 2006, Everall, 2010, Monk *et. al*., 2011 and Mainstone, 2012).

2 Preliminary survey findings

The aquatic invertebrate surveys in the Bossington Estate Home Beat of the River Test indicated varying degrees of sediment, likely sediment associated nutrient, pesticide-herbicide, impacted flows and overall species diversity (invertebrate richness and abundance) plus keystone species abundances for chalk streams, like the freshwater shrimp, in the autumn of 2017 prior to river improvement works. In terms of the conservation status of the aquatic invertebrates found in surveys on the 2nd October 2017 most species were Common i.e. 1 Recorded from >700 10x10 km grid squares in Britain although the scarce soldier fly *Oxycera pygmaea* was noteworthy.

Although best assessed in the Spring of 2018, of greater importance to fly angling and a sustainable food bed for the trout and grayling fishery was the presence in the Autumn of 2017 of representative up-winged mayflies like The Mayfly (*Ephemera danica*), Blue Winged Olive (*Serratella ignita*), Large - Small Dark Olives (*Baetis rhodani - Baetis scambus*) and Small Spurwing (*Centroptilum luteolum*) through the Home Beat section of the River Test in the Bossington estate pre-river habitat work. A few stoneflies were found through the Bossington waters in the form of Willow or Needle Fly (*Leuctra* sp.) and Large Brown Stonefly (*Perla bipunctata*). Amongst the sedges important to fly fishers there were Grannom (*Brachycentrus subnubilis*), Black Sedge (*Silo nigricornis*), Welshman's Button (*Sericostoma personatum*), Cinnamon Sedge (*Limnephilus lunatus*), Black Silverhorn (*Mystacides azurea*), Brown Silverhorn (*Athripsodes aterrimus*), Sand fly (*Rhyacophila dorsalis*), Dark Spotted Sedge (*Polycentropus flavomaculatus*) and Grey Flag (*Hydropsyche pellucidula*). Damselfly (*Calopteryx splendens*), Alder fly (*Sialis lutaria*), Reed Smuts (*Simulium* spp.) and Midges (Orthocladiinae and Tanytarsinii families) were also present throughout the Bossington Estate Home Beat waters. Aside to the importance of the aquatic fauna as a stable base for the fishery food chain, the gnats and other riverflies found through the Home Beat waters also provide a vital food source for local birds and bat populations which hunt over the water like Daubenton's (*Myotis daubentonii*) and Pipistrelle (*Pipistrellus pipistrellus*) bats.

The overall mayfly (Ephemeroptera)-stonefly (Plecoptera)-caddis (Trichoptera) or EPT species richness and abundances will be mapped after the more appropriate required Spring surveys for this measure of ecological condition in 2018.

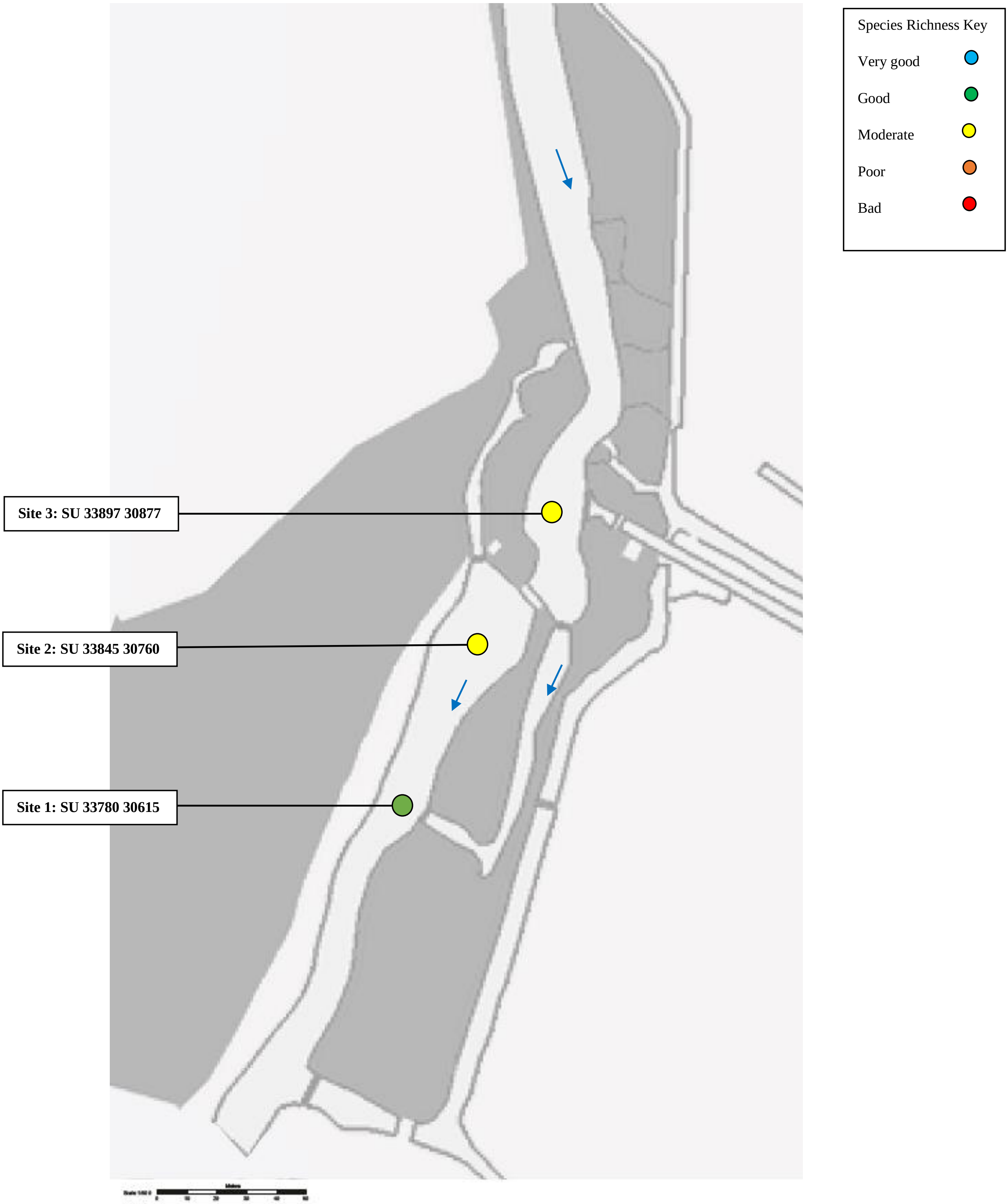
These species-rich river fly (EPT) orders represent a significant proportion of aquatic invertebrate biodiversity in running waters and are an important food resource for other organisms, especially fish and to a lesser extent crayfish. These species rich river fly groups have been reported to be in decline across many UK watercourses in recent decades (Frake and Hayes, 2001, Bennett and Gilchrist, 2010 and Everall, 2013) despite a backdrop of generally improving broad brush water quality criteria. As fly fishing and fishery quality drivers we have chosen the river fly EPT species richness marker as one of the simplest overall indicator of ecological condition through the Bossington Estate waters as part of this benchmark and future fishery assessments. While it is useful to get an early feel for things, as previously stressed, we need the Spring 2018 survey findings for a complete and tempered understanding of both the benchmark ecological condition and the environmental stresses through these waters.

From the maps of environmental stresses for mild organic pollution, sediment, nutrient enrichment and flow over the following pages of this report it was apparent that where these stresses spatially improved through the Bossington Estate waters then so did the mayfly, stonefly and caddisfly species richness. It was of further interest that the raised biological signatures of variable nutrient enrichment showed association with raised sediment fingerprints at many sites. There is growing unison of opinion amongst aquatic toxicologists that the combination of elevated nutrient and sediment levels in rivers may be additively or synergistically toxic to the development and survival of river fly eggs supported by recent studies with blue winged olive egg survival (Everall *et. al*., 2017). Similarly, this combination of environmental stresses is well documented to impair salmon, grayling and trout egg survival in the literature.

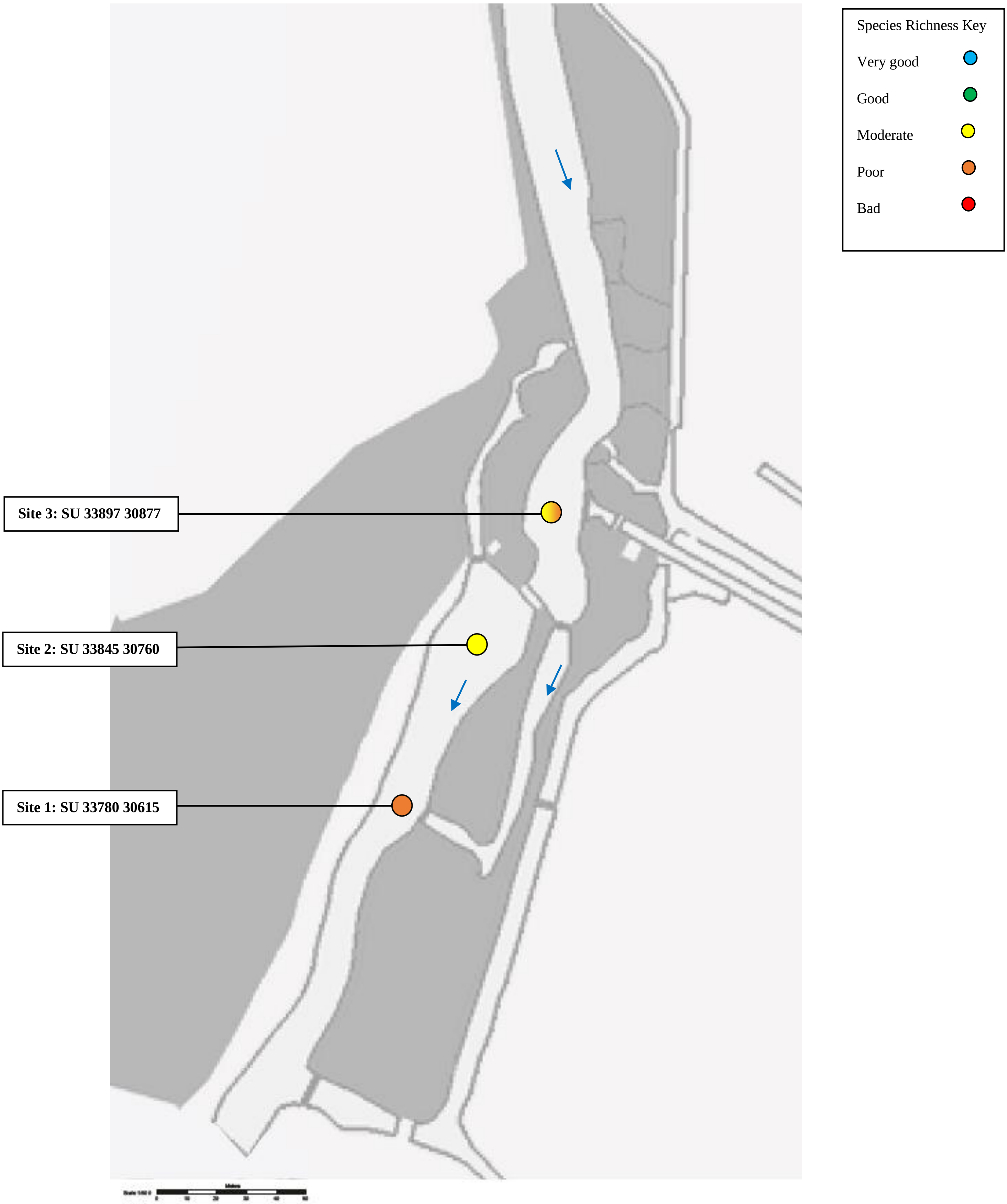
The freshwater shrimp (*Gammarus pulex*) is a stable taxa of healthy chalk rivers with trout and grayling historically seen ‘tailing’ in the Autumn to fatten themselves up for the winter months. Recent work with the Environment Agency because of depauperate *Gammarus* numbers in the River Itchen has generated a WFD type standard of ≥ 500 *Gammarus*/autumn 3 minute kick-sweep net sample indicating a key good component of ecological condition for chalkstreams. This shrimp standard has been used amongst the others to interpret the condition of the Bossington Estate Home Beat waters of the River Test before and after the river works.

The overall aquatic species richness of the watercourse invertebrate communities at each of the sample sites through the Bossington Estate waters on the 2nd October 2017 is mapped overleaf. Depauperate or loss of aquatic species richness at river sites is a key indicator of ‘ecosystem distress’ and conversely good or gain in aquatic species diversity associates with good or improving ecological condition.

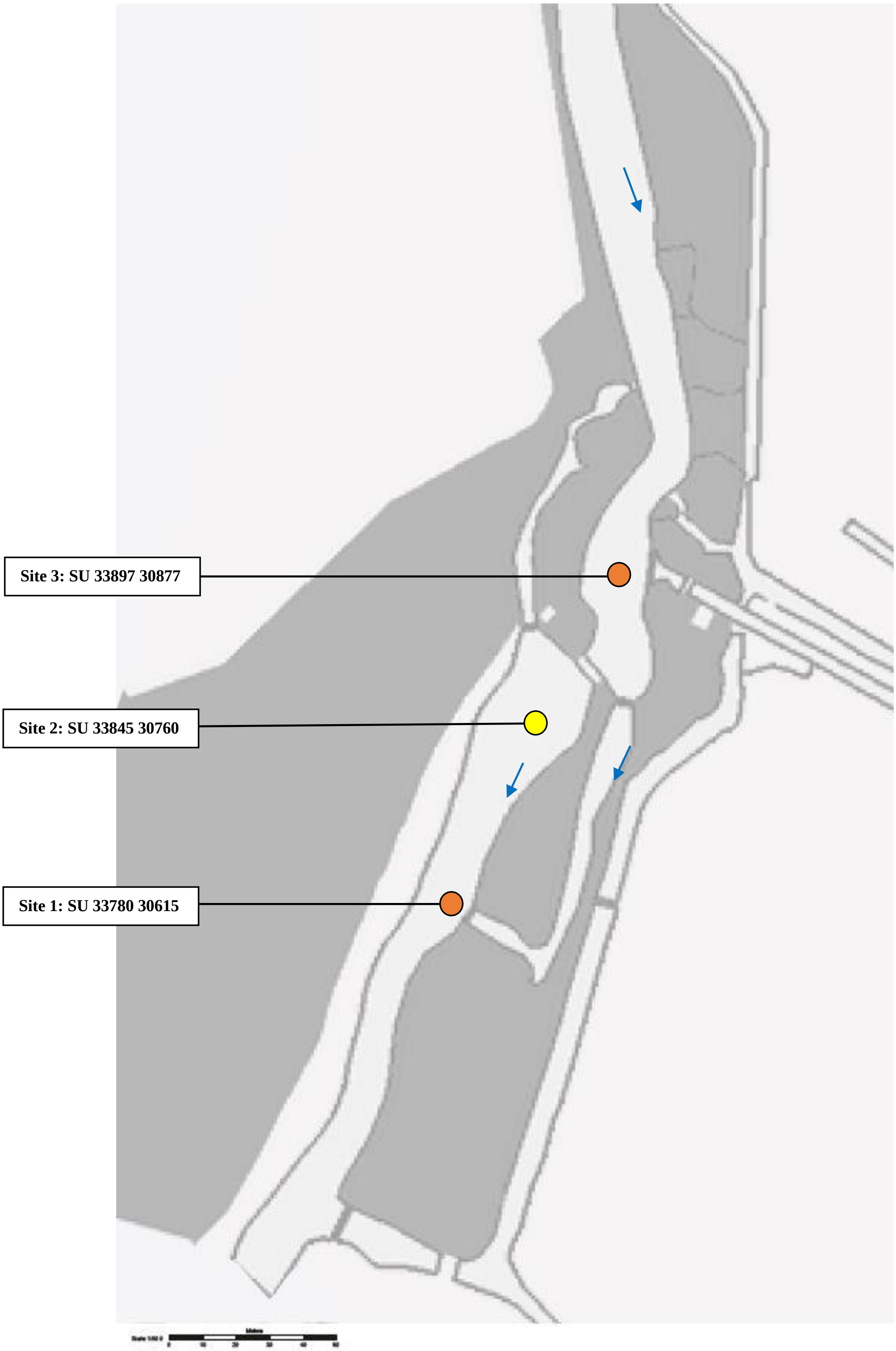
Overall aquatic species richness



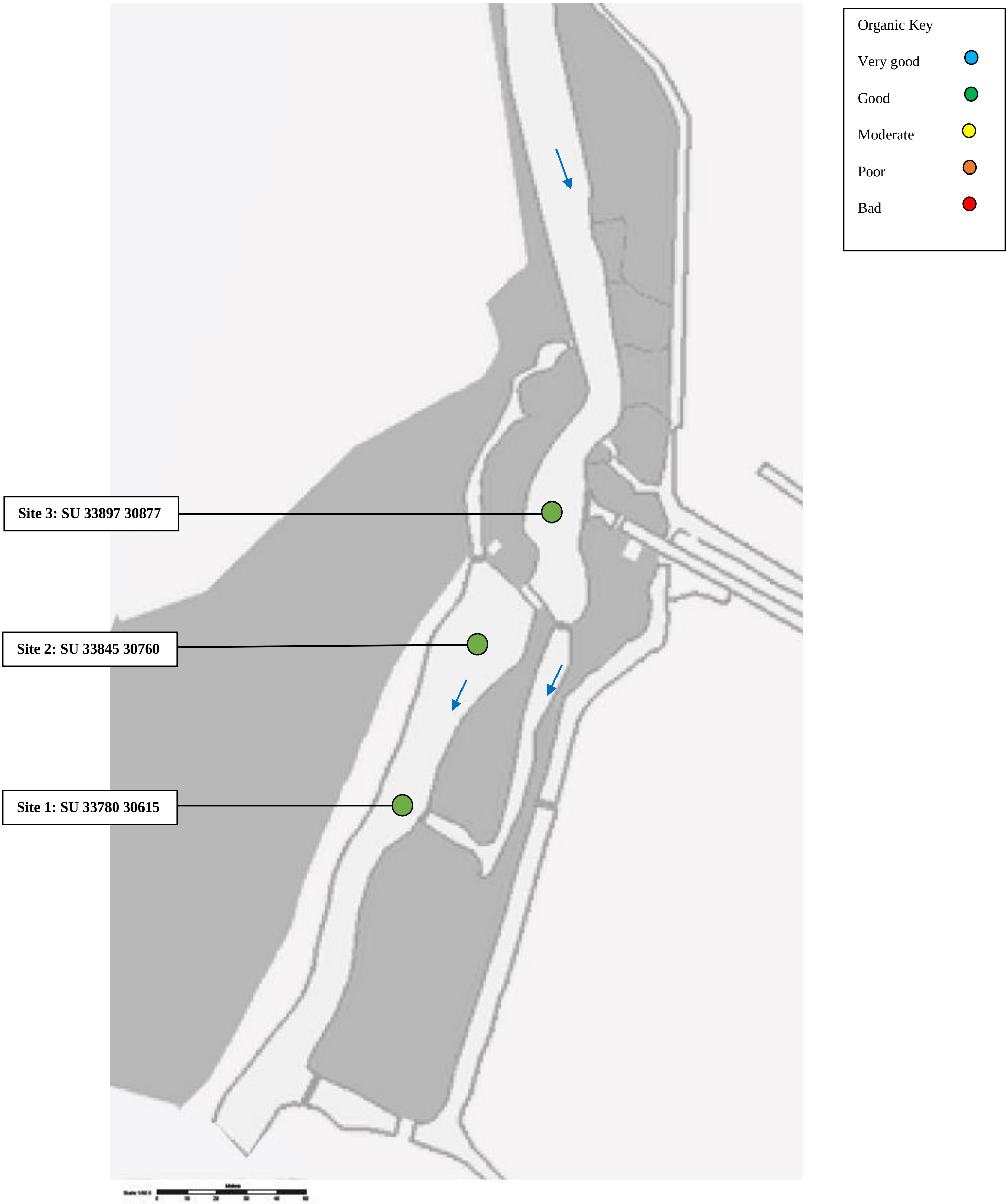
Relative total invertebrate abundance



Autumn shrimp (*Gammarus pulex*) signature (*EA Middle Itchen target)

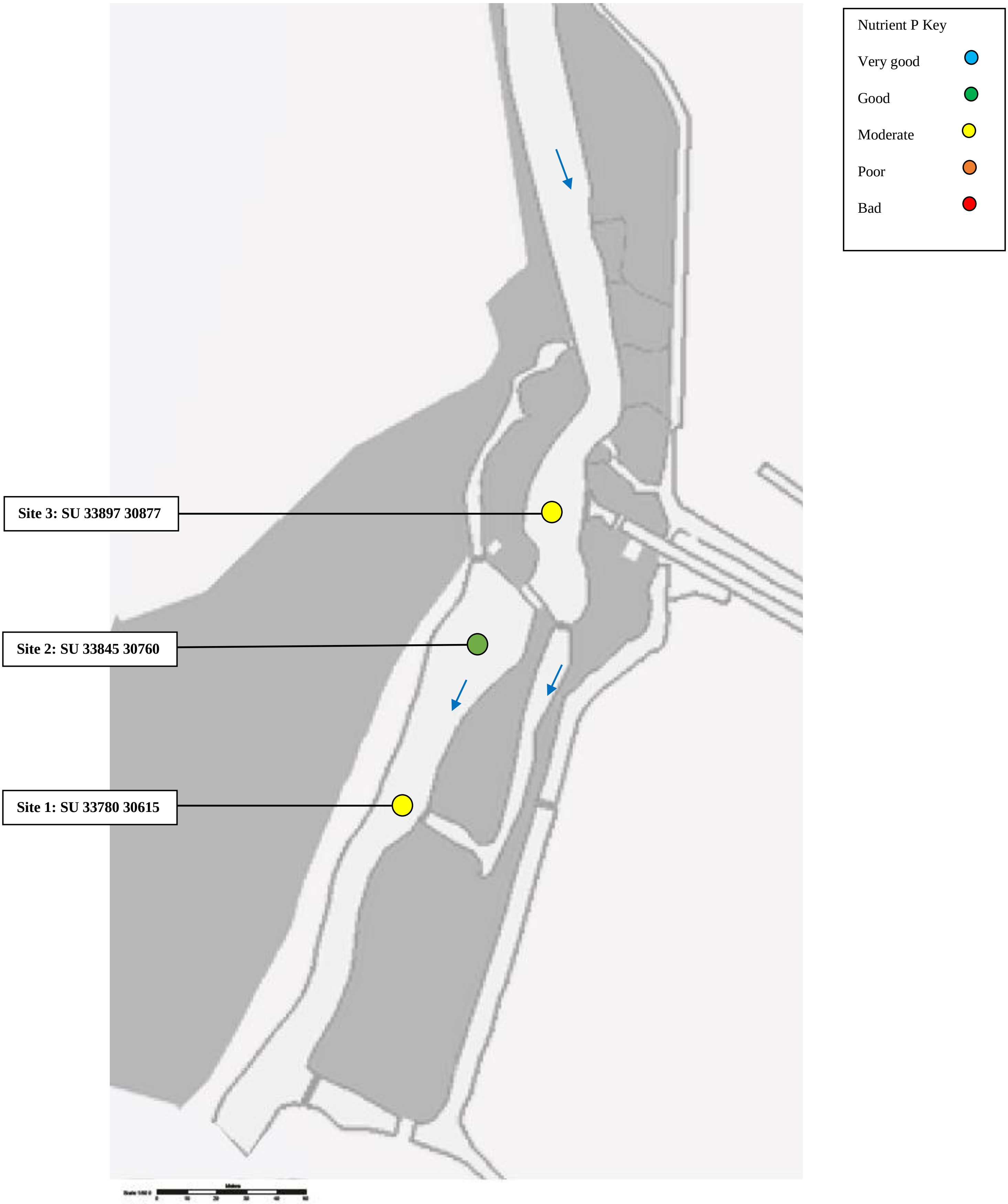


Organic enrichment biosignature (ammonia, lowered dissolved oxygen …)

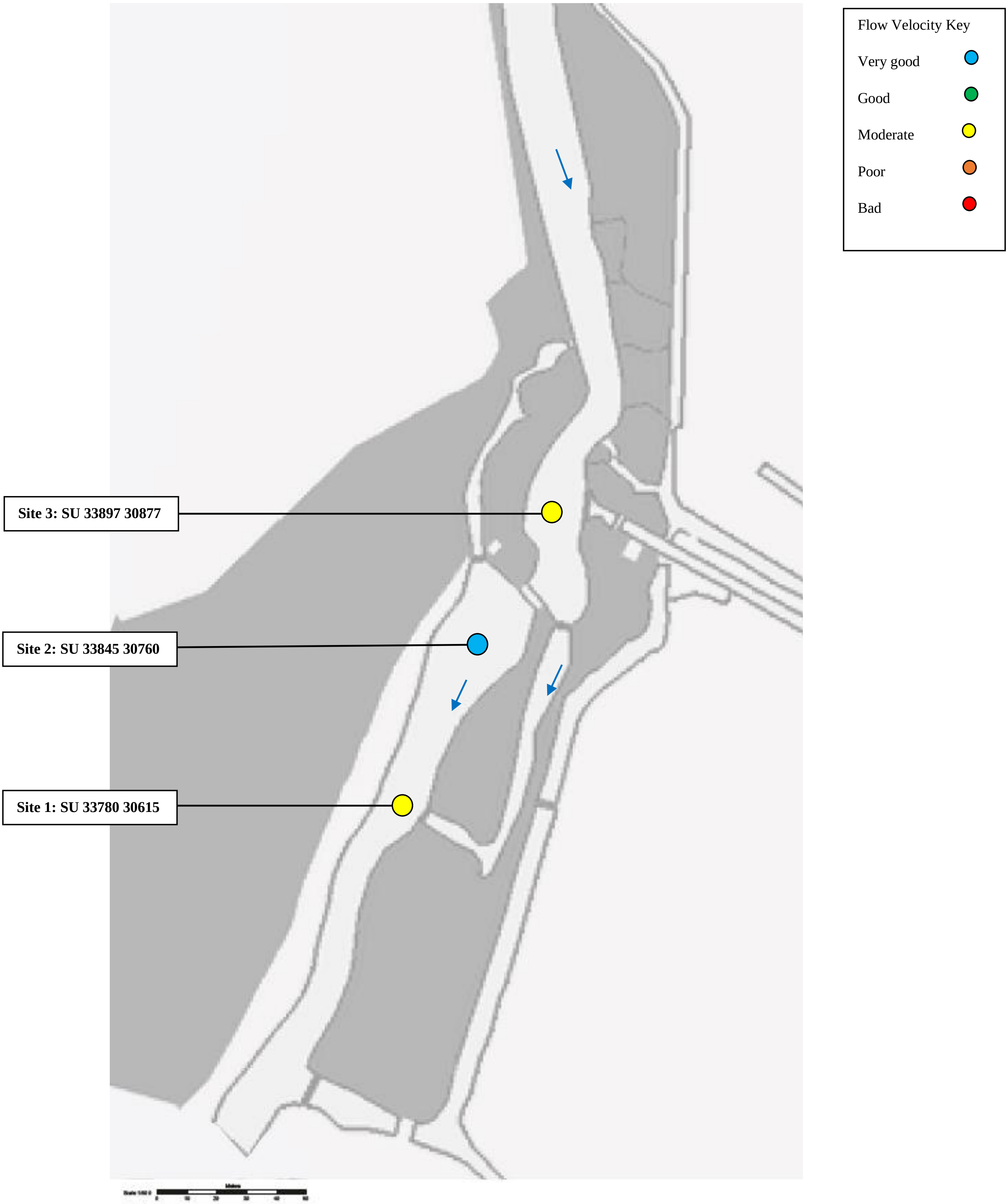


Sediment biosignature

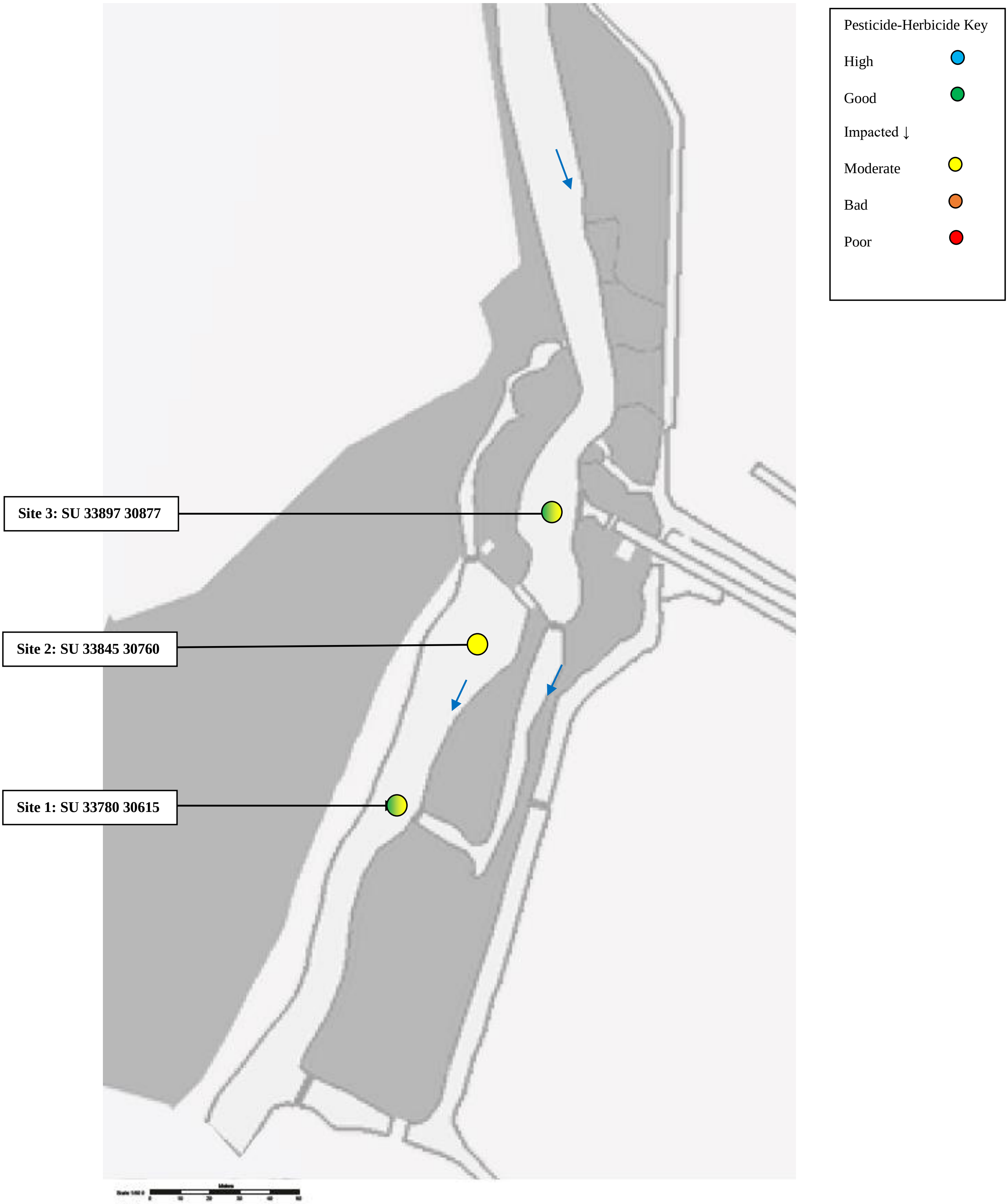
Nutrient (phosphorous) biosignature



Flow (water velocity) biosignature

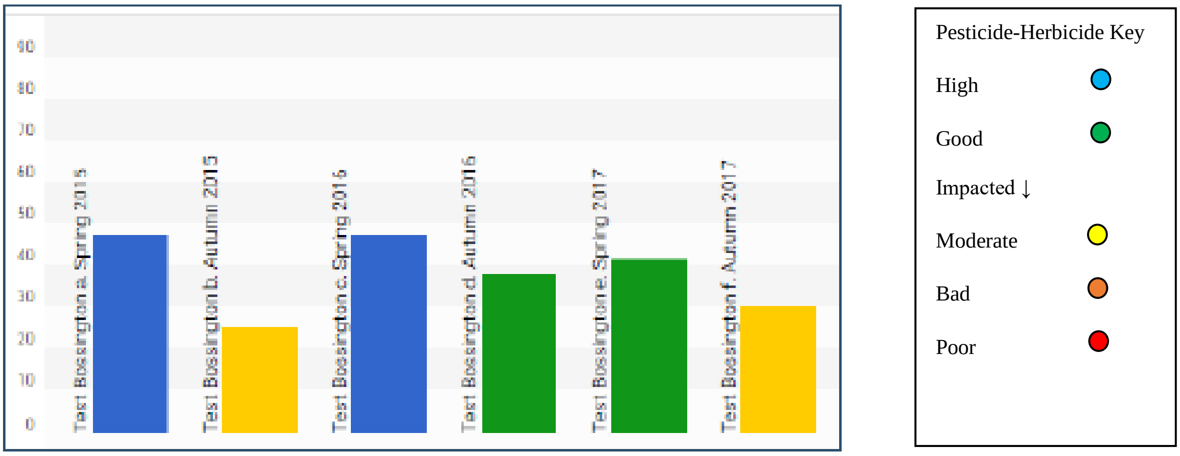


Pesticide-herbicide biosignature



It is likely that the slight pesticide-herbicide signature present through the Bossington Estate Home Beat survey sites was coming from a source upstream of Site 3. It was likely that given the nature of the river corridor that this was some form of plant applied spray drift? Although the sediment signatures may be associated with the relatively depauperate freshwater shrimp (*Gammarus pulex*) numbers the pesticide-herbicide signatures highlighted at the river works survey sites on the previous map may also be associated with the faunal fingerprints. It will be interesting to see how these ecological condition markers respond to the river improvement work alone as the pesticide-herbicide signatures were faint and arguably only just at the threshold for impact.

A similar intermittent picture of pesticide-herbicide signatures was detected at the Salmon & Trout Conservation study site in the Bossington Estate waters ca. 0.5km downstream of the current river works survey area in a River Test carrier at grid reference SU 34059 30707 shown in the fingerprints from 2015-2017 below.



The autumnal biosignatures for pesticide-herbicide in 2015 and 2017 suggested a potential late spring-summer upstream application of pesticide-herbicide to livestock or crop. The nature of the river corridor in this carrier water is shown in the river and watercourse bed photographs below.

It will be very interesting to see what impacts the river works have upon the ecological condition of the receiving River Test in the Bossington Estate Home Beat Waters at the 3 study sites over the next and future years.

References

Arscott, D.B., Jackson, J.K. and Kratzer, E.B. (2006). Role of rarity and taxonomic resolution in a regional and spatial analysis of stream macroinvertebrates. *Journal of the North American Benthological Society*, 25, 977–997.

Bennett, C.J. and Gilchrist, W. (2010). Chapter 22 Riverflies In: Silent Summer The State of Wildlife in Britain and Ireland, Cambridge University Press, New York, 765pp. ISBN 978-0-521-51966

Clements, W.H. and Newman, M.C. (2002). Community Ecotoxicology. Hierarchical Ecotoxicology Series, John Wiley and Sons Ltd., Chichester, 336pp.

Environment Agency (2002). A guide to monitoring the ecological quality of ponds and canals using PSYM. Environment Agency PSYM Manual, Bristol.

Environment Agency (2009a) Freshwater Macro-invertebrate sampling in Rivers. Operational Instruction 018_08. Environment Agency.

Environment Agency (2009b) Freshwater Macro-invertebrate Analysis of Riverine Samples. Operational Instruction 024_08. Environment Agency.

Everall, N.C. (2010). The aquatic ecological status of the rivers of the Upper Dove Catchment in 2009. *Natural England Commissioned Report NECR046*. Natural England: Sheffield.

Everall, N.C. (2013). Environmental pressures impacting river fly populations in UK rivers. Salmon & Trout Association publication, 48pp.

Everall, N.C. *et. al.* (2017). Sensitivity of the early life stages of a mayfly to fine sediment and orthophosphate levels, Environmental Pollution (2017), https://doi.org/10.1016/j.envpol.2017.10.131

Frake, A and Hayes, P (2001). Report on the millennium chalk streams fly trends study. Environment Agency publication. ISBN 1-85-705759-7.

H.M.S.O. (1978). Methods of Biological Sampling Handnet Sampling of Aquatic Benthic Macroinvertebrates. Methods for the Examination of Waters and Associated Materials. London, 8 pp. [Now ISO 7828: 1985 (BS EN 27828: 1994). Water Quality - Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates].

ISO 7828: 1985 (BS EN 27828) (1994). Water Quality - Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates.

Mainstone, C.P. (2012) The use of detailed macroinvertebrate assessment in river SSSI/SAC condition assessment. Paper written on behalf of the UK Common Standards Monitoring group on SSSI/SAC river habitat.

Merritt, R. (2006). Atlas of the water beetles (Coleoptera) and water bugs (Hemiptera) of Derbyshire, Nottinghamshire and South Yorkshire, 1993-2005. Sorby Recortd Special Series, 14, 160pp. ISBN 0260-2032.

Monk, W.A, Wood, P.J., Hannah, D.M., Extence, C.A., Chadd, R.P. and Dunbar, M.J. (2011). How does macroinvertebrate taxonomic resolution influence ecohydrological relationships in riverine ecosystems. Ecohydrology, published online in Wiley Online Library. DOI: 10.1002/eco.192

Appendices

Appendix 1 - Details of biometric testing used on the species macroinvertebrate community data from the Bossington Estate watercourse survey sites on the 2nd October 2017.

Appendix 2 - Macro-invertebrate 3 min kick-sweep survey results for Site 1-3 in the River Test on the 2nd October 2017.

Appendix 1 - Details of biometric testing used on the species macroinvertebrate community data from the Bossington Estate watercourse survey sites on the 2nd October 2017.

Organic pollution (Saprobic indexing)

Many studies have compared the results of different benthic macroinvertebrate metrics used to assess the impact of organic pollution (Hellawell, 1987, Calow & Petts, 1993, Hauer & Lamberti, 1996 and Eurolimpacs, 2004,). The Average Score Per Taxon (ASPT) used by the Environment Agency with the computer model RIVPACS in the UK has been well correlated with the stress gradient in most stream types but the Saprobic Index worked better than ASPT in those countries (e.g. Germany, Austria and the Czech Republic) where macroinvertebrates were generally identified to a lower (species) as opposed to a higher (genus or family) level of identification (Leonard and Daniel, 2004). Saprobic indexing at the species level allowed a greater insight into the nature and quantum of organic pollution in watercourses than other methods since it accounted for species differences in tolerance to organic pollutants (e.g. elevated ammonia and lowering dissolved oxygen regimes) as opposed to generic estimates of whole family responses.

The link between biological water quality and the saprobic system of watercourse classification was because benthic invertebrates are important within the stream community as a fundamental link in the food web between organic matter resources and ecosystem fishery health. A standardised method to assess the biological water quality in European watercourses is the saprobic classification system (saprobity = amount of degradable organic material). This classification system is based upon selected index organisms (indicators), whose appearance is related to the impact of degradable organic material. The saprobic value (s) is a number from 1,0 to 4,0. The category groups of the saprobic values are:

Classification	**s**
oligosaprobic	1,0 - <1,5
oligosaprobic – ß-mesosaprobic	1,5 - <1,8
ß-mesosaprobic	1,8 - <2,3
ß-mesosaprobic – α-mesosaprobic	2,3 - <2,7
α-mesosaprobic	2,7 - <3,2
α-mesosaprobic – polysaprobic	3,2 - <3,5
polysaprobic	3,5 – 4,0

In the calculation of the saprobic classification there are two values that are dedicated to each species:

1. the saprobic value (s) and

2. the indicator value (G)

The saprobic value shows the appearance of the species in a specific range of water quality. Some species have a narrow tolerance range, this means that they are good indicators. The specific tolerance of the species is expressed by the indication value.

The third term to calculate the saprobic classification is:

3. the frequency (A) of a particular species.

Formula for the saprobic index:

$$S = \frac{\sum A*s*G}{\sum A*G}$$

S = saprobic index
A = frequency
s = saprobic value
G = indicator value

The latest Saprobic values (s) and indicator values (G) used throughout Europe were obtained by formal permission in writing from and Dr. Everall was granted (password) access to the EUROLIMPACS database (via www.freshwaterecology.info).

Interpretation of Saprobic indices for levels of organic pollution and inferred chemical status was provided by Laenderarbeitsgemeinschaft Wasser (LAWA), Mainz, Germany, 1976 below:

Quality class	Degree of organic load	Saprobic state	Saprobic index	Usual BOD5 in mg/L	Usual NH4-N in mg/L	Usual O2-minima in mg/L
I	**no or minimal**	**oligosaprobic**	**1,0-<1,5**	**1**	**<0,1**	**8**
I-II	small	oligo-betamesosaprobic	1,5-<1,8	1-2	~0,1	8
II	mild	betamesosaprob	1,8-<2,3	2-6	<0,3	6
II-III	critical	beta-alphamesosaprobic	2,3-<2,7	5-10	<1	4
III	strongly polluted	alphamesosaprobic	2,7-<3,2	7-13	0,5-several mg/L	2
III-IV	very strongly polluted	alphamesosaprobic transition zone	3,2-<3,5	10-20	several mg/L	<2
IV	**extremely polluted**	**polysaprobic**	**3,5-<4,0**	**15**	**several mg/L**	**<2**

Siltation (PSI)

Physical assessment methods have traditionally been used to quantify riverine sedimentation, but Extence *et. al.* (2010 and 2011) have proposed an alternative approach, the use of a sediment-sensitive macro-invertebrate metric, PSI (Proportion of Sediment-sensitive Invertebrates) which can act as a proxy to describe temporal and spatial impacts.

Chris Extence and Richard Chadd from the Environment Agency had very kindly allowed Dr Everall at Aquascience to be one of the first independent workers to trial the macroinvertebrate siltation indexing on the data from the 2009 Upper Dove Catchment Project by Natural England (Everall, 2010 and Extence *et. al.*, 2010).

The PSI score describes the percentage of sediment-sensitive taxa (Table 1 below) present in a sample and the metric is calculated using the matrix shown in Table 2 below and then applying the following formula:

$$\text{PSI } (\Psi) = \frac{\varepsilon \text{ Sediment Scores for Sensitivity Groups A \& B}}{\varepsilon \text{ Sediment Scores for all Sensitivity Groups A, B, C \& D}} \quad \text{X } 100$$

Table 1

Group	Silt Tolerance Definition
A	Taxa highly sensitive to sedimentation
B	Taxa moderately sensitive to sedimentation
C	Taxa moderately insensitive to sedimentation
D	Taxa highly insensitive to sedimentation
E	Taxa indifferent to sedimentation or excluded from the method for other reasons.

Table 2

Group	Sediment Sensitivity Rating (SSR)	Log Abundance. 1-9	10-99	100-999	1000+
A	Highly Sensitive	2	3	4	5
B	Moderately Sensitive	1	2	3	4
C	Moderately Insensitive	1	2	3	4
D	Highly Insensitive	2	3	4	5
E	Excluded	-	-	-	-

From the literature review in Extence *et. al.* (2011), appropriate abundance and affinity weightings have been incorporated into Table 2 to give the final PSI metric better definition. PSI scores range from 0 (entirely silted river bed) to 100 (entirely silt-free river bed). Extence *et. al.* (2011) suggested that when applied to species and family data respectively, the terms PSI (S) and PSI (F) are used. An interpretation scheme for the data is shown in Table 3 below (Extence *et. al.*, 2011).

Table 3

PSI	River Bed Condition
81 -100	Naturally sedimented/Unsedimented
61 - 80	Slightly sedimented
41 - 60	Moderately sedimented
21 - 40	Sedimented
0 - 20	Heavily sedimented

Flow (LIFE)

Many freshwater invertebrates have precise requirements for particular current velocities or flow ranges (Chutter, 1969; Hynes, 1970; Statzner *et al*., 1988; Brooks, 1990), and certain taxa are ideal indicators of prevailing flow conditions. As well as qualitative responses to flow changes, site specific studies also show that most taxa associated with slow flow tend to increase in abundance as flows decline, whereas most species associated with faster flows exhibit the opposite response (Moth Iversen *et al*., 1978; Extence, 1981; Cowx *et al*., 1984; Wright and Berrie, 1987; Boulton and Lake, 1992 and Wright, 1992). Alterations in community structure may occur as a direct consequence of varying flow patterns, or indirectly through associated habitat change (Petts and Maddock, 1994 and Petts and Bickerton, 1997).

The Lotic-invertebrate Index for Flow Evaluation (LIFE) technique is based on data derived from established 3 minute kick-sweep net sampling of macroinvertebrates in order to assess the impact of variable flows on benthic populations (Extence *et. al.*, 1999). The method links qualitative and semi-quantitative change in riverine benthic macroinvertebrate communities to prevailing flow regimes. The higher the LIFE score in comparable flow-habitat sections of watercourse the higher the prevailing flow conditions and *vice versa*.

Nutrient (TRPI)

Eutrophication, defined as the enrichment of waters by nutrients resulting in an array of biological changes, is widespread in the lakes and rivers of industrialised countries (Schindler, 2006 and Lampert and Sommer, 2007). Typical symptoms include increased algae production (Walling andWebb, 1992) and sometimes enhanced growth of higher aquatic plants (Dodds, 2006). Traditionally Water Framework Directive (WFD) biological assessment of nutrient enrichment in watercourses has utilised both plant (macrophyte) and benthic algal (phytobenthos) assessments but these have latterly been found to have some flaws for some watercourse types.

It has long been recognised that nutrient enrichment causes complexation of ecosystems through changes in primary producers (algae and plants) with studies variably recording e.g. a reduction in faunal (consumer) biodiversity following changes in species composition (Smith, 2003 and Hilton *et. al*., 2006) and measurable stress to macroinvertebrate communities (Weitjers *et. al*., 2009 and Miltner, 2010). During the last decade workers have been developing a diagnostic model based upon a Bayesian belief network to detect total reactive phosphorous (TRP) fingerprints from macroinvertebrate community data in receiving watercourses over a wide geographic area of England and Wales (Paisley *et. al.*, 2003, Everall, 2004, Everall, 2005, Everall, 2010 and Paisley *et. al.*, 2011). Eutrophication often occurs in combination with other anthropogenic stresses in rivers in a way that was historically difficult to disentangle, further disrupting simple relationships between nutrient availability and biological response. In the latest TRP diagnostic model developed by the author with Dr. Martin Paisley at Staffs University the benchmark datasets in the model were screened to minimise the confounding effects of organic pollution and split according to site type and season. This is a new biometric developed by Dr. Everall (Aquascience Consultancy) and Dr. Martin Paisley (University of Staffordshire) which is based upon the phosphate and macroinvertebrate studies of Paisley *et. al.* (2003 and 2011) and Everall (2005 and 2006).

The Total Reactive Phosphorous Index or TRPI describes the TRP-sensitive taxa groupings in Table 4 overleaf present in a sample and the metric is calculated from the formula below using the ‘look up’ matrix shown in Table 5overleaf:

$$\text{TRPI} = \frac{\varepsilon \text{ Nutrient Scores for A \& B}}{\varepsilon \text{ Nutrient Scores A\&B\&C\&D}} \text{ X } 100$$

The TRPI, unlike some previous biometrics e.g. PSI (Extence *et. al.*, 2011) has to allow for both positive and negative changes in the abundance of TRP indicator macroinvertebrate families associated with the findings from large field datasets upon the impacts of TRP in watercourses (Paisley *et. al.*, 2011).

Table 4 - Nutrient (TRP) tolerance bandings

Group	TRP Tolerance Definition
A	Taxa very sensitive to [TRP]
B	Taxa sensitive to [TRP]
C	Taxa tolerant to [TRP]
D	Taxa very tolerant to [TRP]
E	Taxa indifferent to [TRP] at P>0.05 or excluded from the method for other reasons.

Table 5 - Nutrient scores based on tolerance bandings and abundance

Group	TRP Sensitivity Rating (PSR)	Log Abundance. 1-9	10-99	100-999	1000+
A	Very Sensitive	2	3	4	5
B	Sensitive	1	2	3	4
C	Tolerant	1	2	3	4
D	Very Tolerant	2	3	4	5
E	Excluded				

A tabular 'look-up' matrix is then used for TRP indicator macroinvertebrate families from Paisley *et. al.* (2011) associated with river site Types, season and alkalinity. For example, Type 1-3 are generally associated with upland rivers and Type 3-5 with increasingly lowland rivers respectively. The model calculation formula then generates the season and river type weighted phosphate-sensitive macro-invertebrate metric, the TRPI and a provisional interpretation scheme for the data is shown in Table 6 below. Effectively, the more TRP sensitive families present the lower the TRPI% and the less chemical TRP present in the watercourse at that site at that time but recording fingerprint from previous temporal exposure as with and other biometric index.

Table 6 - Look up formula results

TRPI	Nutrient Condition
81 -100	Very low [TRP]
61 - 80	Low [TRP]
41 - 60	Moderate [TRP]
21 - 40	High [TRP]
0 - 20	Very high [TRP]

Conservation value (CCI)

Community Conservation Indexing (CCI) after Chadd and Extence (2004) was undertaken for all the macroinvertebrate survey data collected from the study sites in the River Test watercourses in 2014.

Biometric indexing references only

Boulton, A.J. and Lake, P.S. (1992). The ecology of two intermittent streams in Victoria Australia. III. Temporal changes in faunal composition. Freshwater Biology, 27, 123-138.

Brooks, A. (1990). Channelized Rivers Perspectives for Environmental Management. Wiley, Chichester.

Calow, P. & G. Petts: The Rivers Handbook I: Hydrological and Ecological Principles; Wiley and Sons, 1993.

Chutter, F.M. (1969). The distribution of some stream invertebrates in relation to current speed. Int. Revue ges Hydrobiol., 54, 413-422.

Chadd R and Extence C. (2004). The conservation of freshwater macroinvertebrate populations: a community-based classification scheme. Aquatic Conservation: Marine and Freshwater Ecosystems, 14, 597-624.

Chutter, F.M. (1969). The effects of silt and sand on the invertebrate fauna of streams and rivers. Hydrobiologia, 34, 57-76.

Clements, W.H. and Newman, M.C. (2002). Community Ecotoxicology. Hierarchical Ecotoxicology Series, John Wiley and Sons Ltd., Chichester, 336pp.

Commission of the European Communities (CEC), 2000. Directive 2000/60/EC of the European Parliament and of the Council Establishing a Framework for the Community Action in the Field of Water Policy. http://ec.europa.eu/environment/water/water-framework/index_en.html (last accessed August 2008).

Cordone, A.J. and Kelley D.W. (1961). The influences of inorganic sediment on the aquatic life of streams. California Fish and Game, 47, 189-228. California Department of Fish and Game, Inland Fisheries Branch: Sacramento, US.

Cowx, I.G., Young, W.O. and Hellawell, J.M. (1984). The effect of drought on the fish and invertebrate populations of an upland stream in Wales. Freshwater Biology, 14, 165-177.

Davies-Colley, R.J., Hickey, C.W., Quinn, J.M. and Ryan, P.A. (1992). Effects of clay discharges on streams 1, Optical properties and epilithon. Hydrobiologia, 248, 215-234.

Environment Agency (1997). Procedures for collecting and analysing macro-invertebrate samples. Environment Agency, Bristol, 82pp.

Everall, NC. (2010). The aquatic ecological status of the rivers of the Upper Dove Catchment in 2009. *Natural England Commissioned Report NECR046*. Natural England: Sheffield.

Extence, C.A. (1981). The effect of drought on benthic invertebrate communities in a lowland river, Hydrobiologia, 83, 217–224.

Extence C. A., Balbi, D.M. and Chadd R.P. (1999). River Flow Indexing using British benthic macroinvertebrates: A framework for setting hydro ecological objectives. Regulated Rivers Research and Management. 15, 543-574.

Extence, C.A, Chadd, R.P, England, J., Dunbar, M.J., Taylor, E.D. and Everall, N.C. (2010). The assessment of fine sediment accumulation in rivers using macroinvertebrate community response. BHS Third International Symposium, Managing Consequences of a Changing Global Environment, Newcastle, 2010.

Extence, C.A., Chadd, R.P., England, J., Dunbar, M.J., Wood, P.J. and Taylor, E.D. (2011). The Assessment of Fine Sediment Accumulation in Rivers Using Macro-invertebrate Community Response. River Research and Applications, 30pp.

Hellawell, J.M. (1986). Biological indicators of freshwater pollution and environmental management. Pollution Monitoring Series. London, Elsevier Applied Science, 546pp.

Hauer, F. R. and Lamberti, G. A. (1996). Methods in Stream Ecology, Academic Press, 1996.

Hilton, J., O'Hare, M., Bowes, M.J. and Jones, J.I. (2006). How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365, 66-83.

Hynes, H.B.N. (1970). The Ecology of Running Waters. Liverpool University Press, Liverpool, 555pp.

Hynes, H.B.N. (1973). The Effects of Sediment on the Biota in Running water. in Fluvial Processes and Sedimentation, Proceeding of a Hydrology Symposium, University of Alberta, Edmonton: 653-663. National Research Council, Environment Canada.

Moss, D., Furse, M.T., Wright, J.F. and Armitage, P.D. (1987). The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great Britain using environmental data. Freshwater Biology 17, 41-52.

Moth Iversen, T., Wiberg-Larsen, P., Birkholm Hansen, S. and Hansen, F.S. (1978). The effect of partial and total drought on the macroinvertebrate communities of three small Danish streams. Hydrobiologia, 60, 235-242.

Paisley, M.F., Walley, W.J., Nikhade, J. and Dils, R. (2003). Identification of the key biological indicators of nutrient enrichment in rivers for use in predictive/diagnostic models. Proceeding of the 7th International Specialised IWA Conference on Diffuse Pollution and Basin Management, Dublin, Ireland.

Paisley, M.F., Walley, W.J. and Trigg, D.J. (2011). Identification of macro-invertebrate taxa as indicators of nutrient enrichment in rivers. Ecological Informatics, 6, 399–406. doi:10.1016/j.ecoinf.2011.09.002.

Parkhill, K.L. and Gulliver, J.S. (2002). Effect of inorganic sediment on whole-stream productivity, Hydrobiologia, 472, 5–17.

Percival, E. and Whitehead, H. (1929). A quantitative study of some types of stream bed. Journal of Ecology, 17, 282-314.

Petts, G.E. and Maddock, I. (1994). Flow allocation for in-river needs, in Calow, P. and Petts, G.E. (Eds), The Rivers Handbook, Volume 2. Hydrological and Ecological Principles, Blackwell Scientific Publications, London.

Petts, G., Maddock, I., Bickerton, M. and Ferguson, A.J.D. (1995). Linking hydrology and ecology: the scientific basis for river management. In: The Ecological Basis for River Management. Edited by D.M. Harper and A.J.D. Ferguson. John Wiley & Sons, Chichester.

Petts, G.E. and Bickerton, M.A. (1997). River Wissey lnvestigations: linking hydrology & ecology. Execective summary, Environment Agency Project Report, 01: 526 :1A, Environment Agency, Bristol, UK.

Richards, C. and Bacon, K.L. (1994). Influence of fine sediment on macroinvertebrate colonization of surface and hyporheic stream substrates. Great Basin Naturalist, 54, 106–113.

Richards, C., Haro R.J., Johnson L.B. and Host G.E. (1997). Catchment and reach-scale properties as indicators of macroinvertebrate species traits. Freshwater Biology, 37, 219-230.

Ryan PA. 1991. Environmental effects of sediment on New Zealand streams: a review. New Zealand Journal of Marine and Freshwater Research, 25, 207-221.

Statzner, B., Gore, J.A. and Resh, V.H. (1988). Hydraulic stream ecology: observed patterns & potential applications. Journal North American Benthological Society, 7, 307-360.

Walley, W.J. and, Fontama, V.N., 1998. Neural network predictors of average score per taxon and number of families at unpolluted river sites in Great Britain. Water Research 32, 3, 613-622.

Woodiwiss, F.S. (1964). The biological system of stream classification used by the Trent River Board, Chemy. Indust., 11, 443-447.

Wright, J.F. and Berrie, A.D. (1987). Ecological effects of groundwater pumping and a natural drought on upper reaches of a chalk stream. Regulatory Rivers, 1, 145-160.

Wright, J.F. (1992). Spatial and temporal occurrence of invertebrates in a chalk stream. Berkshire, England, Hydrobiologia, 248, 11-30.

Wright, J.F., Sutcliffe, D.W. and Furse, M.T. (2000). Assessing the Biological Quality of Fresh Waters. RIVPACS and Other Techniques. Freshwater Biological Association, Windermere.

Appendix 2 - Macro-invertebrate 3 min kick-sweep survey results for Site 1-3 in the River Test on the 2nd October 2017.

Site 1: River Test - Bossington Estate Benchmark Weir 2-10-17

Saprobic index	1.89
PSI	37.50
LIFE	7.00
CCI	15.35
BMWP	156
ASPT	5.78
NTAXA	27
R (species richness)	35
Mayfly species richness	2
EPT sp (taxa)	9
EPT ab	36
TOTAL ab	142
WHPT	158.1
WHPT ASPT	5.65
TRPI	50
Taxon	**Site 1**
Ephemera danica	10
Silo nigricornis	7
Perla bipunctata	2
Mystacides azurea	3
Brachycentrus subnubilus	1
Sericostoma personatum	3
Calopteryx splendens	3
Physa fontinalis	1
Rhyacophila dorsalis	1
Limnephilus lunatus	5
Theodoxus fluviatilis	1
Ancylus fluviatilis	1
Gammarus pulex	14
Tipula sp.	2
Haliplus flavicollis	2
Haliplidae	2
Dytiscidae	2
Oreodytes sanmarkii	1
Nebrioporus depressus	1
Elmis aenea	10
Limnius volckmari	1
Simulium ornatum group	5
Baetis sp.	4
Sialis lutaria	2
Bithynia leachi	14

Lymnaea palustris	1
Radix balthica	7
Gyraulus albus	10
Anisus vortex	1
Sphaerium sp.	13
Glossiphonia complanata	2
Erpobdella octoculata	1
Orthocladiinae	8
Tabanidae	1

3 Minnow (*Phoxinus phoxinus*)
4 3-spined stickleback (*Gasterosteus aculeatus*)
1 Soldier Fly - Stratiomyidae (*Oxycera pygmaea*)

Site 2: River Test - Bossington Estate Benchmark Weir 2-10-17

Saprobic index	1.83
PSI	76.92
LIFE	8.03
CCI	18.67
BMWP	154
ASPT	6.42
NTAXA	24
R (species richness)	31
Mayfly species richness	4
EPT sp (taxa)	14
EPT ab	110
TOTAL ab	501
WHPT	164.6
WHPT ASPT	6.58
TRPI	61.54
Taxon	**site 2**
Serratella ignita	7
Ephemera danica	1
Leuctra sp.	1
Silo nigricornis	2
Aphelocheirus aestivalis	29
Odontocerum albicorne	1
Brachycentrus subnubilus	1
Sericostoma personatum	9
Rhyacophila dorsalis	12
Rhyacophila septentrionis	1
Polycentropus	2
Theodoxus fluviatilis	5
Gammarus pulex	161
Brychius elevatus	1
Platambus maculatus	1
Gyrinidae	1
Limnius volckmari	45
Elmis aenea	101
Riolus subviolaceus	3
Hydropsyche pellucidula	28
Simulium sp.	17
Cheumatopsyche lepida	5
Baetis scambus	2
Baetis rhodani	38
Potamopyrgus antipodarium	8
Bithynia leachi	2
Gyraulus albus	3

Anisus vortex	5
Sphaerium sp.	7
Erpobdella octoculata	1
Orthocladiinae	1

Site 3: River Test - Bossington Estate Benchmark Weir 2-10-17

Saprobic index	1.83
PSI	27.66
LIFE	7.00
CCI	15.75
BMWP	119
ASPT	5.41
NTAXA	22
R (species richness)	29
Mayfly species richness	2
EPT sp (taxa)	7
EPT ab	108
TOTAL ab	304
WHPT	124.7
WHPT ASPT	5.20
TRPI	50
Taxon	**Site 3**
Ephemera danica	57
Silo nigricornis	2
Mystacides azurea	19
Sericostoma personatum	15
Limnephilus flavicornis	3
Theodoxus fluviatilis	18
Ancylus fluviatilis	2
Hydroptila sp.	2
Gammarus pulex	8
Haliplidae	1
Dytiscidae	3
Limnius volckmari	7
Elmis aenea	17
Riolus subviolaceus	12
Riolus cupreus	3
Polycelis felina	2
Centroptilum luteolum	10
Sialis lutaria	3
Potamopyrgus antipodarium	9
Bithynia leachi	49
Gyraulus albus	32
Anisus vortex	2
Planorbis carinatus	1
Sphaerium corneum	17
Glossiphonia complanata	1
Erpobdella octoculata	1
Asellus aquaticus	2
Orthocladiinae	5

Ceratopogonidae	1