



Impact of untreated and treated effluent discharges from Ilkley STW on the ecology of the River Wharfe:

A Citizen Science Project  
(iWharfe Eco-Ashlands)



# **Impact of untreated and treated effluent discharges from Ilkley STW on the ecology of the River Wharfe: a Citizen Science Project (iWharfe Eco-Ashlands)**

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## **Executive summary**

- We sampled ten sites on the River Wharfe upstream and downstream of the Ilkley Sewage Treatment Works (STW) from Addingham Suspension Bridge to Otley Sailing Club. Samples were analysed at accredited laboratories for water chemistry, diatom algae and aquatic macroinvertebrates.
- We show that discharges of untreated sewage via the STW storm overflow are not the principal cause of the deterioration in the ecological condition of the river downstream.
- We argue that the impact of the dissolved oxygen demand imposed by untreated sewage is mitigated by the ability of the river to replenish its oxygen supply through turbulent uptake of oxygen from the atmosphere.
- Instead, we show that nutrient (phosphorus and nitrogen) pollution derived from the continuous discharge of treated effluent from the STW has a more severe impact on the river.
- Nutrient pollution causes excessive algal growth that changes the structure and function of freshwater ecosystems. Such an effect is clearly indicated in this study by changes in the composition of diatom assemblages found on stones in the riverbed. The changes are coincident with a marked increase in the concentrations of phosphorus and nitrogen immediately downstream of the final effluent outfall close to Beanlands Island.
- Water quality continues to deteriorate from Ilkley downstream, probably caused by the cumulative impact of both organic pollution and nutrient pollution from a succession of Combined Sewer Overflows (CSOs) and STWs serving Ilkley, Ben Rhydding, Burley-in-Wharfedale and Menston.
- These data highlight the importance not only of reducing discharges of raw sewage from storm overflows but also the need to remove phosphorus from STW final effluent.

## Introduction

Over the last three years we have been investigating the water quality of the River Wharfe, both close to Ilkley, from Bolton Bridge to Burley Weir, and more extensively along the full length of the river from its headwaters in Upper Wharfedale to its confluence with the Ouse, south of York (Battarbee et al. 2020, 2022).

The initial focus of our work was on the concentration of faecal bacteria in the river at recreational sites, including the newly designated bathing water site in Ilkley, at the Cromwheel corner.

More recently we have been concerned with nutrient pollution, as indicated by changes in phosphate concentrations and algal (principally diatom) populations along the river (Battarbee et al. 2022). Nutrient pollution has also been the concern of the Environment Agency who have recently completed a review of the ecological status of the full length of the river (Dacombe & Wait 2022) as well as a more detailed study of the river upstream and downstream of the Ilkley STW (Hood 2020).

High concentrations of faecal bacteria and nutrients in the river can be accounted for primarily by discharges from sewage treatment works and runoff from agricultural land. The relative importance of these different sources varies in space from site to site along the river and in time according to differences in weather, principally rainfall frequency and intensity.

In the case of sites such as Beanlands Island we have shown that the Ilkley STW is the major source of both faecal bacteria and nutrient pollution in the river in all weather conditions. What has been unclear, however, is whether the pollution impact is due predominantly to discharges of untreated effluent during intermittent storm overflow events or by discharges of treated effluent that occur continuously.

Most local and national attention in recent years has focused on storm overflows, and there is and has been an implicit assumption that the high frequency of such events, now revealed by the universal deployment of event detection monitors (EDMs) throughout the UK, is mainly responsible for causing high concentrations of faecal bacteria in surface waters and for the poor ecological condition of rivers.

Untreated sewage is potentially damaging not only due to its high concentrations of nutrients and faecal bacteria but due to its high organic content and ammonia concentration. Ammonia can be toxic to fish and organic matter decomposition places an oxygen demand on the water posing a threat to fish and to freshwater invertebrates. The downstream impact of such pollution events depends both on the size of the event and the extent to which oxygen can be replenished from the atmosphere. Fast flowing, turbulent upland rivers, such as the Wharfe, are likely to recover more quickly than slower flowing, deeper lowland rivers.

But poor water quality can also be caused by the discharge of treated (often called “final” effluent). Although fully recognized by the Environment Agency as the principal threat to river ecology, pollution from treated effluent has been largely ignored by the public and by the media, probably because such effluent looks clean. However, treated effluent discharged from most STWs in the UK may meet statutory standards for the removal of organic matter, ammonia, and suspended solids but it is rarely treated to remove faecal bacteria or nutrients. The concentration of *E. coli* in treated effluent can exceed 500,000 cfu/100 ml and the concentration of phosphate-P can exceed 3 mg/l.

Consequently, both untreated effluents (from storm overflows) and treated effluents are major sources of pollution for people and for wildlife in rivers, but they pollute in different ways. Both have high concentrations of faecal bacteria and nutrients but untreated sewage has high concentrations of labile organic matter and is discharged intermittently whereas treated sewage has low concentrations of organic matter and is discharged continuously.

Differentiating their independent and combined effects can be difficult, especially at sites where they are discharged through the same outfall. In Ilkley the storm and final effluent outfalls are separate and the configuration of the STW is such that the final effluent outfall is some 150 m downstream of the storm overflow outfall (Figure 1).



Figure 1. Ilkley STW showing sampling site 5, 6 and 7 with site 6 positioned between the storm overflow outfall and the final (treated) effluent outfall. See Figure 2 for site names.

Whereas the EA has sampled upstream and downstream of the STW (Hood 2020), i.e. at site 5 and 7 (Figure 1) we have collected samples at those sites but also at a site (site 6) between the two outfalls (Figure 1). As such we are able to assess the relative importance of the untreated and treated effluents in explaining any observed change in water quality caused by the STW. Our perhaps counter-intuitive hypothesis is that the treated effluent has a greater impact on the ecology of the river than the untreated storm discharges.

### Sites

Sample sites were chosen on the river from the Addingham Suspension Bridge upstream of the Ilkley STW to the Otley Sailing Club downstream (Figure 2, Appendix A).

Except for site 6 (see Fig. 1 above), all were sites first sampled in the iWharfe project in 2020 (Battarbee et al. 2020). One or more discharges from Pumping Stations Overflows (PSOs), Combined Sewer Overflows (CSOs) and Sewage Treatment Works (STWs) occur between each sample sites (see Appendix A) as follows:

Site 1 – 2: Addingham PSO (2021: 113 spills)

Site 2 – 3: None

Site 3 – 4: Bridge Lane CSO (2021: 5 spills)

Site 4 – 5: Rivadale View (2021: 47 spills)

Site 5 – 6: Middleton (2021: 31 spills); Ilkley STW Overflow (2021: 156 spills)

Site 6 – 7: Ilkley STW Final effluent (continuous flow final effluent)

Site 7 – 8: Leeds Road CSO (2021: 3 spills); Wheatley Lane CSO (2021: 63 spills)

Site 8 – 9: Ben Rhydding STW (2021: 139 spills, and continuous flow final effluent)

Site 9 – 10: Burley Lodge CSO (2021: 6 spills); Burley-in-Wharfedale STW (2021: 60 spills, and continuous flow final effluent)

Samples were taken over four days. On 8<sup>th</sup> August samples were taken for water chemistry and diatoms and on the 9<sup>th</sup> August for macroinvertebrates. On 9<sup>th</sup> and 13<sup>th</sup> August samples were taken for filamentous algae.





Figure 2: iWharfe Eco-Ashlands sampling sites. 1 Addingham Suspension Bridge; 2 Ilkley Golf Course; 3 Ilkley Old Bridge; 4 Ilkley New Bridge; 5 Cromwheel; 6 Ashlands; 7 Beanlands Island; 8 Denton Bridge; 9 Burley Weir; 10 Otley Sailing Club.

## Methods

### Water chemistry

Water temperature, pH and dissolved oxygen were measured in the field using a Hach SL1000 Portable Parallel Analyser (PPA). Samples for nutrient chemistry were collected in 100 ml acid-washed bottles and posted to the James Hutton Institute in Aberdeen for analysis. Laboratory methods are described in Appendix B.

### *E. coli*

Samples for *E. coli* analysis were collected in 350 ml sterile bottles, kept refrigerated with ice in a cool bag and driven to ALS Ltd in Wakefield prior to overnight delivery for analysis in Coventry.

### Diatoms

Epilithic diatoms were collected by brushing the biofilm off three hand-sized cobbles into a container (Figure 3) before transfer to sterilin tubes. Ethanol was added as a preservative. Samples were posted to Henderson Ecology in Newcastle for analysis. Samples were cleaned in the laboratory and diatoms were mounted on microscope slides using Naphrax following standard methods (Battarbee et al. 2001). A minimum of 300 diatom valves was identified at each site. The data were then used to calculate TDI scores for each sample (Kelly et al. 2001).



Figure 3. Diatom sampling

### *Filamentous algae*

Samples of filamentous algae were detached from stones using tweezers or fingers, and the percentage cover of filamentous algae at each site was estimated visually following the RAPPER protocol (Kelly *et al.* 2020). See Appendix F for more details.

### *Macroinvertebrates*



Macroinvertebrate samples were collected by kick sampling for three minutes at each site (Figure 4). Samples were transferred to large volume containers covered in IDS and driven to the APEM laboratory in Salford for analysis. Analysis followed standard WFD protocols (see Appendix C) and the data were used to calculate WHPT and other metrics.

Unfortunately, it has not yet been possible to sample the deep-water benthos in the river due to dangerously high river flows occurring on the planned dates for sampling. A further attempt to obtain such samples will be made in 2023.

Figure 4. Macroinvertebrate sampling

## **Results**

### *Water chemistry*

Water chemistry results are shown in Figure 5. The values align closely with those from the same sites sampled under the iWharfe 2020 project. pH values vary between pH 7 and 8 and dissolved oxygen (DO) between 8 and 11 mg/l. Although there is some variability in both the temperature and DO data there is evidence for DO rising above saturation levels because of algal photosynthesis increasing during the day (Figure 5).

The most striking change occurs between sites 6 (Ashlands) and 7 (Beanlands Island) as the river receives the input of treated effluent from the Ilkley STW. This is not only clearly shown by the phosphate and nitrate data but also by the conductivity values. Sewage effluent has a higher electrical conductivity than unpolluted river water due to its high concentration of dissolved salts principally from ammonium, nitrate and phosphate ions. Its impact on the river is especially marked when river flow is low, providing limited dilution, as on the day of sampling.

The very steep increase in nitrate-N and phosphate-P at this point matches closely the data from August 2020 (Battarbee *et al.* 2022), although upstream nitrate values on that occasion were somewhat higher. These increases are entirely due to the input of treated effluent from the STW. Samples were taken in dry weather during a period of low river flow so no storm overflow discharges were occurring. Consequently, nutrient concentrations at the Cromwheel site and the close by Ashlands site (downstream of the storm overflow) are almost identical.

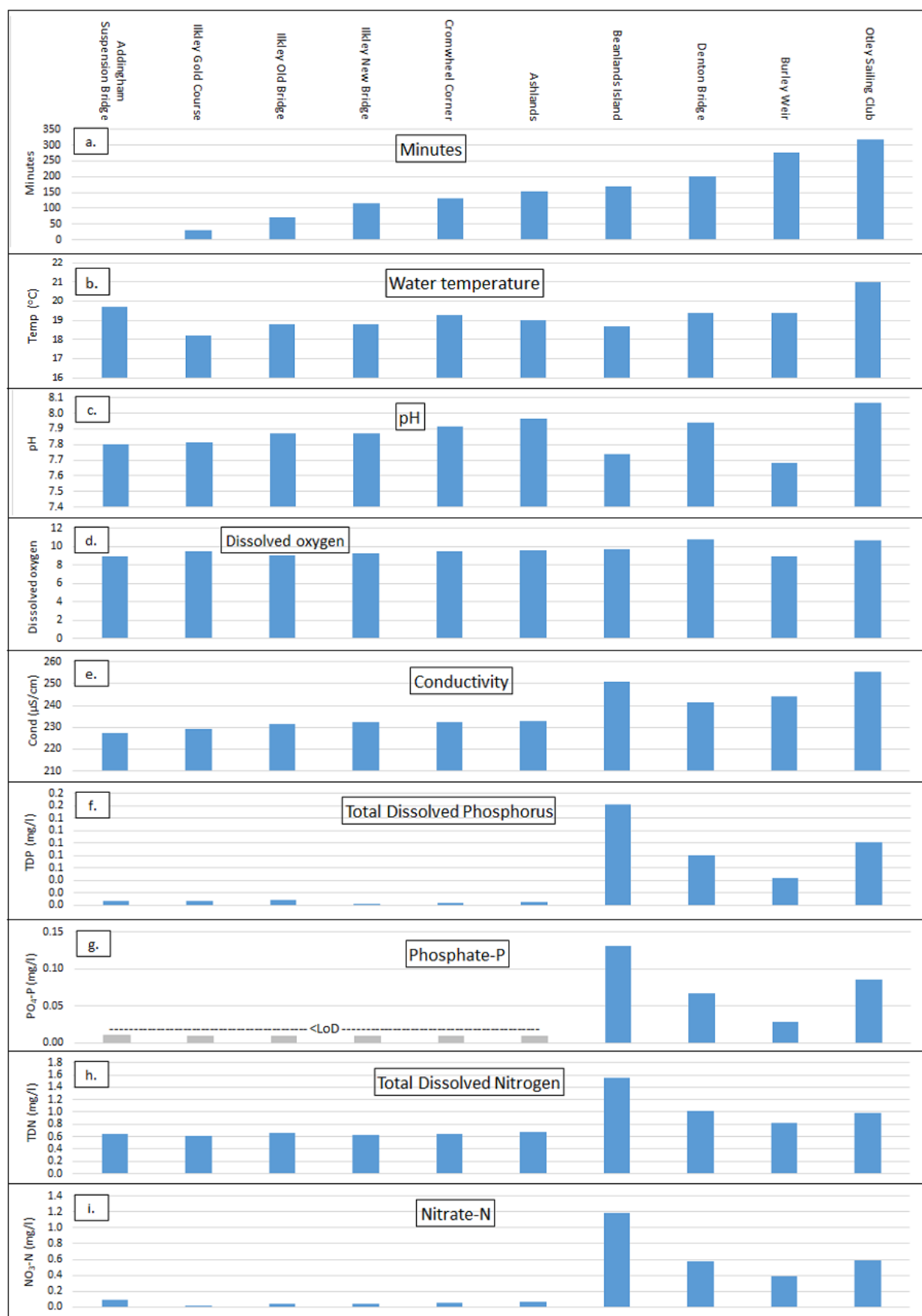


Figure 5. Water chemistry data for Eco-Ashlands sites (see Figure 2 & Appendix A for locations). a. minutes elapsed from the time of the first sample at 08:40; b. water temperature; c. pH; d. dissolved oxygen; e. conductivity; f. total dissolved phosphorus; g. phosphate-P; h. total dissolved nitrogen; i. nitrate-N.

### Faecal bacteria (*E. coli*)

The concentration of *E. coli* in water samples from each site taken at the same time and location as samples for water chemistry (Figure 5) is shown in Figure 6.

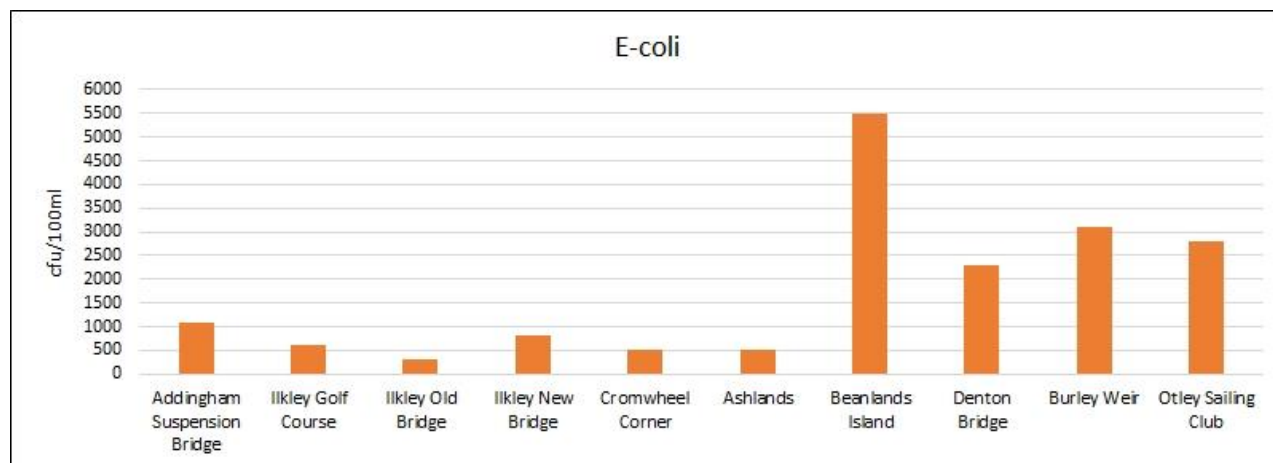


Figure 6. Concentration of *E. coli* (cfu/100 ml) at 10 sites on the River Wharfe from Addingham to Otley

Concentrations upstream of Ilkley STW are low and there is no significant difference between values for the Cromwheel and Ashlands reflecting the absence of storm overflow activity at the sewage works. The high value at Beanlands Island and sites downstream is due to the discharge of final (treated) effluent from the STW at a point located between the Ashlands and Beanlands Island sampling sites. The concentration at Beanlands Island is especially high as the site is close to the outfall and within the mixing zone of the effluent discharge and the receiving water. The lower value at Denton Bridge is partly due to microbial decay (or die-off) but also due to the effluent being fully diluted by the river-water at this point. The higher values at Burley Weir and Otley Sailing Club probably reflect the impact of additional *E. coli*-rich final effluent discharges from the Ben Rhydding and Burley/Menston STWs respectively.

This downstream pattern of *E. coli* concentration has been shown several times before (e.g. Battarbee et al. 2020, 2021) and is typical for this section of the river in dry weather/low river level conditions. It highlights the observation that treated effluent discharges carry very high faecal bacteria loads and that health risks are present for members of the public using the river for recreation downstream from Ilkley at any time.

### Diatoms

Changes in the composition of the diatom assemblage from Addingham to Otley together with changes in pH and nutrient chemistry are shown in Figure 7. The overall pattern for this stretch of river is very similar to that seen in August 2020 (Battarbee et al. 2022). Samples upstream of the Ilkley STW are dominated by nutrient sensitive *Achnanthes* species. Samples downstream include a range of nutrient tolerant species such as *Amphora pediculus*, *Eolimna minima* (Figure 8) and several *Navicula* spp. The nutrient enrichment that occurs by the effluent discharge from the STW is reflected by the decrease in TDI4 scores. TDI stands for Trophic Diatom Index. It is a diatom metric used by the Environment Agency as an indicator of eutrophication in rivers (Kelly et al. 2001). The change in the metric and in diatom assemblage composition aligns perfectly with the data for nutrient chemistry (Figure 5).



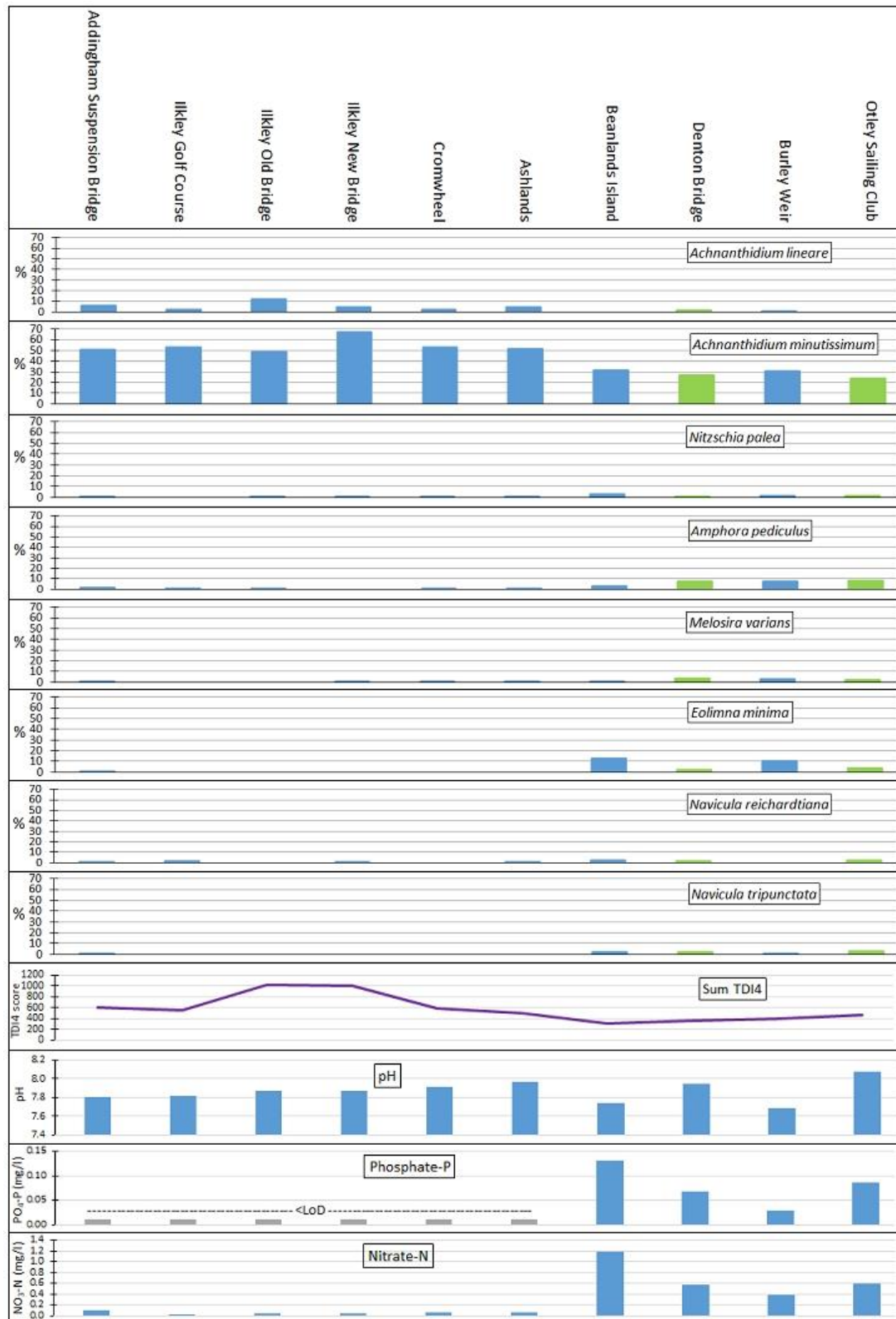


Figure 7. Selected diatom taxa (%), TDI4 and water chemistry data for 10 sites on the River Wharfe between Addingham and Ilkley sampled on the 8<sup>th</sup> of August 2022. Blue = high quality; Green = good quality based on TDI scores.

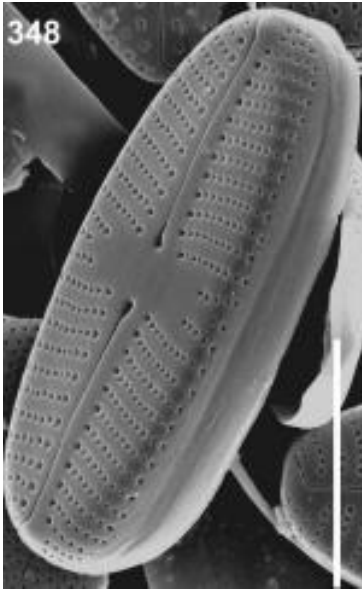


Figure. 8. *Eolimna minima*

Whereas these data confirm the findings of the 2020 study (Battarbee et al. 2022) they additionally indicate the relative importance of the untreated and treated effluent from the STW in causing the change. As explained above this study included a sample site, here called Ashlands, situated between the storm overflow (untreated) and the final effluent (treated) discharge points. It is clear from these data (Figure 7) that the change in both chemistry and diatom biology occurs immediately downstream of the final effluent outfall and not downstream of the storm overflow. Indeed, there is little or no difference between the chemistry and diatom biology of the Cromwheel site, upstream of the STW and the Ashlands site, downstream of the storm overflow point.

Whilst this conclusion is striking it is not surprising. The nutrient status of the river is more influenced by the continuous discharge of the nutrient-rich final effluent than by the equally nutrient-rich effluent from the storm overflow that is only activated intermittently.

### Filamentous algae

Our objective here was to trial a method using filamentous algae as a measure of the relative level of nutrients at each site. The method draws heavily on the RAPPER protocol (Kelly et al. 2020) which assesses evidence for eutrophication through excessive growth of competitive 'C' filamentous algae taxa, which are known to out-compete other taxa where higher nutrient levels are present. These taxa are *Cladophora*, *Vaucheria* spp., *Hydrodictyon* spp., *Rhizoclonium* spp., *Ulva* spp. and *Melosira* spp.

Samples were taken at all sites on Tuesday 9<sup>th</sup> and duplicated on Friday 13<sup>th</sup> 2022. The mean values of the 9<sup>th</sup> and 13<sup>th</sup> August samples were examined in the lab for the relative proportions of competitive and non-competitive taxa, and those proportions applied to the estimated percentage cover.

This method is experimental and further trials are planned. In the meantime, the results are being regarded as inconclusive. However, there is a degree of alignment with the lab measured gradients of phosphate-P and nitrate-N (Figure 5) and an EA survey in 2019 showed an increase in nutrient tolerant taxa downstream of the STW (Hood 2020).

See Appendix F for more details.

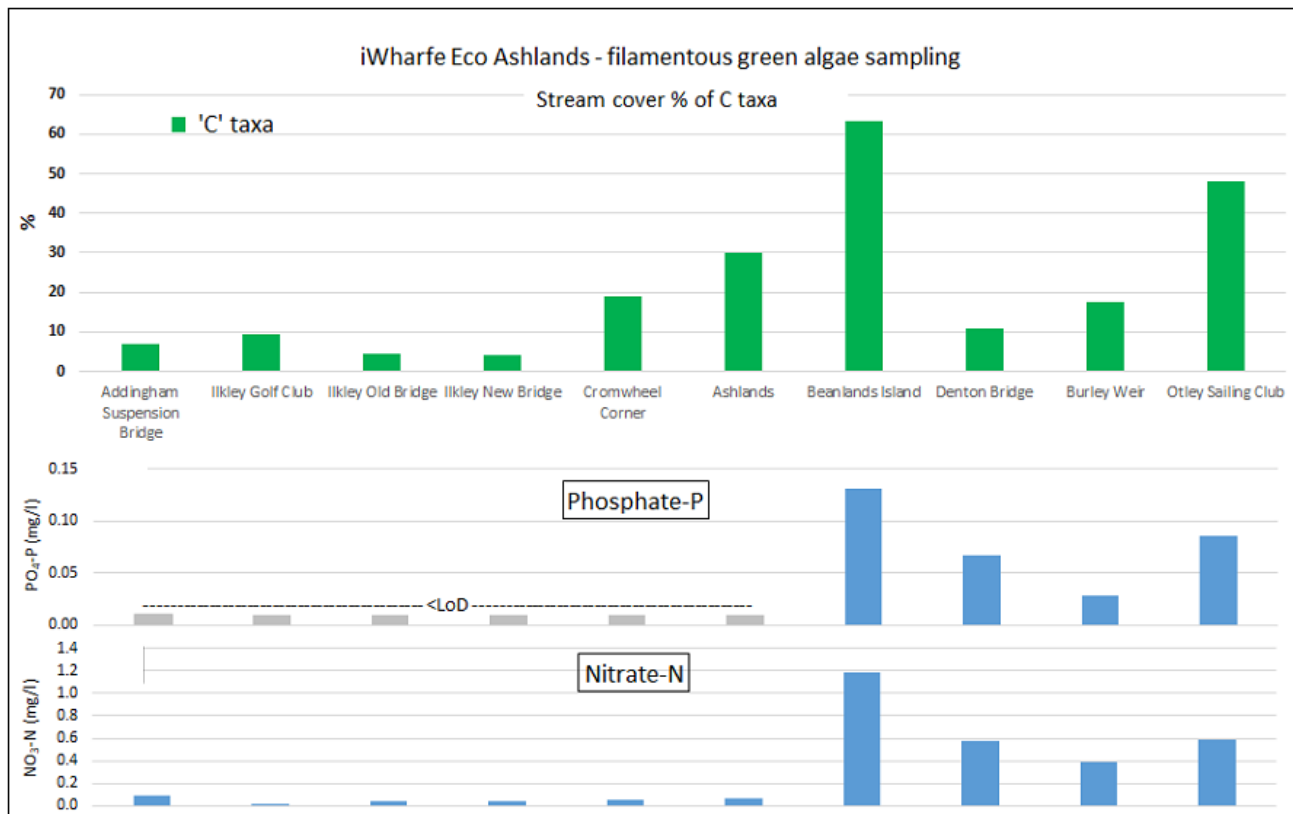


Figure 9. Comparison between % cover of filamentous green 'C' algal taxa and nutrient levels

### Macroinvertebrates

Macroinvertebrate samples from each site were analysed by APEM using the standard Environment Agency protocol which includes species identification where possible. A range of standard metrics based on the ecological preference of taxa with respect to organic pollution and other stresses was also calculated. The numbers of individual animals recovered by a three-minute kick sample varied considerably (Figure 10). Here we have consequently standardised the data by transformation to percentages and grouped taxa into higher taxonomic units (Figure 10).

In contrast to the diatom data there is no significant difference between the Cromwheel, Ashlands and Beanlands Island samples. All samples contain relatively high abundance of mayfly (Ephemeroptera), stonefly (Plecoptera) and caddis fly (Trichoptera) larvae indicative of high water quality. These taxa are often grouped together and expressed as EPT. The data suggest that neither the intermittent discharges of organic matter from the storm overflow nor the continuous discharge of nutrients from the final effluent outfall have an impact on the overall abundance or composition of invertebrate populations along this stretch of the river.

This is also a striking but not surprising finding. It aligns with the EA study from 2019 and 2020 (Hood 2020). The Wharfe is a fast-flowing, turbulent, well-oxygenated river. Although untreated organic-rich effluent is frequently discharged in wet weather via the storm overflow situated between the Cromwheel and Ashlands sites it appears likely that the oxygen consumed by organic matter decomposition is rapidly replenished.

Equally, and in contrast to the diatom response, there is no evidence to indicate that the nutrient-rich effluent discharged between Ashlands and Beanlands Island has an impact on the invertebrate populations. The treated effluent, very low in organic matter, creates little oxygen demand and invertebrates are less affected by nutrient pollution than diatoms.

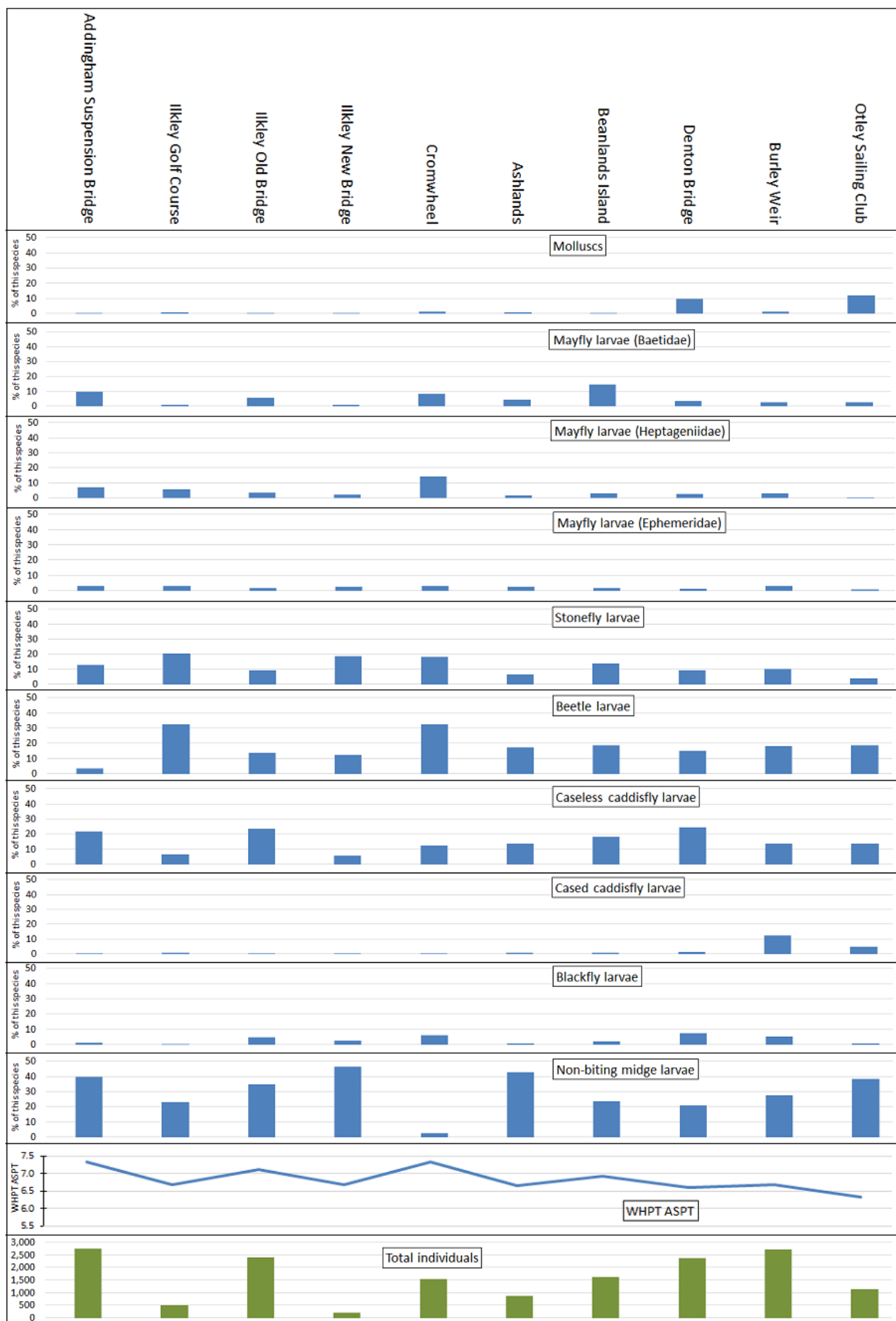


Figure 10. Downstream changes in macroinvertebrate populations (%), WHPT ASPT scores and total individuals in a 3-minute kick sample for sites from Addingham Suspension Bridge to Otley Sailing Club.



Consequently, we have yet to find an adverse ecological effect of the untreated sewage spills immediately downstream of the Ilkley STW storm overflow. However, further work is planned to sample deeper water sediments upstream and downstream of the storm overflow to identify possible changes to the mud-dwelling benthic fauna of the river that might have been brought about by the untreated sewage discharges.



Figure 11. The cased caddis fly larva *Brachycentrus subnubilus* (left) and the mollusc *Anyclus fluviatilis* (right).  
Pictures from <https://eol.org/media/10785692> and <https://www.biolib.cz/en/image/id97859>

Whilst there is little evidence of any clear impact of pollution from Ilkley STW itself on invertebrate life in the river, there is some indication from the macroinvertebrate data that water quality deteriorates downstream from Ilkley to Otley. The percentage of stoneflies, caseless caddis and some mayfly groups remains high, but there is an apparent decrease in heptageniid mayflies, an increase in cased caddis flies and an increase in mollusc numbers (Figure 10). Most notably there is a slight decrease in the WHPT ASPT metric. This metric uses scores for the sensitivity of all individual invertebrate taxa in a sample combined to provide an organic pollution index.

The change is slight and more research is needed. Other factors might be involved such as changes in habitat and flow regimes, but it would be surprising if the cumulative inputs of nutrient rich final effluent and organic rich untreated storm overflows that occur downstream of Ilkley from both CSOs (Wheatley Lane) and STWs (Ben Rhydding, Burley/Menston) when added to the Ilkley STW discharges did not have a negative impact on river ecology.

## Summary and conclusions

In our 2020 iWharfe project (Battarbee et al. 2020) we showed that effluent discharges from the Ilkley STW were the principal cause of deterioration in the ecological health of the river. Data from both nutrient chemistry and diatom assemblage composition showed clear evidence of nutrient enrichment.

In that project samples were collected immediately upstream of the STW (at the Cromwheel Corner) and immediately downstream (at Beanlands Island) but not between the storm overflow and final effluent discharge points. Consequently, we were not able to differentiate between the potential impacts of the intermittent discharge of untreated sewage from the storm overflow and the continuous discharge of treated effluent 150 metres downstream.

In this project we sampled from the same locations as 2020 but added a further site positioned mid-way between the two discharge points (Figure 1), which we called “Ashlands”. We carried out nutrient chemistry and diatom analysis at all sites, as in 2020, but added macroinvertebrate analysis. All analyses were carried out by accredited laboratories. We use diatoms as indicators of nutrient pollution and macroinvertebrates as indicators of organic pollution. We expected to find evidence of organic pollution from the

macroinvertebrate data downstream of the storm overflow (i.e. at the Ashlands site and further downstream) and evidence of nutrient pollution downstream at the Beanlands site and further downstream.

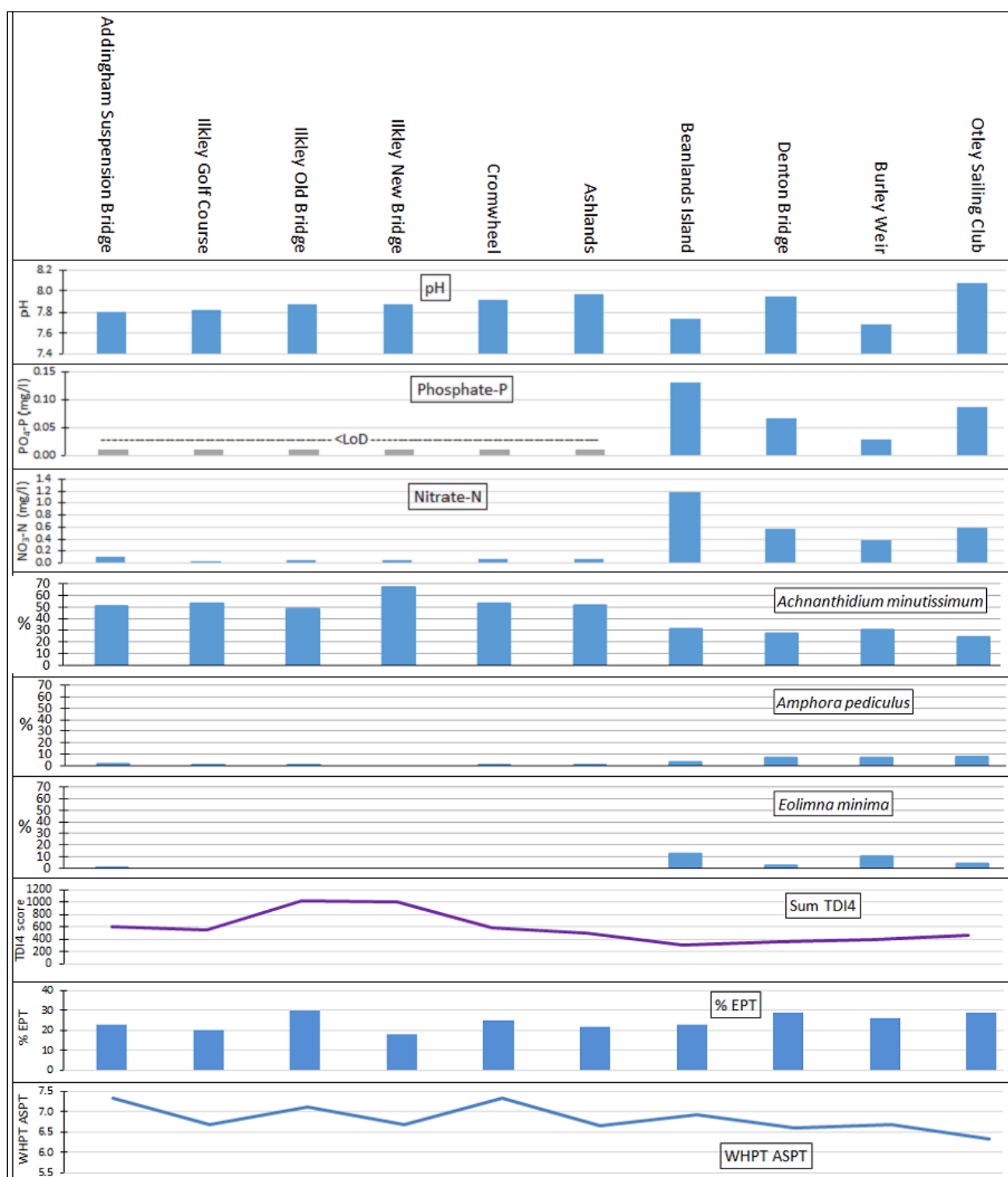


Figure. 12. Summary diagram showing hydrochemistry (pH, phosphate-P, nitrate-N), diatom (selected species, TDI4) and macroinvertebrate data (%EPT, WHPT ASPT) for River Wharfe sites from Addingham Suspension Bridge to Otley Sailing Club

Figure 12 shows a summary of our results, matching water chemistry with the diatom and macroinvertebrate data from the 10 sites between Addingham and Otley. The data show that:

- (i) there is no significant change in water chemistry, diatom composition or macroinvertebrate composition between the Cromwheel Corner and the Ashlands site indicating that the intermittent spills of untreated sewage, despite their frequency, have little apparent adverse impact on the ecology of the river in terms of both nutrient pollution impact and organic pollution impact;
- (ii) there is no significant change in EPT% (the sum of Ephemeroptera, Plecoptera and Tricoptera) downstream but there is a significant change in diatom composition coinciding with the increase in phosphate and nitrate concentration between the Ashlands site and the Beanlands Island site indicating that the biological change is caused by the high nutrient concentration of the final (treated) effluent from the Ilkley STW;
- (iii) whereas there is no detectable local impact of the discharges from the Ilkley STW the decrease in the WHPT ASPT scores from Beanlands Island to the Otley Sailing Club (Fig. 12) indicates a slight but clear deterioration in water quality from Ilkley downstream. This deterioration is probably caused by the cumulative impact of both organic and nutrient pollution not just from Ilkley but also from CSOs and SWTs serving Ben Rhydding, Burley-in-Wharfedale and Menston populations.

Although repeat sampling and analysis at these sites along the river at different times of year would be desirable the overall conclusion, that nutrient pollution from the discharge of treated effluent is the principal threat to the ecology of the river, is robust. It is also in close agreement with the independently obtained findings of the Environment Agency (Dacombe & Wait 2022).

Consequently, these data highlight the importance not only of controlling spills of raw sewage, that occur frequently in Ilkley, but also the need to remove phosphorus from the final (treated sewage) effluent.

We are optimistic that a phosphorus removal stage will be installed at Ilkley STW and possibly also at Burley/Menston STW following an application from the EA to Defra for the Wharfe to be designated as a “sensitive area” for eutrophication under the Urban Wastewater Treatment Regulations (UWWTR). If successful the nutrient reduction requirements will need to be met within seven years of the effective date of the next Urban Wastewater Treatment Identification of Sensitive Areas Notice, so by 2030.

## Acknowledgements

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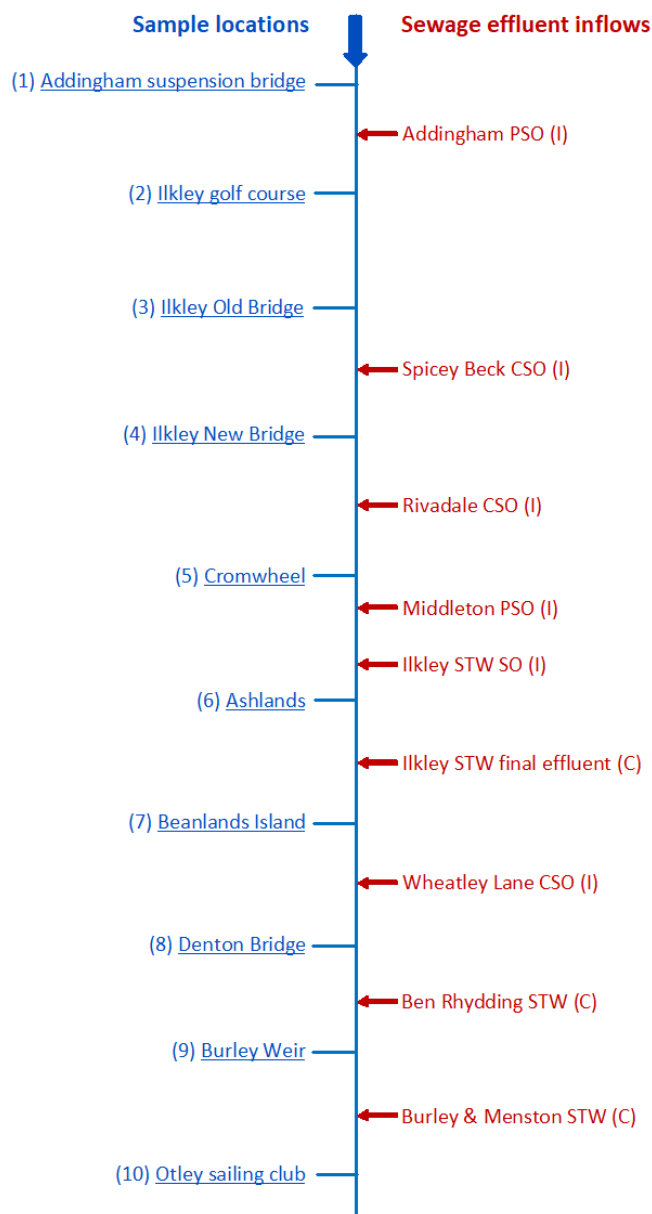
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## Appendix A. Eco-Ashlands sites

Code	Name	Grid Ref	Location
EcoA-1	Addingham Suspension Bridge	SE 08313 50010	53.946134, -1.874828
EcoA-2	Ilkley Golf Course	SE 09586 48446	53.932053, -1.855478
EcoA-3	Ilkley Old Bridge	SE 11096 48131	53.929194, -1.832500
EcoA-4	Ilkley New Bridge	SE 11765 48018	53.928167, -1.822306
EcoA-5	Cromwheel	SE 12153 48412	53.931694, -1.816389
EcoA-6	Ashlands	SE 12525 48402	53.931602, -1.810728
EcoA-7	Beanlands Island	SE 12693 48348	53.931109, -1.808161
EcoA-8	Denton Bridge	SE 13707 48163	53.929417, -1.792722
EcoA-9	Burley Weir	SE 16600 47426	53.922712, -1.748718
EcoA-10	Otley Sailing Club	SE 18694 45584	53.906083, -1.716944

Diagram showing site locations and the position of Yorkshire Water assets along the river. PSO = Pumping Station Overflow; CSO = Combined Sewer Overflow; STW = Sewage Treatment Works; SO = Storm Overflow; C = Continuous; I = Intermittent.

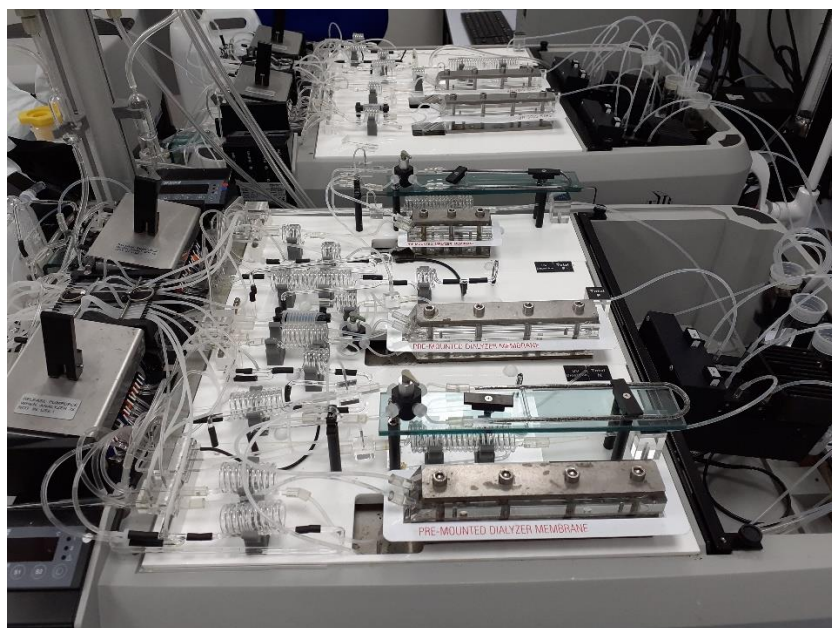


## Appendix B. Hydrochemistry methods (The James Hutton Institute)

pH and electrical conductivity were determined on unfiltered samples using a MeterLab Ion 450 analyser.

Suspended solids (SS), were determined by filtration (under vacuum) of a known volume of sample through pre-weighed GFC filters, which were then air dried and reweighed to derive the weight of suspended material on the papers. The filtrate was then passed through a 0.45 membrane filter (under vacuum) and retained for analysis.

Analysis for the suite of nutrients, DOC,  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$ ,  $\text{NH}_4\text{-N}$ , TDP and TDN was carried out on the filtrate using colorimetry-based methods on the SAN ++ analyser (pictured below) manufactured by Skalar, Breda, Netherlands.



## Appendix C. Macroinvertebrate analysis methods (APEM)

Within the confines of a fume cupboard, a 500µm sieve is placed within a clean plastic container and the sample poured into the sieve to separate preservative from the retained sample fraction. The sample is then thoroughly rinsed using tap water.

Samples may be rinsed through two or more gradations of sieves to separate different size fractions and facilitate the sorting process. This is achieved by using the 500 µm sieve as the bottom sieve and using one or more sieves of greater mesh size on top of it. The sample is poured into the sieves and washed down through each gradation.

A small amount of sample material is transferred to the sorting tray using a clean spoon. The material is diluted with water and spread out to ensure that the amount of sample is manageable.

The tray is systematically scanned for macroinvertebrates, following the grid pattern to ensure that the whole area of the tray is searched.



Using forceps, specimens are picked out from the sample tray and separated into different taxon groups in petri-dishes or an ice-cube tray, for further identification with the use of a microscope (with a 20-50x zoom lens).

Macroinvertebrates are counted by recording abundances directly on the Macroinvertebrate Sample Recording Form, or by using tally counters for the most abundant taxa and recording the abundances upon completion of the sample analysis. Once complete, the sample taxa counts are transferred to APEM's bespoke database.

#### Appendix D. Water chemistry data for Eco-Ashlands sites (The James Hutton Institute)

Code	Date	Lab ID	Site	Time	Minutes	pH	Conductivity	S/S mg/l	Nitrate-N	Phosphate-P	NH4-N mg/l	DOC mg/l	TDN	TDP	pH	DO	Temp °C
Eco A _ 1	2222-08-08	1367319	Addingham Suspension Bridge	08:40	0	7.802	227.4	1.5	0.0915	0.0100	0.0348	8.1981	0.6498	0.0073	8.4	8.96	19.7
Eco A _ 2	2222-08-08	1367320	Ilkley Golf Course	09:10	30	7.811	229	0.0	0.0145	0.0100	0.0208	8.3569	0.6132	0.0076	8.39	9.43	18.2
Eco A _ 3	2222-08-08	1367321	Ilkley Old Bridge	09:50	70	7.872	231.3	1.0	0.0426	0.0100	0.0283	8.4615	0.6657	0.0081	7.61	9.03	18.8
Eco A _ 4	2222-08-08	1367322	Ilkley New Bridge	10:35	115	7.873	232.2	1.0	0.0417	0.0100	0.0198	8.2986	0.6220	0.0013	8.28	9.32	18.8
Eco A _ 5	2222-08-08	1367323	Cromwheel Corner	10:50	130	7.916	232.4	2.3	0.0515	0.0100	0.0195	8.2205	0.6495	0.0033	8.49	9.51	19.3
Eco A _ 6	2222-08-08	1367324	Ashlands	11:14	154	7.966	232.7	0.8	0.0667	0.0100	0.0222	8.2642	0.6699	0.0054	8.53	9.58	19
Eco A _ 7	2222-08-08	1367325	Beanlands Island	11:30	170	7.741	251	2.3	1.1847	0.1310	0.0321	8.5329	1.5582	0.1624	8.33	9.71	18.7
Eco A _ 8	2222-08-08	1367326	Denton Bridge	12:02	202	7.941	241.4	0.8	0.5807	0.0670	0.0232	7.9654	1.0241	0.0811	8.8	10.77	19.4
Eco A _ 9	2222-08-08	1367327	Burley Weir	13:18	278	7.681	243.9	0.0	0.3880	0.0290	0.0313	8.4427	0.8242	0.0444	8.45	8.98	19.4
Eco A _ 10	2222-08-08	1367328	Otley Sailing Club	13:57	317	8.07	255.2	3.1	0.5833	0.0860	0.0172	7.6680	0.9782	0.1015	8.94	10.7	21



## Appendix E. Diatom data (Henderson Ecology)

NBSCode	TaxonName	EcoA-1	EcoA-2	EcoA-3	EcoA-4	EcoA-5	EcoA-6	EcoA-7	EcoA-8	EcoA-9	EcoA-10
NHMSYS0020970806	<i>Achnantheidium pyrenaicum</i>	10.6	7.5	19.4	3.8	3.3	3.3	2.5	1.1	4.9	2.4
NBNSYS0100047734	<i>Karayevia clevei</i>	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NBNSYS0100051134	<i>Planothidium lanceolatum</i>	0.0	0.0	0.1	0.0	0.3	0.0	0.0	0.0	0.2	0.0
NBNSYS0100051130	<i>Planothidium frequentissimum</i>	1.3	0.0	0.0	0.2	0.0	0.0	2.5	0.5	2.0	1.6
NHMSYS0020475041	<i>Achnanthes minutissima</i> var. <i>atomoides</i>	0.0	1.4	1.3	3.0	3.6	1.5	2.8	0.0	0.0	0.0
NHMSYS0021166274	<i>Achnantheidium lineare</i>	5.8	1.9	11.8	4.4	2.3	4.2	0.0	1.6	1.0	0.0
NBNSYS0100041609	<i>Achnantheidium minutissimum</i>	50.8	52.8	48.5	66.8	52.7	51.3	31.0	26.8	30.2	23.9
NBNSYS0100041894	<i>Amphipleura pellucida</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NBNSYS0100041935	<i>Amphora pediculus</i>	1.3	0.5	0.5	0.0	1.0	0.4	2.8	7.0	7.1	7.9
NHMSYS0021180242	<i>Caloneis bacillum</i>	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NBNSYS0100043872	<i>Cocconeis pediculus</i>	1.4	6.3	0.7	1.1	4.3	2.5	4.1	7.9	0.0	5.3
NBNSYS0100043875	<i>Cocconeis placentula</i> var. <i>euglypta</i>	5.5	5.9	2.3	1.9	3.0	4.4	5.0	12.7	1.2	5.7
NBNSYS0100043879	<i>Cocconeis placentula</i> var. <i>pseudolineata</i>	1.4	0.2	0.0	0.0	0.0	0.2	0.6	0.8	0.5	0.2
NBNSYS0100044842	<i>Cyclotella meneghiniana</i>	1.4	2.3	0.2	0.9	2.5	2.9	0.0	0.8	0.0	6.1
NHMSYS0020749137	<i>Cymbella prostrata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
NHMSYS0021177019	<i>Encyonopsis minuta</i>	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NBNSYS0100045694	<i>Encyonema minutum</i>	1.4	2.4	1.3	0.8	1.0	1.7	0.0	1.6	4.4	2.6
NBNSYS0100045702	<i>Encyonema reichardtii</i>	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.4
NBNSYS0100045704	<i>Encyonema silesiacum</i>	1.8	4.0	0.2	3.3	4.8	4.4	2.8	7.0	2.2	7.5
NHMSYS0020953907	<i>Encyonema "ventricosum" ag.</i>	0.0	0.0	0.0	0.4	0.3	0.0	0.0	0.0	0.0	0.8
NBNSYS0100045140	<i>Denticula tenuis</i>	4.0	3.5	7.0	4.5	11.7	8.2	1.3	0.5	0.5	0.4
NHMSYS0020063121	<i>Diatoma ehrenbergii</i>	0.0	0.0	1.1	0.6	0.3	0.8	1.3	0.0	0.0	0.4
NHMSYS0020749132	<i>Diatoma problematica</i>	0.6	0.0	0.2	0.0	0.0	0.4	0.0	0.5	0.0	0.2
NBNSYS0100045227	<i>Diatoma tenue</i>	0.3	0.0	0.0	0.2	0.2	0.6	0.0	0.0	0.0	0.0
NBNSYS0100045229	<i>Diatoma vulgare</i>	0.6	0.0	0.4	0.3	1.5	1.0	0.0	2.2	0.5	2.2
NBNSYS0100046435	<i>Fragilaria capucina</i>	0.0	0.3	0.0	0.0	0.5	0.0	0.3	1.1	0.0	0.4
NHMSYS0000523731	<i>Fragilaria capucina</i> var. <i>gracilis</i>	0.6	0.5	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.2
NBNSYS0100046438	<i>Fragilaria capucina</i> var. <i>mesolepta</i>	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
NHMSYS0021166205	<i>Fragilaria capucina</i> var. <i>pectinalis</i>	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

NBSCode	TaxonName	EcoA-1	EcoA-2	EcoA-3	EcoA-4	EcoA-5	EcoA-6	EcoA-7	EcoA-8	EcoA-9	EcoA-10
NHMSYS0020953943	<i>Fragilaria capucina</i> var. <i>perminuta</i>	0.2	0.5	0.0	0.2	1.6	1.5	1.9	2.2	0.5	0.0
NBNSYS0100053280	<i>Fragilaria construens</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
NBNSYS0100046453	<i>Fragilaria vaucheriae</i>	0.0	0.3	0.0	0.1	0.0	0.0	0.6	1.4	2.0	2.0
NHMSYS0020970882	<i>Fragilaria nanana</i>	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
NBNSYS0100046431	<i>Fragilaria</i> sp.	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
NHMSYS0021167703	<i>Gomphonema olivaceum</i> var. <i>minutissima</i>	0.0	0.3	0.2	0.6	0.0	0.2	0.0	0.5	0.0	0.0
NBNSYS0100046734	<i>Gomphonema olivaceum</i>	1.0	1.7	0.6	0.7	1.1	1.0	0.6	0.8	0.2	1.8
NBNSYS0100046740	<i>Gomphonema parvulum</i>	0.0	0.0	0.1	0.2	0.3	0.0	1.6	0.0	0.5	0.0
NHMSYS0020063128	<i>Gomphonema pumilum</i>	1.4	0.0	0.8	0.0	0.0	0.6	0.0	1.1	0.0	0.0
NBNSYS0100046687	<i>Gomphonema</i> sp.	0.3	0.0	0.2	0.8	0.0	0.8	0.9	0.0	0.0	0.4
NBNSYS0100046751	<i>Gomphonema tergestinum</i>	0.0	0.0	0.3	0.5	0.3	0.0	0.0	0.0	0.5	0.0
NBNSYS0100046752	<i>Gomphonema truncatum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
NBNSYS0100046917	<i>Gyrosigma acuminatum</i>	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
NBNSYS0100048616	<i>Melosira varians</i>	0.8	0.0	0.0	0.4	0.8	0.8	0.3	3.3	2.9	1.8
NBNSYS0100048637	<i>Meridion circulare</i>	0.0	0.0	0.2	0.0	0.7	0.0	0.6	0.0	0.0	0.0
NHMSYS0020954057	<i>Mayamaea atomus</i> var. <i>permitis</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	1.0	0.8
NBNSYS0100049211	<i>Navicula capitatoradiata</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4
NBNSYS0100049233	<i>Navicula cryptocephala</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
NBNSYS0100049239	<i>Navicula cryptotenella</i>	0.3	0.3	0.6	1.0	0.0	0.2	1.3	3.5	0.5	1.8
NBNSYS0100049293	<i>Navicula gregaria</i>	0.6	0.9	0.0	0.2	0.0	0.6	0.9	0.8	1.2	2.0
NBNSYS0100049331	<i>Navicula lanceolata</i>	0.0	0.3	0.1	0.2	0.0	0.4	1.3	0.8	0.7	1.8
NBNSYS0100049351	<i>Navicula antonii</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.4
NHMSYS0020970860	<i>Eolimna minima</i>	0.3	0.0	0.0	0.0	0.0	0.0	12.5	2.2	10.1	3.4
NBNSYS0100049402	<i>Navicula radiosa</i>	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NBNSYS0100049406	<i>Navicula reichardtiana</i>	0.6	1.0	0.0	0.6	0.0	0.4	1.9	1.1	0.0	2.0
NBNSYS0100052466	<i>Sellaphora seminulum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
NBNSYS0100049162	<i>Navicula</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.5	0.4
NHMSYS0020953915	<i>Eolimna/ Craticula subminuscula</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.4	0.0	0.0
NHMSYS0020953767	<i>Navicula suchlandtii</i>	0.0	0.0	0.0	0.2	0.0	0.0	0.3	1.1	0.0	0.0
NBNSYS0100049481	<i>Navicula tripunctata</i>	0.3	0.0	0.0	0.0	0.0	0.0	1.9	2.2	0.5	2.6

[illegible]

## Appendix F. Filamentous algae data and calculations (AEG)

### Notes

*Note 1:* RAPPER taxa classification used here: **Competitive**, Additional classification: **Other**

### Field assessment protocol

Field assessment of the full stream width percentage cover of filamentous algae was carried out twice, first on the 9<sup>th</sup> of August and then the 13<sup>th</sup> of August.

On the 9<sup>th</sup> of August focus was on a 10m x 2m transect from which to collect a representative sample and assess the percentage cover within the transect for extrapolation to the whole river. An attempt was also made to estimate the proportions of 'C' competitive and 'O' other taxa on-site, with, it transpired, little success.

The 9<sup>th</sup> of August survey estimates of cover percentage were subsequently considered inaccurate, and four days later on 13<sup>th</sup> August while the river conditions were unchanged a further survey was carried out using a revised method of assessment. Over a 10m long transect and for as much of the stream width as the depth would safely allow, the percentage cover was estimated, and several growths examined in the field from which a representative sample for each site was collected. No attempt was made to estimate the proportions of 'C' and 'O' taxa abundance. Also noted were the approximate size of the stone the sample was taken from, and its distance from the edge of the wetted zone.

Six of the ten 13<sup>th</sup> August percentage cover estimates were in the main broadly comparable to those from the 9<sup>th</sup> August survey, see the data below. The other four highlighted sites were significantly different, these were where cover was subsequently estimated as 10% or less on 13<sup>th</sup> August - perhaps reflecting the greater likelihood of an error through extrapolation of a low percentage cover in a narrow 2m transect to the whole stream width.

Site	ASB	IGC	IOB	INB	CC	ASH	BI	DB	BW	OSC
9th Aug	60	50	55	10	85	75	90	80	50	90
13th Aug	10	10	5	5	30	50	80	80	40	80

*Whole stream percentage cover estimated percentages on the 9<sup>th</sup> and 13<sup>th</sup> of August*

For the analysis calculations the 13<sup>th</sup> of August percentage cover figures were used.

### Lab analysis protocol

1. Each microscope session was captured on video enabling subsequent review as required.
2. As well as a video capture of microscope sessions a still micrograph of each screen frame was taken where there were either 'C' or 'O' taxa visible (see examples below). Then in review of the micrographs the number for which either C or O taxa were visually dominant were counted and their percentages of the total number of micrographs calculated. In the case of the 9<sup>th</sup> of August when two samples were collected, these were examined separately, and their number of 'C' and 'O' dominant micrographs summed. Examples of screen frame micrographs are at Figure 15.
3. Interpretation using the RAPPER protocol was restricted to using the percentage covers for the whole stream, i.e. those from the 13<sup>th</sup> of August survey.



## Analysis

The table of data below shows from left to right –

- Columns B – G are the screens counted where C or O taxa were dominant
- Column H is the total number of screens assessed in that way
- Columns I and J are the percentages of each of C + O dominant screens
- Column K is the river cover estimated at the second survey on 13<sup>th</sup> August
- Columns L and M are the application of the values in columns I and J to the river cover column K

A	B	C	D	E	F	G	H	I	J	K	L	M
iWharfe Eco Ashlands - filamentous green algae sampling											Stream cover % of C and O taxa	
	Dominant taxa screen count						All taxa total	Percentages of C + O dominant screens		Est. stream cover %	Competitive 'C' taxa	Other 'O' taxa
	9th Aug C taxa	13th Aug Other taxa	13th Aug C taxa	13th Aug Other taxa	9th Aug + 13th Aug C taxa	9th Aug + 13th Aug Other taxa	9th Aug + 13th Aug screens	9th Aug + 13th Aug C taxa	9th Aug + 13th Aug Other taxa	9th Aug + 13th Aug 13th Aug		
Addingham Suspension Bridge	70	33	10	0	80	33	113	71	29	10	7	3
Ilkley Golf Club	62	3	9	1	71	4	75	95	5	10	9	1
Ilkley Old Bridge	49	9	5	0	54	9	63	86	14	5	4	1
Ilkley New Bridge	33	6	4	1	37	7	44	84	16	5	4	1
Cromwheel Corner	40	30	23	7	63	37	100	63	37	30	19	11
Ashlands	14	31	43	7	57	38	95	60	40	50	30	20
Beanlands Island	47	21	70	10	117	31	148	79	21	80	63	17
Denton Bridge	6	18	8	72	14	90	104	13	87	80	11	69
Burley Weir	1	9	21	19	22	28	50	44	56	40	18	22
Otley Sailing Club	19	6	44	36	63	42	105	60	40	80	48	32

Figure 13. Data table and analysis calculations

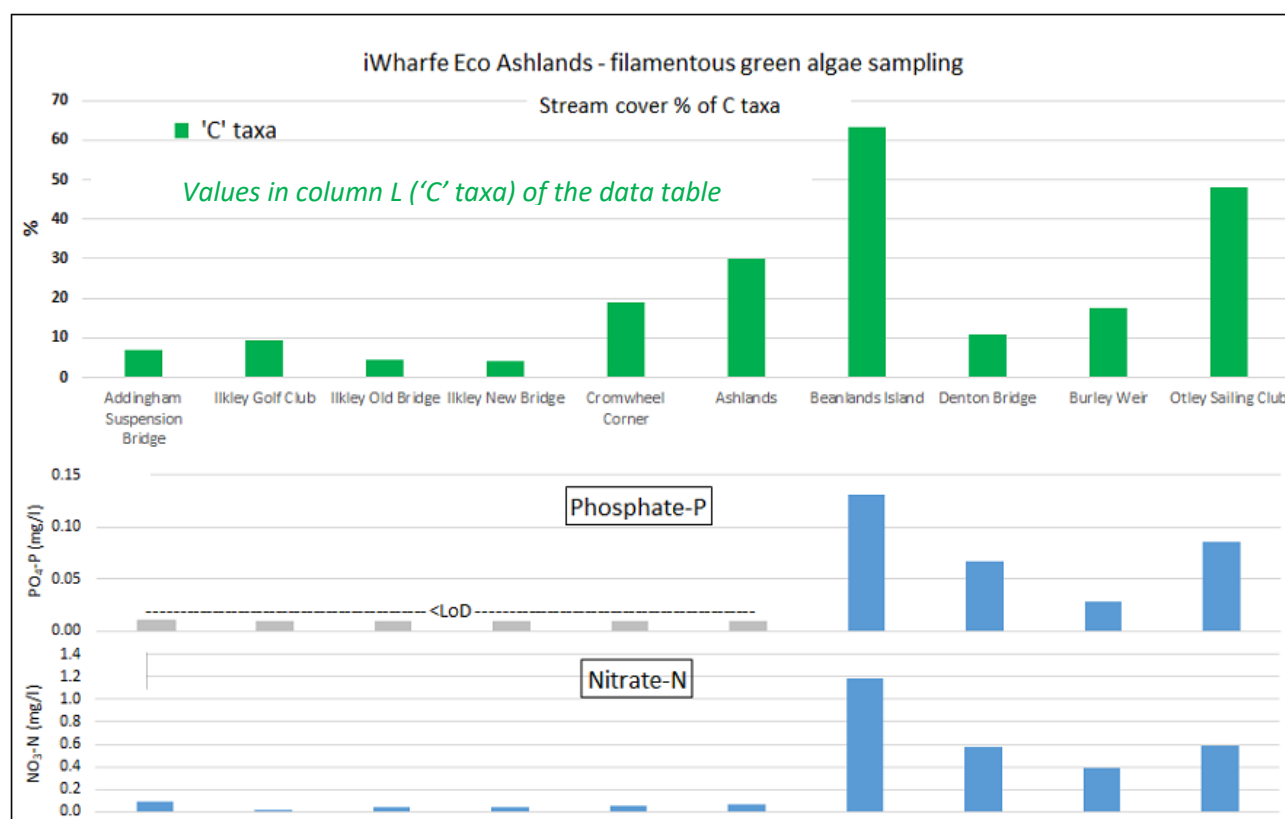


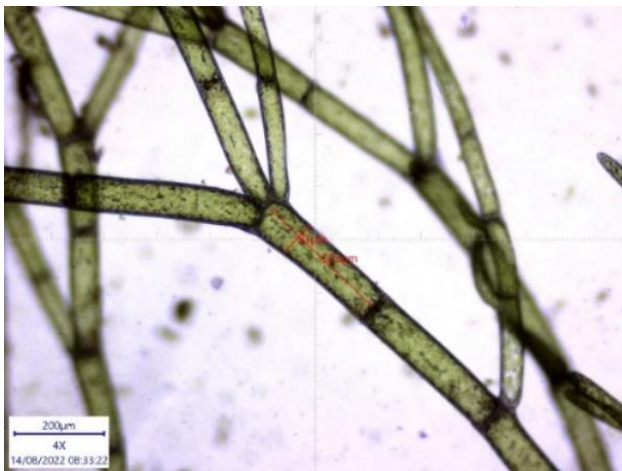
Figure 14. Comparison between % cover of filamentous green 'C' algal taxa and nutrient levels

It was expected that C taxa abundance would be greater in proportion to the level of nutrients available. If the analysis calculations are valid and correct, the results do suggest a degree of relationship to the expected nutrient differences consistent with their locations and distances from each of the STW outflows:

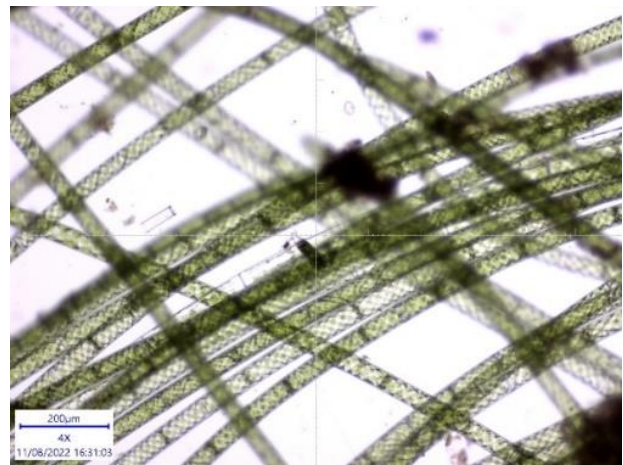
- Beanlands Island 150m downstream from Ashlands STW
- Burley Weir 2,600m downstream from Ben Rhydding STW
- Otley Sailing club 620m downstream from Burley & Menston STW

The Denton Bridge C taxa value appears to be an outlier at 1.2km downstream from Ashlands STW. Outwith the project analysis and by way of experiment and understanding, another survey with five samples collected is planned for Ashlands, Beanlands Island and Denton Bridge on a single day.

Figure 15. Examples of screen frame micrographs with 'C' dominant and 'O' dominant taxa



Addingham Suspension Bridge *Cladophora* sp. (C)



Cromwheel Corner *Spirogyra* spp. (O)

## Appendix G. Macroinvertebrate data (APEM Ltd)

Taxa	EcoA-1	EcoA-2	EcoA-3	EcoA-4	EcoA-5	EcoA-6	EcoA-7	EcoA-8	EcoA-9	EcoA-10
Nematoda								1		
Potamopyrgus antipodarum		3	1	1	3	2	2	13	7	5
Physidae							2			1
Physella sp.						1		1		
Lymnaeidae										1
Ampullaceana balthica										7
Ancylus fluviatilis	1		2		3	5	3	216	25	76
Ancylus group (Ancylus, Ferrissia & Acroloxus)					12					44
Sphaerium sp.										2
Pisidium sp.			1					1		1
Oligochaeta	1		3	1	3	1		18	4	
Helobdella stagnalis		1			1				1	1
Erpobdellidae							4	6	5	3
Erpobdella octoculata							1	1	2	
Hydracarina	4	5	10	9	10	41	6	31	11	21
Oribatei				1						
Pacifastacus leniusculus			2					1		
Gammarus sp.			2							
Gammarus pulex			1							
Gammarus pulex/fossarum		2								
Baetidae						27	6			
Baetis sp.	29	3	40	1		4	177	8	33	30
Baetis atlanticus/rhodani	14		35		73	1	16	51	28	
Baetis scambus/fuscatus	77	1	61	1	48	5	32	21	10	
Baetis vernus					2		6			
Procladius sp.	1									
Baetis muticus	149		2		5				1	
Heptageniidae		15	20	1	19	5	13	18	8	
Rhithrogena sp.	3				81		3	16	27	
Heptagenia group (Heptagenia, Electrogena & Kageronia)		2	21		23	8	13	17	26	
Heptagenia sulphurea	6		4		8				3	
Ecdyonurus sp.	166	12	32	3	85	2	19	14	15	1
Ecdyonurus dispar	16									
Leptophlebiidae		1	4							
Ephemera sp.										1
Ephemerellidae				1						
Serratella ignita	79	16	38	4	47	23	28	29	77	11
Caenis horaria							1			
Caenis luctuosa/macrura			1	1	1	2		12	1	6
Nemouridae			1							
Protonemura sp.			6					1		
Leuctra sp.	56	31	52	13	130	30	70	43	220	30
Leuctra fusca	242	54	88	19	136	19	152	136	44	7
Leuctra geniculata	50	17	76	3	11	6	4	38	11	6
Leuctra inermis				1						
Leuctra hippopus/moselyi				4						1
Perlodes mortoni	1									
Perla bipunctata					2					
Orectochilus villosus							3	2	2	
Hydraena gracilis	1						2			
Elmis aenea	18	75	179	9	371	49	54	45	106	51
Esolus parallelepipedus	64	48	68	10	104	74	121	196	130	66
Limnius volckmari	12	39	60	4	18	16	99	68	248	87
Oulimnius sp.	2	2	15	3	4	12	18	48	2	7
Oulimnius tuberculatus			7		2		9	2	2	4
Trichoptera					1			5	3	1
Rhyacophila sp.	7	3	4	1	9	6	9	5	3	8
Rhyacophila dorsalis	25		5		15	12	47	23	30	8
Glossosoma sp.	1				2			1	2	1
Glossosoma boltoni									4	
Agapetus sp.	2									
Agapetus ochripes	5		1		1					
Hydroptilidae								1		
Hydroptila sp.			1		1	1				1
Psychomyiidae								1		
Psychomyia sp.									1	
Psychomyia pusilla			2					2	1	2

Taxa	EcoA-1	EcoA-2	EcoA-3	EcoA-4	EcoA-5	EcoA-6	EcoA-7	EcoA-8	EcoA-9	EcoA-10
Polycentropodidae	2	9	5	1	5	5	5	4	5	8
Polycentropus sp.	5		15		1				2	
Polycentropus flavomaculatus			18		1			4	1	
Hydropsychidae			96		3	2				
Cheumatopsyche lepida	241	1	26	3	115	31	92	269	159	53
Hydropsyche sp.	140	14		3	6	47	92	10	13	9
Hydropsyche pellucidula	171	5	395	3	29	13	42	217	64	12
Hydropsyche siltalai				1	6		8	41	89	53
Brachycentrus subnubilus	1	4	2	1	1	6	15	25	336	51
Lepidostomatidae			2							
Lasiocephala sp./Lepidostoma sp.	2		4		1					
Sericostomatidae					1					
Sericostoma personatum					1				1	2
Odontocerum albicorne			1							
Athripsodes sp.		1	5			2		1		
Athripsodes albifrons group (bilineatus & commutatus)			1							
Athripsodes cinereus						1		1		1
Ceraclea sp.						1	1			1
Ceraclea annulicornis								1	1	
Ceraclea nigronervosa			1							
Mystacides azurea	1			1						
Tipulidae (Limoniidae, Cylindrotomidae and Pediciidae)	1									
Tipula sp.	1							2	1	
Antocha vitripennis	3	1	13	2	3	19	3	14	12	4
Hexatoma sp.		1								
Dicranota sp.	4	3	2		1		8	4	18	1
Ceratopogonidae	2	1	4		2	2	1	2		
Simuliidae	37	1	117	5	91	8	35	176	140	6
Chironomidae	1093	117	830	99	41	370	384	490	751	438
Atherix ibis	11	11	13	2	1	3	19	9	21	8
Empididae								2		
Clinocerinae	3	3		1	3	5	3	1	3	4
Hemerodrominae									1	1
Chelifera sp.	1		2					3	1	
Limnophora sp.	1								1	3

END