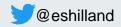
## 

Ewan Shilland London NERC DTP NHM 23/06/2021

Aquatic Macrophyte Change in the UK Upland Waters Monitoring Network

Supervisors: Iwan Jones (QMUL), Anson Mackay (UCL), Anne Jungblut (NHM), Iain Sime (NatureScot, CASE)







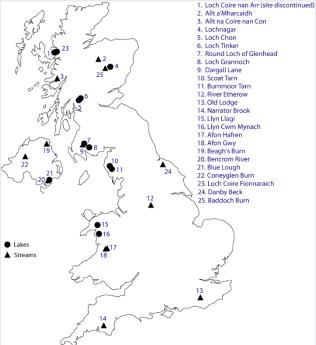






### UK Upland Waters Monitoring Network

- Set up in 1988 to assess the chemical and biological response of acidified lakes and streams to emission reductions.
- Originally 22 sites across UK in N-S, E-W gradients, with afforested and non-afforested pairs and NW "control" sites.
- Water chemistry, fish, invertebrates, macrophytes, diatoms, temperature. Physical variables at some sites e.g. flow.
- Demonstrated the effects of reductions in Sulphur emissions – reduced xSO<sub>4</sub>, also upwards Dissolved Organic Carbon (DOC) trends.
- Despite Nitrogen emission reductions demonstrated variable changes in NO<sub>3</sub>
- Clear recovery evidence from chemistry but biological lags
- Aquatic plants not yet analysed this project.





#### Aquatic Plants (macrophytes)





Higher plants





Stoneworts

Hyocomium armoricum H. flagellare Flagellate Feather-moss

Key 314, 345



Mosses and Liverworts



### Hypotheses

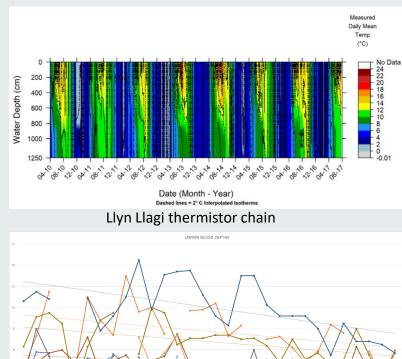


- H1. Upland stream macrophyte species compositions, abundances and ecosystem functions are affected by hydrology, water chemistry and temperature.
- H2. Upland lake macrophyte species compositions, abundances and ecosystem functions are affected by water chemistry, the light environment, wave stress and temperature.
- H3. Macrophyte environmental DNA preserves sufficiently well in lake water, sediment traps and the lake sediments of upland lakes to be identified using molecular techniques.
- H4. Contemporary water eDNA, sediment trap sedDNA and core surface sedDNA faithfully represent the macrophyte species currently growing in upland lakes.
- H5. Preserved macrophyte sedDNA in the lake sediments of upland lakes provides a reliable record, matching known long-term monitoring data, and is sufficient to reconstruct historic macrophyte assemblages.
- H6. Macrophyte dispersal ability limits rapid (re)colonisation of upland waterbodies.
- H7. Aquatic macrophytes are not returning to pre-industrial assemblages due to climate change and nutrient deposition.



## **Drivers of Change**

- Water quality
  - Recovery from acidification
  - Elevated nitrogen levels
- Climate
  - Temperature
  - Hydrology
  - Flow
  - Storminess (aspect)
  - Light environment DOC/Ice Cover
- Hydromorphology
- Others?



#### UKUWMN secchi depths



### Brief Methods - Plant Surveys

#### Streams

Fixed 50 m section of stream with transects every 5 m. Substrates, shade, filamentous algae, macrophyte taxa and cover recorded. Common Standards Monitoring (CSM) Methodology for stream macrophyte sampling also performed since 2019 – 100m sections. Temperature loggers.

#### Lakes

Inshore survey, fixed transect surveys, trawl surveys. Since 2009 also CSM Methodology for lake macrophyte sampling adding additional 100m shoreline transects and strandline species recording. Temperature loggers.







### Brief Methods – DNA. Lake sites only.

#### DNA

- Sampling: eDNA Sterivex water filtering. 3 x filters per site per year. Field blanks. 83 samples. sedDNA Annual sediment traps. 3 x traps per site per year. 66 samples. Shallow and deep-water sediment cores from two sites, Round Loch of Glenhead and Burnmoor Tarn (COVID backup site). Core extrusion into two replicates. 72 core sedDNA samples. Radiometric dating. All samples frozen until analysis.
- Extractions: QIAGEN PowerWater extractions for water eDNA, PowerSoil Pro for cores/traps. Blanks. Nanodrop, Qubit.
- trnL markers rather than rbcL, ITS2 or matK.
- BOLD/Genbank/PhyloNorway/PhyloAlps libraries.
- Voucher specimens collected and in SiO2 for reference library improvement if necessary.
- Metabarcoding PCR methods, Illumina sequencing methods and Bioinformatics pipeline under development.



#### Progress

- Historic data entry/vouchers ongoing
- Fieldwork. 2020 delayed by COVID and trips split into smaller units finished in mid November rather than August. Challenging logistics! All sites visited however. Many thanks to volunteers and CASE partner lain.
- Cores collected and extruded at home due to COVID.
- DNA extractions performed and Nanodrop purity/DNA concentrations measured for all filter, sediment trap and core samples @NHM
- Test PCRs and gels underway, refining recipe
- Core sample dry weights, loss on ignitions and wet densities measured @UCL. In the queue for drying and radiometric dating – funding secured from QRA.









# Examples of plant time-series data RLGH Scores (1-5) Llyn Llagi Scores (1-5)

Juncus

Juncus effusus	-	+	+ + +		+	+ + +	+ + •	+ + + +	+ + •
Nardia compressa	+ + +	+ + +	+ + + •	+ + + +	+ +	+	+ + -	+ + + +	+ -
Racomitrium aciculare	+ + +	+ + +	+ +	+ + + +	+ + + +	+ +	+	+ + + +	+ + -
Scapania undulata		+ +				111	Ш		
Polytrichum sp.	+ + +	+ + +	+ +						
Ranunculus flammula	-			+		+			4
Pellia sp.	-								
Polytrichum commune	-								
ncus bulbosus var. fluitans	-								
Hyocomium armoricum	7				+ +				
Sphagnum sp.	-								
	7								
Lemanaea sp.	7				+ +				
Marsupella emarginata	1						+	+	+ +
	-							11	
	-					lı –	Т		
	-					Ш	.I		
	-								

1991 1993 1995 1997 1999 2001 2003 2005 2007 2009 2011 2013 2015 2017 2019

Filamentous green alga

Afon Hafren % cover

Drepanocladus sp. ]	E
Plectocolea obovata	
Sphagnum (aquatic undet.)	
Fontinalis sp.	
Batrachospermum sp.	
Lobelia dortmanna	
s articulatus/Juncus acutiflorus indet. ]	
Sparganium angustifolium	
Littorella uniflora	
Scapania undulata ]	
	-
Juncus effusus	
Isoetes echinospora	
Juncus bulbosus var. fluitans ]	
Myriophyllum alterniflorum	
Ranunculus flammula	
Potamogeton polygonifolius	
Subularia aquatica ]	
Jungermannia sp. 🧕	E
Elatine hexandra	
Nitella flexilis var.flexilis agg. ]	
	· · · · · · · · · · · · · · · · · · ·

#### 1988 1990 1992 1994 1996 1998 2000 2002 2004 2006 2008 2010 2012 2014 2016 2018

Glyceria fluitans	
Racomitrium aquaticum	
Diplophyllum albicans	
Mnium homum	
Carex rostrata	
Filamentous green algae 🖥 👔 👔 🚛 🖉 👘 👘 🖉 👘	
Eleocharis palustris	
Juncus bulbosus var. fluitans 📲	
Marsupella emarginata	
Nardia compressa	
Scapania undulata	
Lobelia dortmanna 📲 🚺 🚺 🚺 📕 📕 📕 📕 📕 📕 📲 👘	
lsoetes lacustris 🔤 👔 👔 🚺 👔 👔 👔 👔 👔 👔 👔 👔	
Littorella uniflora	
Juncus articulatus/Juncus acutiflorus indet.	
Ranunculus flammula	
Sphagnum (aquatic undet.)	
Sparganium angustifolium 1	
Potamogeton polygonifolius	
Batrachospermum sp.	
Myriophyllum alterniflorum	
Hyocomium armoricum	
Juncus conolomeratus 3	

1988 1990 1992 1994 1996 1998 2000 2002 2004 2006 2008 2010 2012 2014 2016 2018 2020



## Preliminary Results UWMN

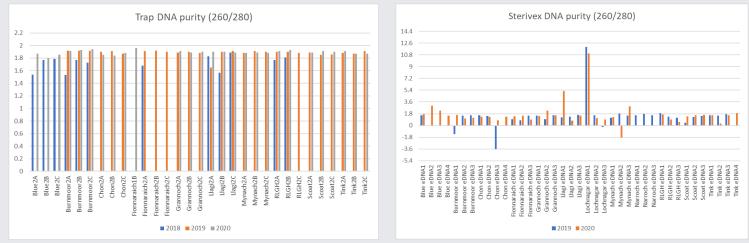
1	Sample Year 95 96 97 98 99 00 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 2																										
LAKES	species	95	96	97	98	99	00	01	02	03	04	05					10	11	12	13	14	15	16	17	18	19	20
Lochnagar	Subularia aquatica	x		x		x		x	•	x		x			•	<b>√</b>		•	<b>√</b>	•	<b>√</b>					<b>v</b>	•
Loonnagai	Elatine hexandra		•			~		x		x		x							1				•			X I	
Loch Chon	Subularia aquatica	1		1		1		$\sim$		$\overline{\checkmark}$		$\overline{\checkmark}$			•			•	1		•		•			x	
	Chara virgata	x		x		1		$\checkmark$		1		1		•	•			•	1		•	•	•		•	x	
Loch Tinker	Subularia aquatica		•			x	•	x		1		$\checkmark$		•	•	$\checkmark$		•	~		•	•	•		•		
Round Loch of Glenhead	Myriophyllum alterniflorum	x		x		x		x		1		1			•	1		•	1		1		•	1		•	1
Round Eden of Olennedd	Elatine hexandra	~		v		v		v		v		v		•	v					-						1	
	Chara virgata	x		x		x		÷		~					~				1		1					1	
Burnmoor Tarn	Nitella translucens	$\frac{1}{x}$		x		x		÷		x		v			1				1		1					1	
Burninoor Fairi	Potamogeton berchtoldii	$\overline{\mathbf{x}}$		x		x		÷		x		x		•	v		•		1		1				•	1	
	Potamogeton alpinus	$\overline{\mathbf{x}}$		x		x		x		x		x		•	x		•	•	x		x		•		•	1	
Llyn Llagi	Callitriche hamulata	v		x				 		- L						<b>√</b>		1		~		<i>√</i>		1		- 	•
	Nitella flexilis agg.	$\overline{\mathbf{x}}$		x		x		x		x		x		•	•	1		1		1		1		1	•	1	
Light Lidgh	Elatine hexandra	x		x		x		x		x		x				1		1		1		1		1		1	•
	Eleogiton fluitans	x	•	X		$\checkmark$	•	$\checkmark$	•			~			$\checkmark$		$\checkmark$	•	$\checkmark$	•	$\checkmark$	•	•	$\checkmark$	•	•	$\checkmark$
Llyn Cwm Mynach	Isoetes lacustris	x	•	x		$\checkmark$	•	$\checkmark$		$\checkmark$		$\checkmark$		•	x		x	•	$\checkmark$	•	$\checkmark$	•	•	$\checkmark$	•	•	$\checkmark$
STREAMS																											
	Hyocomium armoricum	Х	X	Χ	Χ	Χ	Χ	Х	Χ	Χ	$\checkmark$	Χ	Χ	Χ	•	$\checkmark$	Χ	Χ	1	1	Χ	$\checkmark$	$\checkmark$	1	$\checkmark$	$\checkmark$	•
Allt a'Mharcaidh	Marsupella emarginata	х	Х	Х	Х	Х	х	Х	Х	Х	Х	Х	Х	Х	•	Х	Х	$\checkmark$	✓	$\checkmark$	•						
Allt na Coire nan Con	Fontinalis antipyretica	Х	Х	Х	Х	Х	•	Х	Χ	Х	Х	Х	Χ	$\checkmark$	٠	$\checkmark$	•	$\checkmark$	1	$\checkmark$	•	•	•	•	$\checkmark$	$\checkmark$	•
	Blindia acuta	Х	Χ	Χ	Х	Х	Χ	Х	Χ	$\checkmark$	$\checkmark$	$\checkmark$	1	~	1	$\checkmark$	•	•	•	•	•	•	•	•	•	•	•
Dargall Lane	Hyocomium armoricum	х	Х	Х	Х	Х	х	Х	х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	$\checkmark$	Х	1	$\checkmark$	$\checkmark$	$\checkmark$
Diver Ethernov	Hygrohypnum ochraceum	Х	Χ	Χ	Х	Х	Х	Х	Χ	Х	Х	$\checkmark$	Χ	$\checkmark$	1	~	~	1	1	$\checkmark$	1	1	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
River Etherow	Hyocomium armoricum	х	Х	Х	Х	Х	х	Х	х	Х	Х	Х	х	Х	Х	Х	$\checkmark$										
Old Lodge	Hyocomium armoricum	Х	Χ	Х	Х	Х	✓	Х	$\checkmark$	~	√	√	Χ	$\checkmark$	1	~	~	1	1	~	1	1	~	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
	Hyocomium armoricum	X	Х	Х	Х	Х	Х	Х	$\checkmark$	1	Х	$\checkmark$	$\checkmark$	1	$\checkmark$	1	1	$\checkmark$	$\checkmark$	~	$\checkmark$	$\checkmark$	1	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
Afon Hafren	Fontinalis squamosa	x	Х	Х	х	Х	Х	х	Χ	Х	Х	Х	Х	Х	Х	$\checkmark$	Х	Х	Х	1	$\checkmark$	Х	Х	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
	, Marsupella emarginata	x	Х	Х	х	Х	Х	х	х	х	Х	х	Х	Х	Х	Х	Х	Х	$\checkmark$	$\checkmark$	Х	Х	$\checkmark$	$\checkmark$	Х	Х	X
Mar. 0	Hyocomium armoricum	X	Χ	Х	Х	Х	Χ	Х	X	Х	Х	Х	Х	Х	$\checkmark$	1	X	$\checkmark$	$\checkmark$	1	1	1	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
Afon Gwy	Marsupella emarginata	x	Х	Х	х	Х	Х	х	х	х	Х	х	Х	Х	Х	Х	Х	Х	Х	$\checkmark$	Х	Х	$\checkmark$	Х	Х	1	$\checkmark$
Coneyglen Burn	Hyocomium armoricum	X	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	Х	•	•	•	•	$\checkmark$	•	•	•	$\checkmark$	٠	$\checkmark$	✓.	•	$\checkmark$
Bencrom River	Hvocomium armoricum	X	X	X	Х	X	X	Х	X	X	X	X	Х	X	X_	X.	X	X	X.	X	X	X	X	X	X	$\checkmark$	$\checkmark$

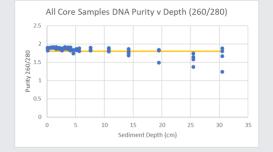
Key. ✓ species recorded during survey, X species not recorded during survey, • no survey

- New aquatic plant species at seven out of eleven lake sites and nine out of eleven stream sites.
- New species in streams tend to be less acid sensitive mosses (especially *Hyocomium armoricum*).
- New species in lakes tend to be those that can't extract carbon from sediments, relying on water-column carbon availability that improves with chemical recovery.
- Macrophytes lag behind diatoms and invertebrates in responding to improved water chemistry
- Little change at "control" sites.



#### Preliminary Results Nanodrop DNA purity

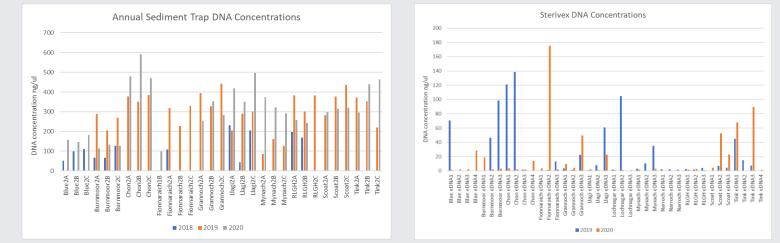


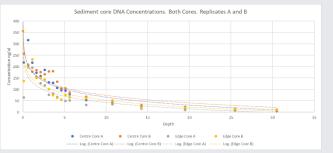


- Aiming for a ratio of 1.8 or above
- Sediment traps good (unfrozen 2018 less so)
- Core good at surface, less good at depth
- Sterivex filtered water less good, and variable



#### Preliminary Results Nanodrop DNA concs

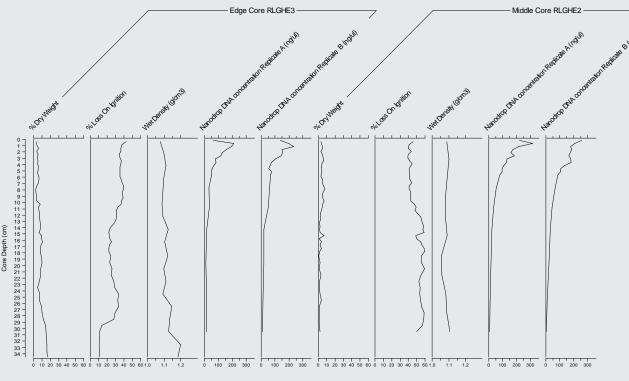




- Sediment traps high, generally ~consistent at a given site in a given year
- Core samples higher at surface, decline with depth
- Sterivex filtered water generally much lower, and variable



#### **Preliminary Results Core Analyses**



- Organic sediments ~50% loss on ignition
- Edge core has greater mineral components, especially at bottom
- DNA concentrations similar between cores and in-core replicates, and suggest undisturbed cores (phew).



#### **Questions?**

## Supervisors: Iwan Jones (QMUL), Anson Mackay (UCL), Anne Jungblut (NHM), Iain Sime (NatureScot, CASE). Support from the Quaternary Research Association and Rick Battarbee/ENSIS Trust Fund gratefully acknowledged.













#### **Expected Outcomes & Impact**

- Contribute to National Emission Ceilings Directive reporting (DEFRA)
- Contribute to SAC/SSSI site condition assessments (SNH/NRW/NE)
- Contribute to Water Framework Directive site condition monitoring (SEPA/NRW/EA) CSM/ LEAFPACS results.
- Produce a calibration dataset for statutory/conservation tool development e.g. LEAFPACS2/DARLEQ2 but for low nutrient/alkalinity sites
- Method development for lake restoration target-setting eDNA cost savings v macrofossils/microfossils?









