Llanishen and Lisvane Reservoirs: Survey of grassland fungi 2019

Dŵr Cymru Welsh Water

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1. Executive Summary

This report presents the findings of a survey and assessment of grassland fungi at Lisvane and Llanishen Reservoirs, Cardiff. The grassland embankments around both reservoirs are designated as a Site of Special Scientific Interest (SSSI) on account of these fungi. This survey follows similar surveys in 2016, 2017 and 2018, and was carried out to inform the ongoing capital works and management by Dwr Cymru Welsh Water (DCWW).

The 2019 survey data recorded a total of 25 species of waxcaps, with a peak of 21 species on 1st November. This demonstrates that the site still exceeds the SSSI selection criteria for fungi. The broad distribution of fungi observations was similar to that in other recent surveys, showing that the embankments around the north-east of Lisvane Reservoir and west of Llanishen Reservoir support particularly high densities of grassland fungi.

The number of fungi seen in 2019 is substantially higher than in 2018, and this is probably due to the very dry conditions during spring and summer of 2018 and the mowing regime implemented by DCWW since 2018. The weather during the summer and autumn of 2019 has been unusually wet and waxcaps were recorded fruiting from August through to December. A small number of species continued fruiting into January 2020.

The number of species of grassland fungi recorded in 2019 was also higher than in 2018, and included the highest number of waxcap species since DCWW took over responsibility for the site. Good numbers of *Microglossum olivaceum* were also recorded this year, with over 100 fruiting bodies counted in early November. Fungi species not recorded for several years were seen during 2019, in particular, *Hygrocybe ingrata* which was observed for the first time since 2010, and *Clavaria incarnata* seen for the first time since 2012.

The vegetation structure of the grassland banks is gradually becoming less tussocky and the increase in numbers of fungi is likely to be linked to the shorter grass sward. Grass cutting involved a total of six cuts through 2019 (the minimum assented number, from Natural Resources Wales, is five), although part of the eastern embankment did not receive the final autumn cut. Grass cuttings were generally left *in situ.* A small-scale trial of raking away cuttings was started on the western bank but there was no detectable difference in the number of fungi fruiting bodies in that area this year.

The DCWW capital works restoration programme has continued through 2019. The removal of the palisade fence and stonework repairs have required the use of excavators and other vehicles on the crest of both reservoirs. This may have affected the appearance of some fungi, especially around Lisvane Reservoir and the south-east and north-west parts of Llanishen Reservoir.

The results of the 2018 investigation of fungi DNA in soil became available during this study. They mostly confirmed field observations, but there were some significant differences including confirmation of the presence of several species that have never been observed fruiting on the site. These included uncommon species such as *Hygrocybe spadicea* and *Clavaria zollingeri*, which appear in the Environment (Wales) Act Section 7 list of species of importance for nature conservation. Only small quantities of DNA were usually detected from the additional species. Limitations of DNA sampling were also evident, and the study did not pick up the presence of several species that are regularly observed by fieldwork in some transects (e.g. *Hygrocybe ceracea* and *H. aurantiosplendens* were not found in any samples). This may be due to the very small soil samples used for the DNA survey.

2. Introduction

Dwr Cymru Welsh Water (DCWW) is currently undertaking works at Llanishen and Lisvane Reservoirs, Cardiff (central OS grid reference approximately ST187818) to restore them for operational purposes, which will include facilities for public recreation and access. DCWW has commissioned this survey of grassland fungi to help inform possible development options and long-term site management. The survey focussed on the Llanishen and Lisvane Reservoir Embankments SSSI, which is designated for its assemblage of grassland fungi.

The 2019 survey follows similar studies undertaken during autumn 2016, 2017 and 2018, and used the same survey method. The fieldwork and assessment was undertaken by Dr Peter Sturgess CEnv MCIEEM, who also carried out the 2016-2018 surveys and several previous studies. For some of the surveys he was accompanied by Dr Richard Cowie, representing the Reservoirs Action Group; who has a special interest in grassland fungi, several staff from the local Natural Resources Wales (NRW) nature conservation team, and Mark Steer representing the Glamorgan Fungus Group.

3. Survey method

The fieldwork involved a total of six survey visits spread out as evenly as possible through the autumn fungi fruiting period. The main survey visits were spread out through October and November, aiming to avoid periods of heavy frost. Additional observations of grassland fungi were also included, where they were made during Dr Sturgess' Ecological Clerk of Works (ECOW) duties through the summer and autumn, and into December 2019.

The main target group for the survey was waxcap fungi (*Hygrocybe* species). Fairy-clubs (Clavariaceae) and earth-tongues (Geoglossaceae) were recorded too, since they are good indicators of grassland habitat quality and had also been recorded during the previous studies. Other indicator species such as pink-gills (*Entoloma* species) were also recorded, but these were generally not identified to species level. Most waxcaps were at least provisionally identified in the field, with specimens collected for confirmation by microscope where necessary. Identification of fairy-clubs and earth-tongues was also confirmed by microscopy.

Waxcap taxonomy has recently undergone significant revision, with many species that were previously classified as *Hygrocybe* species now moved to other genera, largely due to genetic studies. This report has continued to follow the nomenclature of Boertmann (2010) for consistency with previous reports for the site[1].

Each survey used a simple walk-over/ transect-based method to search for grassland fungi, covering all the various grassland types within the SSSI, and aiming to sample as much of the grassland area as possible within a survey day of approximately 5-6 hours. Survey effort was focussed on the slopes, as these have previously been found to have the highest value for grassland fungi. The locations of any waxcap fruiting bodies were marked onto a survey plan during the site visit. This was generally conducted by eye, but the locations of any particularly uncommon species were also checked using a hand-held GPS device.

[1] Boertmann, D. (2010). The Genus Hygrocybe, Fungi of Northern Europe Vol.1, 2nd revised edition. Svampetryk.

Additional records obtained during Ecological Clerk of Works visits were not the result of full surveys and were limited to brief checks of the key areas for fungi.

Notes were made on the condition of the site for fungi, such as approximate sward height and condition, and any other factors that might affect fungi.

4. Survey findings

The species recorded during the fungi visits in 2019 are summarised in Table 1 (with survey dates in bold). The table also includes the additional less intensive observations (survey dates in brackets) seen during ECOW inspections. The observations from September are the result of observations through the whole month rather than on a single date.

The 2019 survey confirmed 25 species of waxcap fungi, with a peak of 21 species on November 1st. The waxcap season began relatively early this year, due to the wet weather in the summer, and was sustained through most of the usual peak season through October and November. Numbers of individual specimens and species dipped slightly in mid-November due to frost.

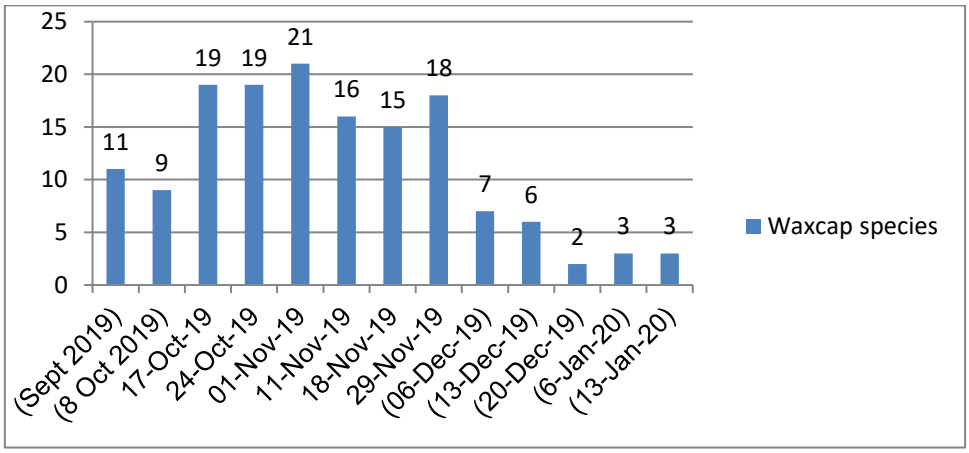


Figure 1 Numbers of waxcap species observed during the 2019 survey season

The grassland fungi species diversity was broadly similar to the previous DCWW surveys, and the number of fruiting bodies was substantially higher than was seen after the prolonged dry weather of summer 2018. Species that typically appear early in the season were especially well represented this year, with *Hygrocybe citrinovirens, H. intermedia* and *H. flavipes* being the best examples. Notable observations included the reappearance of *Hygrocybe ingrata* and *Clavaria incarnata*, which had not been seen for several years. *Clavulina coralloides* was a new species of fairly-club for the site, although this is typically a woodland species and was found adjacent to the hedge at Lisvane Reservoir.

The distributions of recorded waxcap fungi and non-waxcap target species are shown in Figures 2 and 3 respectively. These include observations made during both fungi surveys and other site duties. The scale and degree of overlapping symbols in the figures does not permit the individual species to be seen clearly, so it is mainly intended simply to highlight the locations where the main groups of fruiting bodies were recorded (a more detailed figure is available if required).

Table 1 Summary of 2019 fungi survey observations

Survey date	(Sept 2019)	(8 Oct 2019)	**17 Oct 2019**	**24 Oct 2019**	**1 Nov 2019**	**11 Nov 2019**	**18 Nov 2019**	**29 Nov 2019**	(6 Dec 2019)	(13 Dec 2019)	(20 Dec 2019)	(6 Jan 2020)	(13 Jan 2020)
Waxcaps													
Hygrocybe acutoconica			+	+									
Hygrocybe aurantiosplendens				+	+		+	+					
Hygrocybe calyptriformis	+	+	+	+	+	+	+	+					
Hygrocybe cantharellus	+		+		+								
Hygrocybe ceracea			+	+	+		+	+		+		+	
Hygrocybe chlorophana	+	+	+	+	+	+	+	+					
Hygrocybe citrinovirens	+	+	+		+								
Hygrocybe coccinea		+	+	+	+	+	+	+	+	+			+
Hygrocybe colemanniana			+	+	+	+	+	+	+				
Hygrocybe conica	+	+	+	+	+	+		+					
Hygrocybe flavipes	+												
Hygrocybe fornicata				+	+			+					
Hygrocybe ingrata		+											
Hygrocybe insipida		+	+	+	+	+	+	+					
Hygrocybe intermedia	+		+	+	+								
Hygrocybe irrigata		+	+	+	+	+		+					
Hygrocybe mucronella			+		+	+	+	+					
Hygrocybe pratensis	+		+	+	+	+	+	+	+	+	+	+	+
Hygrocybe psittacina	+	+	+	+	+	+	+	+					
Hygrocybe punicea			+	+	+	+	+	+	+	+	+	+	+
Hygrocybe quieta	+		+	+	+	+	+	+					
Hygrocybe reidii	+		+	+		+	+						
Hygrocybe russocoriacea					+	+	+	+	+	+			
Hygrocybe splendidissima				+	+	+		+	+				
Hygrocybe virginea			+	+	+	+	+	+	+	+			
Total waxcap species	11	9	**19**	**19**	**21**	**16**	**15**	**18**	**7**	6	2	3	3
Other indicator species													
Clavaria fragilis			+	+		+							
Clavaria fumosa		+	+	+	+	+		+	+		+		
Clavaria incarnata					+								
Clavulina coralloides			+										
Clavulina rugosa			+										
Clavulinopsis corniculata			+	+	+	+	+	+	+	+			
Clavulinopsis helvola	+	+	+	+	+	+	+						
Clavulinopsis laeticolor	+		+	+		+							
Clavulinopsis luteoalba	+		+			+							
Dermoloma cuneifolium			+	+	+								
Entoloma incanum			+										
Entoloma porphyrophaeum				+	+	+	+						
Entoloma spp.	+	+	+	+	+	+							
Microglossum olivaceum sl				+	+	+	+	+	+	+	+	+	+
Geoglossum cf fallax					+		+						+
Geoglossum umbratile				+	+								
Geoglossum sp.						+							
Trichoglossum hirsutum				+	+	+	+	+					

Data from the 6 main survey dates are shown in bold text in Table 1. The other records are from less intensive Observations made during ECOW visits. A list of other species recorded during the survey is summarised in Appendix 2.

Figure 2 Distribution of waxcap fruiting bodies seen during 2019

Figure 3 Distribution of other indicator fungi seen during 2019

The distribution maps show a similar pattern to that seen in recent years, with the highest numbers and diversity of fungi seen around the north and north-east margins of Lisvane Reservoir and at the central corner of the western margin of Llanishen Reservoir. Once again, most fungi were seen near the tops of the sloping embankments, with the notable exception of the north east of Lisvane Reservoir where the abundant fungi also extended to the lower parts.

5. Discussion

5.1 Grassland fungi observations in 2019

2019 appears to have been a particularly good year for grassland fungi. This is probably due to the relatively wet spring, summer and autumn weather, which would have allowed good growth of fungi within the soil. Similarly high numbers of waxcaps were also recorded on other local grassland sites. Interestingly, earth-tongues were relatively late appearing this year, both at Llanishen Reservoir and on other local sites.

Llanishen Reservoir continues to meet the SSSI qualifying criteria[2]; the key requirement for waxcaps being the occurrence of 19 or more species. The total is typically the result of several visits through the season, sometimes over several years. However, during the 2019 surveys this 19 species threshold was reached following only three survey visits.

The distribution of fungi at the reservoirs in 2019 showed them reappearing in some locations where they have not been seen for several years. Several were seen in the level ground at the top of the embankment, which had previously become very heavily trampled, but may have since become less compacted in the absence of people. A few species were seen on the eastern embankment of Llanishen Reservoir, especially early in the season. Hardly any have been seen here for several years and the change is probably due to the grass being shorter this year. Unfortunately, the southern part of this bank was not mown in September so had become dense and tussocky by the time the main fruiting season had begun, and no fungi appeared here after early October.

Fungi that tend to appear early in the season were amongst the most abundant this year; particularly *H. intermedia* and *H. citrinovirens*. These had probably been supressed in 2018 when the ground was dry and hard during the summer. They had not been seen before in some of the places they fruited this year. *Hygrocybe ingrata* is also a relatively early species, and has not been recorded at the reservoir since 2010. This was probably also responding to the improved environmental conditions, because it was also recorded at several other local sites for the first time. This is clearly a species that can remain undetected in the soil for several years without fruiting.

Hygrocybe irrigata was observed in several places this year, and these included some locations where it has not been seen for a few years. Other species also reappeared in places where they had only been seen several years before, indicating the presence of a long-lived mycelium within the soil. A possible sign of a younger waxcap mycelium was indicated by a fairy ring of *H. pratensis* on the southern embankment, which was only approximately 2 m in diameter. Waxcaps are rarely seen in such well-formed rings.

[2] Bosanquet, S.D.S., Ainsworth, A.M., Cooch, S.P., Genney, D.R, and Wilkins, T.C. (2018). Guidelines for the Selection of Biological SSSIs. Part 2: Detailed Guidelines for Habitats and Species Groups. Chapter 14 Non-lichenised Fungi. Joint Nature Conservation Committee, Peterborough.

The numbers of fungi seen during 2019 were higher than have been observed for several years, and reappeared over much of the range where they had been recorded previously. In 2004 many species of grassland fungi produced abundant fruiting bodies over a wider area within the site, following a dry previous year. It had been hoped that the damp weather in 2019, which also followed a year of drought, might have resulted in similarly high numbers. However, although observations in 2019 were a significant improvement on those in recent years, they did not reach the levels seen in 2004. This was especially noticeable on parts of the eastern and western banks of Llanishen Reservoir which had produced large numbers of waxcaps in 2004 but very few in 2019 (pers.obs, & R.Cowie pers.comm). Although small numbers of waxcaps were seen in these areas in 2019, it may be that even in the presence of ideal weather conditions, complete recovery from a long period of sub-optimal management may take several more years.

The appearance of small numbers of fungi fruiting bodies continued into January, as in the previous two years. The January fungi appeared to be limited to the north-east of Lisvane Reservoir (also as seen in previous years). This may be because this part of the site is generally the best area for fungi, or perhaps because it is slightly more sheltered than the other areas with high fungi diversity.

5.2 Observations relating to management and construction works in 2019

The mowing regime in 2019 involved a total of six cuts spread through the growing season, using a combination of ride-on mowers, hand-mowers and strimmers. As in 2018 the cuttings were left *in situ* due to the cost and practical difficulties of collection. The agreed cut height was 40-50 mm.

The mowing dates in 2019 were as follows:

1= 10 April

2= 15 May

3= 17 June

4= 19 July

5= 20 August

6 = 1 October

The assent from Natural Resources Wales (NRW) states that a minimum of five cuts per year are required. The additional cut was almost certainly beneficial for fungi by supressing some of the more vigorous grasses (because the damp conditions had been ideal for grass growth) and it meant that the subsequent cut did not generate such large volumes of cuttings. The October cut was carried out in wet weather and some parts (mainly the eastern bank) were not mown. The mild, damp autumn weather resulted in a longer than usual growing season for grass, so much of the sward had become relatively long again by mid-November (especially the southern embankment of Llanishen Reservoir). The shorter grass during 2019 is likely to have been a factor in the reappearance of waxcaps on the eastern embankment and has probably been beneficial throughout the site.

The composition of the grass sward has continued to change through 2019, with a higher proportion of low-growing grasses and herbs. The larger tussock-forming species have declined in most places, so there is now a thicker vegetation cover at ground level and less dead grass thatch. In some places, where there are relatively few coarse grasses, mosses

have begun recolonising the banks. This is probably beneficial for the SSSI because mosses have been found to have a positive correlation with some grassland fungi. However, mosses are still largely absent from many parts of the banks where the grass is tallest.

A small-scale trial of grass raking to remove cuttings was undertaken on part of the western embankment during 2019 but there was no detectable difference in the number of fungi fruiting bodies in that area this year. An area of particularly dense, species-poor grass was selected, and the cuttings were removed after each cut. The initial findings were that the raked area looked different from the adjacent grass, mainly due to the absence of accumulated cuttings. The vegetation composition did not appear very different, but small patches of moss, common cat's-ear, bugle and other low-growing plants are establishing in a few places. These plants have been able to grow in the raked area because they have not been smothered by grass cuttings. The fungi that appeared in the raked area this season were limited to just three species of waxcap (*H. chlorophana, H. calyptriformis and H. pratensis*), so the area was not noticeably different from the adjacent grassland. However, it was one of the few places where *Clavulinopsis helvola* was seen on the western embankment. The others were all in areas of higher fungal diversity where the sward is much shorter, and it is possible that this relatively small species benefitted from the raking.

The locations cleared of trees during 2016 have continued to produce small numbers of waxcap fruiting bodies this season. The newly established grassland also includes several fungi that are more typical of woodlands; possibly because of the dead roots and leaf-litter in these areas.

The narrow strip of grass at the top of the stonework around Lisvane Reservoir produced hardly any fungi during 2018, and it was uncertain whether this might have been due to the summer drought or a result of the herbicide spray drift or clearance of trees and vegetation from the stonework that were carried out in 2018. Further works to repair the stonework were carried out around both reservoirs during spring and summer 2019. This involved use of an excavator and transport of materials around the top of the bank. It was encouraging to see that some grassland fungi appeared around the top of the stonework during the autumn, and although numbers of fruiting bodies here were much lower than had been seen in 2017 it is evidence that the fungal mycelium has persisted through the work. It was encouraging to see *Hygrocybe acutoconica* reappearing in two locations around the top of the stonework this year, as this species has only been recorded in small numbers in previous years, and was almost always limited to this narrow strip. Another species that has only been recorded around the top of the stonework is *Hygrocybe calciphila*, but this did not reappear this year.

The engineering work around Llanishen Reservoir during 2019 mostly avoided any additional excavations within the SSSI grassland. A small area of the grassland was lost to construction near to the Lisvane car-park, but this was mostly on the level ground and lower part of the embankment where only small numbers of waxcaps have been recorded previously. Installation of an additional land-drain west of Llanishen Reservoir was mostly in damp ground that avoided any areas of known value for grassland fungi. An additional land drain south of Llanishen Reservoir was mostly limited to the existing vehicle track, with only a small loss of SSSI grassland. Interestingly several waxcaps appeared within 2 m of the excavation in the autumn.

The removal of the palisade fence from around Llanishen Reservoir involved the use of an excavator and dump truck around the crest of the reservoir. The work was carried out during the summer, while the ground was relatively dry, to try to minimise the possibility of ground compaction. Efforts were made to remove the concrete bases with minimal disturbance to the soil and to re-use the existing turf and topsoil from the site to reinstate the holes. The

fence removal is likely to be beneficial to the grassland fungi because it will allow the grass mowing to be carried out more easily, and because accumulated grass cuttings might dry out and be blown around more easily now that the banks are less sheltered. However, areas previously enclosed by the fence will also inevitably be subject to increased pressure from trampling.

Once again, the land to the south-west of Llanishen Reservoir where *Hygrocybe laeta* had been recorded previously, produced very few fungi fruiting bodies this season. Part of this area was lost to the temporary construction access and the remainder has become more tussocky since the turf was disrupted by extensive badger foraging activity in 2017. *H. laeta* has not been seen at all at the reservoirs since 2016, although the mycelium may still be present in the soil.

5.3 NRW soil fungi DNA survey

In autumn 2018 NRW, in conjunction with Aberystwyth University, carried out a study of fungi DNA in several soil samples from Llanishen Reservoir, as part of a wider study into the effectiveness and possible applications of this new survey technique. The results of the survey[3] became available in autumn 2019, and some of the key findings are discussed here.

Soil samples were taken from 12 areas around the reservoirs (see Figure 4), which were already known to support populations of fungi. An auger was used to collect a total of 36 soil cores from just below the turf surface (each approximately 10 x 1.5 cm) at 5 m intervals in each area. The cores from each area were combined as one sample in a sterile bag (approximately 1 kg of soil per area), and kept in a coolbox prior to freezing within 24 hrs, and subsequent processing at Aberystwyth University. Care was taken to minimise potential contamination by clearing the auger between the different areas.

At the university the samples were processed by freeze-drying, grinding and sieving, followed by extraction of DNA. PCR amplification using fungi-specific primers was then used to amplify specific regions of the DNA which were extracted, purified and analysed using NextGen sequencing. This technique results in huge numbers of DNA sequences (typically around 50,000 per sample), which can then be compared against a database of known sequences, allowing identification of the species present in the samples and providing relative abundance information.

The Llanishen samples produced a minimum of 13,248 fungi DNA sequences (Area 6) and maximum of 72,587 sequences (Area 4). The majority of the sequences were from waxcaps; the lowest proportion of waxcap DNA was 22% in Area 12, and the highest was 69% in Area 8. The proportion of fairy club DNA ranged from 6.1% in Area 5 to 16.4% in Area 8. Earth-tongue DNA was a much smaller proportion of the total, varying from 0.1% in Area 1 to 1.8% in Area 7.

The full survey findings are not reproduced here, but summary data for waxcaps, fairy clubs and earth-tongues from the 12 sampling areas are shown in Tables 2, 3 and 4 respectively.

[3] GW Griffith, L Clasen & AP Detheridge (2019). Use of eDNA analysis of soil samples to evaluate the fungal conservation value of grassland areas in south Wales. IBERS, Aberystwyth University.

Figure 4 DNA sampling areas, 2018 (taken from NRW report)

Table 2 Summary of the presence of waxcap DNA in Llanishen sampling areas

Sample area	1	2	3	4	5	6	7	8	9	10	11	12
Waxcaps												
Hygrocybe acutoconica							+	+				
Hygrocybe aurantiosplendens												
Hygrocybe calyptriformis	+	+		+	+			+	+	+	+	+
Hygrocybe cantharellus				+								+
Hygrocybe ceracea												
Hygrocybe chlorophana	+	+		+	+				+			
Hygrocybe citrinovirens	+	+	+	+	+	+	+	+	+	+	+	+
Hygrocybe coccinea	+		+		+			+	+	+	+	
Hygrocybe colemanniana	+		+	+	+	+	+	+	+	+		
Hygrocybe conica	+	+	+		+	+	+	+	+	+		+
Hygrocybe constrictospora										+		
Hygrocybe flavipes	+	+	+	+						+		
Hygrocybe fornicata		+	+	+	+	+	+	+	+	+		
Hygrocybe glutinipes	+			+			+		+		+	+
Hygrocybe ingrata				+	+							
Hygrocybe insipida	+	+		+			+	+		+	+	
Hygrocybe intermedia	+	+	+	+	+	+	+	+	+	+		+
Hygrocybe irrigata	+		+	+				+	+	+		+
Hygrocybe laeta				+								
Hygrocybe mucronella		+	+	+	+	+	+	+	+		+	
Hygrocybe phaeococcinea											+	
Hygrocybe pratensis	+	+	+	+	+			+		+		+
Hygrocybe psittacina		+						+	+	+		
Hygrocybe punicea	+	+	+		+			+	+			
Hygrocybe quieta	+	+	+	+			+	+	+		+	+
Hygrocybe reidii					+							
Hygrocybe russocoriacea					+		+		+			
Hygrocybe spadicea							+			+		
Hygrocybe splendidissima												
Hygrocybe virginea		+	+	+	+	+	+	+	+	+	+	+
Hygrocybe vitellina								+		+	+	
Total waxcap species per area	**14**	**14**	**13**	**17**	**15**	**7**	**13**	**17**	**16**	**16**	**10**	**10**

It is encouraging to see that the majority of waxcap species detected by DNA analysis correlated with field observations. Waxcap species with the highest proportions of DNA sequences tended to be those that were also recorded by fieldwork; possibly because a mycelium must attain a certain biomass in order to produce fruiting bodies. However, a high proportion of DNA did not always equate to high numbers of fruiting bodies. For example, *H. intermedia* typically represented between 10 and 30% of the DNA in most samples, yet only one fruiting body of this species was seen in 2018.

Surprisingly, the DNA analysis found no trace of some waxcap species that are regularly seen fruiting at the reservoirs. These include *H. aurantiosplendens, H. ceracea and H. splendidissima.* It is possible that the sampling cores had simply missed the mycelium of these fungi, or that perhaps the analysis somehow failed to extract, amplify or recognise their DNA. This highlights that the soil analysis method has its limitations and should not be used in isolation from traditional fieldwork.

The DNA analysis revealed the presence of many species that have never been recorded during field surveys. The DNA of several additional waxcaps was present in the samples, including *Hygrocybe constrictospora, H. phaeococcinea, H. spadicea* and *H. vitellina* (Table 2). All these species are considered uncommon, and there are only a few records of each in

South Wales. *H. spadicea* is protected through inclusion in the Environment (Wales) Act Section 7 list of species of principal importance for nature conservation. These species may only be present at very low density or may fruit so infrequently that they were missed during previous field surveys. However, these additional species were only represented by very small amounts of DNA (typically less than 0.05% of the fungi sequences). Such small numbers of sequences may indicate the presence of only very small mycelial systems incapable of forming fruiting bodies, or potentially ungerminated spores.

Table 3 Summary of the presence of selected fairy club DNA from Llanishen sampling areas

Sample areas	1	2	3	4	5	6	7	8	9	10	11	12
Clavaria acuta	+	+	+	+	+	+	+	+	+	+	+	+
Clavaria argillacea	+	+	+	+	+	+	+	+	+	+	+	+
Clavaria atrofusca		+		+					+			+
Clavaria atroumbrina					+			+				
Clavaria flavipes		+	+	+	+		+	+		+	+	+
Clavaria fragilis	+		+		+		+	+		+	+	
Clavaria fumosa		+	+						+		+	+
Clavaria guilleminii	+	+	+	+	+		+			+	+	+
Clavaria incarnata		+	+	+	+		+	+				+
Clavaria zollingeri	+								+			
Clavicorona taxophila	+	+						+	+			+
Clavulinopsis corniculata	+	+	+	+	+	+	+	+	+	+	+	+
Clavulinopsis helvola	+	+	+	+	+	+	+	+	+	+	+	+
Clavulinopsis laeticolor	+	+	+	+	+	+	+	+	+	+	+	+
Clavulinopsis luteoalba	+	+	+	+	+		+	+	+	+	+	+

The fairy club species with a greater proportion of DNA sequences also correlated with those seen most frequently during fieldwork, and confirmed the presence of species that were not recorded in the field. These included the rare *Clavaria atrofusca, C. atroumbrina, C. flavipes, C. guilleminii and Clavicorona taxophila*, all with very few British records, and *Clavaria zollingeri,* which is listed in the Environment (Wales) Act Section 7 list of species of principal importance for nature conservation. The presence of so many apparently rare species raises the possibility that they are more widespread than previously thought and are under-recorded because they rarely produce fruiting bodies. However, it may also mean that Llanishen Reservoir is even more important than previously thought. Further research using soil DNA analysis at a range of other sites would help to clarify this.

Several other Clavariaceae have not been included in Table 3, including *Hodophilus* and *Ramariopsis* species, and unclassified Clavariaceae. The presence of unclassified species in almost all the samples highlights the problem of species that might be present in soil but have never been found as fruiting bodies (or not been subject to genetic bar-coding).

Table 4 Summary of the presence of earth-tongue DNA found in Llanishen sampling areas

Sample areas	1	2	3	4	5	6	7	8	9	10	11	12
Microglossum olivaceum agg		+						+				
Microglossum fuscorubens		+		+			+	+				
Geoglossum barlae		+	+		+		+	+	+	+		+
Geoglossum dunense					+		+	+				+
Geoglossum fallax	+	+	+	+	+		+	+	+	+	+	+
Geoglossum umbratile		+	+				+					
Glutinoglossum pseudoglutinosum	+	+	+	+			+	+		+	+	+
Trichoglossum hirsutum		+	+		+	+	+	+	+			+
Trichoglossum walteri				+	+					+	+	
Unclassified Geoglossaceae	+	+	+	+					+	+	+	

Earth-tongue DNA was present in most of the sample areas and correlated with the species found during fieldwork, but also revealed the presence of several additional ones. Interestingly, these included the rare *Microglossum fuscorubens*, which was formerly classified as part of the *M. olivaceum* group (the whole of which is included in the Environment (Wales) Act Section 7 list of species of principal importance for nature conservation). Earth-tongue species are very difficult to identify and are currently undergoing taxonomic revision, in parallel with genetic studies, so DNA surveys may be an easier way to identify them in future.

A similar taxonomic problem is seen in the *Entoloma* species, where many are very difficult to identify in the field. Unclassified Entolomataceae were present in small quantities in most of the Llanishen samples. The DNA study confirmed several *Entoloma*s, including the Section 7 species *E. bloxhami* in Areas 2, 4 and 8.

Dermoloma cuneifolium is another grassland fungi indicator species that has been regularly recorded at the reservoirs, and its DNA was found in all of the samples (with a peak of 11% and 12% of the DNA sequences respectively in Areas 10 and 11).

Overall the DNA study has been extremely useful in shedding light on the relative abundance of different fungi species within the soil. The observations have confirmed broadly similar patterns in the areas of highest value as indicated by the occurrence of fruiting bodies, but also identified the presence of species not yet recorded by conventional fieldwork. Importantly, the method has confirmed that fungi are still present in the soil in areas where only a few fruiting bodies have been recorded in the last few years (e.g. Areas 9 and 12). This information can potentially have a predictive role in suggesting where fungi might be recorded by fieldwork. The presence of *Hygrocybe ingrata*, which had not been seen at Llanishen since 2010, was confirmed in the DNA at Area 4, and this is where a single fruiting body was recorded in 2019. The survey findings could potentially be applied to target field survey work, for example to search for fruiting bodies of the Section 7 species *Clavaria zollingeri* in Areas 1 and 9, and *Hygrocybe spadicea* in Areas 7 and 10.

6. Recommendations

The following general recommendations relating to the capital works programme were made after the previous surveys. They are all still applicable and are not repeated in full but summarised as follows:

- Continue to protect and monitor the fungi while construction works are taking place.
- Continue to manage the grassland as short grass, with minimal disturbance of the soil.
- Avoid any significant build-up of grass cuttings, either by removing the cuttings or cutting at a frequency that limits the volume of cuttings being generated.
- Remove trees shading the grass embankments where possible.
- Protect the grassland from vehicle access.
- Any requirement for herbicide close to the SSSI grassland should avoid using spray techniques, and use a weedwipe method or screens to protect the grass.
- Protect the grassland from high levels of pedestrian access/ trampling.
- Restrict access for dog-walking, unless Dog Control Orders are in place and enforced.

- Ensure that any installation or removal of structures (access structures, signage, safety features etc.) within the SSSI grassland is consented by NRW.
- Use the site to promote fungi conservation and education.

Specific recommendations are outlined below.

The 2019 mowing schedule with five cuts (plus the additional June cut) has generally been beneficial for keeping the grass in an improving condition, without the need to remove grass cuttings. Planning a similar frequency of grass cutting for next year is recommended, with provision to include an additional cut in summer or autumn if growth is particularly vigorous. It is especially important that no areas of the embankments are left uncut, as happened on the eastern side of Llanishen Reservoir in autumn 2019.

On several of the mowing dates in 2019 the grass was cut whilst it was wet, which limited the effectiveness of the cut height and meant that the grass cuttings tended to stick together in dense piles. The mowing should always aim for dry conditions to avoid these problems.

The current mowing regime is gradually reducing the dominance of the coarser grasses, but they are still abundant and account for the majority of the grass cuttings. A small-scale trial of cutting an area to a shorter height or with an increased number of cuts in the autumn is recommended, to evaluate whether these grasses could be suppressed more quickly.

The small-scale grass-raking/ removal trial on the west side of Llanishen Reservoir should be continued.

The hedges adjoining Lisvane Reservoir and the eastern side of Llanishen Reservoir should be cut back to the site perimeter, and preferably reduced in height. All hedge cuttings should be removed from the grassland. Creating large piles of wood chips at the edge of the SSSI should not be permitted because this is likely to cause problems with localised high levels of nutrients, leading to growth of coarse grasses, brambles and nettles.

The technique of studying fungi DNA in soil could potentially help to answer several questions relating to the site, in particular:

- Why are so few fungi seen on the north-west bank at Llanishen Reservoir? This was formerly one of the most prolific areas for fungi fruiting, but very few have been seen there in recent surveys. This was the area most affected by years of unauthorised access and dog-walking, so it would be useful to know if the fungi are still present but are just not fruiting at the moment.
- What is the distribution of fungi fruiting bodies across the embankments? This could be determined by conducting DNA sampling along transects from the top to the bottom of the slopes (although this may not explain the observed distribution).
- Could the technique be used to monitor the potential impacts of dogs on grassland fungi? A soil DNA profile could feasibly be recorded for parts of the site where the impacts of trampling/ dog urine etc. might be greatest (e.g. near to the car-park or other entrances) to see whether or not they are changed in several years' time. Sampling from dog-free areas would also be necessary to control for changes unrelated to the presence of dogs.

The traditional field-observation-based fungi survey should be continued through the duration of the construction works, and to monitor how the fungi respond to the gradually

improving conditions created by better grass management. Fieldwork may also be able to observe fruiting bodies of the additional rare fungi recorded by the DNA survey.

Appendix 1: Photographs

1		The grass-cutting through 2019 was carried out on six occasions using a combination of ride-on mowers, hand-mowers and strimmers, with the cuttings being left *in situ*.
2		The cutting was sometimes carried out while the grass was damp, which tended to result in clumping together of dense rows of cuttings.
3		The grass has generally been cut to a good height, but some slopes still produce a substantial amount of dead grass, which tends to form a layer over the ground.

4		Low-growing plants including primroses have benefitted from the shorter grass height (April 2019).
5		A small trial plot on the western bank of Llanishen Reservoir was raked to remove grass cuttings after each cut. Current findings indicate little difference in the vegetation or fungi in the plot, when compared with the adjacent areas, although there is less dead grass/ bare ground and a slightly higher amount of moss.
6		Tall fescue is still locally abundant on the embankments, although the improved mowing has reduced its dominance.

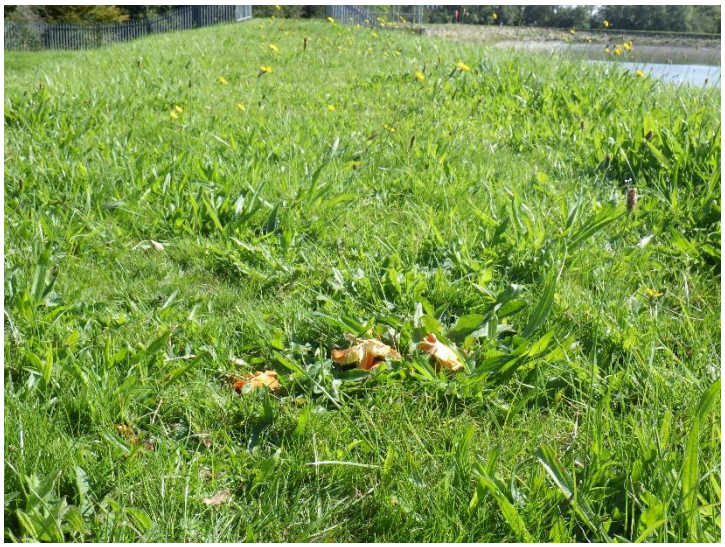
7		The stonework around Lisvane Reservoir was repaired during the early spring, using an excavator working from boards to minimise the ground pressure. (February 2019)
8		A small truck with low ground-pressure wheels was used to transfer materials to and from the work site. The top of the banks became very muddy but ground mats were used in the worst affected areas to prevent deep ruts from forming (February 2019).
9		Ground mats were used around the southern side of Lisvane Reservoir, which became extremely muddy during unusually wet weather in March.

10		In some places boards were left *in situ* for extended periods while the work was suspended, which caused the grass to yellow. The effect of this on the fungi is uncertain.
11		Several areas of grassland were affected by excavations to expose buried pipes. These were generally kept to a minimal area.
12		Common-spotted and twayblade orchids were locally abundant in June, and have probably benefitted from the reduction in grass height. Unfortunately, most were cut down by the unscheduled grass-cut in late-June.

13		The June grass-cut was probably beneficial for the grassland fungi, and a few Orchids were left to set seed.
14		Several patches of dried grass-cuttings around Lisvane Reservoir were set alight by trespassing members of the public during July. The effect of this on grassland fungi is unknown, but the patches were all very small and the heat would not have penetrated deep into the soil.
15		A new land-drain was installed west of Llanishen Reservoir during August. The design was altered to minimise impacts on areas where fungi and rare plants had been recorded. After the drain was installed, the strip was re-covered with the original turf.

16		The old brick headwall structure west of Llanishen Reservoir was broken out and removed. The gap was filled and reprofiled using local subsoil and topsoil to match with the rest of the embankment profile. In due course it is hoped that this will be colonised by grassland fungi like the other parts of the bank.
17		The palisade fencing around Llanishen Reservoir was removed during August. An excavator was used to lift away the panels and pull out the posts and concrete bases. The turf was retained by each hole and replaced after backfilling with local subsoil.
18		A tracked dump-truck was used to carry materials to and from the work site.

19		A new drain was installed along the southern embankment during September. This was mostly confined to the edge of the existing vehicle track.
20		Works in September required use of a tracked excavator along the Llanishen Reservoir eastern embankment in wet weather, which resulted in localised rutting and soil compaction.
21		Repair of the Lisvane Reservoir scour valve during wet weather in November and December required multiple journeys along the top of the reservoir crest, including access by an excavator. This caused localised rutting and compaction.

22		Several overhanging trees are still present around Lisvane Reservoir and the eastern side of Llanishen Reservoir, limiting the grass area of some of the banks.
23		One fallen willow from the bank north of Lisvane Reservoir was cut up and chipped into the boundary hedge, rather than being removed from the site. The chippings were scraped off the adjacent bank but still have the potential to be a source of increased nutrient levels in the surrounding area. Chippings should usually be completely removed from the SSSI.
24		This group of *Hygrocybe intermedia* appeared in the middle of the reservoir crest north of Lisvane Reservoir in September, indicating that the access works earlier in the year had not killed the mycelium. *H. insipida* and *H. pratensis* were also seen along other parts of this track that had been driven over in the spring.

25		*Hygrocybe citrinovirens* appeared in several places this year where it had not been recorded before. This tends to be an early-fruiting species and may have been favoured by the damp conditions through the summer.
26		*Hygrocybe intermedia* was also relatively widespread during the early part of the autumn; appearing in several places where it had not been seen before.
27		*Clavaria incarnata* was recorded for the first time since 2012, north-east of Lisvane Reservoir.

28		This damaged specimen of *Hygrocybe ingrata* (found during the October grass-cut) was the only one recorded this year. This was its first appearance since 2010. It was found in an area where its DNA had been found during the 2018 study. This species also appeared at several other sites in Cardiff during 2019.
29		*Hygrocybe russocoriacea* was locally abundant in part of the grass strip beside the stonework north of Lisvane Reservoir. Very few other species were recorded in the strip this year, which may be due to access by heavy machinery.
30		*Hygrocybe splendisimma* was only found west of Llanishen Reservoir this year. This species did not feature in any of the soil DNA samples.

31		*Hygrocybe irrigata* has been relatively scarce in previous surveys but was found in several places this year. Most notably on the eastern embankment of Llanishen Reservoir where few other species were seen.
32		The 2018 soil DNA survey confirmed the presence of several species of earth-tongue that had not been recorded previously, including another member of the *Microglossum olivaceum* complex.
33		A fairy ring of *Lepista nuda* on part of the grass bank formerly covered by scrub. This species is found in grassland, but more usually in woodland. It may be an indication of changed soil conditions while the ground was covered by trees.

34		*Hygrocybe punicea* was one of the species to continue fruiting late in the season.

Appendix 2: Incidental observations of non-target fungi species

Amanita rubescens
Armillaria mellea
Clitocybe fragrans
Clitocybe geotropa
Clitocybe nebularis
Clitopilus prunulus
Coprinellus micaceus
Coprinopsis cf lagopus
Coprinus commatus
Entoloma cf conferendum
Entoloma cf sericellum
Galerina sp.
Laccaria laccata
Lepista nuda
Lycoperdon excipuliforme
Marasmius hudsonii
Melanoleuca polioleuca
Mycena pura
Stropharia caerulea
Vascellum pratense