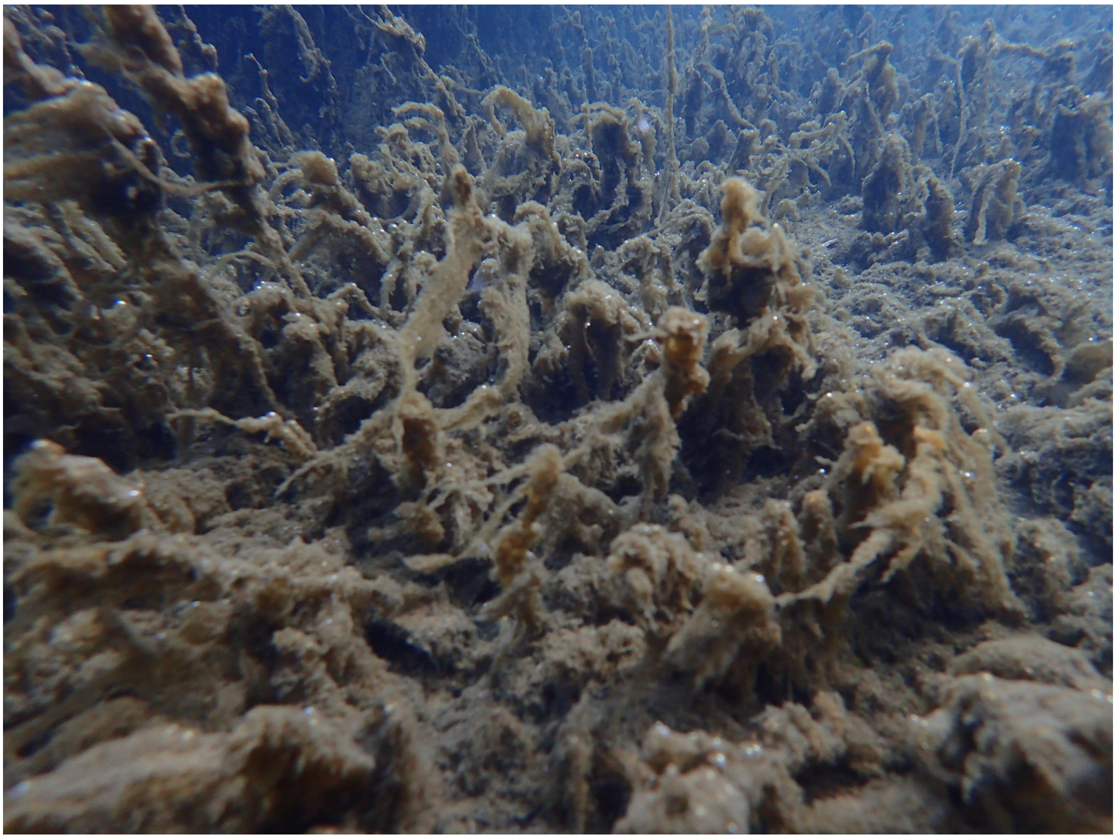
A SHORT REPORT ON THE BENTHIC GROWTH AND MACROINVERTEBRATE COMMUNITY SPECIES PROFILING IN THE UPPER RIVER ITCHEN



Completed on the 11th October 2018

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Executive Summary

The surveyed area of the upper River Itchen below Bakkavor's Alresford Salads' salad washing and packaging factory exhibited faunal and microphyte biological plus reflected chemical water quality signatures which were well below that expected for the headwaters of a SAC chalk river in 2018.
Please note that the term 'below Alresford Salads' is used throughout this report to spatially describe where the survey work took place in the upper River Itchen in 2018. It does not mean that the deleterious biological and associated chemical quality recorded in this reach of the river correlate with the activities at Alresford Salads *per se*.

Contents

Expert's qualifications and experience

I hold a IIi Honours degree in Zoology from the University of Hull (1982) and a Doctorate in Fisheries Ecotoxicology from Trent Nottingham University (1986). I am a registered Member of the Institute of Fisheries Management since 1987 and a Chartered Environmentalist since 2006. I have 35 years operational and research experience in applied aquatic environmental issues. I was a Senior Scientist at DAFS and the Principal Biologist plus environmental impact assessor for Severn Trent Water Ltd. from 1990-2011. For the last 17 years I have worked on ~40 aquatic pollution cases as an Expert Witness for the Courts. I have published ~20 research papers on a number of aquatic environmental issues including invertebrate sampling, sorting and biometric analysis. I am an accredited AQC tester for invertebrate sample analysis for e.g. the Environment Agency and Severn Trent Water Limited. I am an accredited invertebrate sampling and analytical trainer for Riverfly Partnership, Severn Trent Water Limited and SMARTrivers.

Study Limitations

This short report was prepared by Aquascience Consultancy Limited in accordance with the resources allocated by the client to this task. 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.

Background

An initial study of the upper River Itchen below Bakkavor's Alresford Salads factory was carried out on the 31st May 2018 by Dr. Everall when a professional 3-minute kick-sweep net (1- minute hand search) sample of the aquatic invertebrate community was taken at grid ref SU 59476 33411 below Alresford Salads (see Appendix 1). Biometrics for this reach of the River Itchen in the Spring of 2018 showed a biological signature of marked pesticide-complex chemical (SPEAR), sediment (PSI), nutrient P (TRPI), organic (Saprobic) and low flow (LIFE) impacts (see Appendix 1 data).

A biological growth was evident for the entire ~100m length of the River Itchen bed that Dr. Everall had legal access and sampling permission to at that time. Some photographs of the 'growth' and extent of the slime in the Spring of 2018 are shown in the photographs below.




A non-detailed microscopic examination of the growth by Dr. Everall, an expert sewage treatment river biologist and algologist, at the time revealed that the Spring 'growth' was predominantly composed of dead and live algae including blue-greens and aquatic fungi. In essence, it was a 'biological slime' or a 'sewage fungus' according to e.g. Fjerdingstad (1964) and Hellawell (1986).

Methodology

Following a general watercourse inspection 3-minute kick-sweep net sampling and 1-minute hand search sampling of macro-invertebrates was repeated at the survey reach in the upper River Itchen below Alresford Salads on the 5th October 2018. 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 adhered to 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.*, 2018). The samples were then immediately preserved in 70% Industrial Denatured Alcohol (IDA) at the riverside.

The sorting and analysis of all macroinvertebrate samples was in accordance with Environment Agency Best Practices (Environment Agency, 2009b). All the samples were washed and sorted using large stainless-steel sieves down to a final retaining sieve of 500um in size. 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-minute kick-sweep 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. All the sample information and resultant macro-invertebrate identification/counts was entered into the laboratory raw data sheets at that stage. All macro-invertebrate samples were identified to the lowest taxonomic resolution possible and with the exception of a few gnat larvae and worms this was mainly to species and very occasionally genus level.

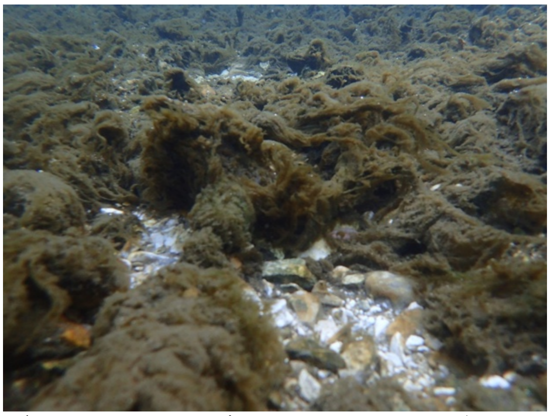
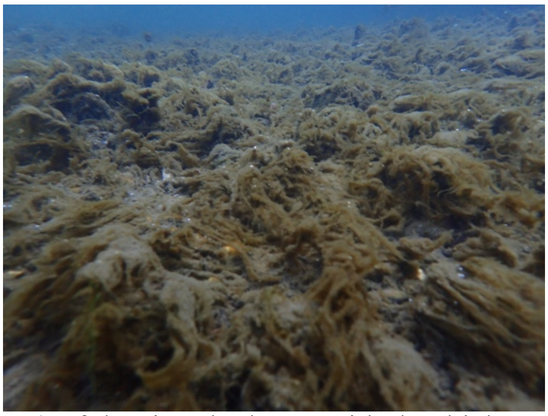
Several samples of the biological slime *per se*, which remained present in the autumn of 2018, were taken at various points throughout the survey reach of the River Itchen shown in part in the photograph below.

The samples of river bed growth were fixed in Lugols iodine, kept cold at 4 degree celsius and examined under high power microscopy within 48 hours of collection. A specimen sample was kept in Lugols iodine.

Findings

The appearance of the surveyed reach of the upper River Itchen below Alresford Salads on the 5th October 2018 was very similar to that in the Spring of 2018 as highlighted in the photographs below.



There was extensive area coverage (over 90%) of the river bed area with the thick biological growth or slime which upon microscopic examination was, aside to a bit of chalk and sediment adhesion, entirely composed of filamentous and attached algal and lesser fungal growth (see Appendix 3). The dominant and major composition of the biological growth covering meters of the bed of the River Itchen below Alresford Salads was, often filamentous, algae (diatoms, blue-greens and green algae) with some fungal component (Appendix 3) which was typical of organic and nutrient enriched benthic 'slime' or sewage fungus' (Fjerdingstad, 1964 and Hellawell, 1986). The middle ground of the pollution signature from the algal fingerprint of organic pollution was alpha-mesosaprobic and thus matched the biosignature of pollution from the invertebrate community analysis discussed below for the same survey site.

Various benchmarking work has been undertaken in interpreting microphyte or periphyton growths with respect to nutrient levels in both the UK with respect to WFD criteria (Mainstone, 2010) and historically in other countries e.g. New Zealand

(Biggs and Kilroy, 2000). I have highlighted below in red in the figure below where the microphyte or periphyton community signature observed at the upper River Itchen survey site in 2018 fits into picture on nutrient levels and therefore why such growth should be of concern.

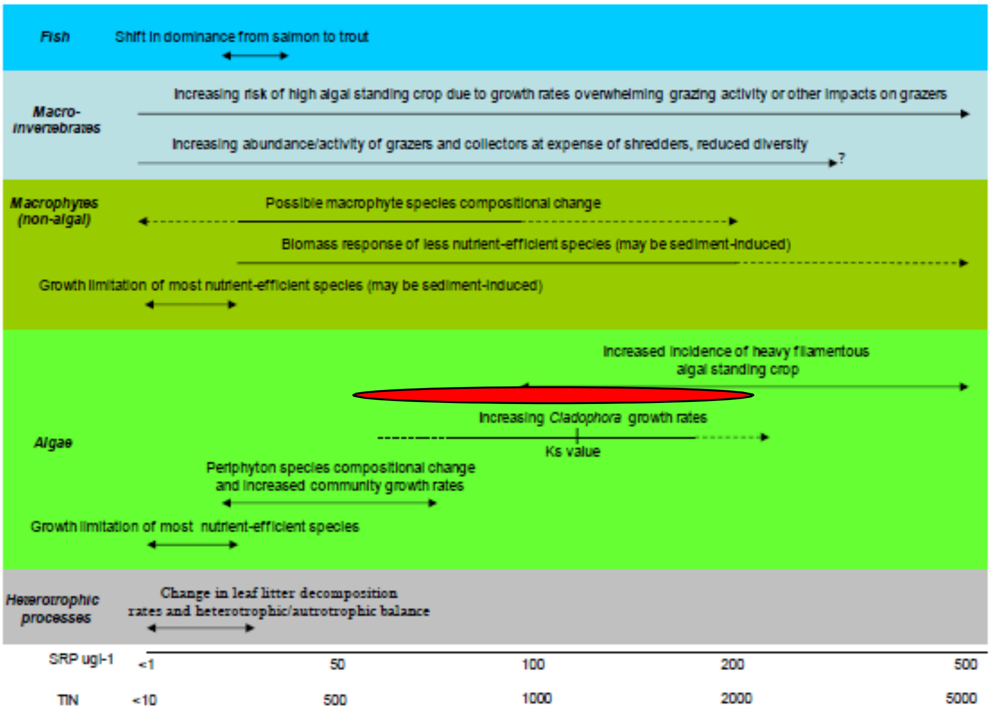


Fig. 1. Synthesis of reported biological changes in streams along a gradient of nutrient availability (from Mainstone, 2010).

Levels of 'phosphate' of 20-36mg/l have been recorded in the trade effluent from Alresford Salads in permit application source documents. However, it was not clear in the permit documentation for Alresford Salads if phosphorous measured in the plant washing discharge was Total phosphorous or soluble phosphorous (e.g. Soluble Reactive Phosphorous or ortho-phosphate) or what. It needed to be orthophosphate or total reactive phosphorous for assessing impact following dilution of trade effluent in the receiving watercourse. Given the modelled receiving watercourse (1.035 m/s) and trade effluent (0.015m/s) flows then, and only following full mixing, the receiving watercourse could contain ca. 290-500 ug/l ortho-phosphate which would be toxic to long-term survival of e.g. blue winged olive eggs (Everall *et. al.*, 2017) and appeared to be in the range of SRP associated with microphyte signatures from Figure 1 above that tallied with the observed benthic biological growth fingerprints in the receiving waters of the River Itchen (Appendix 3 e.g. *Cladophora* and associated heavy filamentous algal crop …. *Ulothrix*, *Oscillatoria* and *Stigeoclonium* species).

There may also be a source of organic and nutrient enrichment from an alleged septic tank adjacent to the study reach of the river. However, the marked biological growth on the bed of the upper River Itchen started well upstream of the alleged discharge location but immediately downstream of Alresford Salads. The alleged septic tank discharge, assuming there is one and it is active, could also be adding to the nutrient and organic enrichment of the receiving waters at this stage. Further field work is required to obtain a fuller understanding of all the organic and nutrient incursions in to this reach of the upper River Itchen. That said, a reduction of the chemical (e.g.

chloramine), organic and nutrient P indirectly or directly associated with the discharge from Alresford Salads would help in alleviating the pollutant signatures given the volumes and known nature of the watercress farm discharges.

The raw results from the 3-minute biological kick-sweep sample of macroinvertebrates from the surveyed reach of the upper River Itchen below Alresford Salads on the 5th October 2018 can be found in Appendix 2 of this short report. The surveyed watercourse exhibited a very similar biological signature to when it was sampled in the Spring of 2018 with marked pesticide-complex chemical (SPEAR), sediment (PSI), nutrient P (TRPI), organic (Saprobic) and low flow (LIFE) fingerprints. Some of the biological signatures have direct reference to associated chemical quality conditions (see Appendix 3 relevant sections).

For example, in both the Spring and Autumn of 2018 the aquatic invertebrate species Saprobic index or degree of organic enrichment in the upper River Itchen downstream of Alresford Salads was 2.70-2.76 which is described as alpha-mesosaprobic or polluted. Such waters typically exhibit Biological Oxygen Demand concentrations of 7-13 mg/l, ammonium levels of 0.5-several mg/l and dissolved oxygen capable of dropping to 2 mg/l. The complete lack of *Gammarus pulex* (freshwater shrimp) and the high numbers of organic (low dissolved oxygen, elevated BOD and ammonia) pollution tolerant *Asellus aquaticus* (water louse) and leeches (e.g. *Helobdella stagnalis*) was indicative of the wider invertebrate community fingerprint of organic enrichment. Other family metrics like the BMWP also reflected the state of the river survey area with a BMWP score of 66 reflecting a General Chemical Quality (GQA) grade of RE3/Fair/Grade C which is a course fishery status with inferred oxygen content of 60% saturation and BOD ~8 mg/l. Similarly, Average Scores Per Taxon (ASPT's) of 3.25 and 3.57 in the upper River Itchen downstream of Alresford Salads in the Spring and Autumn of 2018 are also classed as polluted

The surveyed area of the upper River Itchen below Alresford Salads exhibited faunal and microphyte biological plus reflected chemical water quality signatures which were well below that expected for the headwaters of a SAC chalk river in 2018.

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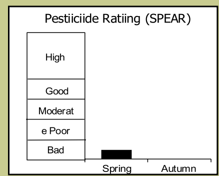
Appendix 1 - Biological water quality and ecological condition results for a 3-minute kick-sweep (1-minute hand search) net sample taken in the upper River Itchen 100-200m below Alresford Salads on the 31st May 2018.

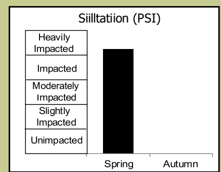
Salmon & Trout Conservation

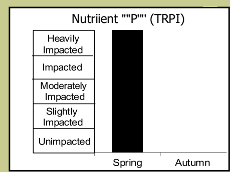
Species	Spring	Autumn
Beetles & Bugs		
Sigara	2	
Dytiscidae	1	
Caddisflies		
Chaetopteryx villosa	1	
Potamophylax	1	
Crustaceans		
Asellus aquaticus	277	
Ostracod	6	
Leeches & Flatworms		
Erpobdella octoculata	6	
Glossiphonia complanata	5	
Helobdella stagnalis	13	
Piscicola geometra	11	
Molluscs		
Radix balthica	17	
Anisus vortex	13	
Sphaeriidae	3	
True flies		
Tanytarsini	13	
Orthocladiinae	17	
Chironominae	4	
Tanypodinae	2	
Eristalis	1	

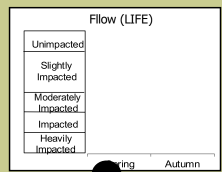
River Itchen - D/S Alresford Salads
31st May 2018
SU 59476 33411

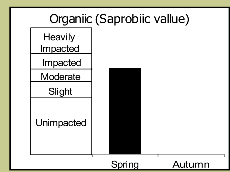
	Spring	Autumn
BMWP	41	.
ASPT	3.73	.
WHPT	39	.
ASPT	3.25	.
Number of Taxa	11	.
Riverfly - species	2	.
Riverfly - numbers	2	.
CCI	4.29	.
LIFE	6.17	.
PSI	15.38	.
SPEAR	5.06	.
TRPI 4	0.00	.
Saprobic	2.76	











Appendix 2 - Biological water quality and ecological condition results for a 3-minute kick-sweep (1-minute hand search) net sample taken in the upper River Itchen 100-200m below Alresford Salads on the 5th October 2018.

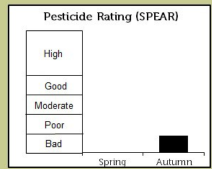
Salmon & Trout Conservation

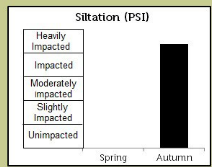
Species	Spring	Autumn
Alderflies		
Sialis lutaria		2
Beetles & Bugs		
Sigara		51
Dytiscidae		1
Oreodytes sanmarkii		1
Caddisflies		
Limnephilidae		1
Limnephilus lunatus		1
Tinodes waeneri		1
Crustaceans		
Asellus aquaticus		317
Crangonyx pseudogracilis		1
Leeches & Flatworms		
Dendrocoelum lacteum		1
Erpobdella octoculata		5
Glossiphonia complanata		3
Helobdella stagnalis		76
Molluscs		
Radix balthica		2
Physa fontinalis		23
Anisus vortex		6
True Flies		
Orthocladiinae		13
Prodiamesa olivacea		3
Tanypodinae		1
Tipula		1
Worms		
Oligochaeta		7
Other		
3-spined stickleback		7
9-spined stickleback		3
Bullhead		1

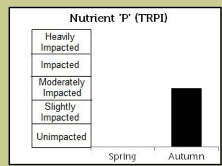
Species	Spring	Autumn

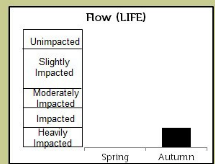
River Itchen - D/S Alresford Salads
5th October 2018

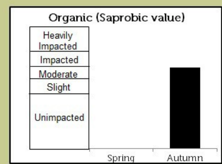
	Spring	Autumn
BMWP	.	66
ASPT	.	4.13
WHPT	.	57
ASPT	.	3.57
Number of Taxa	.	16
Riverfly - species	.	3
Riverfly - numbers	.	3
CCI	.	3.50
LIFE	.	6.00
PSI	.	12.50
SPEAR	.	9.71
TRPI 4	.	50.00
Saprobic	.	2.70











Appendix 3 - Microscopic analysis of slime from bed of upper River Itchen below Alresford Salads on the 5th October 2018.

AQUASCIENCE CONSULTANCY LIMITED ALGAL STATUS REPORT

Date (sample) 5-10-18 Sample Numbers AQ # 1
Site: Upper R. Itchen below Alresford Salads 2140/001/01 See COMMENTS belo

ANALYTICAL RESULTS	Fields of View [50] x 10	
Algal Type	Algae: rel. composition	**Comments**
BACILLARIOPHYTA (Diatoms)		
Melosira varians	F-C	*Associated with 'sewage fungus'
Meridion circulare	C	*Associated with 'sewage fungus'
Navicula sp.	C	*Associated with 'sewage fungus'
Pinnularia sp.	P	
Synedra sp.	C	*Associated with 'sewage fungus'
Nitzschia sigmoidea	F	
Diatoma sp.	C	*Associated with 'sewage fungus'
Gomphonema sp.	P	
Cocconeis sp.	P	
Amphinemura sp.	P	
Staurosirella sp.	C	
CYANOPHYTA (Blue-green algae)		
Oscillatoria sp.	A	*Polysaprobic
Phormidium sp.	C	*Polysaprobic
Merismopedia sp.	F	
CHLOROPHYTA (Green algae)		
Ulothrix sp.	A	*Alpha-mesosaprobic
Cladophora sp. ('blanket weed')	C	*Beta-mesosaprobic
Pediastrum sp.	C	*Beta-mesosaprobic
Stigeoclonium sp.	F	*Alpha-mesosaprobic
Monoraphidium sp.	P	
Aquatic FUNGI (hyphomycetes)	F-C	*Associated with 'sewage fungus'
Rotifers	**F**	
Abundance in fields of view key: VA (Very Abundant), Abundant (Abundant), C (Common), F (Few) and P (Present)		

* Algae and other organisms indicative of varying degrees of organic or nutrient pollution

Received: 6 / 10 / 18 Analysed: 7 / 10 / 18 Analyst: Dr. Nick Everall

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Appendix 4 - Details of biometric testing used on the species macroinvertebrate community data from the upper River Itchen watercourse survey site on the 5th October 2018.

Siltation from Proportion of Sediment-sensitive Invertebrates or PSI

Physical assessment methods have traditionally been used to quantify riverine sedimentation, but Extence *et. al.* (2010) 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. Such techniques have also been used successfully at a large catchment scale to assess the spatial and temporal patterns of siltation in a watershed (Extence *et. al*., 2010, Everall, 2010 and Extence *et. al*., 2011). 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:

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

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.* (2010), 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.* (2010) suggested that when applied to species and family data respectively, the terms PSI (S) and PSI (F) are used. A provisional interpretation scheme for the data is shown in Table 3 below (Extence *et. al.*, 2010).

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 velocity conditions from Lotic Invertebrate Flow Evaluation or 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*. A close correlation anticipated by Extence *et al*., (2011) 'in many instances' was between LIFE and PSI. Extence *et al*., (2011) devised PSI and Extence *et al*., (1999) devised LIFE, but the relationship in the field has not been tested until recently. There was a clear correlation ($r=0.91$, $p<0.01$) between PSI and Life in the Everall (2010) study as recently highlighted in Farmer (2010). It may seem logical that affinity for high flows and low siltation are related, but this was not the complete picture in all preliminary studies to date. Extence *et. al.* (1999) suggested that when applied to species and family data respectively, the terms LIFE (S) and LIFE (F) are used.

Organic pollution and enrichment from Saprobic index

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 and family 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 shown in Table 4 below:

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::

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

$\sum A*G$

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 shown in Table 5 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	**extremeiy polluted**	**polysaprobic**	**3,5-<4,0**	**15**	**several mg/L**	**<2**

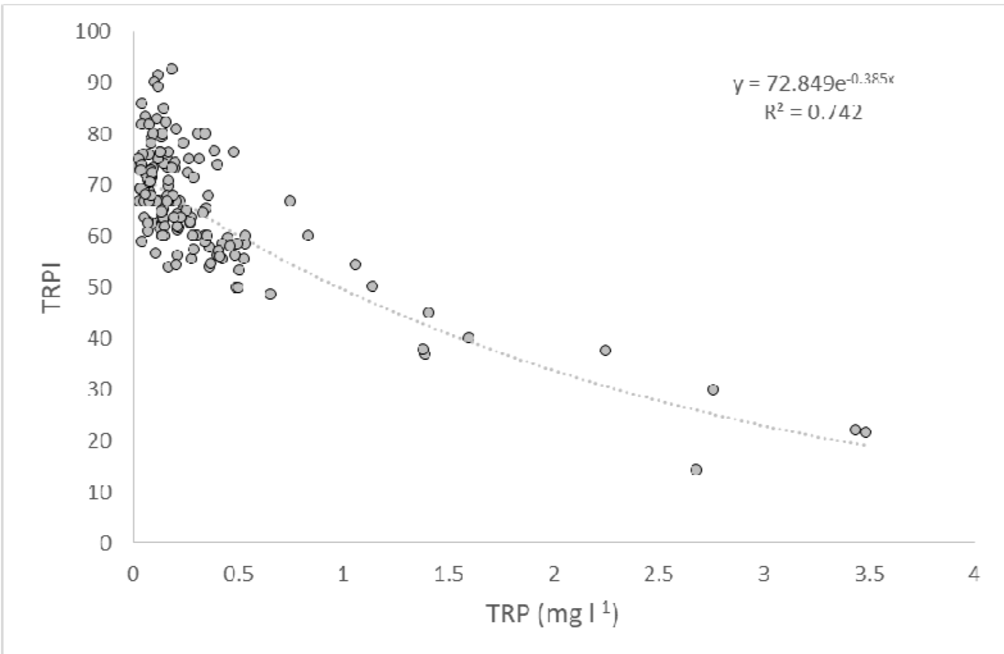
For EA GQA dataset the macroinvertebrate results for organic enrichment expressed as Saprobic index are in fact the best mix of family with species resolution data when and where available.

Inorganic (Phosphorous) enrichment from Total Reactive Phosphorous Index or 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). More recently in Everall *et. al.* (2018) for nutrient P enrichment (TRPI) with the direct chemical relationship shown in the graph below.



The Total Reactive Phosphorous Index or TRPI describes the TRP-sensitive taxa groupings in Table 6 overleaf present in a sample and the metric is calculated from the formula below using the 'look up' matrix shown in Table 6 below:

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

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 6 - 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 7 - 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 8 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 8 - 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]

Aquatic faunal community richness and rarity from Community Conservation Index or CCI

CCI scores account for the community richness and the relative rarity of species present (Chad & Extence, 2004). It is a 'tool to assist in value judgement' and 'must never be allowed to preclude expert opinion' (Chad & Extence, 2004). Conservation scores are assigned to species based on definitions outlined in Chad & Extence (2004), added together and divided by the number of scoring taxa. This figure is then multiplied by a community score. This is based on the highest conservation value species or on the BMWP score, if that gives a higher value.

Organic enrichment from The Walley Hawkes Paisley Trigg (WHPT) index

WHPT is thought to be more accurate than BMWP because it was derived from an analysis of a very large set of field results (more than 100,000 standard samples) from across the UK rather than on expert judgement, sometimes based on limited knowledge available in the late 1970s. However, there is an argument that WHPT category boundaries were derived for clean or 'pristine' sites using reference site data at a later time when few sites remained either clean or 'pristine'. That said, WHPT-ASPT (average score per taxon based on WHPT) responds to the same environmental pressures as BMWP-ASPT (average score per taxon based on BMWP). This includes organic discharges and the pressures associated with them, such as increases in organic loading, the concentrations of nutrients, ammonia and suspended solids, reduction in oxygen concentration and saturation, and habitat degradation, including reduced habitat diversity and increased siltation. It will therefore respond to other activities that cause these pressures, including industrial discharges, reductions in flow, habitat degradation and eutrophication. Unlike BMWP-ASPT, WHPT-ASPT will respond to activities that affect the abundance of different invertebrates, which should improve its ability to distinguish moderate degradation in quality.

WHPT-Ntaxa (number of scoring taxa based on all taxa included in WHPT) responds to the same environmental pressures as BMWP-Ntaxa (number of BMWP-scoring taxa). It responds to most environmental pressures including organic pollution, habitat degradation, acidification and toxic pollution from a wide range of pollutants including metals. Whereas BMWP is based on analysis of 82 taxa, WHPT is based on 106 taxa, so its sensitivity is slightly different.

WHPT better reflects the quality of the invertebrate community because it is based on a wider range of taxa (mostly families), in particular, WHPT includes more families of Diptera and some families that were grouped together in BMWP (known as BMWP composite taxa) are considered separately in WHPT. WHPT index values are on the same scale as BMWP. There is an intentionally very high correlation between BMWP-ASPT and WHPT-ASPT, so anyone familiar with BMWP-ASPT will automatically be familiar with WHPT-ASPT. However, values of indices at individual sites or types of sites may differ because of the improved accuracy of WHPT and its ability to respond to degradation affecting the abundances of each taxon. It should be rememberd that this metric still essentially remains only at family level versus other species and abundance level metrics for assessing organic enrichment like Saprobic index. There are no direct observed water quality bandings for WHPT, unlike BMWP and the score relies on Observed:Expected ratio values generated from family level faunal data via RIVPACS/RICT.

Pesticide fingerprint from SPEcies At Risk or SPEAR

The difficulties in detection and evaluation of pesticide effects on aquatic ecosystems are two-fold. First, specific exposure patterns hinder chemical monitoring of pesticide concentrations - pesticide pollution is mostly diffuse and transient and often occurs at low concentrations. Second, variability in biological communities – there is a high diversity in communities inhabiting freshwaters, and these communities are affected by numerous factors including natural and anthropogenic stressors that may confound effects of pesticides. All this makes attributing any observed changes in biological communities to pesticide contamination complicated. To tackle these difficulties, the pesticide-specific bioindicator system SPEAR (SPEcies At Risk) was developed (Liess et al., 2001; Liess and Von der Ohe, 2005) and successfully employed to link pesticide exposure and effects (namely insecticide toxicity of pesticides) (Scha¨fer et al., 2007; Liess et al., 2008). The main advantage of the SPEAR system is that this system is based on biological traits of stream invertebrates, and not on taxonomic composition or abundance parameters like many conventional bioassessment indices (e.g. EPT, Lenat, 1988). Therefore, it is relatively independent from confounding factors, and application of this system is not constrained by geographical and geomorphological factors and associated differences in biological communities. Furthermore, missing information on biological traits of species can be extrapolated from closest phylogenetic relatives for which such information is available. The SPEAR bioindicator is based on biological traits responsive to the effects of pesticides (i.e. insecticide toxicity of pesticides – physiological sensitivity, spatio-temporal co-occurrence of organisms and toxicants) and post-contamination recovery (generation time, migration ability).

After defining the ‘‘species and families at risk’’ the SPEARpesticides index was computed as relative abundance of these taxa for each site and date as follows:

$$\mathrm{SPEAR}_{\mathrm{pesticides}} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \cdot y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100$$

where n is the number of taxa, xi is the abundance of the taxon i and y is 1 if taxon i is classified as ''at risk'', otherwise 0. These calculations were performed for the lowest possible identified taxonomic levels (downto species level) to define SPEAR(sp)pesticides and for the families to define SPEAR(fm)pesticides.

Stress bandings for SPEAR classes have been defined (Von der Ohe *et. al*., 2007; Beketov *et al*., 2009) that allow the application of the indicator within the framework of the European Water Framework directive (CEC, 2000). They are provided in the sample data result graphs for Severn Trent Water biological samples.

Other useful info:

Organic enrichment from The Biological Monitoring Working Party score or BMWP)

BMWP scores are generally used by the Environment Agency as a proxy measure of the levels of organic pollution acting upon a site where a sample was taken. There are equivalents in other countries for example in Spain it is called iBMWP, the Iberian BMWP. It is a biotic index calculated by scoring taxa, mostly at a family level, in terms of organic pollution sensitivity. The scores number from one to ten, with ten being the least tolerant to organic pollution. It takes no account of abundance or resolution to species level and each taxa only scores once. The scores are accumulative and high scores indicate better water quality as reflected by the presence of scoring taxa of the invertebrate community. BMWP scores are highly dependent on number of taxa (Alvarez-Cabra et al., 2010). High scores represent both tolerance and taxonomic richness (Environment Agency, 2009) within the limits set by the methodology.

The Average Score Per Taxon (ASPT) can be calculated from the BMWP score by dividing by the number of BMWP scoring taxa (NTAX). This gives an index from 0 to 10 and takes more account of low scores to moderate the index instead of contributing to it. It also reduces the effects of different sampling efforts. This metric is used by RIVPACS (River Invertebrate Prediction And Classification System) (Wright et al., 1984; Wright et al., 2000) to help predict the expected community structure of a clean river based on reference sites with similar abiotic conditions.

These metrics are used by the E.A. as they are relatively simple to collect and will pick up any large changes in water quality.

Further useful biometric info:

Interpretation of 'old' BMWP with old river classes and water chemistry

BMWP Score	River Ecosystem Class	Inferred General Chemical Quality (GQA) Grade & Typical Use		Old RPS Class	Inferred O_2 content 90% readings above :	Inferred BOD (ATU) 90% readings below:	Inferred NH_3 95% below:
>96	RE 1	Good - A	Potable, Game Fishery, High Amenity Value	1A	80% Saturation	2.5 mg/l	0.25 mg/l
71 - 95	RE 2	Good - B	Potable, Game Fishery, High Amenity Value	1B	70% Sat.	4.0 mg/l	0.6 mg/l
51 - 70	RE 3	Fair - C	Potable (After Advanced Treatment) Coarse Fishery	2	60% Sat.	6.0 mg/l	1.3 mg/l
36 - 50	RE 4	Fair - D	Poor Industrial use . No Fishery / Little Amenity Value	3	50% Sat.	8.0 mg/l	2.5 mg/l
13 - 35	RE 5	Poor - E	Not Useable , Grossly Polluted , May Cause Nuisance	4	20% Sat.	15 mg/l	9.0 mg/l
0 - 12	--	Bad - F	Not useable. Nuisance likely in Urban Areas	--	--	--	--

Appendix 4 -