Milton-under-Wychwood Neighbourhood Plan

Appendix 10

Environment Agency review of monitoring data from the Littlestock Brook

March 2021



A review of macroinvertebrate and water quality monitoring data from the Littlestock Brook.

Date: March 2021

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Executive summary

In late June 2020 a Riverfly survey reported a target level breach, alerting us to a potential issue with water quality in the Littlestock Brook, downstream of the Milton-under-Wychwood sewage treatment works (STW). The breach was investigated by the Environment Agency, examining the macroinvertebrate community present at multiple sites along the Brook, collected in 3-minute kick samples. Assessment was by *in-situ* field identification and later laboratory analysis of the same samples. This report presents the findings of this work and examines other relevant long-term monitoring data collected by the Environment Agency and Riverfly volunteers.

Although a breach in the Riverfly score was not confirmed in our field study, it returned a low score relative to an upstream reference point. The subsequent laboratory analysis showed a reasonably diverse community to be present and a comparable Riverfly score to that recorded upstream. However, there were notable increases in the abundance of some taxa (e.g. Gammarus, mud snails and true flies) downstream of the STW. These increases, relative to upstream are indicative of organic enrichment, associated with sewage effluent. The persistence of pollution-sensitive taxa downstream (e.g. Blue-winged olive) suggest the impact is a chronic one, rather than an acute pollution.

The habitat at the Riverfly breach site contrasts with conditions upstream of this point. The former is a little wider and shallower, with more sediment and algal cover. These conditions, whilst partially a function of the contribution of enriched effluent, are also likely to be influencing the observed macroinvertebrate community. It may also help explain why field and laboratory observations differ so widely, with taxa harder to spot with the naked eye amongst the weed and sediment.

Continued monitoring is recommended to help disentangle the relative influences of organic enrichment and habitat, downstream of the STW. The use of an extended Riverfly taxa list which also considers the presence and relative proportions of pollution tolerant taxa, may also help in providing a clearer signal of any future pollution events.

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1. Background

The Littlestock Brook is a small tributary of the River Evenlode in West Oxfordshire, situated in a predominately rural catchment. The Brook is approximately 5km in length flowing east from its source and joining the River Evenlode just north of the villages of Milton and Shipton-under-Wychwood. For the majority of its length the brook is underlain by Lias clay (Charmouth Mudstone Formation). A sewage treatment works (STW) serving the villages of Milton and Shipton-Under-Wychwood has a permit to discharge sewage effluent in to the brook approximately 700m above the confluence with the River Evenlode.

In recent years local concerns have been raised regarding the functioning of the STW and the impact on the effluent discharge and storming on the brook. In response the Environment Agency increased the frequency of water quality sampling at its monitoring point on the brook (downstream of the STW) in 2019. A local Riverfly group has also carried out routine monitoring for river flies since 2017 at the same site, reporting this against a trigger level established in consultation with the Environment Agency.

On 25 June 2020, a breach of the Riverfly trigger level was detected and, following a second confirmatory sample, on 14 July 2020 the Environment Agency were informed. The Environment Agency attended the site on 24 July, where they repeated the sampling and returned additional samples for laboratory analysis. The field results found a low Riverfly score at the same site but above the breach level. The results were provided to the Riverfly group as a brief report. This report builds on these initial findings, examining the laboratory analysis in combination with the routine water quality monitoring data.

2. Monitoring

Site and sampling details

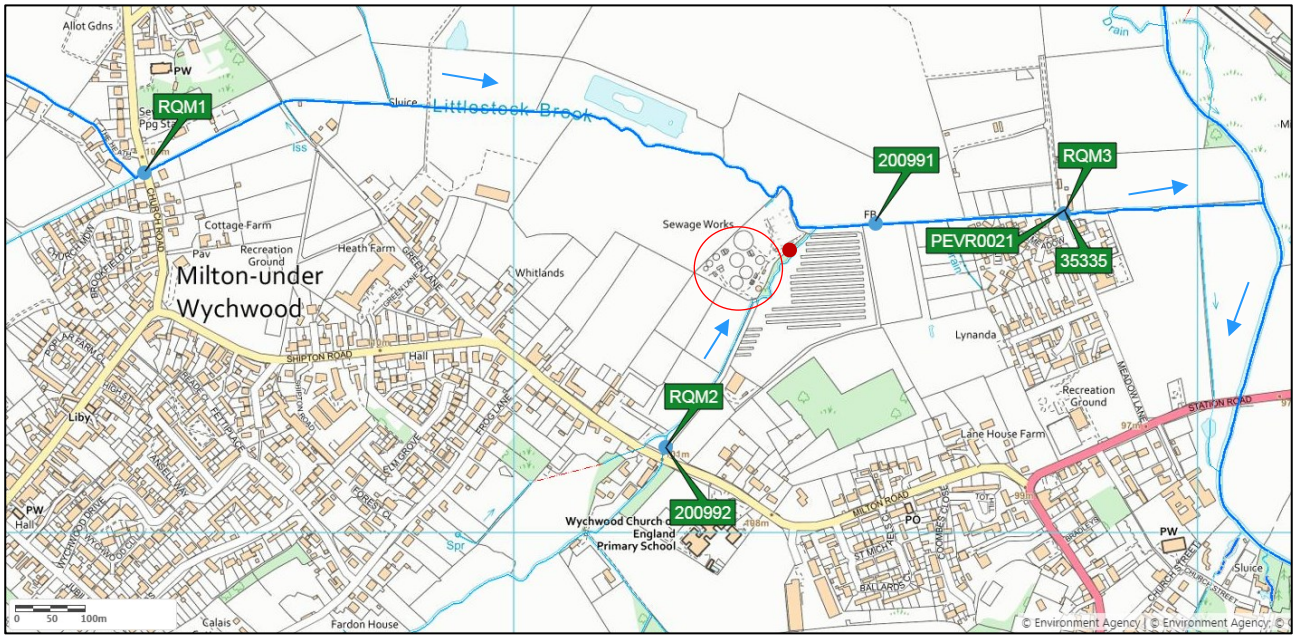
The Environment Agency has a routine monitoring point on the Littlestock Brook (site ID: 35335 / PEVR0021) located 370m downstream of the STW outfall and approximately 300m upstream from the confluence with the River Evenlode. The site is used for the collection of ecology (macroinvertebrate, diatoms and plants) and water chemistry samples. Data from this site are used in the determination of the Water Framework Directive (WFD) classification for the Littlestock Brook water body (GB106039029910). In response to the recent Riverfly trigger level breach, two additional monitoring sites, one upstream (site ID: 200992) and one 90m downstream (site ID: 200991) of the STW, were established and surveyed for macroinvertebrates (Table 1, Figure 1).

Ecology: All Environment Agency macroinvertebrate samples are collected using a standardised 3-minute kick sample with a 1-minute hand search technique (Murray-Bligh., 1999). On the Littlestock Brook, in line with standard practice, samples have been collected from sample point 35335 in spring (Mar-May) and autumn (Sept–Nov). This sample record dates back to 1989, and monitored since on a roughly 3 year biannual cycle. A few samples have also been collected) in the summer (Jun-Aug). All samples are preserved and returned to the laboratory for analysis.

Water Chemistry: The Environment Agency routinely collects water chemistry samples from site PERV0021. Samples have been collected since the early 1980s, although the frequency of sampling has varied. Since 2013 sampling has mostly been undertaken on a quarterly basis (exceptions being 2017 & 2018) but in 2019 more frequent sampling was established with a total of 9 monthly samples analysed over the year. Field measurements including dissolved oxygen, temperature and pH are undertaken in-situ using a hand held meter (YSI Pro DSS or Pro Plus handheld). Water samples are returned for laboratory analysis for other determinants including ammonia and orthophosphate.

Riverfly monitoring is carried out by the local Riverfly group at three locations on the Littlestock Brook: two sites upstream of the STW at sites (RMQ1 & RMQ2) and one downstream (RMQ3), since 2017 (Table 1, Figure 1). Trained volunteers collect macroinvertebrate samples using the same kick sample technique as the Environment Agency, referenced above. All samples are analysed in the field. The focus of this field assessment is on the presence and abundance of specific river fly larvae and freshwater shrimps. Water quality samples are also collected by volunteers who use colorimetric field test kits to detect phosphate and nitrate, within concentration ranges.

Figure. 1. Environment Agency and Riverfly monitoring locations on the Littlestock Brook.



Note: RQM codes refer to Riverfly sample points, all other codes refer to EA points. Some sample points are collocated (see Table 1). Blue arrows - direction of river flow. Red circle – location of STW. Red dot – approximate location of STW outfall. Ordnance Survey map produced under licence 100024198. Crown copyright and database rights 2021.

Table 1. Details of monitoring site on the Littlestock Brook.

Site ID	OS Grid Ref	Location relative to STW	Source	Purpose	Dataset	No. of samples
RQM1	SP2646018526	Upstream	Riverfly group	Macro-invertebrates	2017 - 2020	17
RQM2*	SP2722218124	Upstream trib.	Riverfly group	Macro-invertebrates	2017 – 2020	17
200992*	SP2722218124	Upstream trib.	EA	Macro-invertebrates	2000	1
200991	SP2752918451	Down-stream	EA	Macro-invertebrates	2000	1
RQM3**	SP2780418466	Down-stream	Riverfly group	Macro-invertebrates	2017 - 2020	19
35335**	SP2780418466	Down-stream	EA	Macro-invertebrates #	1980 – 2020	20
PEVR0021	SP2780418466	Down-stream	EA	Water quality	1980 - present	302

Note1: The length of the dataset does not imply samples collected in all years.
*Note2: * Denotes co-located sites RQM2 & 200992, ** denotes co-located sites RQM3 & 35335.*
Note3: # sample record for site 35335 - includes one macrophyte survey (2014) & one diatom sample (2008).
Note4: RQM2 / 20092 located on small tributary of Littlestock Brook, approx.400m upstream of confluence with Brook.

Sample processing & assessment

Environment Agency

All routine 3-minute kick samples collected by the Environment Agency are sorted in a laboratory and macroinvertebrates identified using a light microscope. Since 2002 all fauna have been identified to 'mixed taxon' level, meaning that most taxa are taken to species level with the exception of certain groups, harder to identify, such as midges and worms. Prior to 2002, taxa were usually identified to Family level. The total abundance of each taxa identified in a sample is recorded as the exact number found (where there are less than 10 individuals) or estimated from count data where greater than 10 individuals are detected. Additionally, formal quality assurance was introduced to the laboratory sorting and identification process in 2000. Prior to this, checks on identification and abundance, were less rigorous.

The Environment Agency uses standard indices to help assess and classify the ecological condition of a water body. The Walley Hawkes Paisley Trigg (WHPT) index is used for river

macroinvertebrate assessment. The index works by assigning scores to different taxa based on their tolerance to environmental pressures, with the most sensitive taxa scoring highest. The index is usually expressed in terms of the average score per taxon (WHPT ASPT) or the number of scoring taxa (WHPT N-taxa). These metric are abundance weighted and tuned to detecting organic enrichment as well as other pressure and influences such as habitat degradation and the impact of toxic substances. In addition to these, other indices have been developed, designed to response to different pressures. The Proportion of Sediment-sensitive Invertebrates (PSI) index (Extence *et al.*, 2013) is used to gauge the impact of excess fine sediment and suspended solids on macroinvertebrates communities. It is an inverse index where low values equate to more fine sediment being present.

Water chemistry samples are routinely collected from rivers for a variety of purposes that include WFD status monitoring. The range of chemical determinands that are measured depends on the purpose and scope of the monitoring programme. For the WFD status monitoring this usually comprises; field measurements for dissolved oxygen, temperature, pH and conductivity and laboratory analysed water samples for phosphate, ammonia, nitrogen and alkalinity. All analysed is carried out at one of the Environment Agency's national laboratories using UKAS certified methods.

Riverfly partnership

Riverfly samples are analysed in the field, and the abundances of eight key pollution sensitive taxa recorded. The sum of these taxa gives a combined Riverfly score which can be used to assess water quality. Site specific 'trigger levels', set in consultation with the Environment Agency, act as an alert to when a score falls below the total score expected for that site, under normal conditions. The trigger score is set by reviewing Riverfly and Environment Agency monitoring data to identify trends and natural variation over time. For the Littlestock Brook Riverfly sites the trigger value is currently set at 4. This is a fairly low score which, in part, reflects the nature of the Brook, a small low energy stream where, under natural conditions, not all Riverfly taxa would be expected to be present.

3. Riverfly trigger level breach

Event

On the 25 June 2020 a trigger level breach was recorded downstream of the STW at Riverfly monitoring site RMQ3 (EA Site ID: 35335) by a Riverfly volunteer. A score of 2 was logged (trigger level = 4). In line with the Riverfly survey protocol the volunteer alerted their Riverfly coordinator and the Environment Agency on 14 July. The Riverfly volunteer repeated the sampling on 15 July, confirming the breach (a score of 2) and alerting the Environment Agency. The event was logged via the Environment Agency incident hotline by the volunteer on 22 July 2020.

Response

The Environment Agency visited the site on 24 July 2020, 10 days after the confirmation of the trigger level breach and less timely than we would have liked. However, we were working in line with our national policy and safety guidelines for incident response during the COVID pandemic. The aim of the visit was to examine the macroinvertebrate community up and downstream of the STW to ascertain if the trigger level breach was continuing and to assess any impact from the STW on the macroinvertebrate community. Standard 3-min. kick samples were taken at the Riverfly site, where the breach had been recorded (RQM3, EA Site 35335), and at two locations upstream (Figure 1, Table 1). Each sample was sorted on the bankside and Riverfly scores derived (Table 2). Samples were placed in separate containers and preserved in industrial methylated spirits, for later laboratory analysis. A summary report, which documented a low Riverfly score at RQM3 but not at an additional site downstream of the STW, was produced and provided to the Riverfly group. The full taxa lists from the 3-minute kick samples, analysed in the laboratory, are provided in the Appendix A1. The main groups of taxa recorded and their abundance are summarised in Table 3, with the key biological indices and Riverfly scores.

Table 2. Riverfly monitoring results from bankside assessment of 3 min. kick samples collected by the Environment Agency on 24 July 2020

	Site ID: 200992 (RQM2)		**Site ID: 200991**		**Site ID: 35335** (RQM3)	
Distance from STW	350m upstream of STW outfall		90m downstream		370m downstream	
Riverfly taxa	**Abundance**	**Riverfly score**	**Abundance**	**Riverfly score**	**Abundance**	**Riverfly score**
Cased caddis	5	1	1	1	1	1
Caseless caddis	1	1	1	1	-	-
Mayfly (Ephemeridae)	-		2	1		
Blue winged olive (Ephemerellidae)	8	1	3	1	3	1
Flat bodied (Heptageniidae)	-	-	-	-	-	-
Olives (Baetidae)	10	2	15	2	1	1
Stoneflies	-	-	-	-	-	-
Freshwater Shrimp	500	3	100	3	22	2
Total Riverfly score		**8**		**9**		**5**

Table 3. Biological indices derived from laboratory analysis of 3-min. kick samples collected by the Environment Agency on 24 July 2020

	Site ID: 200992 (RQM2)	**Site ID: 200991**	**Site ID: 35335** (RQM3)
Distance from STW	350m upstream of STW outfall*	90m downstream	370m downstream
Taxa groups	**Abundance**	**Abundance**	**Abundance**
Cased caddis flies	15	14	52
Caseless caddis flies	5	3	1
Mayflies (all)	67	73	75
Stoneflies	2	3	4
Shrimps	1,170	550	93
Beetles	28	29	53
Snails	0	275	383
Pea mussels	9	10	39
Water hoglouse	0	13	1
Leeches	0	2	0
True flies (Blackfly)	8	45	5
True flies (Chironomid)	47	100	400
True flies (others)	20	27	17
Worms	10	11	31
Other	8	15	8
Total abundance	1389	1169	1162
Biological Indices			
WHPT	121.8	134.8	143.1
WHPT-NTaxa	19	26	25
WHPT-ASPT	6.41	5.18	5.72
PSI	88.57	52.08	60
Riverfly score**	12	10	11

Note - Site located on small tributary of Littlestock Brook, approx.400m upstream of confluence with Brook.*
*Note** - The Riverfly scores presented in the above table, are calculated from laboratory analysis. Due to the greater level of scrutiny possible under laboratory conditions these scores are notably higher than those recorded in the field (Table 2). Laboratory derived scores are therefore not directly comparable to bankside data but are helpful in providing a more accurate account of the Riverfly community present at the sampled sites.*

4. Data review

In assessing the severity of the Riverfly breach, 3 datasets were examined:

a) Data collected by the Riverfly group at 3 sites on the Littlestock Brook, since 2017.

b) Data collected by the Environment Agency on macroinvertebrates in the Littlestock Brook at 3 sites in July 2020.

c) Data collected by the Environment Agency on water quality and macroinvertebrates since 2003[1] at the Littlestock Brook long-term monitoring site, downstream of the STW.

a) Riverfly data from 2017 – 2020

Data collection started in the autumn of 2017, at the 3 sites identified in Table 1, Figure 1 (RQM1, 2 &3). A total of 53 Riverfly records are documented up to December 2020, with the majority of data concentrated between 2018-2019. Examining the Riverfly scores and abundance data the following trends are apparent:

i. Over the duration of the monitoring period the highest Riverfly scores are from the upstream (control) site on the Littlestock Brook (RQM1). The difference between scores recorded at RQM1 and at RQM3 (downstream of the STW) is significant (Appendix B). The difference in these data appears to be driven by higher seasonal scores in the spring (Figure 2). However, there is no significant difference between scores at RQM1 and RQM2, or between RQM2 and RQM3. Variation in scores between years is not significant (Appendix B).

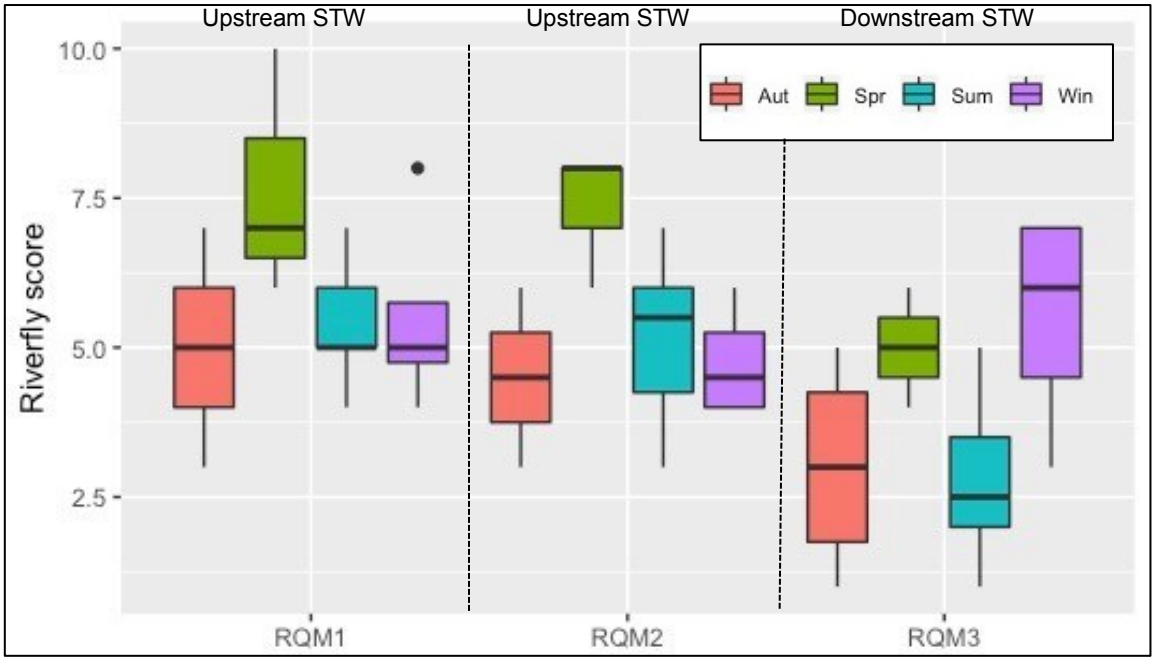


Figure 2: Boxplot of Riverfly scores by season at the 3 monitoring sites on the Littlestock Brook (RQM1 & RQM3) and tributary (RQM2), 2017-2020. These plots display the median value (thick black line) with the 1st (25%) and 3rd (75%) quartiles (coloured boxes), and the extent of the largest and smallest values within a range from the quartile (whisker). Beyond this range values are considered statistical outliers and plotted as points.

[1] Whilst more historic data is available, method changes and quality assurance processes prior to this date make this data less suitable for assessment. These data are provided as appendices.

ii. Freshwater shrimps (*Gammarus*) are the most abundant taxa, with highest abundance recorded at the upstream sites RQM1 & RQM2. This contrasts starkly with significantly lower abundance downstream at RQM3. There is a slight seasonal pattern across all sites, with higher abundance in autumn and winter (Figure 3).

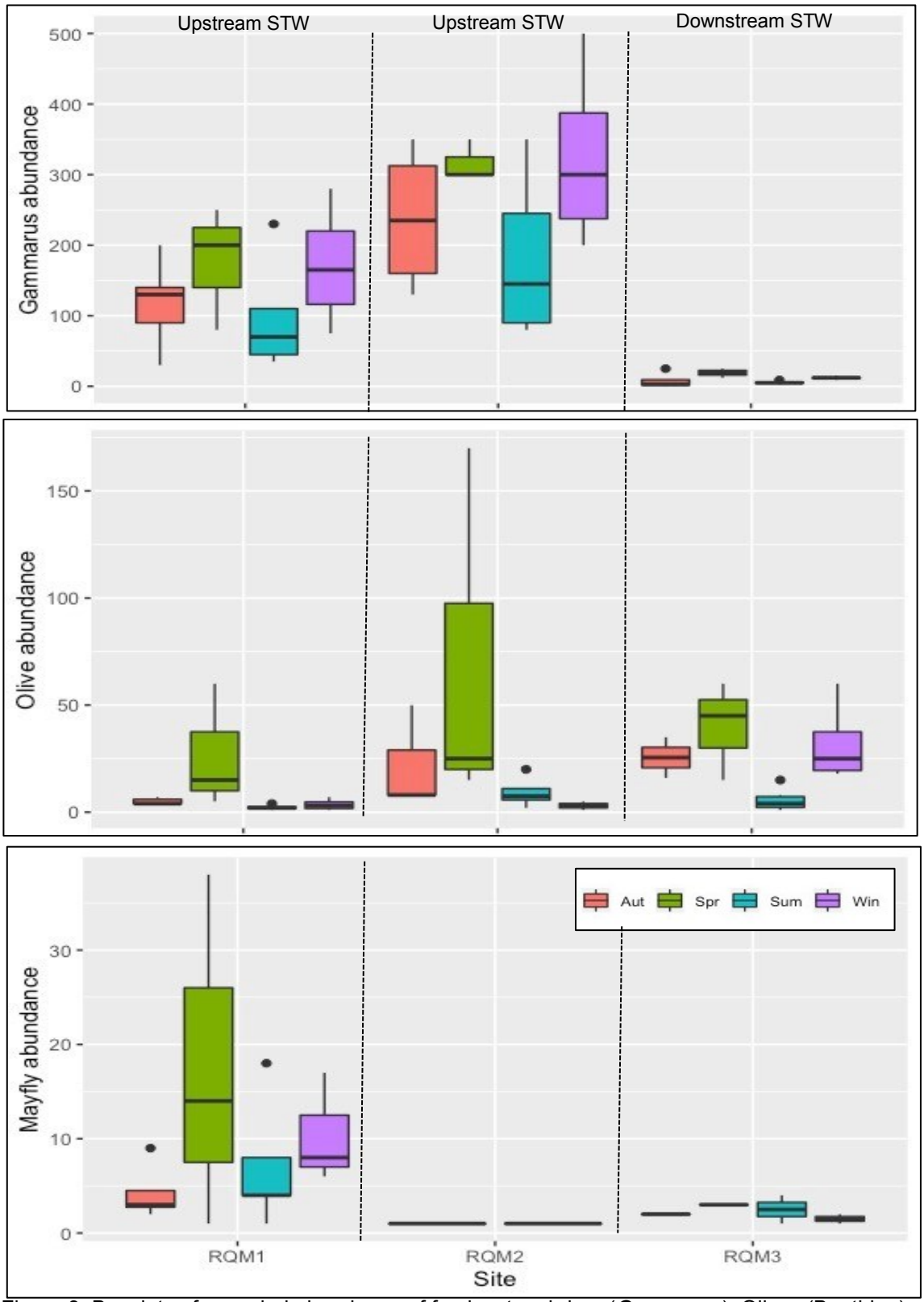


Figure 3: Boxplots of recorded abundance of freshwater shrimp (*Gammarus*), Olives (Baetidae) and Mayfly (Ephemeridae) by season at the 3 monitoring sites on the Littlestock Brook (RQM1 & RQM3) and tributary (RQM2), 2017-2020. (See Figure 2 for explanation of boxplot)

iii. Of the other Riverfly taxa recorded Olives (Baetidae) are the next most common taxa. Whilst highest abundance is reported from RQM1, there no significant difference in reported abundance between the sites (Figure 3).

iv. The more sporadic occurrence of other taxa recorded makes comparison less clear, although Mayfly (Ephemeridae) are clearly most common at RQM1 (Figure 3). All other taxa are less common and usually recorded in low numbers (<10). There are occasional records of Flat-bodied (Heptagenaiidae) reported from all sites but most frequently downstream at RQM3. The Blue-winged olive (*Serratella ignita*) is rarely reported and only from RQM2 & RQM3. Whereas, of the few records for Stoneflies (Plecoptera) none are from RQM3. Cased and caseless caddis flies (Trichoptera) are reported from all sites.

b) Environment Agency macroinvertebrate data from July 2020

These data comprise abundance of macroinvertebrate taxa recorded from the bankside assessments carried out on 24 July 2020 and from subsequent laboratory analysis of the same samples. Whilst a Riverfly trigger level breach was not detected by the Environment Agency, any short term acute impact may potentially been missed due to the enforced delay in attending the site following the reported breach.

Data from the bankside assessments (Figure 4), shows the highest Riverfly score and taxa richness were recorded at the site 90m downstream of the STW outfall (200991). The difference in scores between this site and the upstream site (200992) is marginal with the latter recording a greater overall abundance, most notably for freshwater shrimp. The site furthest downstream (Site ID 35335/RQM3 - the location of the Riverfly breach) contrasts sharply with these two sites, with a much lower overall abundance and lower taxa richness. Whilst the Riverfly score here was above the trigger level on this occasion it was much lower (about half) relative to the two other sites.

Reasons for the differences observed between the sites may in part be due to habitat, although water quality, discussed later, may also be responsible. Both the upstream site and the site 90m downstream of the STW have similar gravel/pebble substrates and a heterogeneous habitat providing multiple niches. In contrast, the site of the Riverfly breach is more uniform, slightly wider and shallower with a reduced stream velocity, relative to the preceding downstream site. Whilst both downstream sites might expect[2] to experience a similar nutrient load from the sewage effluent, the impact further downstream appears greater, illustrated by the observed widespread growth of filamentous algae. Whereas at the site 90m downstream of the STW, the faster flow conditions may help ameliorate some of the impact from the effluent on the macroinvertebrate community.

[2] Whilst the length of a mixing zone may extend some distance downstream of a discharge, the small size and relative flow within the Littlestock Brook would suggest that a similar level mixing should be achieved at the two downstream sites.

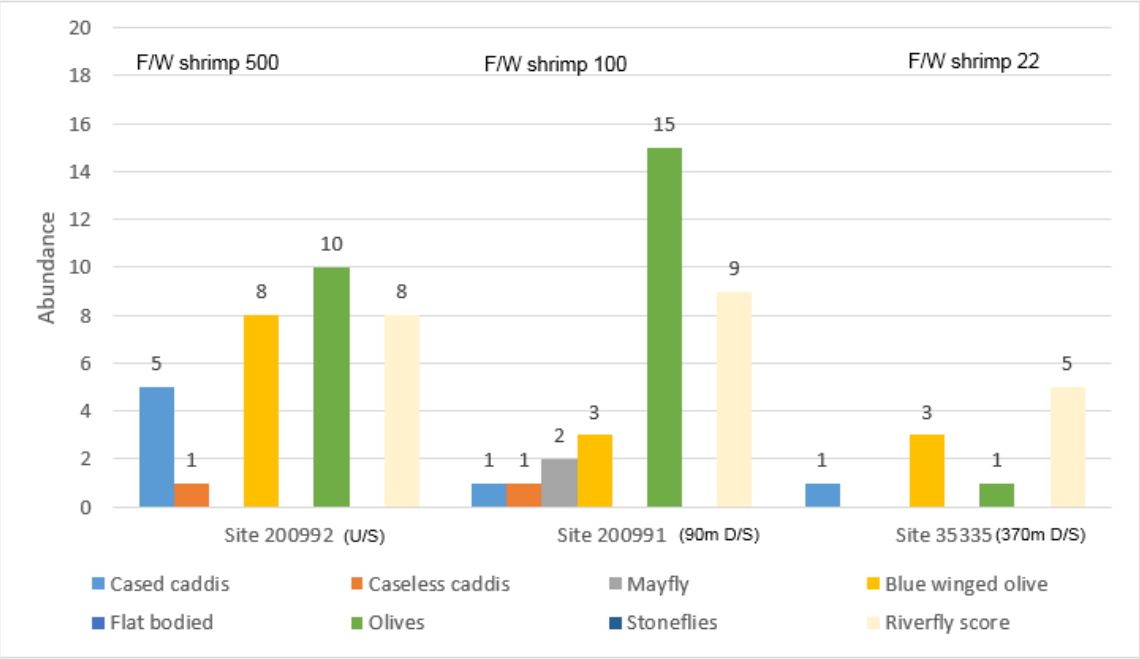


Figure 4: Abundance of Riverfly taxa recorded in bankside analysis of 3-min. kick samples taken at 3 sites on the Littlestock Brook by the Environment Agency on 24 July 2020. (Freshwater shrimp numbers included as values to avoid scaling issues).

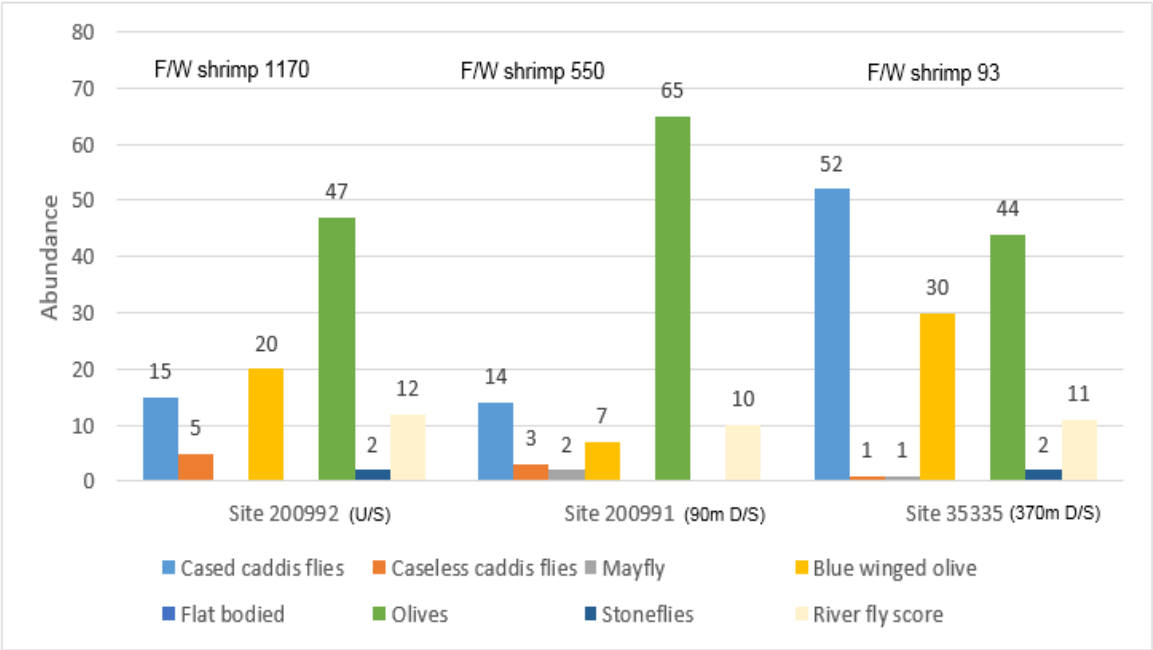


Figure 5: Abundance of Riverfly taxa recorded in laboratory analysis of 3-min. kick samples taken at 3 sites on the Littlestock Brook by the Environment Agency on 24 July 2020. (Freshwater shrimp numbers included as values to avoid scaling issues).

The laboratory analysis provides a more detailed picture of the macroinvertebrate communities sampled across the 3 sites as the samples are sorted with a higher level of scrutiny over a longer time of time, than possible in the field. Unsurprisingly, at all sites Riverfly taxa were recorded in higher abundance and greater taxa richness than on the bankside (Figure 5).

When examined as proportions, the abundance of Riverfly taxa recorded from field and laboratory analysis at each site show similar patterns (Figure 6). Ignoring the contribution of freshwater shrimps (discussed later), Olives, including the Blue-winged olive (BWO) largely dominate the Riverfly community at all sites. Whilst Olives as a group are moderately pollution sensitive, the BWO is highly sensitive and was found in highest abundance downstream of the STW at the trigger breach site, by laboratory analysis. Additionally, this site has the highest taxa richness with a comparable Riverfly score to the upstream control site. Representatives from the stonefly family, another highly pollution sensitive taxa, were also recorded from both these sites, albeit in low numbers. But no flat-bodied (Heptageniidae), which are also highly sensitive and detected in previous Riverfly monitoring, were recorded in any of the samples.

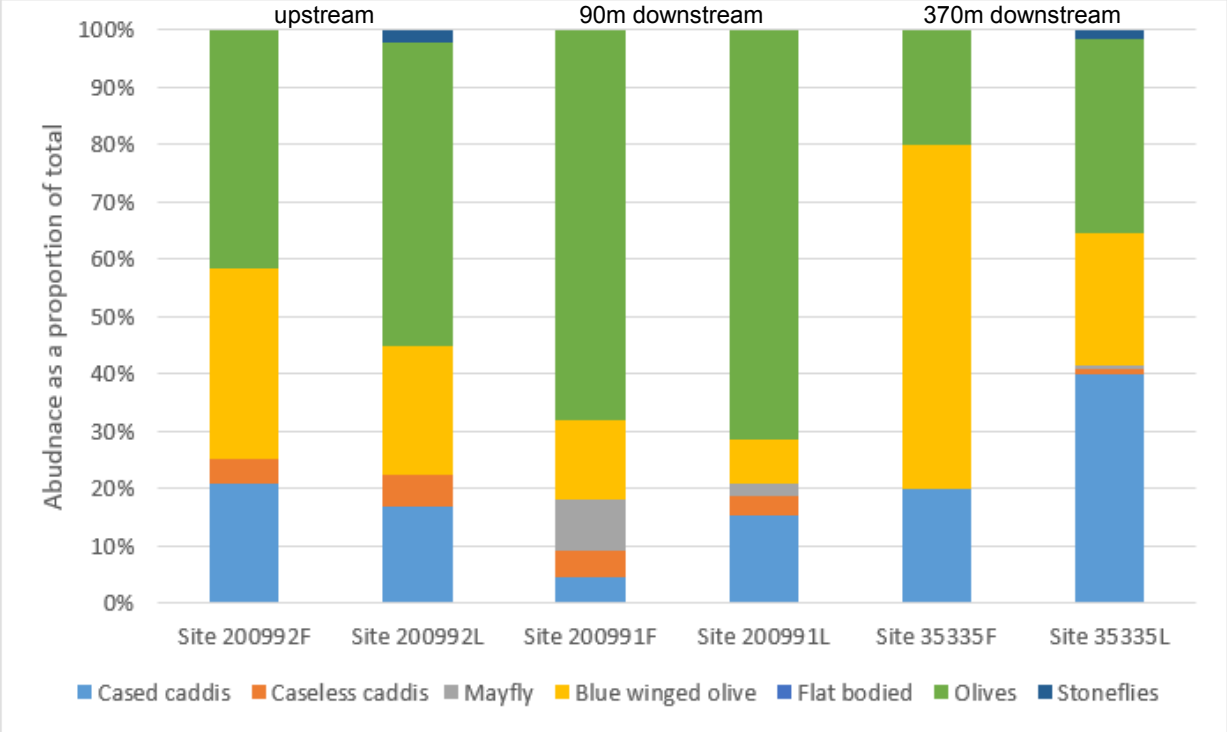


Figure 6: Abundance of Riverfly taxa expressed as a proportion of the total Riverfly taxa recorded in each 3-min kick sample (excluding freshwater shrimps) from the field (F) and the laboratory (L). Data from kick samples taken at 3 sites on Littlestock Brook by the Environment Agency on 24 July 2020.

Examining the wider macroinvertebrate community across the three sites, taxa groups found and total abundance were similar across all sites (Table 3). However, the sites differ markedly in the abundance of individual groups of taxa (Figure 7): *Gammarus* abundance (sensitive to ammonia) declines sharply downstream of the STW, relative to the upstream sample, whereas snails and true flies (principally chironomids) increase downstream (67% of the community d/s at site 35335, compared with 3.4% u/s at site 20099). Caddis numbers are also more common furthest downstream, mainly driven by the cased micro-caddis *Hydroptila*. Conversely, Olive numbers remain similar across all sites. These findings indicate differences in both water quality and habitat between the upstream and downstream sites. The higher numbers and proportions of chironomids, together with the occurrence of other taxa such as blackfly and water hoglouse (e.g. site 20991 – 90m d/s of the STW) indicate organic enrichment. Whereas the appearance of

more snails and the micro-caddis *Hydroptila*, might suggest differences in habitat. However, these differences may in part be driven by the nutrient enrichment (e.g. promoting more algal growth, providing suitable habitat for grazers such as snails and *Hydroptila*). The differences in *Gammarus* numbers up and downstream of the STW may also suggest effluent related issues. However, differences in substrate and flow between the two downstream sites (i.e. more silty, slower flowing conditions furthest d/s) are also relevant.

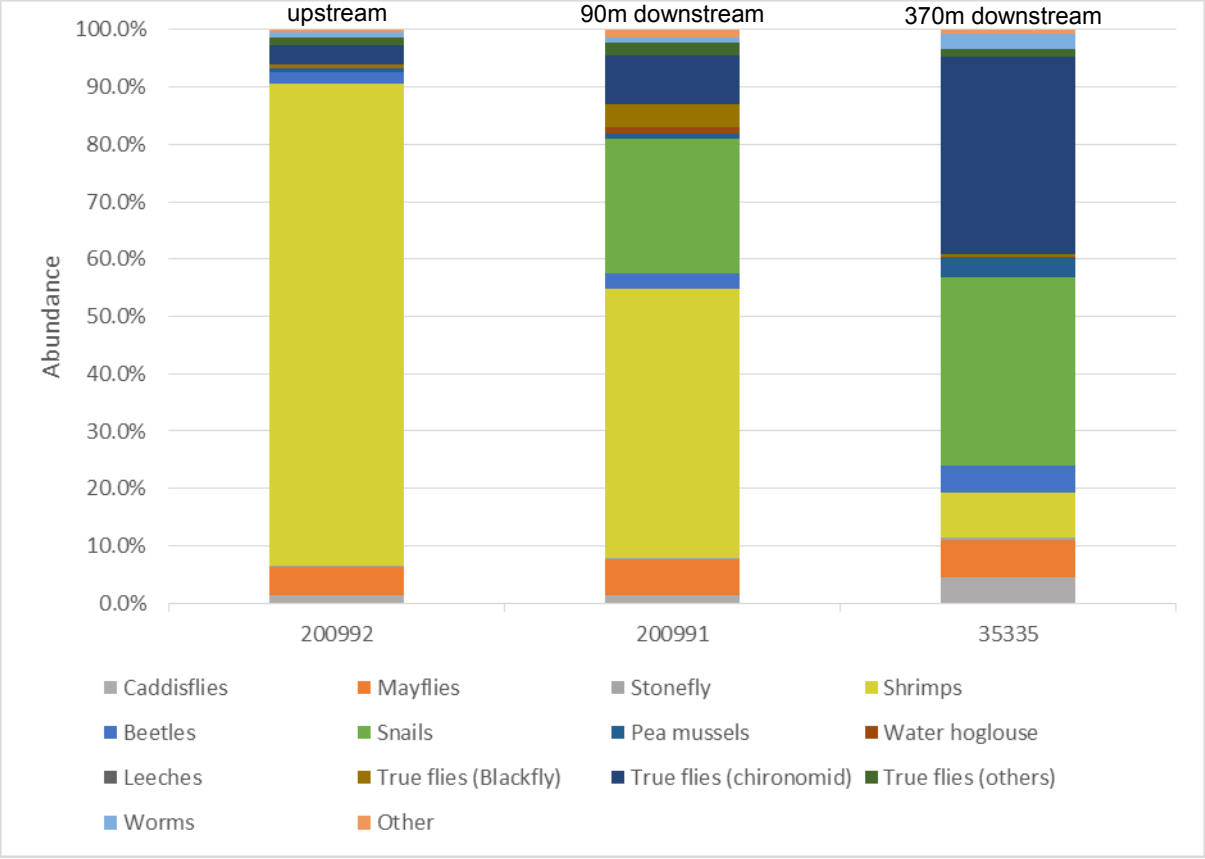


Figure 7: Abundance of all taxa groups expressed as a proportion of the total abundance of taxa recorded in laboratory analysed samples. Data from kick samples taken at 3 sites on Littlestock Brook by the Environment Agency on 24 July 2020.

The WHPT indices, derived from the laboratory analysed samples (Table 2), do not indicate the macroinvertebrate community of the Littlestock Brook is acutely compromised. But WHPT-ASPT, a signal for water quality, indicates water quality is better upstream. This contraction highlights a fundamental issue - that effluent discharges cause a level of ecological disturbance. Interestingly, the downstream samples recorded higher numbers of scoring taxa (WHPT-NTAXA), likely indicating differences in habitat between sites. The slightly lower overall WHPT score upstream is probably due to the high number of *Gammarus*. This is because the WHPT score is abundance weighted so that excessive numbers of certain taxa (such as *Gammarus*) will reduce the overall score. PSI scores indicate both downstream sites are effected by moderate sedimentation, the upstream site being relatively unaffected.

c) Environment Agency long term macroinvertebrate and water quality data (2003-2019)

This dataset comprises macroinvertebrate and water chemistry data recorded 370m downstream of the STW on the Littlestock Brook (site 35335/PVER0021) by the Environment Agency from 2003-2019. Data prior to this was not included due to changes in methods and quality assurance procedures. The data is provided in Appendices A2&3.

Over the period reviewed, the macroinvertebrate community has remained similar in composition but varied in abundance and in the relative proportions of taxa recorded (Figure 8). There is some seasonality within the data, illustrated by higher numbers of mayfly and caddis fly usually recorded in the spring.

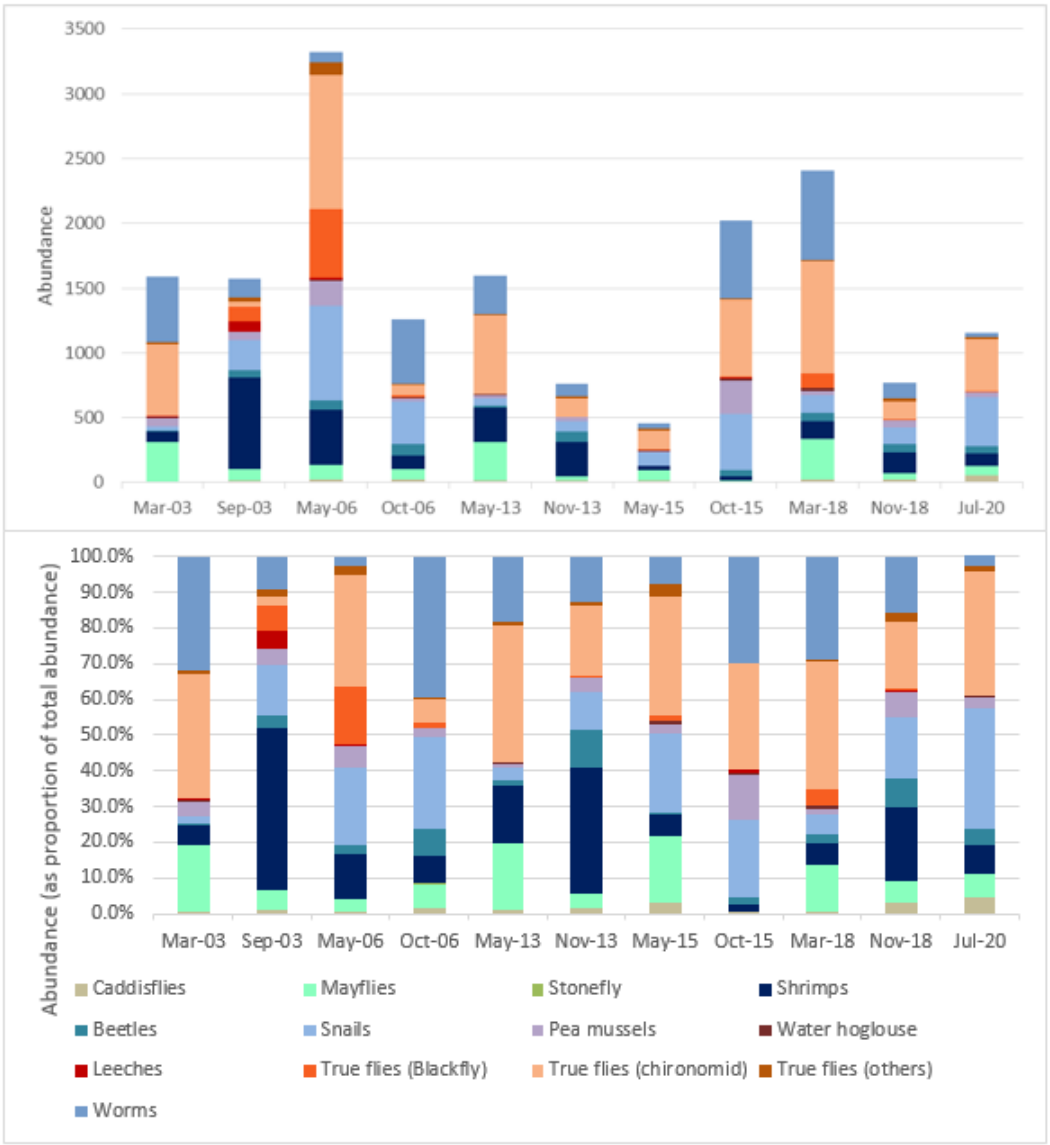


Figure 8: Recorded abundance of all taxa and their relative proportions, recorded in laboratory analysed samples from kick samples at site 35335/PEVR0021, taken by the Environment Agency from 2003-2020.

In general, the proportion of true flies (principally chironomids) and worms equates to roughly half the overall abundance of macroinvertebrates in most samples from this site.

Whilst equitable numbers of taxa are not expected, the dominance of true flies and worms highlights potential water quality issues. The abundance of the freshwater shrimp (*Gammarus*) varies seasonally being more common in autumn, potentially due to greater amounts of leaf litter. However, total numbers are considerably lower at this site than at either of the upstream sites (discussed earlier) and appear to have declined in recent years. Collectively, these observations are symptomatic organically enriched watercourse.

Water quality determinands (dissolved oxygen, ammonia and phosphate) measured at this site indicate stable/improving conditions since 2003 (e.g. ammonia shows step change improvements from 2007 and orthophosphate from 2006, whilst DO has remained largely stable, Figure 9). The biological indices (WHPT-ASPT and WHPT-Ntaxa) are also stable with a slight upward trend since 2003 (Figure 10). This trend is more pronounced when data from 1989 onwards is included (Appendix C). However, it is important to note that monitoring frequencies for water quality and macroinvertebrates have reduced since 2003. From these data the following observations are drawn:

- Concentrations of ammonia were highest between 2003-2007, with peaks in autumn 2003 and spring 2006. There is a corresponding drop in dissolved oxygen in 2003 but not in 2006. The spring 2006 event is associated with an increase in the abundance of true flies and suggests an impact on the macroinvertebrate community. In autumn 2006 the proportion of true-flies drops but worms increase, suggesting continued impact, potentially from an increase in sediment and silt.

- Between 2013-2016 phosphate concentrations fluctuated widely compared to the preceding period 2007-2009 (no data 2010-2012). This suggests effluent quality from the STW was inconsistent. An ammonia spike and drop in dissolved oxygen in October 2015 corresponds with reduced abundance of Riverflies and *Gammarus*.

- Taxa abundance dropped markedly in 2013 and was at its lowest in spring 2015. However, proportions of taxa were largely similar and Riverfly taxa relatively abundant. Fluctuations in water quality in combination with changes in flow (e.g. 2013-14 floods) may offer a partial explanation. Additionally, a drop in the biological index for sediment (PSI) in autumn 2015 (Figure 10) points to possible low flows and/or greater sediment inputs, and is accompanied by large numbers of molluscs.

- In spring 2018 the proportions of true flies and worms was the highest recorded. Unfortunately, there is no supporting water quality data. However, it similar to the pattern observed in 2006 and so plausibly likely linked to a drop in effluent quality.

- In mid-2019 phosphate levels rose slightly, relative to 2017 (no data 2018). Riverfly monitoring recorded particularly low numbers of *Gammarus* around this time, possibly exacerbated by low flows (although there are no concurrent flow data at this location to substantiate this suggestion). Limited subsequent data shows improved conditions.

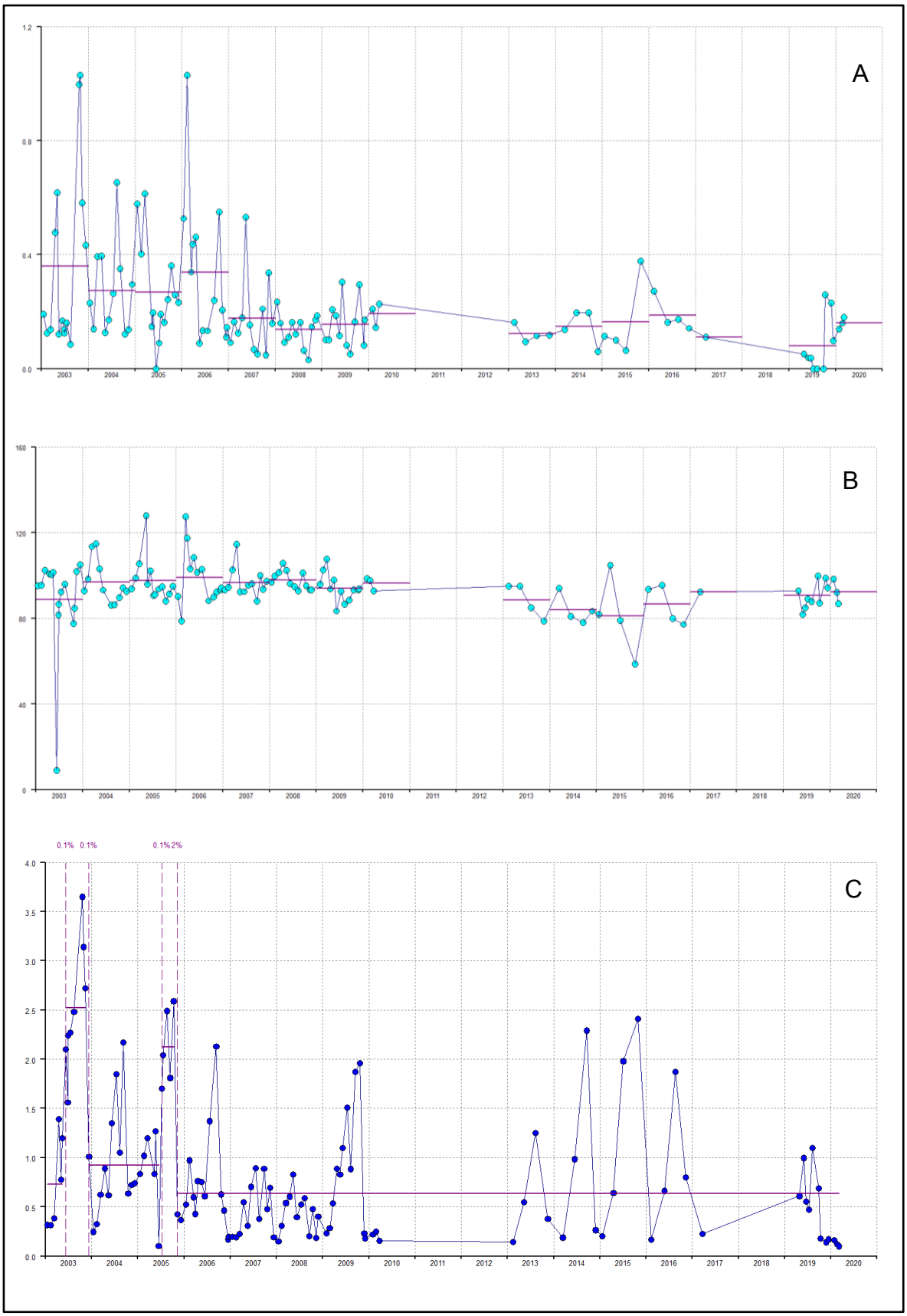


Figure 9: Water chemistry monitoring data from the Littlestock Brook at site 35335/PEVR0021, taken by the Environment Agency from 2003-2020. [A] Ammonia (mg/l); [B] Dissolved oxygen (% saturation); [C] orthophosphate (cusum plot). The solid horizontal purple line indicated a step change using a sequential analysis of the mean. The dashed purple vertical lines indicate a statistical difference in the concentration.

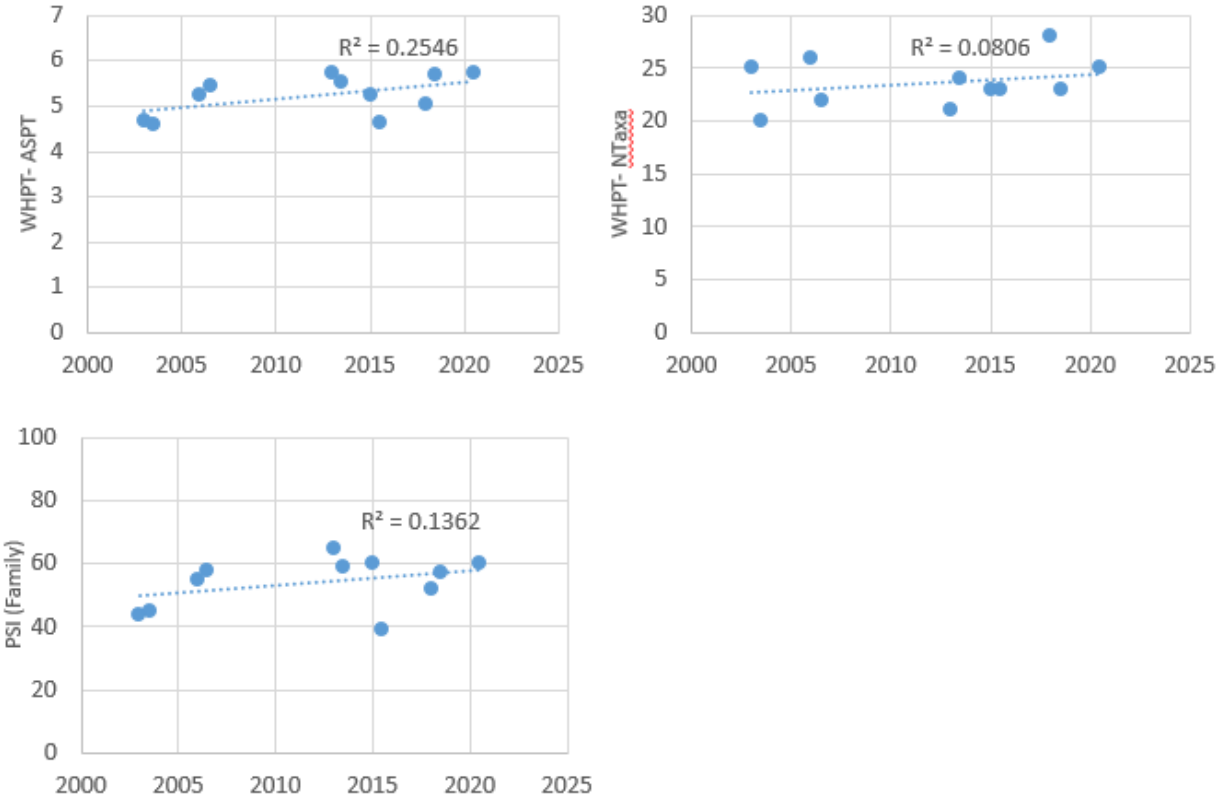


Figure 10: Biological indices calculated from macroinvertebrate monitoring data from the Littlestock Brook at site 35335, taken by the Environment Agency from 2003-2020. [A] WHPT-ASPT; [B] WHPT-NTaxa. Dotted line – linear regression line, R^2 indicated strength of fit (R^2=1 best fit). PSI scores: 0-20 heavily sedimented, 21-40 sedimented; 41-60 moderately sedimented; 61-80 slightly sedimented; 80-100 unsedimented.

5. Discussion

The Riverfly trigger level breach event in June 2020 highlighted a potential issue with the effluent quality from the Milton-under-Wychwood sewage treatment works (STW). Our initial field observations did not confirm the breach but we could have missed the 'peak' impact due to the enforced delay in our response. Our field observations and laboratory analysis of 3-minute kick samples taken up and downstream of the STW post the breach event do not indicate an acute impact on the macroinvertebrate community: there were no dead macroinvertebrates and a reasonably diverse community present throughout. The biological indices (WHPT-ASPT and WHPT-NTaxa) derived from these data, support this assertion.

However, Riverfly data collected by volunteers, reveals a significant difference in scores between the most upstream site (RQM1) and the furthest downstream site (RQM3/35335). The abundance of certain taxa; *Gammarus* and to a lesser extent mayfly (Ephemeridae), are also much lower downstream. These trends are however, are not repeated across all Riverfly taxa with Olives (Baetidae) abundant downstream, and rarer taxa such as caddis and the highly pollution sensitive flat-bodied mayfly (Heptageniidae) recorded at all sites.

Our field data from July 2020 shows a similar trend between upstream (RQM1) and downstream (RQM3/35335). But there is no significant difference in scores between RQM1 and our site located 90m downstream of the STW (200991). Additionally, Riverfly scores derived from our laboratory analysed samples were similar across all sites (ranging from 10-12). Taxa richness and abundance was also similar, with the notable exception of *Gammarus* (higher upstream). These scores suggest that differences in the Riverfly communities up and downstream of the STW are not as acute as field observations indicate.

Examining the whole macroinvertebrate community, there are clear differences in the relative abundance of certain taxa up and downstream. Both downstream sites examined in our investigation were characterised by fewer *Gammarus* and much larger numbers of snails (dominated by the New Zealand mud snail) and true-flies (mainly Chironomidae), relative to upstream. Our long-term monitoring (since 2003) downstream at RQM3/35335 shows that whilst caddis, stonefly, mayfly and beetles are all present, overall community abundance is dominated by chironomids, snails and to a lesser degree worms. These findings strongly point to the influence of organic enriched conditions associated with sewage effluent. However, the persistence of pollution sensitive taxa demonstrate that the impact on the community is chronic rather than acute. Recent annual (Arp19 - Mar-20) concentrations of ammonia (<0.3mg/l) and dissolved oxygen (>80% saturation) are not indicative of gross toxic pollution. Whereas, mean annual phosphate concentrations (0.44mg/l) suggest nutrient enrichment, associated with high organic loading.

The sediment index (PSI) indicates that the two downstream sites are moderately impacted by sediment. This links to the contribution of suspended solids from the effluent, sediment associated with storming events as well as from run-off, flowing in to the brook. The addition of sediment has likely helped modify the downstream habitat, facilitating conditions for sediment tolerate taxa (e.g. chironomids, snails and worms) to thrive. However, the two sites subtly differ in their morphology which may explain differences in taxa abundance. RQM3/35335 has a marginally wider channel, with slightly shallower, slower flowing waters. Such conditions have likely resulted in more silt deposition and aided the proliferation of filamentous algae in nutrient rich waters. This is manifest through the macroinvertebrate community shown by an increase in the abundance of the micro-caddis *Hydroptila* and the New Zealand mud snail, which attach and feed on algae. The decrease in *Gammarus*, which tends to prefer gravel substrates, is also likely due to the more silt rich environment.

In summary the macroinvertebrate community downstream of the STW is influenced by an increase in sediment and organic loading, compared to upstream. The input of sewage effluent (including storming events) has likely helped modify the habitat, increasing sedimentation and promoting algal growth, shaping the macroinvertebrate community. These changes, which appear most pronounced furthest downstream (at RQM3/35335), are also likely exacerbated by channel morphology. Relative to upstream, this site is more uniform in cross section reducing the velocity of the stream, resulting in deposition of silt. Elsewhere, channel shape is more varied enabling faster flowing conditions which help maintain clean gravels. The low Riverfly scores recorded in the field by both volunteers

and the Environment Agency, at this site might be explained by the excessive algal cover and silty conditions, making it harder to spot taxa amongst the weed. The higher laboratory derived Riverfly scores tend to support this suggestion.

Whilst no evidence was found to indicate an acute pollution, the prevailing conditions are indicative of a chronic impact. Further monitoring is needed to help improve our understanding of the relative impacts of sewage effluent and channel morphology in shaping the macroinvertebrate community downstream of the STW. Improvements in effluent quality and habitat are likely to improve both species evenness and richness.

6. Forward look

We will recommence macroinvertebrate monitoring on the Littlestock Brook in 2021, collecting samples from our long-term site (35335) in spring, summer and autumn. In this way we hope to capture seasonal variation and particularly the impact of low summer flows upon the macroinvertebrate community, when effluent dilution is low. We will also consider deploying an in-situ multi-parameter sonde to detect water quality changes.

In 2021/22 we plan to carry out a series of bankside macroinvertebrate assessments, similar to a Riverfly survey, along the Evenlode and selected tributaries. The purpose of this exercise will be to get a better 'picture' of the macroinvertebrate community across the catchment and help pin-point other potential water quality issues.

We welcome the opportunity to continue to working with the Riverfly monitoring volunteers, and remain committed to sharing and reviewing our collective data. This investigation and data review has highlighted the value of Riverfly monitoring in triggering this investigation and possible issues with the Riverfly field survey approach when blanket weed is present. We suggest that an additional survey site (e.g. site 200991: 90m d/s of the STW) is added to the Riverfly programme or perhaps visited as a secondary check site when a low score at RQM3 is recorded. We would also recommend consideration is given to using the expanded Riverfly taxa list so that the presence and abundance of pollution tolerant taxa are also captured.

7. References

Extence, C. A. et al., 2013 'The assessment of fine sediment accumulation in rivers using macroinvertebrate community response', River Research and Applications, 29(1), pp. 17–55. doi: 10.1002/rra.1569.

Murray-Bligh, J. A. D., 1999. Procedure for collecting and analysing macro-invertebrate samples. Quality Management Systems for Environmental Biology: Biological Techniques, BT001 Version 2.0. Bristol: Environment Agency

8. Appendix

A1 – Macroinvertebrate data 2020 (Site IDs: 200992, 200991, 35335)

Site ID	200992	200991	35335		
Catchment	EVENLODE	EVENLODE	EVENLODE		
Waterbody	LITTLESTOCK BROOK	LITTLESTOCK BROOK	LITTLESTOCK BROOK		
Site/Station name	SHIPTON ROAD ABOVE STW	90 M BELOW STW	ABOVE EVENLODE AT SHIPTON UNDER WYCHWOOD		
NGR	SP2721718117	SP2752918451	SP2780418466		
Sample Date	24/07/2020	24/07/2020	24/07/2020		
BMWP ASPT	6.19	5.14	5.76		
BMWP N Taxa	16	22	21		
BMWP Total	99	113	121		
WHPT APST	6.41	5.18	5.72		
WHPT N Taxa	19	26	25		
WHPT Total	121.8	134.8	143.1		
LIFE Family Index	8.4	7.24	7.19		
PSI Family Score	88.57	52.08	60		
Taxa & Sort Code	Estimated + Number found	Estimated + Number found	Estimated + Number found		common name
Simuliidae	8	40			black fly larave
Simulium ornatum/intermedium/trifasciatum		5	4		black fly larave
Simulium lundstromi			1		black fly larave
Cottus gobio	4	1	2		bullhead
Agapetus fuscipes	2				cased caddis
Hydroptila		9	39		cased caddis
Hydropsyche			1		cased caddis
Halesus digitatus			2		cased caddis
Chaetopteryx villosa	2	1			cased caddis
Limnephilus lunatus			1		cased caddis
Goeridae		1			cased caddis
Silo		2			cased caddis
Silo nigricornis	10		7		cased caddis
Sericostoma personatum	1				cased caddis
Mystacides azurea			2		cased caddis
Rhyacophila		1			caseless caddis
Rhyacophila dorsalis	4	1			caseless caddis
Lype	1	1			caseless caddis
Polycentropus			1		caseless caddis
Ancylus fluviatilis	1	10	2		freshwater limpet
Gammarus fossarum	1170	400	87		freshwater shrimp
Gammarus pulex/fossarum agg.		150	5		freshwater shrimp
Gammarus pulex			1		freshwater shrimp
Glossiphonia complanata		2			leech
Ephemera danica		1	1		mayfly
Serratella ignita	20	7	30		mayfly (blue winged olive)
Baetidae		25			mayfly (olive)
Baetis			4		mayfly (olive)
Baetis vernus			8		mayfly (olive)
Baetis rhodani/atlanticus	37	40	12		mayfly (olive)
Baetis scambus/fuscatus	10		20		mayfly (olive)
Oribatei		2			mite
Brillia bifida			4		non biting midge larvae
Chironomidae		30	96		non-biting midge larvae
Tanypodinae		20	76		non-biting midge larvae
Prodiamesinae	44	10	32		non-biting midge larvae
Orthocladiinae	3		4		non-biting midge larvae
Chironomini			20		non-biting midge larvae
Tanytarsini		40	168		non-biting midge larvae
Sphaerium corneum		2			pea-mussel
Pisidium	9	8	39		pea-mussel
Elmis aenea	25	8	25		riffle beetle
Limnius volckmari	2				riffle beetle
Oulimnius		21	28		riffle beetle
Riolus	1				riffle beetle
Pacifastacus leniusculus			1		signal crayfish
Gyraulus crista		33	1		snail
Potamopyrgus antipodarum		240	382		snail
Radix balthica		1			snail
Physa		1			snail
Leuctra fusca	2		2		stonefly
Velia			1		true bug
Microvelia		2			true bug
Antocha vitripennis			1		true fly larvae
Dicranota	17	20	8		true fly larvae
Pericoma		1	1		true fly larvae
Pericoma blandula			1		true fly larvae
Pericoma trivialis			1		true fly larvae
Dixa maculata	1	1			true fly larvae
Dixa nebulosa	1				true fly larvae
Empididae			5		true fly larvae (dance fly)
Chelifera	1	5			true fly larvae (dance fly)
Asellus aquaticus		13	1		water hoglouse
Hydracarina	2		2		water mite
Curculionoidea	1				weevil
Oligochaeta	8	11	30		worm
Eiseniella tetraedra	1		1		worm

A2 – Macroinvertebrate data 2003-2020 (Site ID: 35335)

Site ID	35335	35335	35335	35335	35335	35335	35335	35335	35335	35335	35335	35335	35335
Sample ID	360804	360804	364616	539031	548042	578801	660442	680090	719124	729198	767741	787603	810871
Sample Date	24/03/2003	24/03/2003	16/09/2003	31/05/2006	12/10/2006	25/09/2008	18/05/2013	15/11/2013	06/05/2015	28/10/2015	07/03/2018	16/11/2018	24/07/2020
Analysis ID	368230	368230	370449	537411	543146	580167	652809	668075	713926	725102	759899	783579	815220
BMWP ASPT	4.48	4.48	4.21	5	4.8		5.84	5.57	5.32	4.83	4.92	6.05	5.76
BMWP N Taxa	21	21	19	23	20		19	21	19	18	24	21	21
BMWP Total	94	94	80	115	96		111	117	101	87	118	127	121
WHPT APST	4.69	4.69	4.57	5.23	5.45		5.73	5.52	5.25	4.62	5.03	5.69	5.72
WHPT N Taxa	25	25	20	26	22		21	24	23	23	28	23	25
WHPT Total	117.3	117.3	91.4	136.1	119.8		120.4	132.4	120.8	106.3	140.8	130.9	143.1
LIFE Family Index	6.95	6.95	6.94	7.09	7.33		7.65	7.45	7.41	6.94	7.12	7.35	7.19
PSI Family Score	43.9	43.9	44.9	54.9	57.5		64.86	58.97	60	39.02	52	57.14	60

Sample Date	24/03/2003	24/03/2003	16/09/2003	31/05/2006	12/10/2006	25/09/2008	18/05/2013	15/11/2013	06/05/2015	28/10/2015	07/03/2018	16/11/2018	24/07/2020
Brillia bifida											110		4
Caenis luctuosa/macrura												1	
Galba truncatula											1		
Gyraulus crista										1	3		1
Pediciidae					2		5	3					
Potamopyrgus antipodarum				706	300				95	400	120	120	382
Dendrocoelidae													
Nematoda				2	1								
Hydrobiidae	32	32	105				50	60					
Physidae										1	1		
Lymnaeidae	1	1	20										
Lymnaea peregra				2									
Planorbidae	11	11	80		4		2	2					
Gyraulus albus				3						1	2		
Ancylidae	1	1	23		20		2	20					
Ancylus fluviatilis				21					5	27	5	12	2
Zonitidae											1		
Sphaeriidae (Pea mussels	64	64	68		30		23	29					
Sphaerium corneum				10									
Pisidium				115					10	260	34	53	39
Pisidium casertanum				3									
Pisidium nitidum				12									
Pisidium subtruncatum				56									
Oligochaeta	506	506	142	81	500		295	96	35	600	680	121	30
Lumbriculidae											8		
Naididae											1		
Tubificidae											8		
Eiseniella tetraedra													1
Piscicolidae	1	1											
Glossiphoniidae	7	7	13		1			1					
Glossiphonia complanata				1					1		2		
Erpobdellidae	7	7	67		1								
Erpobdella octoculata				17						14	3	2	
Hydracarina	100	100		19			11		2	3	1		2
Oribatei				3	2								
Ostracoda			1										
Astacidae			1										
Pacifastacus leniusculus													1
Asellidae	2	2	18		2		8	1		2			
Asellus aquaticus									5	10	23		1
Asellus meridianus				3									
Crangonyx pseudogracilis/floridanus										1	4		
Gammaridae	87	87	706				262	265					
Gammarus fossarum											30		87
Gammarus pulex/fossarum agg.											91	156	5
Gammarus pulex sens. lat.				424	100				28	33			
Gammarus pulex											15		1
Baetidae	298	298	90	77	80		167	28					
Baetis									80				4
Baetis rhodani												35	
Baetis vernus													8
Baetis rhodani/atlanticus				1						5	310		12
Baetis scambus/fuscatus													20
Leptophlebiidae	1	1			4		113	1	3		1		
Paraleptophlebia									1	1	2		
Paraleptophlebia submarginata											1		
Ephemeridae								2					
Ephemera												3	
Ephemera danica										1	2	10	1
Ephemerellidae							17						
Serratella ignita				35					1			1	30
Caenis luctuosa				2									
Nemouridae					1								
Leuctra fusca													2
Calopteryx												1	
Velia													1
Notonectidae													
Haliplidae													
Dytiscidae			1										
Gyrinidae					2								
Hydrophilidae	1	1											
Hydraena				1									
Elmidae	4	4	55		90		20	81					
Elmis aenea				66					3	34	60	39	25
Limnius volckmari												1	
Oulimnius				3						15		21	28
Oulimnius tuberculatus											2	4	
Riolus				1									
Riolus subviolaceus											1		
Curculionidae							1						
Sialidae													

Sample Date	24/03/2003	24/03/2003	16/09/2003	31/05/2006	12/10/2006	25/09/2008	18/05/2013	15/11/2013	06/05/2015	28/10/2015	07/03/2018	16/11/2018	24/07/2020
Rhyacophilidae			1				1	5					
Rhyacophila									3				
Rhyacophila dorsalis										6	2		
Hydroptilidae							6	1					
Hydroptila				3					6		5		39
Ithytrichia											1		
Psychomyiidae	1	1											
Lype											1		
Tinodes waeneri										1			
Cyrnus trimaculatus											2		
Polycentropus													1
Polycentropus flavomaculatus												1	
Polycentropus irroratus												3	
Hydropsychidae	1	1	16		10			2					
Hydropsyche													1
Hydropsyche instabilis									1		1		
Hydropsyche pellucidula												4	
Hydropsyche siltalai				5								1	
Lepidostoma hirtum												2	
Limnephilidae	8	8		9	1		5				4	8	
Halesus digitatus									1				2
Halesus radiatus											2		
Glyphotaelius pellucidus											1		
Limnephilus lunatus													1
Goeridae				2	10		1	3					
Goera pilosa				1					2			1	
Silo nigricornis													7
Beraeodes minutus													
Sericostomatidae							1	1					
Leptoceridae							2	1					
Mystacides azurea												1	2
Mystacides longicornis				1								1	
Adicella reducta				1									
Lepidoptera				1									
Diptera							1						
Tipulidae	7	7	32	1				2					
Tipula										3		3	
Tipula montium											1		
Antocha				3									
Antocha vitripennis											1		1
Dicranota				23					8		1	8	8
Psychodidae	2	2	1	6				1				2	
Pericoma									1	2			1
Pericoma blandula													1
Pericoma trivialis													1
Ceratopogonidae	2	2		20	1		3	1	3	1	2		
Simuliidae	1	1	112	532	20			3	7	1			
Simulium angustipes/velutinum										1	5		
Simulium ornatum/intermedium/trifasciatum											78		4
Simulium lundstromi											22		1
Simulium ornatum												7	
Chironomidae	552	552	40	1,035	80		611	147	150		54		96
Tanypodinae											54	5	76
Prodiamesinae											54	26	32
Orthocladiinae										14	435	62	4
Metriocnemus											54		
Chironomini										14	54	26	20
Tanytarsini										570	54	22	168
Stratiomyidae	1	1							1				
Empididae	8	8		22	1		2	3	3	2			5
Chelifera											1		
Hemerodromia				18									
Muscidae													
Limnophora											1	8	
Limnophora riparia										1			
Cottus gobio								1	8				2

Note: data available at https://environment.data.gov.uk/ecology-fish/

A3 -Water chemistry data 2003-2020 (Site ID: PEVR0021)

PTCODE	Date	Time	Ammonia (N), mg/l	Ortho-phosphate, mg/l	Dissolved O2, %satuartion		PTCODE	Date	Time	Ammonia (N), mg/l	Ortho-phosphate, mg/l	Dissolved O2, %satuartion
PEVR0021	15/01/2003	1010	0.191	0.317	95.2		PEVR0021	15/08/2007	1140	0.051	0.38	96.2
PEVR0021	12/02/2003	1025	0.125	0.315	95.5		PEVR0021	24/09/2007	1219	0.21	0.886	88
PEVR0021	10/03/2003	950	0.137	0.382	102.4		PEVR0021	20/10/2007	1130	0.048	0.476	100
PEVR0021	14/04/2003	1120	0.478	1.39	100.7		PEVR0021	12/11/2007	1235	0.336	0.696	93.5
PEVR0021	01/05/2003	1015	0.617	0.776	100.4		PEVR0021	10/12/2007	1220	0.159	0.191	97.3
PEVR0021	13/05/2003	1010	0.121	1.2	101.5		PEVR0021	16/01/2008	1135	0.234	0.151	97
PEVR0021	09/06/2003	955	0.169	2.1	9		PEVR0021	11/02/2008	1140	0.16	0.309	99.7
PEVR0021	24/06/2003	1049	0.137	1.56	81.7		PEVR0021	14/03/2008	1010	0.093	0.54	101.5
PEVR0021	26/06/2003	910	0.124	2.24	86.7		PEVR0021	14/04/2008	1155	0.11	0.604	105.8
PEVR0021	14/07/2003	900	0.161	2.27	92.3		PEVR0021	12/05/2008	1420	0.162	0.828	102.4
PEVR0021	13/08/2003	1055	0.085	2.48	96		PEVR0021	09/06/2008	1315	0.122	0.395	96.1
PEVR0021	20/10/2003	1350	0.997	3.65	77.5		PEVR0021	15/07/2008	1250	0.163	0.523	94.7
PEVR0021	29/10/2003	935	1.03	3.14	84.8		PEVR0021	11/08/2008	1345	0.064	0.588	92.9
PEVR0021	12/11/2003	1325	0.581	2.72	101.9		PEVR0021	17/09/2008	1155	0.031	0.201	101.2
PEVR0021	10/12/2003	1230	0.433	1.01	105		PEVR0021	13/10/2008	1153	0.147	0.478	95.2
PEVR0021	13/01/2004	1045	0.23	0.247	92.8		PEVR0021	11/11/2008	1035	0.171	0.185	93.2
PEVR0021	11/02/2004	1040	0.14	0.324	98.3		PEVR0021	25/11/2008	1310	0.186	0.402	93.3
PEVR0021	10/03/2004	1125	0.394	0.625	113.5		PEVR0021	30/01/2009	1003	0.102	0.233	95.9
PEVR0021	14/04/2004	1222	0.395	0.89	114.9		PEVR0021	24/02/2009	1030	0.101	0.284	102.7
PEVR0021	12/05/2004	1130	0.127	0.617	103.2		PEVR0021	21/03/2009	1235	0.208	0.535	107.7
PEVR0021	08/06/2004	835	0.171	1.35	93.2		PEVR0021	20/04/2009	815	0.186	0.887	93.7
PEVR0021	12/07/2004	915	0.265	1.85			PEVR0021	16/05/2009	1111	0.116	0.829	97.9
PEVR0021	10/08/2004	1010	0.653	1.05	86.1		PEVR0021	06/06/2009	950	0.304	1.1	83.4
PEVR0021	06/09/2004	1110	0.35	2.17	86.4		PEVR0021	11/07/2009	955	0.082	1.51	92.7
PEVR0021	15/10/2004	935	0.122	0.635	89.7		PEVR0021	08/08/2009	1400	0.051	0.884	86.7
PEVR0021	12/11/2004	945	0.137	0.723	94.2		PEVR0021	14/09/2009	1321	0.165	1.87	88.6
PEVR0021	07/12/2004	800	0.296	0.743	92.7		PEVR0021	20/10/2009	1047	0.295	1.96	93.4
PEVR0021	17/01/2005	1010	0.578	0.836	93.8		PEVR0021	24/11/2009	1135	0.082	0.23	93.3
PEVR0021	18/02/2005	1040	0.403	1.02	98.9		PEVR0021	01/12/2009	1320	0.171	0.182	93.9
PEVR0021	16/03/2005	1155	0.614	1.2	105.5		PEVR0021	02/02/2010	1145	0.209	0.219	98.5
PEVR0021	10/05/2005	1300	0.148	0.836	128		PEVR0021	24/02/2010	1205	0.145	0.25	97.7
PEVR0021	19/05/2005	948	0.197	1.27	95.9		PEVR0021	25/03/2010	1235	0.227	0.154	92.9
PEVR0021	13/06/2005	1115	0	0.103	102.1		PEVR0021	13/02/2013	901	0.163	0.144	95
PEVR0021	07/07/2005	1120	0.09	1.7	90.7		PEVR0021	09/05/2013	825	0.094	0.547	95
PEVR0021	18/07/2005	1120	0.191	2.04	91.1		PEVR0021	08/08/2013	817	0.115	1.25	85
PEVR0021	17/08/2005	1215	0.162	2.49	93.7		PEVR0021	15/11/2013	1247	0.118	0.378	78.8
PEVR0021	12/09/2005	1300	0.243	1.81	94.7		PEVR0021	13/03/2014	1033	0.138	0.187	94
PEVR0021	10/10/2005	1230	0.362	2.59	88		PEVR0021	13/06/2014	802	0.196	0.984	81
PEVR0021	08/11/2005	1225	0.26	0.425	91.4		PEVR0021	18/09/2014	906	0.197	2.29	78
PEVR0021	05/12/2005	1305	0.233	0.366	95		PEVR0021	28/11/2014	1125	0.061	0.264	83.4
PEVR0021	16/01/2006	1330	0.527	0.524	90.3		PEVR0021	20/01/2015	1456	0.114	0.203	81.9
PEVR0021	13/02/2006	1240	1.03	0.973	78.7		PEVR0021	17/04/2015	1331	0.1	0.642	104.7
PEVR0021	15/03/2006	1300	0.339	0.598	127.4		PEVR0021	03/07/2015	1016	0.064	1.98	78.9
PEVR0021	28/03/2006	1256	0.436	0.427	117.5		PEVR0021	28/10/2015	1154	0.378	2.41	58.6
PEVR0021	18/04/2006	1340	0.462	0.761	103.1		PEVR0021	10/02/2016	1122	0.272	0.166	93.6
PEVR0021	17/05/2006	1300	0.089	0.753	108.4		PEVR0021	26/05/2016	1220	0.163	0.662	95.4
PEVR0021	12/06/2006	1505	0.134	0.61	101.4		PEVR0021	19/08/2016	1043	0.173	1.87	79.9
PEVR0021	20/07/2006	1325	0.133	1.37	102.8		PEVR0021	10/11/2016	1458	0.142	0.801	77.2
PEVR0021	11/09/2006	1310	0.239	2.13	88.2		PEVR0021	20/03/2017	1116	0.111	0.227	92.3
PEVR0021	19/10/2006	1205	0.55	0.627	90		PEVR0021	26/04/2019	1302	0.051	0.61	92.8
PEVR0021	13/11/2006	1327	0.205	0.463	92.4		PEVR0021	31/05/2019	1339	0.038	0.996	81.9
PEVR0021	13/12/2006	1239	0.11	0.171	93.4		PEVR0021	19/06/2019	1019	0.037	0.552	84.9
PEVR0021	18/12/2006	1211	0.145	0.197	94.3		PEVR0021	10/07/2019	1118	0	0.47	89
PEVR0021	17/01/2007	1200	0.092	0.196	93.3		PEVR0021	09/08/2019	1142	0	1.1	87.8
PEVR0021	14/02/2007	1300	0.165	0.192	94.5		PEVR0021	26/09/2019	1118	0	0.69	99.7
PEVR0021	14/03/2007	1200	0.125	0.225	102.7		PEVR0021	11/10/2019	1215	0.26	0.18	87.2
PEVR0021	16/04/2007	1359	0.179	0.547	114.5		PEVR0021	26/11/2019	1251	0.23	0.14	98.8
PEVR0021	16/05/2007	1335	0.531	0.306	92.3		PEVR0021	12/12/2019	858	0.098	0.17	94.3
PEVR0021	14/06/2007	1345	0.154	0.704	92.5		PEVR0021	28/01/2020	1303	0.14	0.16	98.3
PEVR0021	19/07/2007	1050	0.067	0.893	95.5		PEVR0021	20/02/2020	847	0.16	0.12	92.1
							PEVR0021	05/03/2020	1050	0.18	0.1	86.8

B – Statistical outputs

General linear model outputs for Riverfly scores between sites (RQM1, RQM2 & RQM 3)

```
glm(formula = Score ~ Site + Season, family = "gaussian", data = data1)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-2.3123  -1.1872  -0.3123   1.0612   2.8578

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   4.9388     0.5017   9.844  5.3e-13 ***
SiteRQM2     -0.4264     0.5196  -0.821 0.416063
SiteRQM3     -1.8749     0.5082  -3.689 0.000583 ***
SeasonSpr     2.4949     0.6561   3.803 0.000412 ***
SeasonSum     0.2484     0.5475   0.454 0.652187
SeasonWin     1.0782     0.6058   1.780 0.081543 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
glm(formula = Score ~ Year + Season, family = "gaussian", data = data1)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-3.0029  -1.0029  -0.2938   1.3333   3.3333

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -583.04361  530.31751  -1.099  0.27707
Year           0.29093    0.26271   1.107  0.27364
SeasonSpr      2.32400    0.74401   3.124  0.00303 **
SeasonSum     -0.04888    0.61623  -0.079  0.93710
SeasonWin      1.05280    0.68117   1.546  0.12877
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C – WHPT –ASPT & NTaxa long term trends

