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Developing a sentinel monitoring network for Scotland's rivers and lochs

Daniel Chapman, Laurence Carvalho, Iain Gunn, Linda May, Mike Bowes, Matthew O'Hare

NERC Centre for Ecology & Hydrology

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Executive summary

- The Scottish Environment Protection Agency's (SEPA) surveillance monitoring networks for rivers and lochs were established over a decade ago to help assess the state of Scotland's freshwater environment and detect environmental change. This long-term monitoring is integral in formulating evidence-based policy and evaluating whether land and water management aimed at improving environmental quality is effective.

- SEPA and Scottish Government have commissioned this review of the surveillance networks to better understand their national representativeness, optimal size and sampling intensities.

- The review also considered new and innovative monitoring technologies, and assessed where these may help SEPA to more cost-effectively assess long-term trends in the environment.

- The specific aims of this report are: (1) to assess how well the SEPA river surveillance network represents Scotland's environment; (2) to identify possible changes in the river surveillance network to improve its representativeness; (3) to estimate the ability of the existing river and loch surveillance networks to detect long-term environmental change, and investigate how this might be affected by changes in sampling regimes; (4) to analyse environmental changes detectable since the inception of the surveillance networks; and (5) to analyse the benefits of adopting new sampling methods.

- In Section 1, the national representativeness of the current river surveillance network was analysed, with respect to a range of pressure and habitat gradients. Univariate and multivariate tests for the equality of distributions were used to assess whether the distributions of those gradients in the monitored water bodies were similar to their distributions across Scotland's water bodies. The analysis showed that although the current river surveillance network spans a wide range of pressure and habitat conditions, it was designed to over-represent major downstream river water bodies. This was reflected in pronounced biases towards water bodies with large catchments, shallow channel slopes, high mean flow rates (Q_{mean}) and high sinuosity. The monitored water bodies were also disproportionately exposed to anthropogenic pressures, especially with respect to nutrient loads from pollutant sources but also for nutrient concentrations, morphological modifications and modifications to flow regimes. Due to these biases, if used in isolation, environmental statuses and long-term trends obtained from the network will provide an unrepresentative assessment of the overall national situation.

- Section 2 considered options for improving the representativeness of the river surveillance network with respect to the pressure and habitat gradients. A stepwise algorithm was developed to prioritise removal

or addition of water bodies from or to the current network, based on maximising representativeness across those gradients. This showed that the best gains in representativeness were achieved by removing a large proportion of existing water bodies from the network, and then selecting new ones to replace them with. However, since doing this degrades the legacy of existing long-term monitoring, there is a need to balance improvements in the representativeness of existing surveillance monitoring networks with the retention of existing monitoring. It was also beyond the scope of the current analysis to consider the logistical challenges of sampling individual sites.

- In Section 3, power analysis was used to estimate the ability of the current river and loch surveillance networks to detect trends over time at the scale of the entire monitoring network. This suggested that the existing surveillance networks are generally less powerful than is desirable, in that the probability of detecting 5% change over 10 years was lower than 80% for nearly all the monitored variables analysed. The power analysis also evaluated how modified sampling strategies for the river surveillance network may influence power to detect trends. For all scenarios considered, reduced resourcing was incompatible with improving the power to detect trends. However, the best way to maximise power for a given level of resourcing was to avoid repeat sampling of water bodies in the same year. This is because it allows sampling of a greater number of sites in a greater number of years, averaging out the spatial and annual variation obscuring the trend more efficiently. Therefore, it may be possible to improve trend detection power at the current level of resourcing by adding more water bodies to the networks but sampling water bodies less frequently.

- Section 4 reported trends in the existing river and loch surveillance networks from 2007-2016 using with both linear and nonlinear trend models. Linear trend models detected statistically significant upward trends in the Ecological Quality Ratios (EQRs) for river invertebrates and diatoms and for loch invertebrates, phytoplankton and cyanobacteria, equivalent to percentage increases between 2 and 12% over ten years. There were significant negative trends in river total reactive phosphorus (down 35% over ten years) and loch total phosphorus (down 7%), but an increase in loch ammonia (up 43%). We note that these large changes in chemical determinands had very wide confidence intervals, and could be much smaller than these estimates. Since we previously found that the networks had relatively low power to detect trends in most parameters, it is not surprising that more significant trends were not found, while the large estimated chemical trends were estimated imprecisely and may have been affected by changes in laboratory analytical techniques. Many of the monitored parameters appeared to exhibit non-linear trends that fitted the data better than the linear trend models. As such it is useful to use both linear and non-linear models for analysis and interpretation of monitoring trends.

- In Section 5, we considered refinements to existing SEPA methods for sampling in the current river and loch surveillance networks, focusing on alternative inter-calibrated methods as well as novel and emerging methodologies. Current sampling methods are well established and have been inter-calibrated with methods used in other EU member states. Many of the methods pre-date the Water Framework Directive and provide excellent long-term records of river and loch health in Scotland. However, new methods may still offer improvements. For each method the following characteristics were scored; efficiency, cost effectiveness, data quality, suitability for Scotland, compatibility with existing data and stage of development. Methods for all biological quality elements and supporting elements were considered. While specific recommendations are made on an element by element basis, the best novel methods overall were forms of eDNA analysis, where a sample of water is analysed for DNA from fish, invertebrates or algae (benthic diatoms or phytoplankton) and meta-barcoding, where a sample of invertebrates, diatoms or macrophytes is identified using barcoding techniques rather than traditional microscopic approaches. These eDNA approaches have been of interest for over two decades but their practical development has accelerated in recent years. Some are close to practical deployment (diatoms) while others require further research and the development of skills and infrastructure within the agency. Other recommendations include the adoption of fluorometers to measure Chlorophyll a in the field, preparing and adjusting river hydromorphology methods to align with the new European Committee for Standardization (CEN) standard and considering the use of inter-calibrated, rapid invertebrate sampling techniques.

- Our recommendations arising from this work are as follows:

    - SEPA should define clear goals for the sentinel monitoring programme and ensure that changes to the existing surveillance networks improve performance for all of these goals. The analyses in this report were restricted to the single goal of estimating overall trends across the network to provide a representative picture of long-term change across Scotland. Therefore, our conclusions about representativeness and power to detect trends should be interpreted for that goal alone. Other potential goals of a sentinel network include estimation of overall status, attribution of trends to pressures, assessment of restoration and remediation measures and local-scale water body classification, all of which were outside the scope of this research. SEPA must decide on the exact goals of the new sentinel networks and assess the compatibility of alternative goals. Designing sentinel networks for multiple goals is likely to mean it is not structured optimally for any one goal. In this case, SEPA should investigate the best ways to design networks that balance these competing demands and use statistical approaches to correct for biases in the networks when estimating national trends.

- Related to the above, SEPA should define the minimum requirements for surveillance monitoring in the new sentinel network. Detectable trends should be able to demonstrate planned improvements to Scottish freshwaters. In the context of networks designed for national trend analysis, we recommend a minimal requirement is for the network to be statistically representative of pressures and habitat gradients across the country and have sufficient statistical power to detect changes in the monitoring data. SEPA will need to clearly define this minimum detectable trend for the network, for example, 80% power to detect 5% change over 10 years was used in this report.

- If sentinel networks are to be used to estimate overall trends and other environmental patterns for the whole of Scotland, then improving representativeness of the networks should be done to reduce bias in the resulting evidence base. The approaches developed here can be used to select water bodies to remove or add to the current surveillance networks to improve representativeness. However, it may be beneficial to refine our approach to include additional criteria in the prioritisation. For example, SEPA may wish to prioritise sites by their amount of existing long-term monitoring data or their accessibility from SEPA offices.

- To design sentinel monitoring networks that make robust estimates of overall national trends, we recommend that SEPA: (a) maintain as large a network as possible, in terms of numbers of water bodies; (b) sustain this large network by sampling water bodies less frequently, and specifically conduct less repeat sampling within years; (c) allocate sampling resources efficiently among monitored variables, using power analysis to equalise power across variables; and (d) investigate whether there is potential to improve trend estimates by combining data from the sentinel network with data from operational and other SEPA monitoring without introducing bias.

- SEPA should review performance of the sentinel networks periodically and continue to adapt and improve them over time. This is partly because pressures and habitat gradients might change over time, affecting the representativeness of the network at a given time. It is also because power analyses used to refine monitoring strategies are always approximate. Furthermore it is possible that the statistical power could change over time. This could occur if patterns of 'noise' in the monitoring data change through adoption of different monitoring methods or changes in climatic variability, seasonality or other factors.

- SEPA should look to new technologies for improving the cost effectiveness, consistency and precision of measurement data. In the short term, this may include considering application of rapid invertebrate sampling techniques and the use of fluorometers for chlorophyll. Over the medium

term, investing in the development of eDNA methods for biological monitoring may prove effective. We also recommend that SEPA align hydromorphological assessment methods with the new emerging European Committee for Standardization (CEN) standard.

- The benefits to SEPA of adopting these recommendations are that their sentinel surveillance monitoring should be more cost effective and provide more robust evidence of long-term trends in the state of Scotland's rivers and lochs.

Introduction

Long-term environmental monitoring is vital for assessing the state of the environment, detecting environmental change and ecological responses to change (Lovett et al. 2007, Lindenmayer and Likens 2010). It is also integral in formulating evidence-based environmental policy and evaluating whether land and water management aimed at improving environmental quality results in its intended effects. For example, it is a legal requirement of the EU Water Framework Directive, transposed into Scottish Law in 2003 by the Water Environment and Water Services Act, that long-term changes in water quality are monitored and that management improves chemical and ecological quality indicators to achieve 'good status' by 2026.

The Scottish Environment Protection Agency's (SEPA) surveillance monitoring networks for Scotland's rivers and lochs were established over a decade ago for this purpose. Their primary function is to detect long-term changes in water quality from both natural and man-made sources in order to inform policy decisions. As the SEPA surveillance networks have been operational for approximately ten years, it is timely to review the performance of the current network and identify ways in which resources can be prioritised to make the monitoring more cost effective (Levine et al. 2014). Indeed, in the future SEPA wish to alter their surveillance monitoring strategy through the development of Sentinel networks for monitoring the quality of Scotland's freshwater environment. The new Sentinel networks should be designed to provide robust evidence in a cost effective manner, which motivates a consideration of their national representativeness, optimal size and sampling intensities, and adoption of new and innovative monitoring technologies. However, to maintain the legacy of the existing long-term monitoring evidence base, it is also desirable to base the Sentinel networks on existing surveillance networks, in as much as this is compatible with the former goals. Therefore, it is important that changes to surveillance monitoring programmes should be based on rigorous statistical evidence.

As such, this research addresses three major questions:

1. How representative are current monitoring networks of the wider environment in Scotland, and can their representativeness be improved?
2. How good are the current surveillance networks at detecting trends, and what trends are evident?
3. Can innovative monitoring techniques be adopted by SEPA to improve the quality and cost effectiveness of freshwater monitoring?

Importantly, the research does not consider other important questions for which surveillance networks may provide evidence. These include the attribution of overall trends to changes in pressures, classification and trends of individual water bodies, and assessment of restoration and remedial measures, all of which were beyond the scope of this research.

The remainder of the report comprises five sections, with the following specific objectives:

- Section 1 – Analysis of the representativeness of the river sentinel network with respect to the range of major anthropogenic pressures and habitat gradients found across Scotland.
- Section 2 – Identification of water bodies that could be removed or added to the river surveillance network to give a more representative and efficient network.
- Section 3 – Estimation of the power of the river and loch surveillance networks to detect change, and present options for increasing the power of the network.
- Section 4 – Identification of long-term changes from baseline conditions already monitored by the surveillance networks.
- Section 5 – Identification of innovative monitoring techniques that might provide similar information at lower cost and recommendation of options for their application in Scotland.

The outputs from this research will be used by SEPA firstly to prioritise resources to ensure they are used to the optimum benefit and secondly to design an efficient and innovative monitoring system to allow Scotland to meet its WFD objectives and maintain a safe and healthy water environment.

1. Representativeness of the existing river surveillance network

Summary

1. This analysis evaluated whether water bodies within the existing SEPA river surveillance network are representative of the profile of key pressure gradients and habitat factors found across all river water bodies in Scotland. A representative surveillance network is desirable because it would provide unbiased evidence on the overall long-term changes in Scotland's freshwater environment.
2. Univariate and multivariate tests for the equality of distributions were used to demonstrate that water bodies in the river surveillance network are not a statistically representative sample of all Scotland's rivers.
3. Although the river surveillance network spans a wide range of pressure and habitat conditions, it was designed to over-represent major downstream river water bodies. This was reflected in pronounced biases towards water bodies with large catchments, shallow channel slopes, high mean flow rates (Q_{mean}) and high sinuosity. The monitored water bodies were also exposed to higher levels of anthropogenic pressures, especially with respect to nutrient loads from pollutant sources but also for nutrient concentrations, morphological modifications and modifications to flow regimes.
4. Improving the representativeness of the river surveillance network would produce more accurate evidence on the overall status and trends in Scotland's rivers.

Introduction and aims

The existing SEPA river surveillance network was designed to assess long-term changes in natural conditions and long-term changes in ecological and chemical status due to widespread anthropogenic activity (SEPA 2007). It also supplements and validates Water Framework Directive (WFD) impact assessment procedures and ensures efficient and effective design of future monitoring programmes. To be most effective at these goals, SEPA have recognised the need for the river surveillance network to be as representative of Scotland's rivers as possible. Specifically, the river surveillance network should represent the major anthropogenic pressure gradients driving ecological change in Scotland's rivers and the major habitat factors mediating ecological sensitivity to those pressures. As a national network, it should also provide a representative spatial coverage of monitoring sites.

It is not clear how representative the existing river surveillance network is. Its historical development was founded on long-established sites for The Convention for the Protection of the Marine Environment of the

North-East Atlantic (OSPAR), older EC directives, UK Environmental Change Network, UK Harmonised Monitoring and long-term quality trend assessment. However, these did not provide a representative coverage and were considered biased towards large, lowland catchments. Therefore, around 2007 additional sites were added to the river surveillance network to increase representation of smaller catchments and to ensure the network better represented WFD risk categories, WFD typologies and major pressure profiles acting on Scotland's water bodies.

Ten years on, it is now timely re-appraise the representativeness of the existing river surveillance network. The aim of this analysis is to test statistically whether river water bodies within the network represent the profile of key pressure gradients, habitat factors and spatial distributions found across all water bodies in Scotland.

Methods

Data

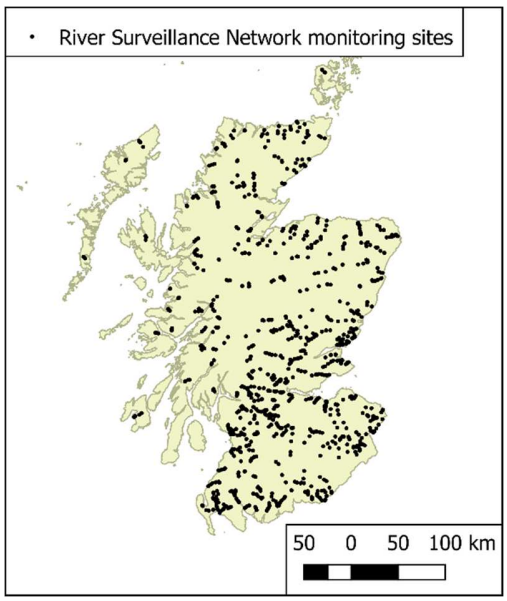
The analysis operated at the level of the SEPA river water body (Table 1.1), which are a subset of all the surface and ground water bodies monitored and assessed by SEPA (Figure 1.1). The river water bodies do not cover every river in Scotland. Instead they principally represent significant main stem channels in the river network. SEPA have already defined the catchment polygon of each river water body (Table 1.1). We identified which water bodies formed part of the river surveillance network by a spatial overlay of the sampling point locations for inorganic chemistry, invertebrates, diatoms and macrophytes. Of the 2273 river water bodies, 258 were identified as being within the river surveillance network for inorganic chemistry, invertebrates, diatoms and macrophytes.

Table 1.1. Spatial data layers used to define the SEPA river surveillance network.

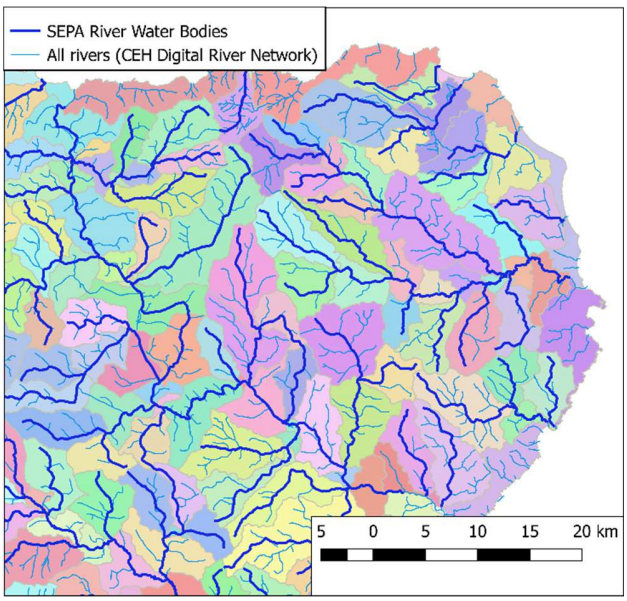
Spatial feature	Details
River waterbodies	Shapefile of main river lines supplied by SEPA.
River waterbody catchments	Shapefile of catchment polygons supplied by SEPA as 'Baseline water body intercatchments' and available from http://map.sepa.org.uk/atom/SEPA_WB_Inter_Catchments.atom.
Water monitoring sites	Shapefile of sampling points supplied by SEPA as 'SEPA Water Monitoring sites in Scotland' and available from http://map.sepa.org.uk/atom/SEPA_Water_Monitoring_Sites.atom. Separate point shapefiles were derived for the monitoring sites for inorganic chemistry, invertebrates, diatoms and macrophytes.

Figure 1.1. (a) Map of the monitoring sites within the SEPA river surveillance network. Each monitoring site was spatially attributed to a SEPA river water body, some of which are illustrated in (b). These are a subset of all rivers in Scotland, preferentially selecting sections of main stem rivers. The unique (inter) catchment of each river water body is shown as the coloured background polygons, each containing a single river water body.

(a)



(b)



To evaluate the representativeness of the river water bodies within the Surveillance Network, we compiled relevant information about each water body or its catchment (Table 1.2). The selected variables are grouped into measures of the pressure gradients that the Surveillance Network should represent, as well as important physical gradients that mediate the sensitivity of rivers to the pressures.

Table 1.2. The gradients for which the river surveillance network representativeness was assessed. These were selected to capture key gradients in the pressures on river ecosystems, the habitat factors mediating ecological sensitivity to those pressures and the spatial distribution of rivers in Scotland.

Group	Gradient	Details
Pressures	Phosphate concentration from diffuse sources (mg l^{-1})	Modelled mean phosphate concentration at the water body outflow that are apportioned to diffuse sources (arable, livestock, urban and highways). All nutrient modelling was performed by SEPA using the SAGIS model.
	Phosphate concentration from point sources (mg l^{-1})	As above but for point sources (sewage works, intermittent discharges and onsite wastewater treatment works).
	Nitrate concentration from diffuse sources (mg l^{-1})	Modelled mean nitrate concentration at the water body outflow that are apportioned to diffuse sources (arable, livestock, urban and highways).
	Nitrate concentration from point sources (mg l^{-1})	As above but for point sources (sewage works, intermittent discharges and onsite wastewater treatment works).
	Phosphate load from diffuse sources (kg day^{-1})	As above, but for load.
	Phosphate load from point sources (kg day^{-1})	As above, but for load.
	Nitrate load from diffuse sources (kg day^{-1})	As above, but for load.
	Nitrate load from point sources (kg day^{-1})	As above, but for load.
	Morphology pressure to channel (%)	Summed % of the channel assessed by SEPA as being affected by a range of morphological pressures[1]. Note the % can sum to more than 100 as these pressures are not necessarily exclusive.
	Morphology pressure to bank and riparian zone (%)	As above but for the bank and riparian zone.

[1] Bed Reinforcement, Boat Slips, Bridges, Croys, Groynes and other Flow Deflectors, Dredging, Embankments and Floodwalls no Bank Reinforcement, Embankments and Floodwalls with Bank Reinforcement, Green Bank Reinforcement and Bank Reprofiling, Grey Bank Reinforcement, High Impact Channel Realignment, Impoundments, Intakes and Outfalls, Low Impact Channel Realignment, Pipe and Box Culverts, Riparian Vegetation, Set Back Embankments and Floodwalls.

Group	Gradient	Details
	Low and medium flow modification pressure	Classification for the reduction in flow at flow rates lower than the natural Q_{70} (the flow rate exceeded 70% of the time under natural conditions). Natural flow regimes were estimated by SEPA hydrologists using Low Flow Enterprise modelling (LFE) and assuming no artificial influences on flow. Realised flow regimes were modelled based on SEPA-licenced abstractions and impoundments. Based on the modelled reduction in natural flow, the river water bodies were classified as having high, good, moderate, poor or bad status (The Scottish Government 2014), which we rescaled as an integer pressure scale from 1 (high status) to 5 (bad status).
	High flow modification pressure	As above but for modelled reduction in flow at flow rates greater than the natural Q_{70}. These will principally reflect impact of impoundments.
Sensitivity	Catchment mean elevation (m)	Used to define the WFD river typologies. Calculated from the nested catchment polygons and a 50 m digital elevation model.
	Catchment area (km^2)	Used to define the WFD river typologies. Calculated from the nested catchment polygons.
	Catchment peat coverage (%)	Used as a measure of alkalinity to define the WFD river typologies. 1:625k percentage peat coverage within the water body catchment, as supplied by SEPA.
	Catchment siliceous bedrock coverage (%)	Used as a measure of alkalinity to define the WFD river typologies. 1:250k percentage siliceous bedrock coverage within the water body catchment, as supplied by SEPA.
	Catchment calcareous bedrock coverage (%)	Used as a measure of alkalinity to define the WFD river typologies. 1:250k percentage calcareous bedrock coverage within the water body catchment, as supplied by SEPA.
	Mean channel slope (%)	Slope is one of the key factors mediating habitat structure. Estimated from the river water body lines and a 50 m digital elevation model.
	Natural Q_{mean} flow ($Ml\ day^{-1}$)	Flow is one of the key factors mediating habitat structure and SEPA use proxies for flow (precipitation and base flow) in determining standards for river flows (The Scottish Government 2014). SEPA supplied estimated mean river flow rates at the river water body outflow point, assuming no artificial influences on flow. These were produced by SEPA hydrologists using Low Flow Enterprise modelling (LFE).
	River sinuosity index	Used by SEPA to define morphological condition standards (The Scottish Government 2014). The index measures the deviations from a path defined as the maximum downslope direction, e.g. bedrock streams that flow directly downslope have a sinuosity index of 1.
Spatial	Easting (m)	British National Grid easting of the water body inter catchment centroid.
	Northing (m)	British National Grid northing of the water body inter catchment centroid.

Most of the gradients used in the analysis were available for all river waterbodies, with the greatest amount of missing data for nutrient pollution (Table 1.3). The water bodies that were missing data tended to be small and were thus not covered in the network of points used in the SAGIS nutrient modelling by SEPA. Overall, fully complete data were available for 2271 of the 2377 water bodies (95.5%). Of the 258 water

bodies in the river surveillance network, four did not have complete data (10266 River Add/Abhainn Bheag an Tunns (u/s Kilmartin Burn), 20622 Sandside Burn, 20652 Abhainn Ghriomarstaidh - d/s Loch Faoghail Charrasan and 20690 Burn of Hillside). Water bodies with missing data had to be omitted from the analysis.

Table 1.3. Completeness of the data, showing the percentage of all river water bodies with valid data for each gradient.

Pressure gradient	**Data completeness (% of water bodies)**	**Sensitivity or spatial gradient**	**Data completeness (% of water bodies)**
Phosphate concentration from diffuse sources (mg l^{-1})	96.1%	Catchment mean elevation (m)	100%
Phosphate concentration from point sources (mg l^{-1})	96.1%	Catchment area (km^2)	100%
Nitrate concentration from diffuse sources (mg l^{-1})	96.1%	Catchment peat coverage (%)	100%
Nitrate concentration from point sources (mg l^{-1})	96.1%	Catchment siliceous bedrock coverage (%)	100%
Phosphate load from diffuse sources (kg day^{-1})	96.1%	Catchment calcareous bedrock coverage (%)	100%
Phosphate load from point sources (kg day^{-1})	96.1%	Mean channel slope (%)	99.9%
Phosphate load from diffuse sources (kg day^{-1})	96.1%	Natural Q_{mean} flow (Ml day^{-1})	99.9%
Phosphate load from point sources (kg day^{-1})	96.1%	River sinuosity index	99.9%
Morphology pressure to channel (%)	99.6%	Easting (m)	100%
Morphology pressure to bank and riparian zone (%)	99.6%	Northing (m)	100%
Low and medium flow modification pressure	99.6%		
High flow modification pressure	99.6%		

Representativeness with respect to individual gradients

The representativeness of the river surveillance network for each gradient in Table 1.2 was assessed individually using two-sample two-sided Kolmogorov-Smirnov (KS) tests. This is a non-parametric test that evaluates whether two continuous variables come from the same underlying (but unknown and unspecified) distribution. As a test statistic, it uses the maximum absolute difference between the empirical cumulative density functions of both variables, *D*.

Here, KS tests were applied to compare the gradient distributions among water bodies in the river surveillance network with those not in the network. If the river surveillance network was a representative sample of Scotland's river water bodies then we would expect both groups to have similar gradient distributions. As such, the KS test statistic *D* is a measure of how strongly the river surveillance network deviates from a nationally representative sample on the focal gradient, and its *P* value indicates the statistical significance of this deviation. The *P* values were estimated by a permutation test, which accounts for ties in the data and the discrete nature of two of the gradients (both flow pressure scores). The permutation involved randomly shuffling which water bodies were inside or outside of the network, that is it represents what a representative network created by random sampling would look like. For each of 10^6 random permutations *D* was calculated and the *P* value for the observed *D* value was estimated as the proportion of permutations that exceeded the observed value of *D*, including the observed data as one permutation (Good 2013).

Regardless of the gradient being assessed, the maximum value of *D* is 1 and this indicates that the distributions of the two samples do not overlap at all, while the minimum value of $D = 0$ indicates the two samples have exactly the same values. The critical value of *D* yielding $P = 0.05$ is approximately $1.36\sqrt{\frac{n_X+n_Y}{n_X n_Y}}$, where n_X and n_Y are the sizes of the two samples. With 258 water bodies in the river surveillance network and 2017 water bodies outside the river surveillance network, the critical value of *D* is approximately 0.090, indicating a high power to detect departures from representativeness.

Representativeness with respect to all gradients

Representativeness across all gradients was jointly assessed in a similar way to the univariate tests described above. For this, the two-sample Cramér test (Baringhaus and Franz 2004) was used to evaluate whether the multivariate gradient distributions differed among water bodies inside and outside of the network. The Cramér test is a powerful non-parametric test that evaluates whether two continuous multivariate datasets come from the same underlying (but unknown and unspecified) multivariate distribution. It is sensitive to differences in the locations, variances and covariances of the two multivariate datasets. The test statistic *T*

is based on the sum of all Euclidean distances between all data points in the two samples, minus half of the corresponding sums of distances within each sample. It is calculated as:

$$T = \frac{n_X n_Y}{n_X + n_Y}\left[\frac{1}{n_X n_Y}\sum_{j=1}^{n_X}\sum_{k=1}^{n_Y} d_{X_j Y_k} - \frac{1}{2n_X^2}\sum_{j=1}^{n_X}\sum_{k=1}^{n_X} d_{X_j X_k} - \frac{1}{2n_Y^2}\sum_{j=1}^{n_Y}\sum_{k=1}^{n_Y} d_{Y_j Y_k}\right]$$

where X and Y are the two multivariate datasets, n are their sample sizes and d_{jk} is the multivariate Euclidean distance between two data points j and k. We evaluated T for all gradients in Table 1.2 to compare water bodies inside vs outside of the network. To standardise the influence of each variable on T, we first used a rank-transformation on each gradient so that they conformed to Gaussian distributions with means of zero and standard deviations of one. Thus, T is a measure of how strongly the river surveillance network deviates from a nationally representative sample, with respect to all the gradients. As above, we assessed the statistical significance of T using 10^6 permutations.

Results

The distributions of all individual gradients in Table 1.2 within the river surveillance network were significantly different to those across Scottish water bodies not in the river surveillance network, according to the two-sample KS tests (Table 1.4). However, as the KS test is highly powerful, statistical significance can result from relatively small differences in the gradient profiles (see Figure 1.2).

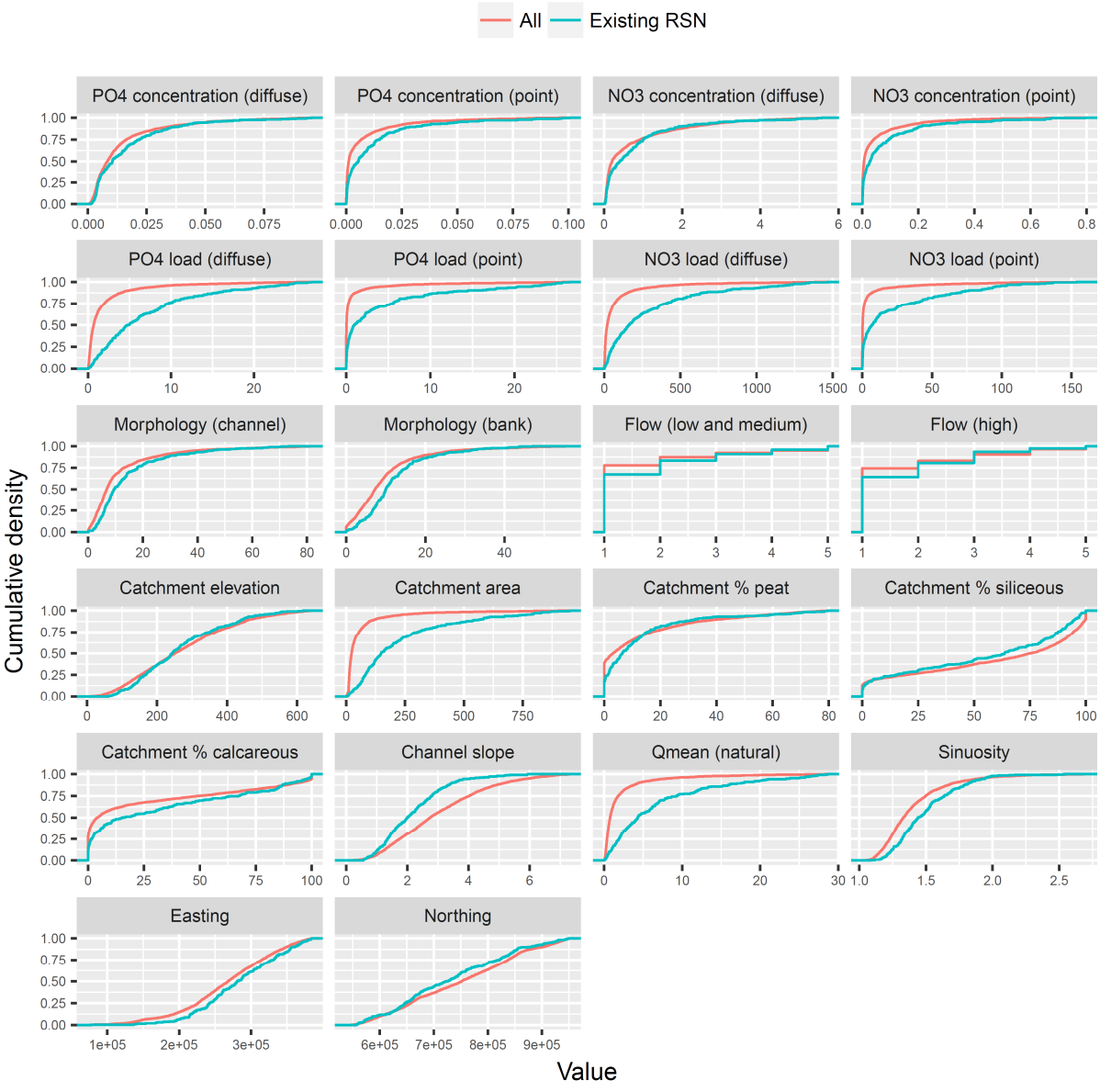
Among the pressures, the river surveillance network was least representative of nutrient loads, with a major bias towards water bodies with high loads (Figure 1.2). The river surveillance network was also very strongly biased towards water bodies with large catchments and high natural flow rates (Table 1.4, Figure 1.2). There were less strong, but still clear, biases towards water bodies with higher nutrient concentrations from point sources, higher morphological pressures, shallower slopes, higher sinuosity, more peat, less siliceous bedrock and more calcareous bedrock. Lesser biases for higher nutrient concentrations from diffuse sources and higher flow modification pressures were evident, while catchment elevation was relatively well represented by the river surveillance network. Spatially, a weak bias for over-representing south-easterly water bodies was found.

The multivariate two-sample Cramér test confirmed the strong biases found for the individual gradients (T = 219.8, P = 0.0001).

Table 1.4. Tests for lack of representativeness of each individual gradient in the river surveillance network (two-sample Kolmogorov-Smirnov tests for differences between water bodies within and outside the network). The test statistic *D* indicates the degree of departure from a representative sample. *P* values were estimated by permutation.

Group	**Gradient**	**Departure from representativeness (*D*)**	***P***
Pressures	Phosphate load from diffuse sources of pollution (kg day-1)	0.6228	<0.0001
	Nitrate load from diffuse sources of pollution (kg day-1)	0.5603	<0.0001
	Phosphate load from point sources of pollution (kg day-1)	0.5363	<0.0001
	Nitrate load from point sources of pollution (kg day-1)	0.5199	<0.0001
	Phosphate concentration from point sources of pollution (mg l-1)	0.2869	<0.0001
	Nitrate concentration from point sources of pollution (mg l-1)	0.2728	<0.0001
	Morphology pressure to bank and riparian zone (%)	0.2347	<0.0001
	Morphology pressure to channel (%)	0.2148	<0.0001
	Nitrate concentration from diffuse sources of pollution (mg l-1)	0.1538	<0.0001
	Phosphate concentration from diffuse sources of pollution (mg l-1)	0.1272	0.0012
	Low and medium flow modification pressure	0.1176	<0.0001
	High flow modification pressure	0.1148	0.0001
Sensitivity	Catchment area (km^2)	0.7134	<0.0001
	Natural Q_{mean} flow ($Ml\ day^{-1}$)	0.5995	<0.0001
	Mean channel slope (%)	0.3072	<0.0001
	River sinuosity index	0.2653	<0.0001
	Catchment peat coverage (%)	0.2383	<0.0001
	Catchment calcareous bedrock coverage (%)	0.1983	<0.0001
	Catchment siliceous bedrock coverage (%)	0.1623	<0.0001
	Catchment mean elevation (m)	0.0930	0.0376
Spatial	Easting (m)	0.1402	0.0003
	Northing (m)	0.1100	0.0078

Figure 1.2. Comparison of gradient profiles across all Scottish water bodies with those for water bodies within the existing river surveillance network. The distributions of the gradients in Table 1.2 are displayed as empirical cumulative distributions functions. As such the x-axes represent the values of the gradients (see Table 1.2 for explanation and units) and the y-axes show the proportion of water bodies with values less than or equal to the x-axis value. To enhance visualisation, upper extreme values beyond the 97.5th percentile were excluded from the plots.



Discussion and conclusions

The existing river surveillance network was found to strongly over-represent water bodies subject to anthropogenic pressure, relative to pressure profiles across all Scottish river water bodies. This was most clear when considering nutrient loadings, but biases towards high nutrient concentrations (especially from point sources), morphological pressures and flow modification pressures were also found. This likely results from an over-sampling of major rivers in their downstream reaches, given the additional biases we found in the river surveillance network towards water bodies with large catchments, high natural flow rates, low slopes and high sinuosity. There was also a slight bias towards sampling in the south and east of Scotland. This may reflect accessibility and proximity to SEPA offices, as well as the historical legacy of network development. When the current network was founded in 2007, there were a greater number of rivers in the south and east of Scotland with long-term historical monitoring data, leading these to be disproportionately included.

Despite these biases, the river surveillance network does span a wide range of pressure and habitat conditions, with some representation of water bodies across most of the ranges of the national gradient profiles shown in Figure 1.4. Therefore, there should be potential to modify the existing network to increase its representativeness, by either selectively removing existing monitoring sites from the network or selectively adding new water bodies. The quantification of deviation from multivariate representativeness using the *T* statistic offers a potential way to prioritise site addition and removal, and this will be explored in Section 2.

2. Designing a more representative and efficient river sentinel network

Summary

1. Section 1 highlighted the unrepresentativeness of the SEPA river surveillance network with deliberate bias towards downstream major river water bodies that are disproportionately exposed to anthropogenic pressures. The aim of this section is to provide options for changing the water bodies in the river surveillance network to maximise its representativeness, while maintaining data continuity in terms of the numbers of currently monitored water bodies retained in the network.
2. A stepwise algorithm for iteratively removing or adding water bodies to the river surveillance network was developed. At each iteration, the algorithm selected the water body whose removal or addition would most improve representativeness of the range of habitat and pressure gradients analysed in Section 1. The result is a ranking of water bodies in terms of removal or addition priority, which can be used by SEPA to decide on revisions to the river surveillance network.
3. When reducing the overall size of the network, the best results in terms of representativeness were achieved by removing existing water bodies and then adding new ones. Indeed, more representative networks always resulted from a higher proportion of currently-monitored water bodies being removed. This highlights the need to balance improvements in representativeness with maintaining the legacy of long-term monitoring by retention of currently-monitored water bodies.
4. R code for running the stepwise site selection can be found in Appendix 2.3 of this report.

Introduction and aims

The analysis in Section 1 established that SEPA's river surveillance network over-represented major rivers in their downstream reaches, skewing the network towards water bodies subject to anthropogenic pressures. Making changes to the river surveillance network so that it is more representative should improve its efficiency for detecting long-term changes in natural conditions and in ecological and chemical status caused by widespread anthropogenic pressures. This is because a more representative network would contain less redundancy and better cover the full profile of current pressure gradients and habitat factors mediating ecological sensitivity to those pressures.

Deciding which water bodies to remove or add to the river surveillance network is analogous to existing approaches for spatial conservation prioritisation in which computational tools support the spatial allocation

of conservation effort (Lehtomäki and Moilanen 2013). For example, in the Zonation framework a set of candidate habitat patches are ranked in terms of their perceived conservation value, and therefore priority for inclusion within a network of conservation action (e.g. a reserve network) (Moilanen et al. 2005). A similar framework could be applied to redesigning the river surveillance network with the objective of increasing its representativeness. From the analysis presented in the previous section, a metric to quantify the lack of representativeness is available, namely Cramér's *T* statistic (Baringhaus and Franz 2004). This was used to compare the multivariate distributions of environmental gradients across water bodies within and outside the river surveillance network. Potential changes to the water bodies within the river surveillance network could therefore be compared in terms of their effects of network representativeness, with prioritisation given to changes that minimise *T*.

Additionally, decisions about changes to the river surveillance network should consider a secondary goal of maintaining as much continuity of monitoring as possible. This reflects a need to preserve the legacy of existing long-term monitoring at existing water bodies to better quantify and interpret future trends in their ecological or chemical state. Since the existing river surveillance network is highly unrepresentative, re-designing the river surveillance network will require a trade-off between increasing representativeness and maintaining data continuity.

The aim of this section is to provide SEPA with options for changing the number of water bodies in the river surveillance network in such a way as to maximise its representativeness, while maintaining data continuity in terms of the numbers of currently monitored water bodies retained in the network. To achieve this we developed new stepwise algorithms for removing or adding individual water bodies to the river surveillance network to minimise the Cramér's *T* statistic. The result is a ranking of water bodies by their priority for removal or addition. We also suggest a strategy for determining the optimal balance between water body removal and addition, when the goal is to result in a monitoring network of a given size.

Methods

An algorithm for prioritising the removal or addition of water bodies was developed using the statistical programming language R (R Core Team 2017). Prioritisation was based on increases in the network's representativeness for all the gradients in Table 1.2 of Section 1. These represent major anthropogenic pressures (phosphate and nitrate loadings and concentrations from diffuse and point sources of pollution, morphological pressures to the bank and channel, and modifications to both low and high flow regimes), habitat factors influencing ecosystem sensitivity (catchment elevation, area and geology, natural flow and sinuosity) and the spatial distribution of sites (easting and northing).

Changes in the network's representativeness for these gradients was assessed jointly by calculating two-sample Cramér's *T* statistic between water bodies inside and outside of the network (see Section 1 for details of its calculation). This measures the multivariate difference between both groups of water bodies, so that larger values indicate a more biased and less representative network. Therefore, removal or addition of water bodies was done on the basis of minimising *T*. Specifically, in a removal step all possible removals of single water bodies in the current network were tried and the one resulting in a smaller network with the lowest value of *T* was chosen. Likewise, in an addition step all possible single water body additions to the current network were tried and the one causing the lowest *T* value of the new larger network was selected.

Through this stepwise process, the existing river surveillance network was first reduced in size iteratively from its current 254 water bodies to as few as five. The order of water body removal provides a prioritisation ranking for reducing the existing river surveillance network to any given size, solely on the basis of representativeness. Then stepwise additions of up to 250 water bodies were simulated from the existing river surveillance network and from networks of sites reduced in size to 50, 100, 150 and 200 water bodies. As in the network reduction simulations, the order of water body addition provides a prioritisation.

At each stage in the stepwise simulations, the statistical significance of *T* was estimated by 1,000 permutations (see Section 1 for full details of the permutation test). The network size at which *T* is not statistically significant represents the point at which the network cannot be statistically distinguished from a random sample of Scotland's water bodies, with respect to the evaluated gradients.

R code for running the stepwise site selection can be found in Appendix 2.3 of this report.

Results

The stepwise water body removal and water body addition algorithms resulted in new river surveillance networks that were substantially more representative of Scotland's river water bodies than the existing river surveillance network is (Figure 2.1). Priority rankings for water body removal and selected priority rankings for water body addition are given in Appendix 2.1.

This is illustrated for two scenarios of network change, namely reducing the existing river surveillance network to 100 water bodies (Figure 2.2) or reducing the river surveillance network to 100 water bodies followed by addition of 50 new water bodies (Figure 2.3). Both plots show the distributions of the assessed gradients across all water bodies in Scotland, in the existing river surveillance network and in the modified networks.

In the reduction-only scenario (Figure 2.2), 12 out of the 22 assessed gradients showed less deviation from their national distributions in the modified river surveillance network than in the current network of 254 water bodies, according to two-sample Kolmogorov-Smirnov tests. However, the network remained highly unrepresentative ($T = 19.44$, $P < 0.001$) and many of the gradients still had large deviations from the national profile (e.g. catchment area, natural Q_{mean} flow rate, phosphate and nitrate loads from diffuse sources). It was also clear that the algorithm had selected to remove sites from the south of Scotland, causing a bias towards representing northern catchments.

In the removal-then-addition scenario (Figure 2.3), the modified network did not significantly differ from a representative sample ($T = 2.61$, $P = 0.710$) and the two-sample Kolmogorov-Smirnov tests indicated smaller deviation than for the current network in 18 of the 22 assessed gradients. However, although the network was made much more representative, some substantial deviations remained. For example, the network still over-sampled large catchments, high natural Q_{mean} flow and northern water bodies.

According to the Cramér tests, selective reduction of the river surveillance network to 58 or fewer water bodies was needed to result in a statistically representative network ($T < 5.10$ indicating $P > 0.05$). Therefore, to establish a new network of more than this number of sites, it would be desirable to combine both removal and addition of water bodies to result in a more representative network.

Figure 2.1. Performance curve for modifications to the existing river surveillance network based solely on increasing its representativeness. Deviation from representativeness was quantified by Cramér's *T* statistic. Reductions in network size from the existing river surveillance network were achieved by stepwise removal of water bodies (WBs) to minimise *T* (blue line). Increases in network size were achieved by an equivalent stepwise addition of water bodies from varying starting points (orange lines). The dashed horizontal line shows the critical value of *T*, below which the network cannot be distinguished statistically from a random sample of Scotland's water bodies.

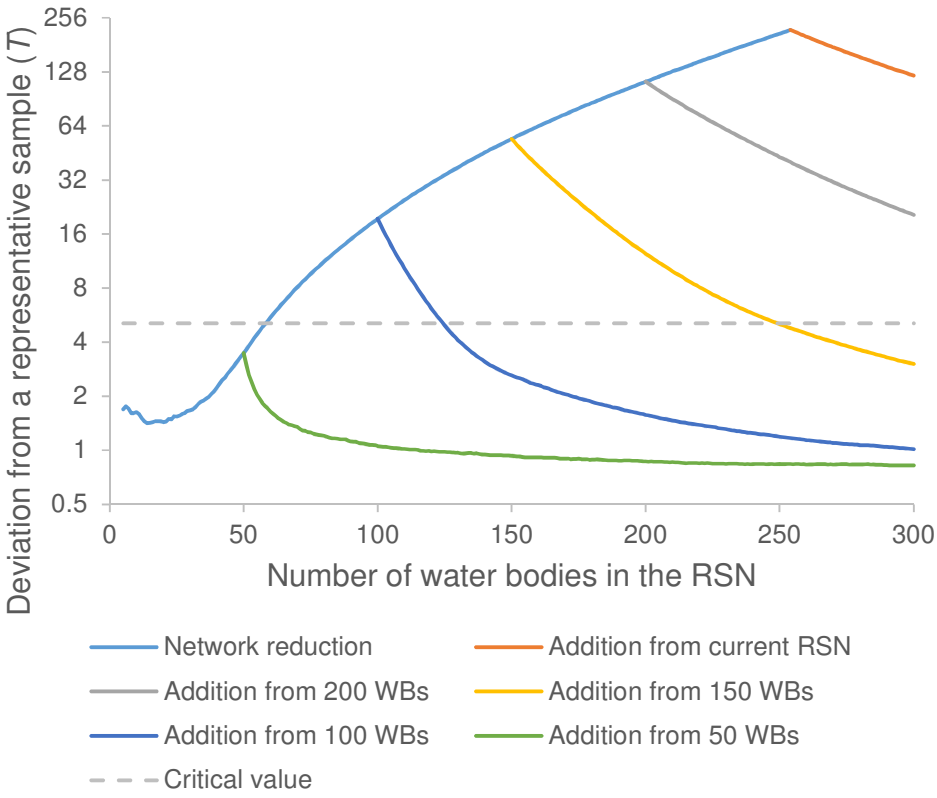


Figure 2.2. Effect of reducing the number of water bodies in the river surveillance network to 100, by stepwise removal to maximise representativeness. Panels show cumulative distribution functions of the gradients under consideration across all water bodies in Scotland (red), the existing river surveillance network (green) and the reduced network (blue). As such the x-axes represent the values of the gradients (see Table 1.2 for explanation and units) and the y-axes show the proportion of water bodies with values less than or equal to the x-axis value. To enhance visualisation, upper extreme values beyond the 97.5th percentile were excluded from the plots.

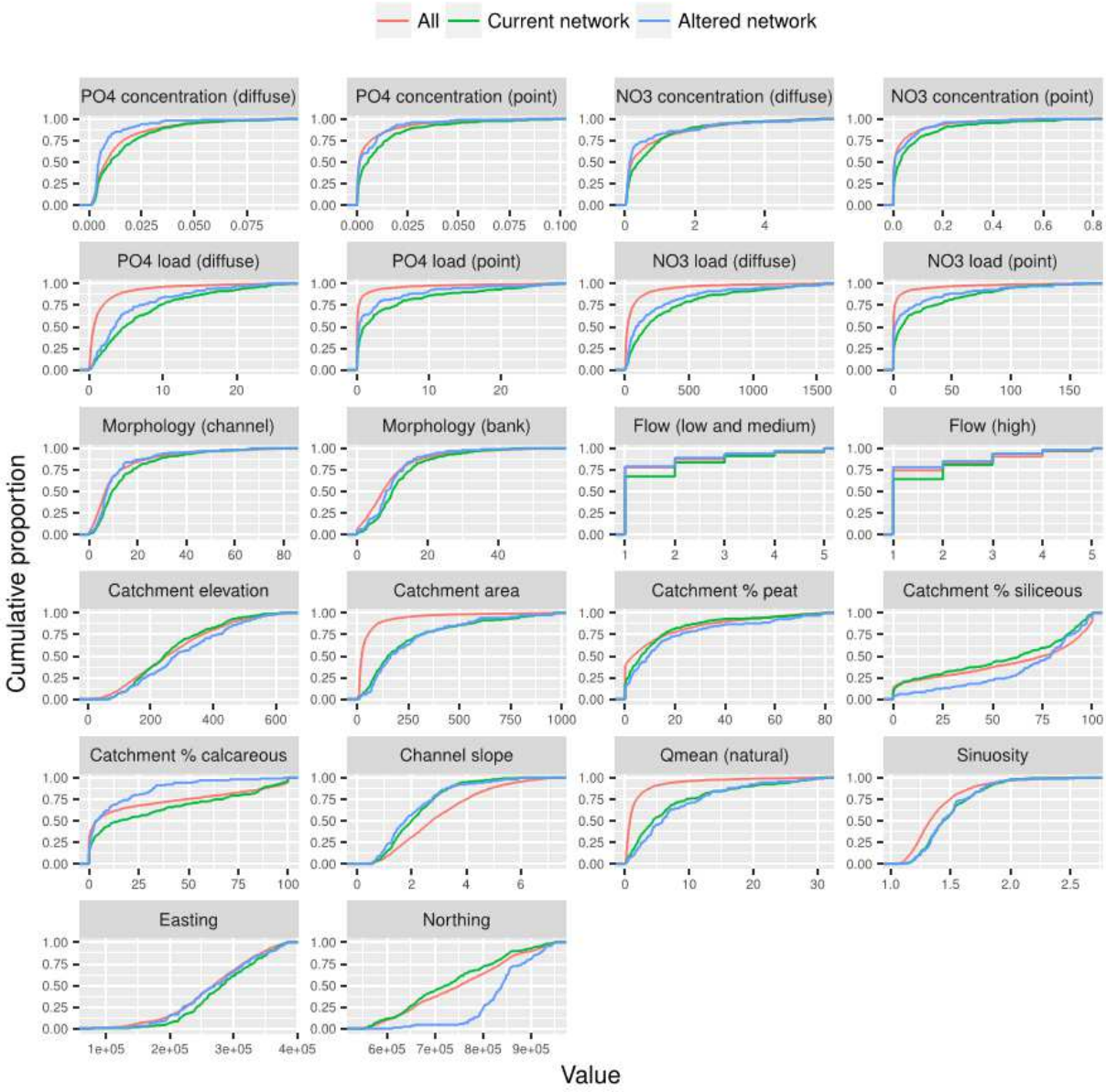
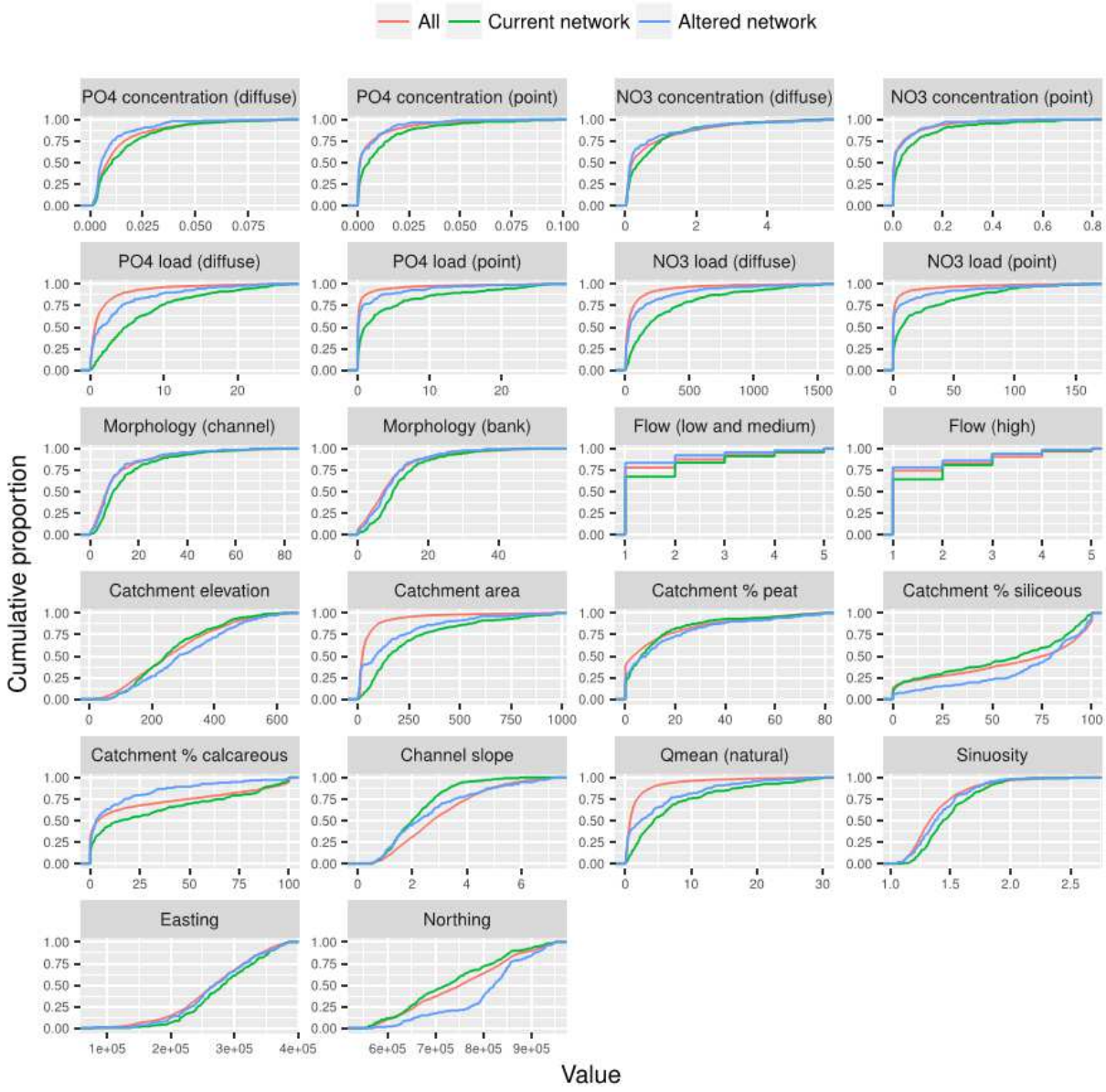


Figure 2.3. Effect of modifying the river surveillance network by reducing the number of water bodies to 100 and then adding 50 water bodies to maximise representativeness. Panels show cumulative distributions of the gradients under consideration across all water bodies in Scotland (red), the existing river surveillance network (green) and the reduced network (blue). As such the x-axes represent the values of the gradients (see Table 1.2 for explanation and units) and the y-axes show the proportion of water bodies with values less than or equal to the x-axis value. To enhance visualisation, upper extreme values beyond the 97.5th percentile were excluded from the plots.



Discussion and conclusions

The stepwise algorithm developed for the prioritisation of water body removal and addition demonstrated the scope for changing which river water bodies are monitored in the river surveillance network to achieve a more representative coverage of key national pressure and environmental gradients. However, the analysis also showed that achieving a statistically representative sample of Scotland's water bodies will require a combination of water body removal and addition, unless resources are so constrained that only 58 or fewer water bodies can be maintained. Given this, SEPA will need to decide on the balance between water body retention and water body addition in determining the new structure of the river surveillance network.

Retaining fewer water bodies from the existing river surveillance network will result in a more representative network, but at the cost of lower long-term data continuity. Therefore, SEPA will need to decide on the balance in importance between these two factors. Our recommendation would be to first decide on the number of sites that can be supported in the network, given the current budget, and then calculate the maximum number of currently-monitored water bodies that can be retained and the minimum number of new water bodies that needs to be added to result in a statistically representative network ($T <$ 5.10 indicating $P > 0.05$) of the desired size. For example, if only 125 water bodies can be monitored, Figure 2.1 indicates that the way to achieve $T < 5.10$ while maximising existing river surveillance network water body retention is to first reduce the existing river surveillance network to 100 water bodies and then add 25 new water bodies.

The analysis here only considered network representativeness and data continuity in terms of retention of currently-monitored water bodies as a criteria to evaluate modifications to the river surveillance network. We did not consider factors such as how long each currently-monitored site has been monitored for, or whether candidate sites for addition to the network have been subject to existing monitoring for Operational or Investigative purposes. Other factors important in determining optimal network structure, such as the spatial balance of the network and logistical or access considerations, were also not taken account of. In principal the exercise here could be extended to include factors such as the history of monitoring and cost of sampling each water body and the minimum travel time from SEPA offices. Doing this was beyond the current project scope, but would be sensible for future investigation and to make the final decision on how to redesign the river surveillance network.

This analysis provides SEPA with options for increasing the representativeness of the river surveillance network by altering its composition. However, the impact of such changes on the ability of the network to detect long-term changes in ecological and chemical state caused by effects of multiple, potentially

interacting stressors remains unclear. Our expectation is that for a given network size, its efficiency will be increased by taking a more representative sample of Scotland's water bodies. To test this, power analysis of trend-detection models on differently-configured monitoring networks will be performed in Section 3.

3. Power of the river and loch sentinel networks to detect change

Summary

1. Power analysis was used to evaluate the ability of the current river and loch surveillance networks to detect trends over time at the scale of the entire monitoring network, and to evaluate how modified sampling strategies for the river surveillance network may influence power to detect trends. Power analysis for modified sampling regimes in the lochs network was outside the scope the study. The results relate only to the power to detect trends. Other potential evidence needs from surveillance networks, including classifying overall status and trends at individual water bodies, were not considered.
2. The results indicate that the existing surveillance networks are generally less powerful than is desirable. Using a benchmark of 80% power to detect a 5% change over 10 years, only one out of the seven monitored parameters achieved this in the river surveillance network (that is the invertebrate ASPT EQR) and only one out of ten of the monitored parameters in the loch surveillance network (that is the diatom LTDI2 EQR).
3. The power analysis of modified sampling regimes for the river surveillance network considered a range of scenarios for the number of water bodies in the network, the interval between sampling years and the number of samples per year during sampling years. The best way to maximise power for any given level of resourcing was to avoid repeat sampling of water bodies in the same year. This is because it allows sampling of a greater number of sites in a greater number of years, which means the data averages out the spatial and annual variation obscuring the trend more efficiently.
4. The analysis also showed that reduced resourcing of the river surveillance network was incompatible with boosting its power to detect trends in all monitored parameters at the benchmark level of 80% power for a 5% change over 10 years. However, it did highlight parameters for which the current (low) power can be maintained at lower levels of resourcing, indicating that efficiency savings to the current monitoring programme are possible.
5. Some sampling regimes with low to moderate levels of resourcing did not yield sufficient information to robustly characterise trends over a 10-year period. These included monitoring water bodies very rarely (e.g. one in six years with only one sample per sampling year) so that there was insufficient replication at water body level in a 10-year period. Additionally, networks with very small numbers of water bodies (that is 50) selected to be more representative of Scotland than the current network, also performed poorly. This was because very small representative network did not include the full range of

rare water body typologies found across Scotland, allowing network trends to be skewed by trends in the more common typologies.

6. Overall, this research shows that power analysis is a very valuable approach to design and revise environmental monitoring programmes, and provides SEPA with recommendations for modifying its surveillance networks for rivers and lochs to improve or maintain their ability to detect change at the level of the whole network.
7. R code for running the power analysis can be found in Appendix 3.1 of this report.

Introduction and aims

The purpose of long-term environmental monitoring programmes is to produce reliable evidence about the monitored ecological indicators or quantities. This can comprise information on the state of the environment, environmental changes and ecological responses to change (Lovett et al. 2007, Lindenmayer and Likens 2010). Crucially, whether or not a monitoring programme delivers this evidence satisfactorily depends on the way sampling is conducted (Irvine et al. 2012). Therefore, it is important to consider how the design of monitoring programmes influences the quality of evidence that can be delivered, and whether this can be improved.

Evaluation of monitoring programme performance is generally done within the framework of power analysis, which is an attempt to quantify the probability of rejecting an untrue null hypothesis, that is detecting a true effect, using a particular statistical model and data structure (Cohen 1988). This approach is especially valuable when existing monitoring programmes are being revised or redesigned, as is currently the case for SEPA's surveillance monitoring networks. For example, Irvine et al. (2012) conducted power analysis of trends in water quality monitoring in Greater Yellowstone, USA. This allowed for evaluation of the impact of choice of length of the monitoring programme, sampling frequency and sampling locations on power to detect trends of different magnitude, while accounting for confounding factors such as seasonality.

Given limited resourcing, SEPA must make decisions about which locations should be monitored and how often should they be sampled, both in terms of annual sampling frequencies and how many samples are taken during sampling years. To understand how these decisions are likely to influence evidence from the monitoring network, recent monitoring data can be used to quantify the factors influencing power. Essentially this boils down to quantifying the relative strength of the signal and the noise in the monitoring data, and how that noise is structured. The noise in environmental monitoring data arises from factors such as seasonality, variation among sampling locations, variation among years, and unexplained sample-level

variation through measurement imprecision. Analyses of recent monitoring data can be used to quantify the structuring of noise by such factors. Then, this information can be used in power analysis to estimate the performance of ongoing monitoring under alternative sampling designs and data scenarios, assuming that future noise will be similarly structured (Irvine et al. 2012, Johnson et al. 2015).

The scope of this chapter was restricted to the power of SEPA's river and loch surveillance networks to detect changes in terms of statistically significant trends over time in monitored parameters, at the scale of the entire monitoring network. Therefore, all results and conclusions presented here preclude other potential goals of monitoring networks, including assessing the current state across the whole network, attributing changes to particular pressures, or characterising changes at individual monitoring locations. The specific aims of the research were:

1. To estimate the minimum detectable trends in recent data from SEPA's river and loch surveillance networks.
2. To evaluate how altered monitoring strategies could change the power of the river surveillance network to detect trends.

Methods

Overview

The first step in the power analysis was to fit models for trends in the monitoring data over a recent 10-year period. These estimated the strength of the recent trend and characterised the structure of the noise obscuring the trend. This noise was modelled as arising through seasonality, variation among water bodies and types of water body, variation among years and other unexplained (residual) sample-level variance.

Based on these models, power analysis simulation techniques (Johnson et al. 2015) were used to estimate the minimum detectable trends in the recent monitoring data from the river and loch networks, and also to estimate the effect of modified monitoring network structures and sampling regimes on power to detect trends in the river network.

Trend models

The power analysis was based on linear mixed effects (LME) models fitted to data from the surveillance monitoring networks for rivers and lochs from 2007-2016, as provided by SEPA (Table 3.1). LMEs provide a suitable analytical framework for monitoring data because of their ability to accommodate multiple levels of variation as 'random effects' as well as trends of interest as 'fixed effects' (Bolker et al. 2009).

Separate LME models were fitted to each monitored parameter, as described in Table 3.1. Fixed effects were specified for:

1. Year, to model the annual trend of interest. To aid model-fitting, year values were centred on their midpoint.
2. Day of year, to account for seasonality in the monitored parameters. To model seasonality with a flexible periodic function, linear terms were fitted for the first two harmonics of the Fourier series for day of year, centred on zero and scaled to the same variance as the year variable, that is:

$$h_1 = \left(\frac{Y-1}{2}\right)\cos\left(\frac{2\pi d}{365}\right)$$
$$h_2 = \left(\frac{Y-1}{2}\right)\sin\left(\frac{2\pi d}{365}\right)$$
$$h_3 = \left(\frac{Y-1}{2}\right)\cos\left(\frac{4\pi d}{365}\right)$$
$$h_4 = \left(\frac{Y-1}{2}\right)\sin\left(\frac{4\pi d}{365}\right)$$

   where Y=10 is the number of years of data and d is the day of year of the sample. Seasonal terms were not included in models for macrophytes since these were sampled once per year and sampling dates were not supplied.

Random effects were specified as:

1. Random intercepts for year, to model annual divergence from the overall trend.
2. Random intercepts for Water Framework Directive (WFD) river or loch typology, since similar types of water body might have similar monitoring parameters. Typologies were defined based on all permutations of the factors in Table 3.2.
3. Random intercepts for water body, nested within typology.
4. Where possible, random slopes for the annual trend were also specified for WFD typologies and water bodies, nested within typology. This was only possible for models for the river network data, as the lochs network contained too few water bodies for this. It was also not possible to include random trends for river macrophytes, as there was insufficient data.

Prior to model fitting, response variables were transformed to meet model assumptions about the distribution of residuals. For the chemistry parameters, logarithmic transformation was applied, to exclude negative model predictions. Logarithmic transformations were also used for the ecological quality ratios (EQRs) when supplied as precise values. However, when EQRs were supplied as 'capped' values with an

upper bound of 1, empirical logit transformations was applied as this is the preferred approach to model proportion data (Warton and Hui 2011). The equation for the empirical logit transform of a proportion x is:

$$x^* = \ln\left(\frac{x+\epsilon}{1-x+\epsilon}\right)$$

where ϵ is a small constant to avoid errors when $x = 0$ or $x = 1$. The value of ϵ was set at $(1\text{-}x_{max})/2$, where x_{max} is the largest value of x that is less than 1.

The LME models were developed using the R packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2015). Model fitting used restricted maximum likelihood (REML) and fixed effect statistical significance was estimated using Satterthwaite's approximation of the numbers of degrees of freedom.

Table 3.1. Monitored parameters for which power analysis was performed, a summary of their recent monitoring intensity and transformations used in their analysis. The monitoring parameters were supplied by SEPA at the level of an individual sample. For classification purposes, SEPA aggregates most of the parameters over longer time periods.

Water body type	Monitoring type	Monitored parameter	Number of samples (2007-2016)	Number of water bodies (2007-2016)	Typical annual sampling frequency (post- 2010)	Median number of samples per year, when sampled (post-2010)	Transformation for analysis
River	Chemistry	Ammonia as nitrogen (mg/L)	23510	246	every year	12	$\log_{10}$
		Dissolved oxygen (mg/L)	22807	245	every year	11	$\log_{10}$
		Total reactive phosphorus (mg/L)	23477	246	every year	12	$\log_{10}$
		Total phosphorus (mg/L)	22695	246	every year	11	$\log_{10}$
	Ecology	Invertebrate EQR (Average Score Per Taxon, ASPT abundance)	3202	252	1 in 2 years	3	$\log_{10}$
		Macrophyte EQR (River Macrophyte Nutrient Index, RMNI)	488	256	1 in 6 years	1	empirical logit
		Diatom EQR (River Trophic Diatom Index, TDI4)	3662	252	2 in 3 years (mix of 1 in 3 and 2 in 3)	2	empirical logit
Loch	Chemistry	Ammonia as nitrogen (mg/L)	4317	39	every year	12	$\log_{10}$
		Dissolved oxygen (mg/L)	4265	39	every year	11	$\log_{10}$
		Total reactive phosphorus (mg/L)	4182	39	every year	11	$\log_{10}$
		Total phosphorus (mg/L)	4146	39	every year	11	$\log_{10}$
	Ecology	Invertebrates (ASPT abundance)	249	40	1 in 6 years	2	Scores from 0 to 8, so divided by 8 then logit
		Invertebrates EQR (Chironomid Pupal Exuviae Technique, CPET)	267	40	1 in 6 years	4	empirical logit
		Macrophyte EQR (Lake Macrophyte Nutrient Index, LMNI)	83	42	1 in 6 years	1	empirical logit
		Diatom EQR (Lake Trophic Diatom Index, LTDI2)	493	40	1 in 2 years	2	empirical logit
		Phytoplankton EQR (Phytoplankton trophic index, PTI)*	868	81	1 in 2 years	3	$\log_{10}$
		Cyanobacteria EQR (PLUTO EQR)*	820	81	1 in 2 years	3	$\log_{10}$

* Data missing for 2007 and 2008

Table 3.2 Characteristics used to define water body typologies for rivers and lochs. These are based on the Water Framework Directive definitions applied in the UK. However, loch size not used because all monitored lochs were in the 'large' category.

Rivers		**Lochs**	
Mean catchment altitude	Low: < 200 m	Mean altitude	Low: < 200 m
	Mid: 200-800 m		Mid: 200-800 m
	High: >800m		High: >800m
Catchment area	Small: <100 km^2	Dominant geology	Siliceous
	Medium: 100-1000 km^2		Calcareous
	Large: > 1000 km^2		Organic
Catchment dominant geology	Siliceous	Depth	Shallow: < 3m
	Calcareous		Deep: > 3m
	Organic		

Minimum detectable trends in the current monitoring data

To estimate the minimum detectable trend for each monitored parameter over the past 10 years (2007-2016), a power analysis was conducted on LMEs for the existing data using a range of annual trend values. The procedure for the power analysis followed recently-developed protocols (Johnson et al. 2015) and was as follows:

1. Decide a value of the annual trend to assess the power of the model. In this analysis, trend values between 0 and values greatly exceeding the observed trends from 2007-2016 were tested. The maximum trend values for each monitored parameter were chosen in a pilot study to ensure the analysis included values with very high statistical power.
2. Simulate a large number of randomly generated response variables from the model, using the specified value of the annual trend. For each assessment, 500 simulations were performed using the 'simulate.merMod' function of the lme4 R library (Bates et al. 2015). Simplified R code for running the power analysis can be found in Appendix 3.1 of this report. Simulations used:
    a. The data used to fit the model. This defined the sampling regime in terms of the dates at which each water body was sampled.
    b. The specified value of the trend being tested.
    c. Other fixed effects of the model (that is intercept and seasonality) at their fitted values.

d. Random effect variances and covariances, from which new multivariate normally-distributed random effect values are generated at each simulation.
e. Residual (unexplained) variance from the model, from which normally-distributed errors are included in the stochastic simulation.

3. Fit the same LME model to each simulated response variable and estimate the statistical power, which is the proportion of simulations yielding a statistically significant annual trend.

The above procedure established a 'power curve', that is power to detect a significant trend as a function of trend size (Johnson et al. 2015). The minimum detectable trend was estimated using linear interpolation between points on the power curve to calculate the trend size where the power curve crossed 80% power (that is a Type II error rate of 20%). This is a commonly used value for minimum adequate power, though one that has been criticised (Di Stefano 2003).

Trend sizes here were obtained in terms of LME slope coefficients. For interpretation, they were converted into the proportion change over a 10-year period using the following expressions:

$$10^{b(Y-1)} \qquad \text{if the variable was } \log_{10} \text{ transformed}$$

$$\frac{10^{a+b\left(\frac{Y-1}{2}\right)}-1}{10^{a-b\left(\frac{Y-1}{2}\right)}-1} \qquad \text{if the variable was } \log_{10}(x+1) \text{ transformed}$$

$$\left(\frac{(1+\epsilon)e^{a+b\left(\frac{Y-1}{2}\right)}-\epsilon}{1+e^{a+b\left(\frac{Y-1}{2}\right)}}\right)\left(\frac{1+e^{a-b\left(\frac{Y-1}{2}\right)}}{(1+\epsilon)e^{a-b\left(\frac{Y-1}{2}\right)}-\epsilon}\right) \qquad \text{if the variable was empirical logit transformed}$$

where a is the LME intercept, b is the LME slope for year, Y=10 is the number of years and ϵ is the empirical logit offset. Note that these expressions are derived based on year values being centred on their midpoint (that is $(Y-1/2)$). Coefficients for other fixed and random effects are not needed since the other fixed effects were centred on zero and the random effects were modelled with zero mean.

Power of modified sampling regimes to detect trends in the river surveillance network

For the river surveillance network, power analysis experiments were performed to investigate how modified network structures and altered sampling regimes may influence the ability of the model to detect trends over a 10-year period (see Table 3.3). For this, the power analysis procedure described above was repeated with two modifications:

1. The trend value was fixed at either the estimated trend from 2006-2017, or set to a value leading to ±5% change over 10 years. The former was used to consider realistic trend values consistent with recent data. The latter was chosen as a 'target' for good performance by the network. To implement this, values of the trend leading to +5 and -5% changes differ slightly from one another, so both were used and the results averaged.
2. In data simulations, instead of defining the sampling regime by inputting the historical monitoring data, new sampling regimes were created and entered into the model. The modified sampling regimes consisted of choices about which water bodies to include in the network and when to sample them, as summarised in Table 3.3.

Table 3.3. Settings for the power analysis experiment. All factorial combinations of the treatment levels were evaluated with 500 replicate simulations each.

Sampling regime feature	Treatment levels
Trend in the monitored data	Fixed at recent trend, or set to a value giving +/- 5% change over 10 years
Selection strategy for water bodies in the network	Random subsets of the current water bodies or a more representative set of water bodies
Number of water bodies in the network	50, 100, 150, 200 and either all current water bodies (for the random selection) or 250 (for the representative selection)
Annual sampling interval	1, 2, 3, or 6 years
Number of samples per year	1, 6, or 12 for chemistry parameters; 1, 2 or 3 for ecology parameters*

* except macrophytes, which are monitored with only 1 sample per year.

In the simulated sampling regimes, effort was distributed evenly across and within years. For example, if sampling employed a 1 in 3-year rotation, then one third of the water bodies would be visited in each year. Likewise, if sampling were done two times per year, then sampling dates for each water body would be drawn randomly from the distribution of sampling dates in the 2007-2016 data, with one from the first half of the year and one from the second half of the year.

To determine more representative subsets of water bodies to include, we used the results from Section 2 to identify the stepwise strategy resulting in a statistically representative network (Cramér test with $P > 0.05$) with the smallest loss of currently monitored water bodies. These were:

1. To select 50 water bodies – a stepwise reduction from the current network to 50 water bodies.

2. To select 100 water bodies – a stepwise reduction from the current network to 50 water bodies and then a stepwise addition of 50.
3. To select 150 water bodies – a stepwise reduction from the current network to 100 water bodies and then a stepwise addition of 50.
4. To select 200 water bodies – a stepwise reduction from the current network to 100 water bodies and then a stepwise addition of 100.
5. To select 250 water bodies – a stepwise reduction from the current network to 150 water bodies and then a stepwise addition of 100.

Some of the treatment combinations in the experiment resulted in simulated data sets to which the models could not then be fitted. For the calculation of power, these were considered to yield non-significant trends, since they represent sampling schemes not providing sufficient information to fit an appropriate trend model.

Results

Trend models

The fitted models on which power analysis was performed are summarised in Table 3.4. From the river surveillance network, the analysis indicated a statistically significant decrease in total reactive phosphorus concentrations and significant improvements in the invertebrate and diatom EQRs. In the loch surveillance network, there was a statistically significant increase in ammonia, a significant decrease in total phosphorus and increases in the phytoplankton, cyanobacteria and invertebrate (CPET) EQRs.

Generally, the model fixed effects explained only a small amount of the variation compared to the random effects or residual unexplained variation. Across all monitoring parameters, the marginal R^2 (from fixed effects only) had a median value of 0.015 in rivers and 0.021 in lochs, while median values of the conditional R^2 (from fixed and random effects) were 0.660 in rivers and 0.477 in lochs. Therefore, in general, less than 2.1% of the variation in the data was explained by the annual trend (since marginal R^2 combined effects of the annual trend as well as seasonality). Examination of the random effects (Table 3.4) suggests that spatial (among water body) variability was greater than year-to-year variations, and that there was more variability among water bodies within a typology than between typologies.

Minimum detectable trends in the current monitoring data

Power curves from the analyses of the river and loch surveillance networks are shown in Figures 3.1 and 3.2. From these, the minimum detectable trends were estimated as the trend size yielding 80% power (Figure 3.3). Generally, the power for the observed trends was below 80%, meaning that observed trends from 2007-2016 were weaker than the minimum detectable trends. The sole exception to this was river invertebrates (ASPT EQR), for which the current significant trend was estimated to have 85% power.

Only two parameters had minimum detectable trends less than ±5% over 10 years, seen as a 'target' for good network performance by SEPA. These were for river invertebrates (ASPT EQR) and loch diatoms (LTDI2 EQR). By contrast, minimum detectable trends greater than ±10% were observed in the river surveillance network for total reactive phosphorus , Ammonia as nitrogen and total phosphorus, and in the loch surveillance network for Ammonia as nitrogen, total reactive phosphorus , total phosphorus, EQRs for macrophytes, cyanobacteria and phytoplankton and invertebrates (ASPT). These can be considered the parameters for which the network seems to have performed most poorly.

Table 3.4. Summary of the LME models for which power analyses were performed. The table shows the estimated annual trends (in bold where $P < 0.05$), an indication of whether the model found significant seasonality and the random effect and residual standard deviations. All results are on the scale of the transformed monitoring parameters.

Water body type	Monitoring type	Monitored parameter	Annual trend on transformed scale (SE)	*P*	Significant seasonality	Random intercepts and residuals (standard deviations)		Random slopes for year (standard deviations)	
River	Chemistry	Ammonia as nitrogen	$6.87x10^{-3}$ ($4.67x10^{-3}$)	0.160	yes	Year:	0.027		
						Typology:	0.236	Typology:	0.0102
						WB within typology:	0.268	WB within typology:	0.0282
						Residual:	0.306		
River	Chemistry	Dissolved oxygen	$2.83x10^{-4}$ ($9.02x10^{-4}$)	0.761	yes	Year:	0.00793		
						Typology:	0.00497	Typology:	0.0006065
						WB within typology:	0.01495	WB within typology:	0.0015582
						Residual:	0.03369		
River	Chemistry	Total reactive phosphorus	$-1.42x10^{-3}$ ($5.71x10^{-4}$)	0.027	yes	Year:	0.00447		
						Typology:	0.01688	Typology:	0.0007276
						WB within typology:	0.0269	WB within typology:	0.0024204
						Residual:	0.03043		
River	Chemistry	Total phosphorus	$-5.90x10^{-3}$ ($3.29x10^{-3}$)	0.099	yes	Year:	0.02625		
						Typology:	0.29274	Typology:	0.003195
						WB within typology:	0.29815	WB within typology:	0.015847
						Residual:	0.2305		

Water body type	Monitoring type	Monitored parameter	Annual trend on transformed scale (SE)	*P*	Significant seasonality	Random intercepts and residuals (standard deviations)		Random slopes for year (standard deviations)	
River	Ecology	Invertebrate EQR	$1.06x10^{-3}$ ($3.22x10^{-4}$)	0.010	yes	Year:	0.00193		
						Typology:	0.01373	Typology:	0.0004635
						WB within typology:	0.02594	WB within typology:	0.0019569
						Residual:	0.02176		
River	Ecology	Macrophyte EQR	0.0565 (0.021)	0.074	-	Year:	0.117		
						Typology:	1.139		
						WB within typology:	0.859		
						Residual:	1.035		
River	Ecology	Diatom EQR	0.0383 (0.0132)	0.022	yes	Year:	0.042		
						Typology:	0.446	Typology:	0.0275
						WB within typology:	1.023	WB within typology:	0.0717
						Residual:	1.202		
Loch	Chemistry	Ammonia as nitrogen	0.0174 ($7.06x10^{-3}$)	0.040	yes	Year:	0.06248		
						Typology:	0.12065		
						WB within typology:	0.16596		
						Residual:	0.3012		
Loch	Chemistry	Dissolved oxygen	$4.82x10^{-4}$ ($8.89x10^{-4}$)	0.603	yes	Year:	0.0079		
						Typology:	0		
						WB within typology:	0.00956		
						Residual:	0.03515		

Water body type	Monitoring type	Monitored parameter	Annual trend on transformed scale (SE)	*P*	Significant seasonality	Random intercepts and residuals (standard deviations)		Random slopes for year (standard deviations)
Loch	Chemistry	Total reactive phosphorus	-1.42×10^{-3} (4.43×10^{-3})	0.757	yes	Year:	0.03858	
						Typology:	0.06682	
						WB within typology:	0.1446	
						Residual:	0.23276	
Loch	Chemistry	Total phosphorus	-3.71×10^{-3} (1.55×10^{-3})	0.045	yes	Year:	0.0102	
						Typology:	0.2052	
						WB within typology:	0.2612	
						Residual:	0.2002	
Loch	Ecology	Invertebrates ASPT EQR	-4.86×10^{-4} (0.0130)	0.972	no	Year:	0.0673	
						Typology:	0.2672	
						WB within typology:	0.3696	
						Residual:	0.4474	
Loch	Ecology	Invertebrates CPET EQR	0.0397 (0.0176)	0.025	no	Year:	0	
						Typology:	0.2405	
						WB within typology:	0.5984	
						Residual:	0.7069	
Loch	Ecology	Macrophyte EQR	0.0411 (0.0247)	0.170		Year:	0.07733	
						Typology:	0.11644	
						WB within typology:	0.51632	
						Residual:	0.58057	

Water body type	Monitoring type	Monitored parameter	Annual trend on transformed scale (SE)	*P*	Significant seasonality	Random intercepts and residuals (standard deviations)		Random slopes for year (standard deviations)
Loch	Ecology	Diatom EQR	$3.52x10^{-3}$ (0.0321)	0.913	yes	Year:	0	
						Typology:	0	
						WB within typology:	1.699	
						Residual:	1.811	
Loch	Ecology	Phytoplankton EQR	$5.24x10^{-3}$ ($1.73x10^{-3}$)	0.003	yes	Year:	0	
						Typology:	0.07445	
						WB within typology:	0.12854	
						Residual:	0.10757	
Loch	Ecology	Cyanobacteria EQR	$4.09x10^{-3}$ ($1.58x10^{-3}$)	0.041	no	Year:	0.00921	
						Typology:	0.0096	
						WB within typology:	0.0208	
						Residual:	0.0422	

Figure 3.1. Power curves for the 2007-2016 data from the current river surveillance network, showing how power increases with the magnitude of the trends (% change over 10 years). The vertical red line is the value of the trend in the data, while the grey lines show the minimum detectable trend, resulting in 80% power.

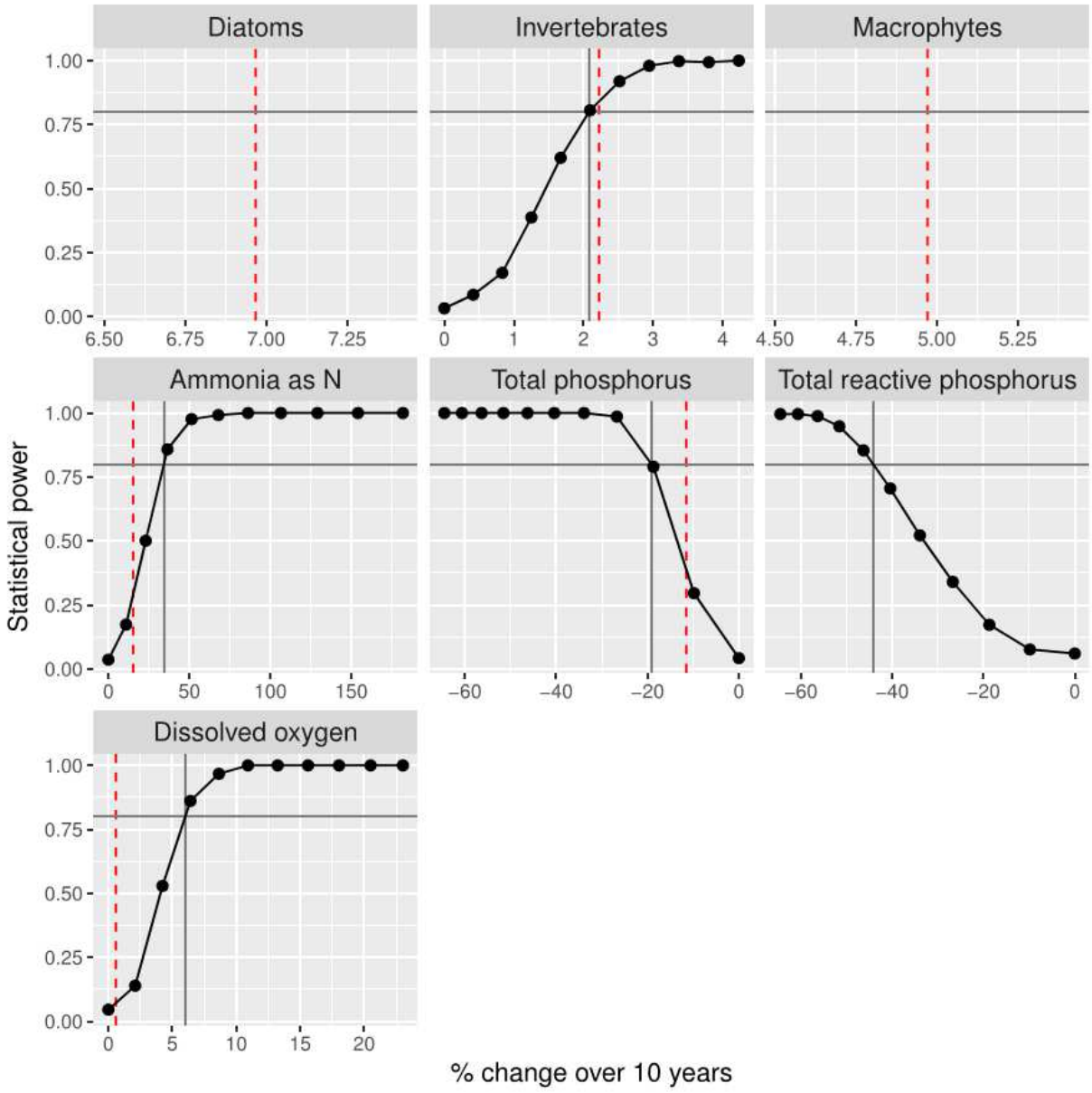


Figure 3.2. Power curves for the loch surveillance network, equivalent to Figure 3.1.

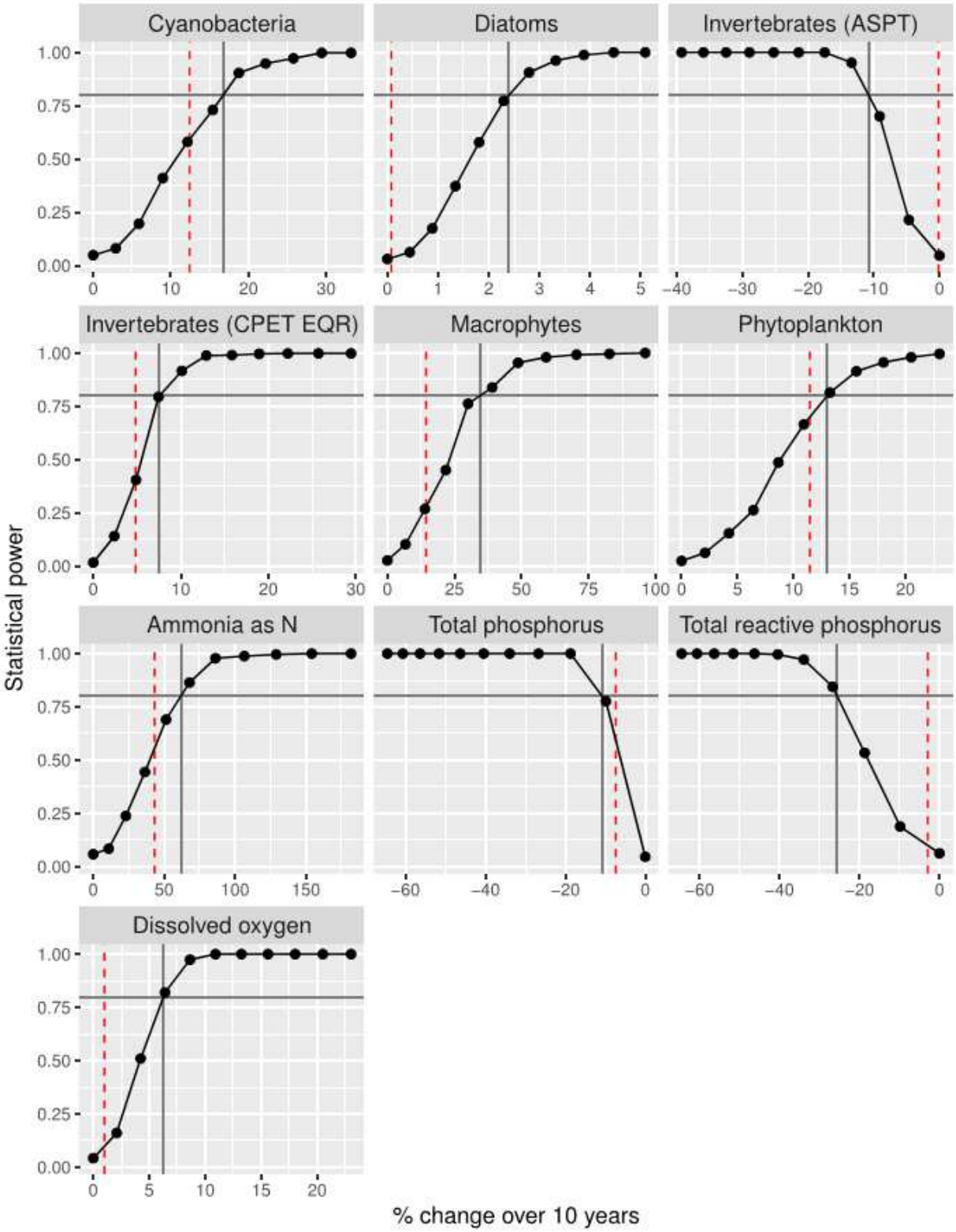
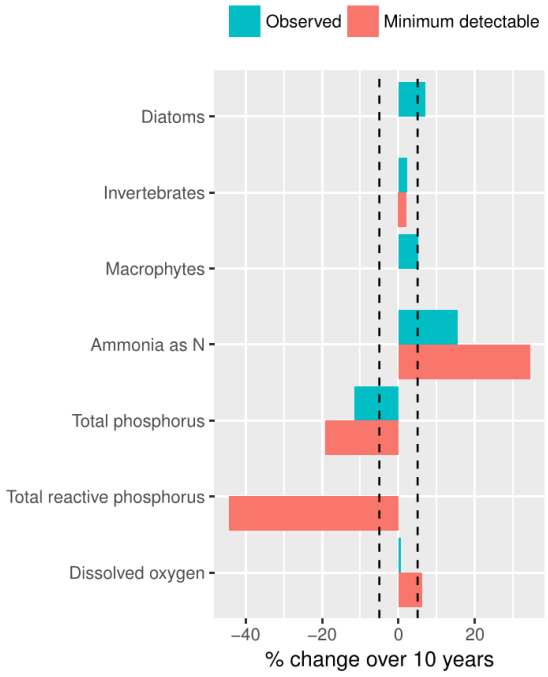
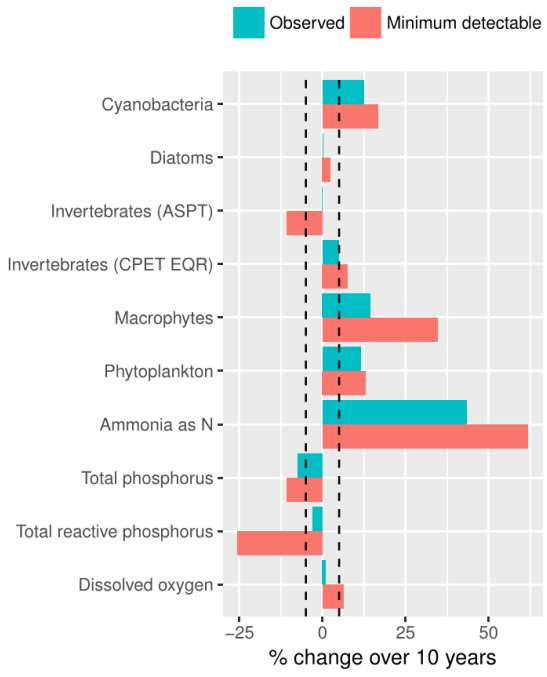


Figure 3.3 Observed and minimum detectable (80% power) trends in the 2007-2016 monitoring data from (a) rivers and (b) lochs. Dashed lines show ±5%, considered an upper limit for the minimum detectable trends in a monitoring network.

(a)



(b)



Power of modified sampling regimes to detect trends in the river surveillance network

Power experiments with simulated data from modified sampling regimes were performed assuming a 10-year period with both the estimated 2007-2016 trends (Figure 3.4) and with trend values resulting in an overall change of ±5% in each parameter (Figure 3.5). In both cases, an increase in the number of samples collected generally resulted in higher power. However, there appeared to be some scope to reduce the number of samples with minimal power loss. Furthermore, the best way to reduce resourcing, while maintaining the network's ability to detect trends, is to reduce repeat sampling within years while maintaining as many sites as possible and sampling them in as many years as possible. For any given level of resourcing, the highest power was obtained with the lowest level of repeat sampling within years considered in the experiment.

The plots also identify two clusters of points with distinctly low power, representing sampling regimes that should be avoided. First, there was a cluster of sampling regimes at zero power. These were sampling regimes with monitoring in 1 in 6 years and only 1 sample per year. Such schemes yielded data that did not allow the trend model to be fitted because there was insufficient replication at water body level to estimate variation among water bodies as well as seasonality and year effects. In addition, there was a group of sampling regimes yielding low power from the more representative networks. These were representative networks of only 50 water bodies, the smallest network sizes considered. It is likely that this resulted from the small representative networks not including the rarer river typologies, and, therefore, having a high chance of the trends being obscured by among-typology variation in a small number of common typologies. For larger networks, however, there was little effect of representativeness on network performance at trend detection, although it is to be expected that the representative networks would yield more representative trend estimates.

Figure. 3.4 The estimated power of modified river surveillance sampling regimes to detect the recent (2007-2016) trends over a 10-year period. Statistical power is plotted as a function of total resourcing per parameter (number of samples taken over 10 years). The point shapes and shading indicate the effects of choice of water body selection strategy and within-year sampling intensity, as these appeared to be the two key factors affecting power, in addition to the number of samples. The grey lines indicates the number of samples taken from 2007-2016.

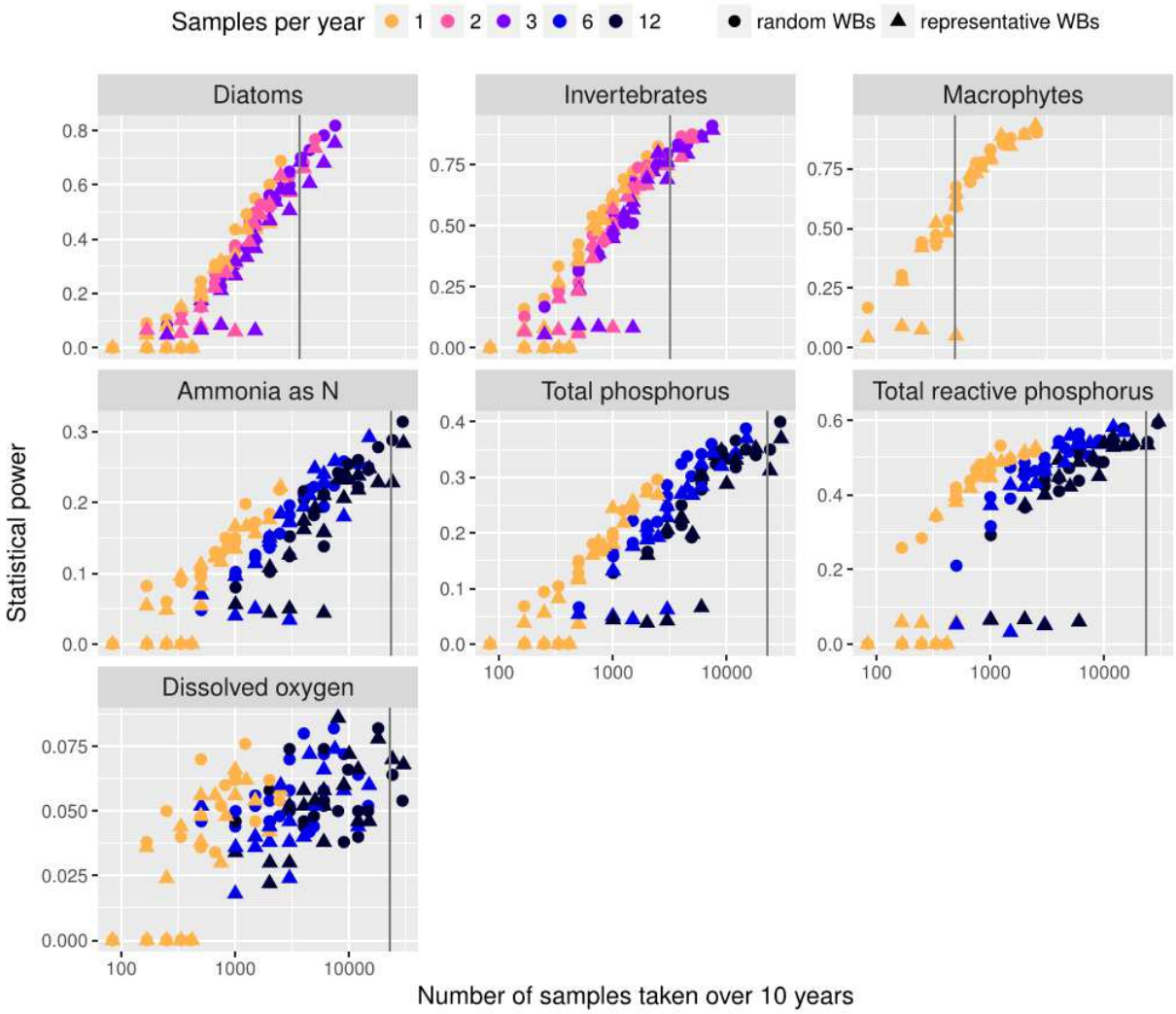
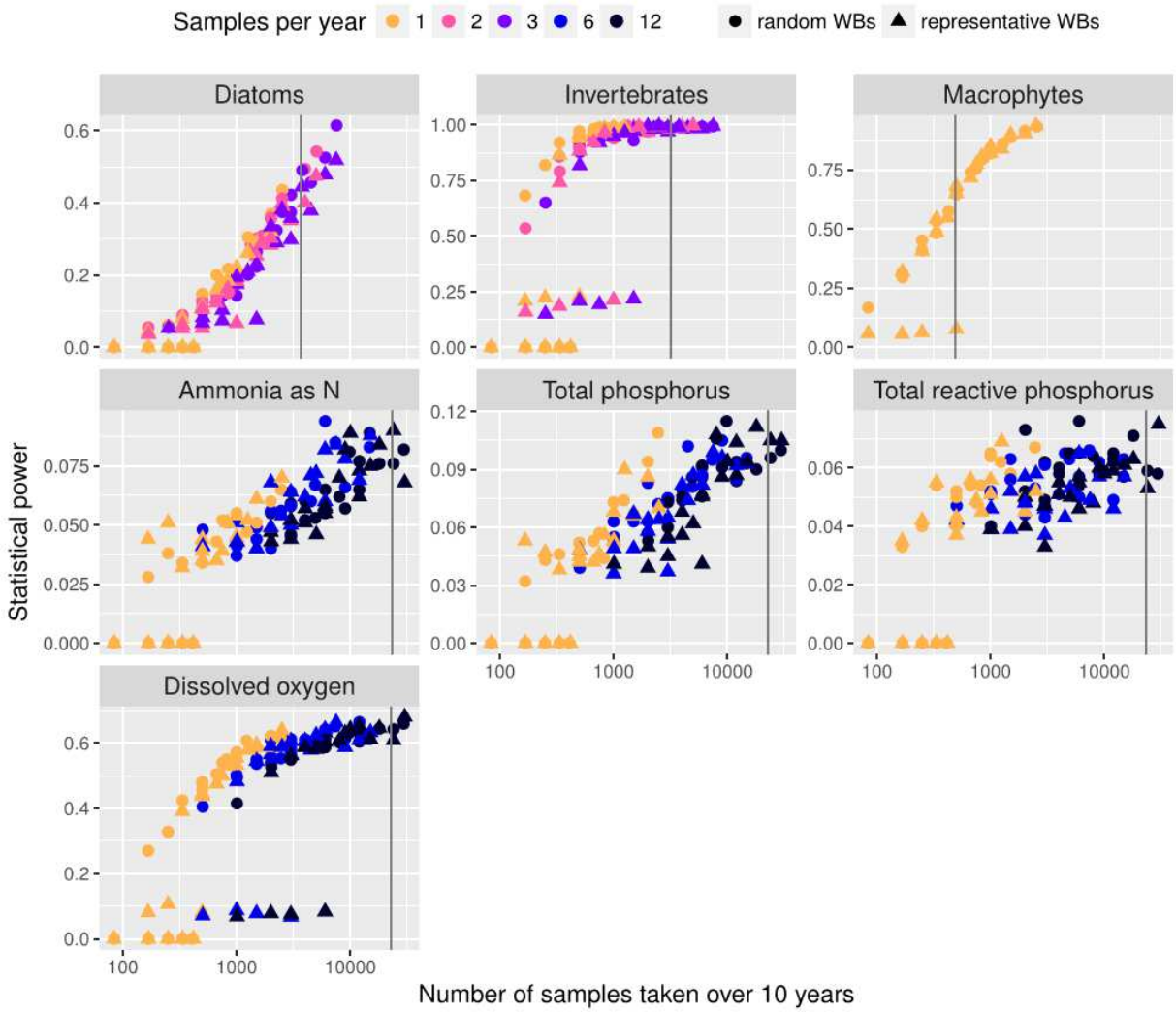


Figure. 3.5 The estimated power of modified river surveillance sampling regimes to detect a trend of ±5% change over a 10-year period. The plot is otherwise equivalent to Figure 3.4.



Discussion and conclusions

This research was motivated by SEPA's need to operate a freshwater surveillance network that delivers high quality evidence in the most cost effective manner. We used power analysis to evaluate the ability of the river and loch monitoring networks to detect trends over a 10-year period. Therefore, all our results and recommendations should be interpreted in the context of trend detection ability at the level of the whole network, and may not apply to other evidence needs from the surveillance monitoring networks, such as managing the condition of individual river or loch water bodies.

The power analysis indicated that the current river and loch surveillance networks did not achieve sufficient power for detecting trends of the observed magnitude. Trends in monitoring data from 2007-2016 were generally below the estimated minimum detectable trends for each parameter, defined as the trend value giving 80% power. This would not necessarily be interpreted as a problem, since it may be that the monitored parameters were not exhibiting strong trends. Of more concern, however, is that these minimum detectable trends were often large. For example, we found <80% power to detect changes of more than ±5% over a 10-year period in six out of seven monitored parameters in rivers and nine out of ten monitored parameters in lochs. The two exceptions were trends for river invertebrates (ASPT EQR) and loch diatoms (LTDI2 EQR), where the minimum detectable trends were <±5%, even though no actual trend was detected for diatoms.

This variation among monitoring parameters in both recent trends and the minimum detectable trends, suggests current sampling effort is not distributed optimally across parameters. Potentially, it would be desirable to shift effort from parameters with a small minimum detectable trend to those with a high one, in order to equalise the minimum detectable percentage change across parameters. For example, Ammonia as nitrogen and total reactive phosphorus would benefit from improved monitoring to bring down their large minimum detectable trends in both rivers and lochs.

The power analysis also considered modified sampling regimes for the river surveillance network, informed by SEPA's need to modify their current surveillance monitoring. This demonstrated the value of power analysis for re-designing monitoring networks and sampling regimes (Irvine et al. 2012). In particular, it showed that for a given level of resourcing, power was maximised by moving towards sampling only once per year. This likely reflects an ability of the statistical models to characterise seasonality across water bodies, and remove its influence on apparent trends without a need to intensively sample across the year in all monitored water bodies. Sampling only once per year also means resources can be spread more widely

across a range of water bodies and typologies, so the model averages out spatial and between-typology variability more effectively and produces a more accurate estimate of the average trend.

It should be noted however, that while this improves power for detecting trends at the level of the whole network, it may not deliver sufficient information on the status or trends of individual water bodies or typologies. This includes WFD classification for the condition of individual water bodies. Consideration of these goals were outside the scope of the analysis performed here, but should be undertaken by SEPA when making decisions on how to adjust their surveillance networks. This could be done using similar power analysis approaches to the one we developed here.

The power analysis on modified sampling regimes also highlighted the need for clearly defined goals and targets for trend detection by the modified network. Below, the results are evaluated in terms of three alternative goals for a modified river surveillance network:

1. If the goal is to maintain current power to detect trends of similar magnitude to those of the past 10 years, then Figure 3.4 suggests that substantial reductions in sampling of chemical determinands can be made without substantial loss of their current (low) power. By contrast, reduced sampling effort for the ecological parameters results in greater power loss, suggesting their sampling should be maintained or intensified.
2. If the goal is to maintain current power to detect trends of ±5% over 10 years, then Figure 3.5 suggests reduced sampling of invertebrates, as well as chemistry. This is because the current network has relatively high power for river invertebrate trends, with a minimum detectable trend of ~2%. Therefore, invertebrate sampling intensity can be reduced to the point where the minimum detectable trend is ±5%.
3. If the goal is to achieve a minimum adequate level of power across all monitored parameters, then the level of resourcing will determine how much power can be achieved. For example, if the goal is to achieve 80% power for a ±5% change in all parameters, then Figure 3.5 suggests this cannot generally be done with current or reduced resourcing. However, since current sampling is more than adequate for invertebrates, we would recommend redistributing some of the effort towards the parameters currently having the lowest power to detect ±5% change (Ammonia as nitrogen, total phosphorus and total reactive phosphorus). Based on the arguments above, greater sampling of these parameters is likely to be most effective at trend detection if achieved by adding new water bodies to the network and sampling the expanded network only once per year. Although, the power experiment did not evaluate the potential power gain under this scenario it seems likely that improvements on current power could be achieved with similar or improved resourcing.

Power analysis is always approximate and subject to a number of caveats (Johnson et al. 2015). One caveat comes from the assumption that the structure of noise in future monitoring data will follow patterns from the last ten years. This may not be true because emerging technologies for monitoring may improve accuracy (that is reduce sample-level residual variation), there may be better standardisation of sampling and laboratory methods, or factors such as climate change may alter seasonality, among-water body, among-typology and among-year variability. Another caveat is that assumptions of the trend model may not have been fully met in the data. Nevertheless, the power analysis approaches developed and applied here should be considered an important element in the design of environmental monitoring programmes. In this context, our results provide SEPA with information to interpret evidence about trends from their existing surveillance networks as well as suggest options for modifying their networks under different levels of future resourcing.

4. Long-term changes detectable in the existing river and loch sentinel networks

Summary

1. In this section, trends over time in the river and loch surveillance networks from 2007-2016 were evaluated with both linear and nonlinear trend models.
2. Linear trend models for the river surveillance network detected upward trends in invertebrates and diatoms and a decline in total reactive phosphorus. For the loch network, there were significant increases in EQRs for invertebrates (CPET), phytoplankton and cyanobacteria, a reduction in total phosphorus and an increase in ammonia. Since we previously found that the networks had relatively low power to detect trends in most parameters, it is not surprising that more significant trends were not found.
3. Trend variation among river water body typologies was present, but the patterns of variation were not consistent across river typologies. This probably reflects both a relatively small number of water bodies representing many of the individual typologies in the river surveillance network and a general finding that there was more variation among water bodies within typologies than between typologies.
4. Many of the monitored parameters appeared to exhibit non-linear trends that fitted the data better than the linear trend models. This was particularly evident for larger monitoring datasets, where the greater amount of data allowed the use of more parameter-rich models, such as those were the trend is modelled with a smoothing spline. These allow testing and visualisation of non-linear trends, but do not yield a direct estimate of the form or magnitude of the trend. As such they complement the use of the linear models for analysis and interpretation of monitoring trends.

Introduction and aims

The identification of trends is one of the key goals of environmental monitoring programmes (Lindenmayer and Likens 2010). It is especially important for so-called 'mandated' monitoring where the focus is on determining whether the state of the environment is changing over time, rather than trying to attribute that change to a particular cause. SEPA's surveillance monitoring networks for rivers and lochs can be considered to fall into this category, with operational and investigative monitoring designed to identify and manage drivers of local change.

As demonstrated in the previous section, trend detection in noisy monitoring datasets requires large volumes of data, ideally collected across many sites and over a long time period. Therefore, after around ten years of operation in their current form, there is an opportunity to evaluate long-term trends across both surveillance networks for a range of ecological and chemical parameters. However, ability to detect trends depends not only on the sampling regime and volume of data, but also on whether the chosen trend model is appropriate for the type of trend present in the data. Specifically, linear trend models are commonly applied but could perform poorly at detecting more complex nonlinear trends. Therefore, it is beneficial to complement the linear models with application of more flexible nonlinear trend models.

In Section 3 we used linear trend models to perform power analysis aimed at evaluating the ability to detect changes in current and modified surveillance networks for rivers and lochs. This section focuses on the actual trends revealed in that analysis. The specific aims were:

3. To summarise the linear trends in the river and loch surveillance networks from 2007-2016, including variation in trends among water body typologies, where possible.
4. To evaluate whether linear trends are an appropriate model for observed changes in the surveillance network data, or whether nonlinear models are required.

Methods

Overview

Linear and nonlinear trend models were fitted to data from the river and loch surveillance monitoring networks from the ten year period 2007-2016. The data used in the modelling is fully described in Section 3. For the river network, trends were estimated for ammonia, dissolved oxygen, total reactive phosphorus, total phosphorus, invertebrates (ASPT EQR), macrophytes (RMNI EQR) and diatoms (TDI4 EQR). For the loch network, trends were estimated in ammonia, dissolved oxygen, total reactive phosphorus, total phosphorus, invertebrates (CPET EQR and raw ASPT abundance), macrophytes (LMNI EQR), diatoms (LTDI2 EQR), phytoplankton (PTI EQR) and cyanobacteria (PLUTO EQR).

Linear trend models

The trend models reported here are those fitted for the trend power analysis in Section 3. For full details of the methods, see Section 3. In overview, linear mixed effects (LME) models were fitted in R package 'lme4' (Bates et al. 2015) to surveillance monitoring data for rivers and lochs from 2007-2016 as provided by SEPA (see Table 3.1). Fixed effects were specified for the covariates year (the trend of interest) and harmonics of the day of year (to model seasonality), except that seasonality could not be modelled for

macrophytes which are only sampled once per year. Random intercept effects were specified for year (to model annual divergence from the overall trend), and water bodies nested within river or loch Water Framework Directive (WFD) typology (see Table 3.2 for definitions). For models fitted to the river surveillance network data, random slope effects for the year trend were also specified for water bodies nested within typology, except in the analysis of macrophytes where there was insufficient data to do this. To summarise these models here, the magnitude and statistical significance of the annual trends are reported, as well as the estimated variation in trends among river typologies.

Nonlinear trend models

The above trend models were designed to detect a linear trend through the specification of a fixed effect of year, centred on its midpoint. To examine whether there may have been pronounced deviation from a linear trend, two approaches were used to visualise and characterise nonlinear trends.

First, LMEs were fitted with year as a fixed factor rather than linear covariate. The LME specification included:

1. Fixed effect of year as a factor, rather than as a covariate and random effect.
2. Fixed terms for day of year, capturing seasonality and modelled using harmonics as described above.
3. Random intercepts for water body nested within typology, capturing site effects.

Note that the random year trends in the previous models are not specified since no trend is actually estimated. To visualise departures from a linear trend, patterns in the fitted year coefficients were examined.

Second, we fitted generalised additive mixed models (GAMMs) to the data, using flexible smoothing splines for the responses to year and day of year. The GAMM models were fitted in the R package 'gamm4' (Wood and Scheipl 2017) which estimates the appropriate flexibility in the spline responses supported by the data. The spline degrees of freedom is therefore a measure of trend complexity and deviation from a linear trend (one degree of freedom). The GAMM specification included:

1. Smoothing spline for year, centred on its midpoint and restricted to a maximum of 6 degrees of freedom (since there were only a maximum of 10 years of data).
2. Smoothing spline for day of year, centred on the year midpoint, to model seasonality.
3. Random intercepts for water body nested within typology, capturing site effects.

Results

Linear trend models

The annual trends estimated by LMEs for the monitoring networks from 2007-2016 are summarised in Table 4.1 (see also Table 3.4 for full model details). For the river surveillance network, the LMEs identified statistically significant increases in the EQRs for invertebrates and diatoms, and a significant decrease in total reactive phosphorus. The increase in the macrophyte EQR and decrease in total phosphorus concentrations almost achieved statistical significance and these are consistent with the significant trends in the other ecological parameters and total reactive phosphorus. In the loch surveillance network, there were significant increases in the phytoplankton, invertebrate (CPET) and cyanobacteria EQRs (Table 4.1). These were accompanied by a significant increase in ammonia concentrations and a significant decrease in total phosphorus concentrations.

Variation in trends among river typologies was modelled as a random slope effect in the LMEs for the river surveillance network. The random effects are plotted as standardised trends in Figure 4.1. This gives little indication that the analysed parameters had similar trends across typologies, even for related parameters such as total phosphorus and total reactive phosphorus. An explanation for this may lie in the relatively small numbers of water bodies representing each typology. Although generally adequate for LME modelling, where a rule of thumb is to have more than five replicates of each random effect level (Bolker et al. 2009), the relatively low site-level replication variation among typologies probably precludes meaningful comparison of trends among typologies.

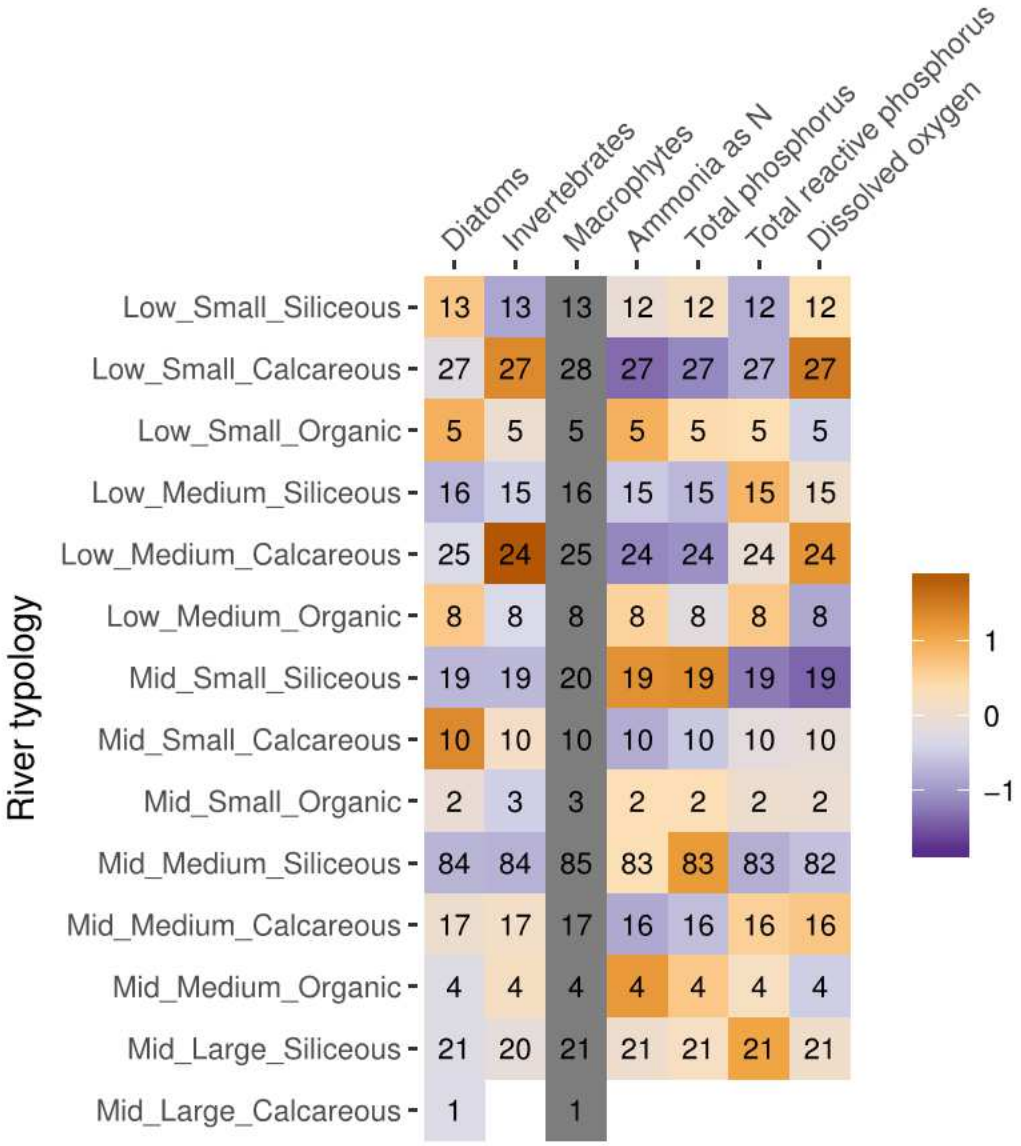
Nevertheless, there is a suggestion that catchment geology type may have influenced ecological trends. In particular, the upward trend in invertebrates appeared strongest in typologies with calcareous geology (Figure 4.1). Conversely, the upward trends in both diatoms and invertebrates appeared weaker than average in siliceous typologies.

Table 4.1 Summary of long-term trends in the monitoring parameters in the river and loch surveillance networks from 2006-2017. Annual trends are the fitted LME coefficients for year, showing annual change in the monitoring parameters on their transformed scales and their standard errors (see Table 3.4 for full details of the models). Statistically significant trends are displayed in bold. Trends have been converted into equivalent expected changes over the ten year period of the data, with 95% confidence intervals calculated from the standard error of the trends.

Water body type	Monitoring type	Monitored parameter	Annual trend on transformed scale (SE)	*P*	Equivalent % change in 10 years (95% CI)
Rivers	Chemistry	Ammonia as nitrogen (mg/L)	$6.87x10^{-3}$ ($4.67x10^{-3}$)	0.160	+15.3 (-4.6 to +39.4)
		Dissolved oxygen (mg/L)	$2.83x10^{-4}$ ($9.02x10^{-4}$)	0.761	+0.6 (-3.0 to 4.3)
		Total reactive phosphorus (mg/L)	**$-1.42x10^{-3}$ ($5.71x10^{-4}$)**	**0.027**	**-34.6 (-54.3 to -6.4)**
		Total phosphorus (mg/L)	$-5.90x10^{-3}$ ($3.29x10^{-3}$)	0.099	-11.5 (-22.6 to 1.1)
	Ecology	**Invertebrate EQR (Average Score Per Taxon, ASPT abundance)**	**$1.06x10^{-3}$ ($3.22x10^{-4}$)**	**0.010**	**+2.2 (+0.9 to +3.6)**
		Macrophyte EQR (River macrophyte nutrient index, RMNI)	0.0565 (0.021)	0.074	+5.0 (+1.3 to +8.9)
		Diatom EQR (River Trophic Diatom Index, TDI4)	**0.0383 (0.0132)**	**0.022**	**+7.0 (+2.2 to +12.0)**
Lochs	Chemistry	**Ammonia as nitrogen (mg/L)**	**0.0174 ($7.06x10^{-3}$)**	**0.040**	**+43.3 (+7.6 to +90.9)**
		Dissolved oxygen (mg/L)	$4.82x10^{-4}$ ($8.89x10^{-4}$)	0.603	+1.0 (-2.6 to +4.7)
		Total reactive phosphorus (mg/L)	$-1.42x10^{-3}$ ($4.43x10^{-3}$)	0.757	-2.9 (-18.9 to 16.2)
		Total phosphorus (mg/L)	**$-3.71x10^{-3}$ ($1.55x10^{-3}$)**	**0.045**	**-7.4 (-13.1 to -1.4)**
	Ecology	Invertebrates (ASPT abundance)	$-4.86x10^{-4}$ (0.0130)	0.972	-0.1 (-6.0 to +6.1)
		Invertebrates (CPET EQR)	**0.0397 (0.0176)**	**0.025**	**+4.9 (+0.6 to +9.3)**
		Macrophyte EQR (Lake Macrophyte Nutrient Index, LMNI)	0.0411 (0.0247)	0.170	+14.4 (-2.3 to +34.3)
		Diatom EQR (Lake Trophic Diatom Index, LTDI2)	$3.52x10^{-3}$ (0.0321)	0.913	+0.1 (-1.3 to +1.5)
		Phytoplankton EQR (Phytoplankton trophic index, PTI)*	**$5.24x10^{-3}$ ($1.73x10^{-3}$)**	**0.003**	**+11.5 (+3.9 to +19.6)**
		Cyanobacteria EQR (PLUTO EQR)*	**$4.09x10^{-3}$ ($1.58x10^{-3}$)**	**0.041**	**+12.4 (+2.9 to +22.9)**

* Data missing for 2007 and 2008

Figure 4.1 Visualisation of standardised variation in trends among typologies in the river surveillance network. Typology labels reflect altitude (low or mid), catchment area (small, medium or large) and geology type (siliceous, calcareous or organic) (see Table 3.2). Shading indicates the random year slopes for each monitored parameter, standardised by dividing by the overall standard deviation for the typology effect, multiplied by the sign of the trend. Large positive values indicate that trends were more extreme than average (more positive or more negative), while large negative values indicate trends that were closer to zero or even reversing the main trend direction. The number show the number of water bodies monitored in each typology. Trend variation could not be estimated for macrophytes, which are shaded in grey.



Nonlinear trend models

When LMEs were fitted with year as a fixed factor, rather than a linear covariate and random effect, ANOVA's on the year effect found statistically significant year-to-year variability in most parameters (Table 4.2). The exceptions to this were loch invertebrates (ASPT), loch diatoms and loch macrophytes. Plots of the estimated year coefficients (Figures 4.2 and 4.3) show that some of the variation over years could be well approximated with linear trends. For example, trends in river invertebrates and loch phytoplankton and total phosphorus. However, many of the parameters exhibited strong fluctuations from year-to year or apparent step changes in their trends (e.g. river macrophytes, loch ammonia), suggesting more complex trend models may be required.

These impressions were borne out by the GAMM models, where the optimal splines for the year effect tended to degrees of freedom greater than one. This indicates that a nonlinear trend tended to fit better than a linear one (Table 4.2). The main exceptions to this pattern were ecological parameters in the loch network, which mostly supported a linear trend model (d.f. = 1). The spline curves are not plotted here, as they follow the form of Figures 4.2 and 4.3, with some smoothing of the year-to-year variation.

Table 4.2. Summary of the year effects in two types of nonlinear trend model for the river and loch surveillance networks from 2007-2016. First, year was entered as a fixed factor in LME models and the year effect tested by ANOVA with the Satterthwaite's approximation to the denominator degrees of freedom (d.f., given as numerator, denominator). Second, GAMM models with smoothing splines for year were fitted. The estimated d.f.s indicate spline complexity and, when greater than 1, deviation from a linear trend.

Water body type	**Monitoring type**	**Monitored parameter**	**Year factor effect in LME**			**Year effect in GAMM**	
			ANOVA *F*	**d.f.**	***P***	**Spline complexity (d.f.)**	***P***
Rivers	Chemistry	Ammonia as nitrogen	23.35	9, 23271	<0.001	4.765	<0.001
		Dissolved oxygen	97.1	9, 22609	<0.001	4.896	<0.001
		Total reactive phosphorus	230.20	9, 23231	<0.001	4.957	<0.001
		Total phosphorus	37.56	9, 22447	<0.001	4.896	<0.001
	Ecology	Invertebrate EQR	10.1859	9, 2976.9	<0.001	2.750	<0.001
		Macrophyte EQR	2.6121	9, 383.77	0.006	3.041	0.001
		Diatom EQR	3.1228	9, 3420.6	<0.001	2.293	<0.001
Lochs	Chemistry	Ammonia as nitrogen	28.758	9, 4266.4	<0.001	4.764	<0.001
		Dissolved oxygen	20.56	9, 4219.0	<0.001	3.589	<0.001
		Total reactive phosphorus	11.133	9, 4131.1	<0.001	4.514	<0.001
		Total phosphorus	3.179	9, 4094.3	<0.001	1.961	0.001
	Ecology	Invertebrates (ASPT abundance)	1.28309	9, 217.54	0.247	1.669	0.551
		Invertebrates (CPET EQR)	0.85752	9, 236.14	0.5640	1	0.039
		Macrophyte EQR	1.310	9, 54.327	0.2536	1	0.087
		Diatom EQR	0.6924	9, 445.06	0.71597	1	0.838
		Phytoplankton EQR	1.4480	7, 796.29	0.18277	1	0.002
		Cyanobacteria EQR	9.6001	7, 694.24	<0.001	4.100	<0.001

Figure 4.2 Estimated annual variation in the monitored variables in the river surveillance network on their transformed scales (LME coefficients ± 1 standard error), as estimated by LME models with year as a fixed factor effect. The coefficients represent deviation in the mean transformed value from that in the first year of the data.

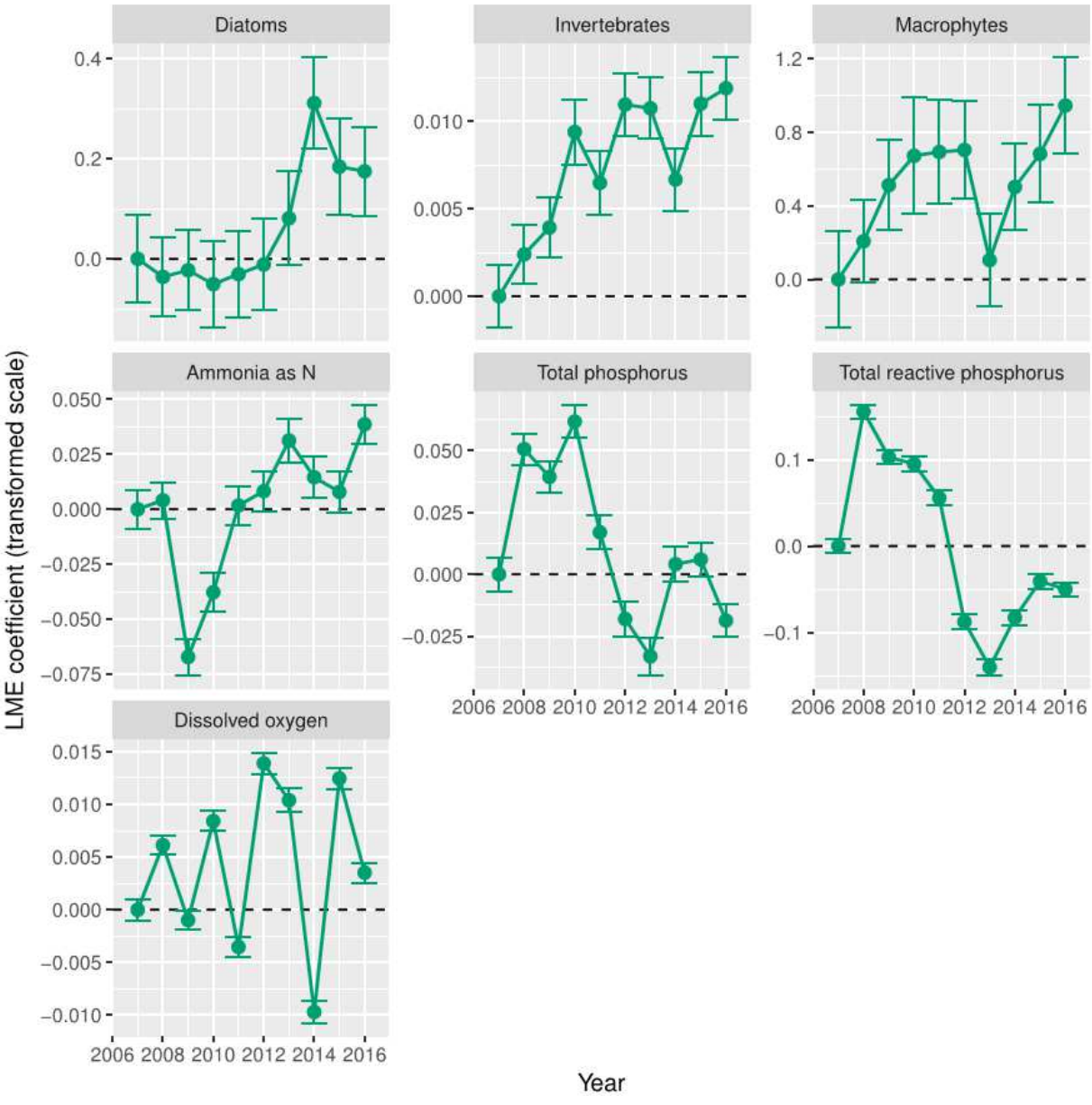
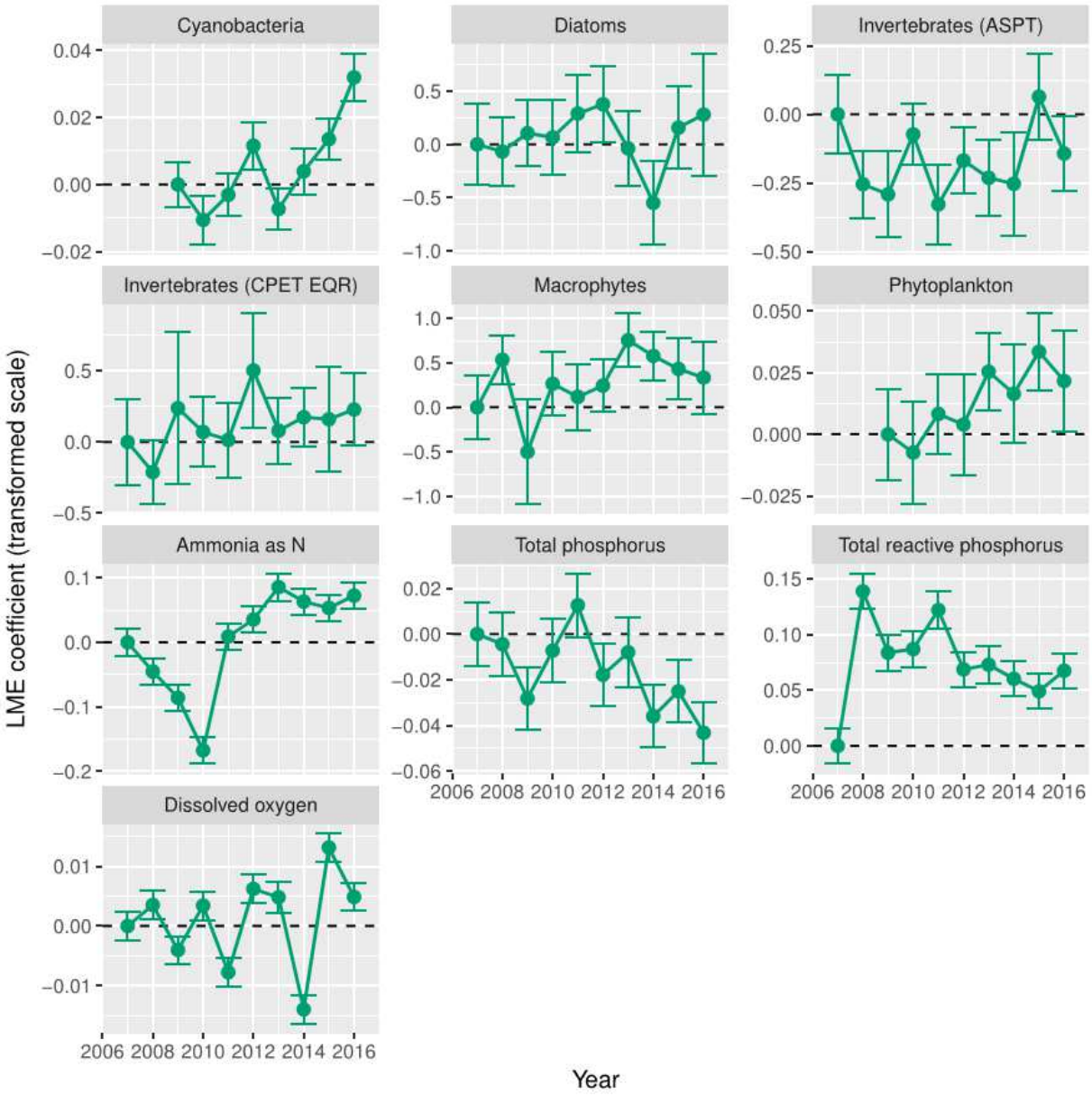


Figure 4.3 Estimated annual variation in the monitored variables in the loch surveillance network on their transformed scales (LME coefficients ± 1 standard error), as estimated by LME models with year as a fixed factor effect. The coefficients represent deviation in the mean transformed value from that in the first year of the data.



Discussion and conclusions

Linear trend models fitted to SEPA's surveillance network detected a number of statistically significant trends over time in a recent ten-year period. In the river network there were upward trends in river invertebrates and diatoms, and a decline in total reactive phosphorus. In the loch network, the models found significant increases in EQRs for invertebrates (CPET), phytoplankton and cyanobacteria, accompanied by a reduction in total phosphorus but an increase in ammonia. The lack of significant trends in other parameters should be viewed in the context of the power analysis in Section 3, which found that the networks had relatively low power to detect reasonable changes of ±5% in most parameters. Thus it is not surprising that all trends did not reach statistical significance. Nevertheless, the trends evident in the data do suggest an improving state of Scotland's freshwater environment, with the exception of the increase in loch ammonia concentrations.

From the linear trend models of the river surveillance network, it was possible to examine between-typology variation in trends, as estimated in the random effect structure. This did not reveal any clear mediation of trends by typology across monitoring parameters, although there was some suggestion that ecological trends may have been influenced by geology type. This lack of a clear consistent typology effect was probably caused by the relatively low number of water bodies represented in the river network for many of the typologies, especially since trend variation among water bodies within a typology was much larger than the trend variation among typologies. For future analyses, it would be desirable to see whether alternative water body groupings could provide a stronger structuring of trend variation. For example, if pressure gradients drive the trends, but these are not tightly correlated to typologies, then using groupings based on pressures (pressure typologies) may have been a better strategy. If so, this could also help in determining the ability of surveillance monitoring to attribute ecological and chemical trends to particular pressures, although that goal was beyond the scope of this study.

The analysis also supported the use of nonlinear trend models for analysis of long-term changes in the surveillance networks. Both the LMEs with year as a fixed factor and the GAMMs where the trend was modelled with a smoothing spline generally indicated significant changes over time following more complex trends than were represented by the linear models. These nonlinear trend models are more parameter-rich than the linear trend models, making them especially suitable for complex trends in larger monitoring data sets. This was evidenced by their relatively poor performance for the ecological data from the lochs network, which had the lowest sample sizes. As such, they would be especially suitable for even longer time series of monitoring data, where departures from linear trends are likely to be even more apparent. However, unlike the linear trend models, they do not provide a direct estimate of the type of trend

and so require visualisation for interpretation. This, along with the computational intensiveness of GAMMs, will likely preclude their use in power analyses aimed at evaluating current monitoring or designing new monitoring strategies. Therefore, we suggest linear and non-linear models should both be used for future trend analyses.

The strongest estimated trends were for monitoring variable that required modelling on a logarithmic scale and tended to be those that were estimated the least precisely (see Table 4.1). For example, the estimated 43% increase in loch ammonia had a 95% confidence interval of being an 8 to 91% increase, while the 35 % decrease in river total reactive phosphorus had a 95% confidence interval of being a 6% to 54% decrease. Part of this uncertainty was because these trends were modelled on a $\log_{10}$-scale, meaning that uncertainty in the fitted trend coefficient propagates exponentially into the estimates of percent changes. The large uncertainty also resulted from these variables being among those for which the non-linear trend models identified the strongest departures from linear trends. For example, loch ammonia decreased sharply from 2007 to 2010, then increased until 2014 before stabilising (Figure 4.3), a pattern also seen in the rivers (Figure 4.2). River total reactive phosphorus increased in 2008 from 2007 levels, but then fell until 2013, after which increases were apparent. Similar patterns were seen in river total phosphorus, though trends in lochs appeared qualitatively different.

A further caveat to all conclusions about the chemistry monitoring data is that these data may have been substantially influenced by changes in laboratory analytical methods over time and between regions. These may have caused step changes in the monitoring data from individual water bodies, aggregated up to regional levels, which would not necessarily have been well captured in the statistical modelling. Similarly, step changes in the ecological monitoring data from local water bodies could also occur through major management interventions, such as the removal of an impoundment or removal of a point source of pollution. Although the statistical analyses here used annual random effects that can represent coherent variation between years such as step changes across the whole monitoring network, these are likely to be insufficient to capture local or regional step changes. To improve the statistical analysis of trends in the presence of such step changes will require information on factors such as changes in laboratory analytical methods or management interventions, which can be entered into the models as fixed factor effects.

Where linear or nonlinear trends are evident in the current monitoring networks, it is important to evaluate these in the light of the unrepresentativeness of the current networks. In Section 1 we demonstrated that the rivers network was over-sampling major downstream river water bodies. As such, trends found in the existing monitoring data do not directly extrapolate to trends across Scotland. There are two ways in which estimated trends could be made more representative of Scotland as a whole. First, as demonstrated in

Section 2, selective removal or addition of water bodies to the networks can be used to improve their representativeness. However, Section 2 also highlighted a conflict between improving network representativeness and retaining existing water bodies to maintain the legacy of long-term monitoring. An alternative approach would be to develop statistical approaches to weight the trend estimation by representativeness of the water bodies, so that a more nationally representative trend estimate is made. For example, water bodies from over-represented typologies would be downweighted, while those from under-represented typologies would be upweighted. It seems likely that in practice a combination of both approaches would yield the best results, especially if there is a need to reduce the size of the surveillance networks due to resource constraints.

5. Review of innovative monitoring methods

Summary

1. This chapter reviews innovative monitoring methods and compares them to methods currently used by SEPA.
2. Methods were scored on the following criteria; efficiency, cost, data quality, stage of development, suitability for Scotland and compatibility with existing data.
3. Detailed assessments are given for all Biological Quality Elements (BQEs) and Supporting Elements (SEs) sensu the Water Framework Directive.
4. SEPA's current monitoring approaches could not be bettered by available alternatives.
5. There is potential to improve data quality by sampling all parameters at single sites and the use of single summer invertebrate samples to replace 2 season sampling. However there are trade-offs in terms of compatibility with existing data and other on-going monitoring.
6. eDNA has the potential to revolutionise the field of biological monitoring but few methods are fit for purpose yet. However there is an acceleration in applied research and the field is expected to develop rapidly.
7. Other recommendations are made for specific BQEs and SE and should be viewed individually.

Introduction

Purpose

The purpose of this report is to review freshwater monitoring methods that are suitable for deployment in Scotland. The work was carried out in conjunction with a statistical review of the Scottish Environment Protection Agency (SEPA)'s Water Framework Directive surveillance network and the primary application of these methods is to that network.

Background

This is a general review of the efficiency and effectiveness of SEPA's surveillance network. It follows a commissioned review of innovative approaches to monitoring the aquatic environment that identified a number of novel methods that had potential application in Scotland (Helwig et al. 2015). This report complements that review as it focuses on the practical suitability of the available methods and compares them directly with methods currently used by SEPA.

Approach

Data were collected from the literature and through direct contact with colleagues in the UK, Europe and elsewhere. The Centre for Ecology & Hydrology (CEH) made extensive use of its active involvement in EU Framework projects that addressed the implementation and application of assessment methods for the Water Framework Directive (WFD) e.g. STAR, REBECCA, REFORM and WISER. We also incorporate relevant research from internally funded research on eDNA (for macrophytes) and research council funded work on benthic invertebrates eDNA (LOFRESH). All relevant freshwater biological quality and supporting elements were considered.

Report Structure

A summary of findings is provided below, detailing the recommendations for the improvement of current methods. It is based primarily on detailed analyses of individual methods that are presented in Appendix 5.1.

Appendix 5.1 is divided into river and loch sections and structured by quality element. Methods are scored on a 1 (bad) – 5 (good) scale and the results tabulated. An overall score for each method is given; this is not by default an average of the individual criterion scores. There is a natural hierarchy among the criterion, which the final score reflects, for example, a method may be highly commendable but if it is unsuitable for application in Scotland then its final score is down weighted. A brief description of each method is also given with a summary of its suitability and state of development.

Methods, which are already in use or close to practical deployment, were considered most relevant. To address the issue of readiness we considered three categories for all monitoring elements:

1. refinements to existing monitoring methods;
2. alternative intercalibrated methods; and
3. novel and emerging methodologies.

Methods were assessed and scored on the following criteria: efficiency, cost, data quality, stage of development, suitability for Scotland and compatibility with existing data.

Refining existing SEPA monitoring methods: There is the potential to refine existing SEPA monitoring methods. Often savings can be made in the logistics of sample collection. We consider some of the more novel solutions to logistics that have been used in the past, for example, sampling lakes from helicopters. Members of the team have also been directly involved in developing the sampling methodologies for the

assessment of the Glastir programme in Wales. This is a large multisite study based on a rolling programme. In that study an effective means of improving data quality and reducing costs was achieved by adjusting standard BQE sampling methodologies and habitat assessments while retaining their intercalibrated status.

Alternative intercalibrated methods: There are over 600 monitoring techniques identified from across Europe (e.g. Poikane et al. 2015). We focus on monitoring techniques that have been successfully intercalibrated for the WFD. These methods are already accepted by the European Commission and would, therefore, be the simplest methods to implement. CEH staff in the project team (O'Hare, Carvalho) have been involved in intercalibration exercises and in the independent expert review of Member State methods, so have first-hand knowledge of the efficacy and practicality of the most appropriate methods. As many of these methods have been put into practice since the inception of SEPA's modern monitoring network in 2006 they are worth considering in the review. For the most promising methods we interviewed our colleagues from European monitoring agencies regarding the practical application of the methods.

Novel and emerging methodologies: SEPA/Scottish Government have previously identified a number of novel methodologies that have undergone initial assessment. We compare these methods with intercalibrated methods. We have used many of these methods in our research and we summarise our experience and their suitability for application in a large-scale field campaign: remote sensing lake water quality, high frequency water chemistry samplers, novel biological monitoring techniques, eDNA, and hydro-acoustics of macrophyte and fish populations. We consulted with SEPA and interviewed colleagues from across CEH and other institutes who have applied these methods. For example, our team include staff working on the NERC GloboLakes Project who have 10 years of satellite data from the larger Scottish lochs to review the suitability of Earth Observation for WFD monitoring purposes. They are also familiar with the pros and cons of new satellite products soon to be made available from ESA's Sentinel satellites.

The surveillance network has been in place in Scotland for ten years and has evolved over that time with some significant changes to sampling strategy. We provide a summary of the evolution of the network to reflect the lessons learned from that process presented in Appendix 5.2. In addition to assessing individual methods, we also considered how sampling logistics and strategy has influenced the development of surveillance monitoring in other EU countries. Two case studies of efficient monitoring are provided, one from the Republic of Ireland and another from Finland, also in Appendix 5.2.

Results of the project were presented at a UKEOF meeting and feedback received from participants. The outcomes from that workshop are incorporated into the report in Appendix 5.3.

Findings

Recommendations for improving methods are given below and are reported by habitat, loch or river. However, the method that has the best overall potential is applicable to BQEs in both rivers and lochs and is considered first. It is eDNA /metabarcoding analysis.

eDNA application to monitoring in Scotland

The best methods overall were eDNA analysis, where a sample of water is analysed for DNA from fish, invertebrates or algae (benthic diatoms or phytoplankton) and metabarcoding, where a sample of invertebrates, diatoms or macrophytes is identified using barcoding techniques rather than traditional microscopic approaches. These approaches have been of interest for over two decades but their practical development has accelerated in recent years. Existing research requirements include the compilation of comprehensive DNA libraries for target species and the reduction of false positives. Robust field sampling strategies and the development of new metrics are areas of active research. Our review indicates that research developments are positive. Field-testing for fish and diatoms is most advanced and is close to practical deployment. Work is somewhat less advanced for invertebrates but results are encouraging to date and the relevant supporting research is underway , particularly in the EU DNAqua-net COST action – where invertebrate methods for WFD monitoring are being developed. Fish eDNA and diatom metabarcoding methods are more or less ready for implementation, while invertebrate methods require some more development and are closer to 2+ years before deployment. For invertebrates metabarcoding is closer to deployment than eDNA due to the complicating factors of eDNA transport and persistence. Significant investment in these new methods must be undertaken for comparison with existing data and, possibly may require the development of new metrics. They are likely to require intercalibration with existing methods from other EU countries.

The use of eDNA from macrophytes is not considered effective but the analysis of material collected from the plants to confirm identification has potential but requires species to be bar coded. This work is underway. This approach has potential in rivers where the key characteristics for identifying species, flowers or fruit occur at different points in the growing season, for example June for water crowfoot species and July-August for most others. This necessitates multiple site visits if an accurate species level identification list is to be attained. This rarely happens. By taking samples for DNA analysis, a single site visit would be possible. For lochs, the main application would be to confirm the identification of specimens from the more challenging groups; Potamogetons and Charophytes. The number of taxonomic experts who can confirm identifications of these groups is dwindling and DNA analysis could provide a long-term, robust alternative.

Our recommendation would be to build capacity in eDNA analysis in preparation for deployment in the near to medium term. Commercial laboratories can provide sample analysis very cost effectively, but these services would need to be complemented by the skills of biometricians who can interpret and quality check the resulting data. Biometricians who have an in-depth understanding of freshwater ecology are rare and the staff with the right mix of skills would need to be developed.

Rivers – general monitoring review

Sampling logistics

Currently, only diatoms and invertebrates are routinely collected at the same sites. In many other EU countries BQEs and supporting hydromorphology elements (but not chemistry) are collected at the same location at the same time. There are significant time savings to be made with this approach. There are also advantages in terms of data quality. Many of the existing BQE methods record some habitat characteristics as part of the survey process, acknowledging that local, as well as regional landscape variables influence the signal from the BQE sample. Sampling all parameters at a single location makes this process more robust and removes ambiguity caused by convoluted processes of matching sites in space and time during analytical processes. Arguably this disconnect in sampling locations has previously caused significant, costly, problems in tool development. In Countryside Survey BQE and RHS sampling are conducted in a spatially nested manner. This approach was adopted and refined in the GMEP project in Wales, where simple changes increased the usefulness of the data.

Our recommendation is that if SEPA reduces the size of its surveillance network it focuses on sites where as many parameters as possible are collected at a single location and adopts the general GMEP strategy of spatially nesting BQE and RHS samples.

Biological Quality Elements (BQEs) sampling recommendations

eDNA analysis is considered above, in addition we have identified that SEPA's invertebrate sampling protocol is relatively time-consuming requiring spring and autumn sampling compared to that used in countries such as Finland and Ireland where single season samples are taken. The Irish EPA sampling protocol is based on a summer sample, which can be taken at the same time as macrophyte samples, an attractive option for some systems in Scotland. As the Finnish and Irish methods are intercalibrated, by inference, their ability to detect degradation and improvement are sufficient for WFD reporting purposes. However there would still need to be an exercise that showed that when it is applied to Scottish waters it gives comparable results; it would be necessary to demonstrate that the EQR results – specifically the High / Good & Good / Moderate boundaries are comparable. SEPA's method was refined over time and there are known statistical advantages and robustness of signal associated with the two-season method (Wright

2000). The question is, therefore, whether the benefit of sampling two seasons is sufficiently cost effective to warrant its continuance. The decision to go for a single or two-season sample is relevant to eDNA sampling as well as traditional sampling techniques.

Our recommendation is for SEPA to consider a more rapid invertebrate sampling technique, as a cost saving solution to surveillance monitoring. The decision to proceed should be based on an analysis of the trade-off in compatibility with data collected using the existing kick sampling method (Historic data, operational and investigative monitoring sites) and the sensitivity of the new method to detect change. In addition, to its application to surveillance monitoring, rapid assessment techniques should also be considered for use in investigative and operational monitoring.

Supporting elements

The CEN standard for hydromorphology is changing and SEPA is likely to be obliged to follow the new standard. The new standard is designed to be flexible, but provide a more comprehensive hierarchical approach to hydromorphological assessment. MIMAS and RHS, in combination, should fit within the new framework of assessment with some modification. This approach has been applied to the River Tweed - see Appendix 5.1 for details. There have been a number of exciting developments in process understanding and the advent of sophisticated survey and visualisation techniques. These technical advances are of potential application to investigative monitoring and improving process understanding but have limited application to surveillance monitoring.

ECOSTAT are concerned at the lack of detectable sensitivity of BQEs to hydromorphological impact. Two simple improvements to hydromorphological monitoring would potentially prove beneficial. Downstream of impoundments, the reduction in wetted width from pre-dam conditions should be routinely calculated and a scaled reduction in invertebrate abundance noted in BQE assessments. Data on routine channel maintenance should be recorded for all surveillance sites, including the date, type and extent of the activity.

The development of analytical chemistry has gone in three directions, automatic *in situ* samplers, low cost kits and high frequency sampling. There are clear benefits of using high frequency samplers for nutrient load apportionment and identification of key release events. As such, they are more attractive for operational monitoring of eutrophication and/or water quantity impact than surveillance monitoring. The cost and reliability of *in situ* samplers remains in question, however, should costs drop and routine maintenance reduce they would form an attractive alternative to current methods.

Our recommendation is that SEPA actively engage with the development of the new CEN standard for hydromorphology and prepare for its deployment. They should consider minor adjustments to the

methodologies to improve diagnostic capability. Water chemistry should remain unchanged but the frequency of data collection should be reduced at surveillance sites in light of the limited influence current sampling rates have on statistical power. Due consideration should be given to other applications of high frequency sampling.

Lochs – general monitoring review

The part of SEPA's current surveillance network that has most potential for beneficial change is the room to increase the number of loch sites thereby improving the networks representativeness and statistical power. Of the emerging methods, the use of remotely sensed satellite data provides the best means for rapid and significant expansion of the network. However, data could only be provided for water level fluctuations and phytoplankton abundance. Complementing this with a single visit blitz type campaign to sample and record nutrients, and in the future, fish and phytoplankton diversity could be measured using eDNA, would provider a fuller suite of parameters. A successful blitz campaign has been carried out in Northern Ireland using helicopters to visit multiple lakes in a short period of time. Sampling is recommended after the 'spring clear water phase', which can extend to June, July through September is ideal.

The development of hand-held fluorometers is both cost and quality advantages over existing methods chlorophyll methods of measuring phytoplankton abundance. There are some benefits to adopting components of intercalibrated methods for loch invertebrates and macrophytes. For example, the inclusion of emergent vegetation in surveys allows for the creation of metrics that indicate response to water level management. More novel techniques, such as hydroacoustics, are better suited to detailed investigative studies. There are also sampling methods for invertebrates that indicate sensitivity to hydromorphological alteration but these would require significantly more field sampling effort and are not directly compatible with existing methods.

The recommendations for chemical analysis are the same as those for rivers.

Our recommendation is to consider changes that allow the loch surveillance network to be expanded. In addition to the promising development of eDNA techniques, the use of hand held fluorometers for measuring chlorophyll is encouraged.

Hydromorphological assessment for standing waters has lagged behind developments for other supporting elements. SEPA's current methods are as good as available alternatives.

General observations

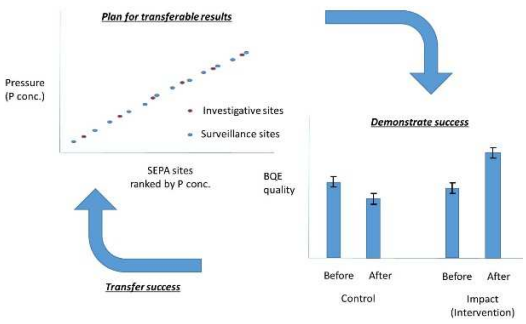
The main aim of this review was to assess alternative methodologies that could provide more cost effective sampling and improved data quality. The underlying principal is to facilitate the demonstration of successful improvement to the Scottish environment with clear and reliable evidence.

Efficiency in the field and laboratory is straightforward to assess in general terms and the capabilities and application of available methods has been assessed. There are some clear trade-offs in terms of data quality and efficiency and here data quality refers to sensitivity of the sample to anthropogenic impact. The emergence of rapid methods may help SEPA in achieving the statistical requirements for sampling, in terms of both representativeness and statistical power.

SEPA has already identified that all BQEs are not all sensitive to all pressures at all sites and have curtailed their sampling accordingly. This is in line with research that indicates, for example, that macrophytes are unlikely to be sensitive to nutrient pollution in upland systems, as the main control on species richness and growth are physical habitat characteristics that select for relatively nutrient insensitive assemblages of bryophytes (O'Hare submitted).

Future challenges for surveillance networks

Using the surveillance network to build on success – Improving the freshwater environment can be relatively straightforward; for example, there is little doubt regarding the efficacy of removing barriers to migration in extending the available habitat for salmonids across most of Scotland's river types. More often, though, moves at positive intervention require a leap of faith with doubts about applying new approaches or the efficacy of established methods across river types, for example, buffer strips. This uncertainty has contributed to limited success in tackling problems such as diffuse pollution and the process of remediation has been so protracted that some issues are considered intractable.

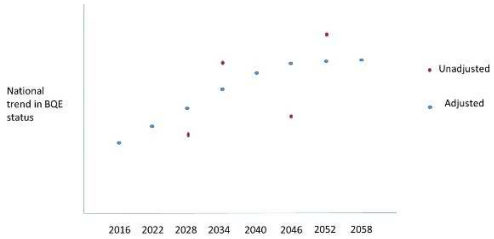


Inevitably, there is always concern about a method's efficacy when applied to new sites. This is especially so in Scotland where the biotic communities, the range of river types and the stressor complexes vary significantly. The Surveillance Network is nationally representative and, as such, it provides a template for transferring success from investigative trial sites. A cost-effective solution is to establish the success of a remediation method at a small number of sites (10

approx.) representative of the range of sites the method could potentially be applied to. The surveillance network provides a source of such sites as well as control sites and 'before' intervention data for comparison. The approach produces clear and reliable evidence that is transferable. The approach can also be applied to understanding how drivers and pressures effect state and help set targets.

The approach can be simple; focusing on a single driver or stressor, or it can be multifactorial and include measures of a wide range of factors such as interaction mechanisms with key actors. The design is also robust enough to allow for the piecemeal inclusion of sites over time as resources become available.

Critically this approach is particularly cost effective; it makes excellent use of the surveillance network data and facilitates the swift transfer of knowledge and success to new sites.



Climate change and surveillance networks – BQEs are known to respond to climatic conditions and in future it is likely climate change will alter BQE status at surveillance sites. For management purposes it will be important to disentangle this effect from that of other pressures. Climatic impacts could mask the success of environmental improvements. This task becomes complicated as climate change impacts are mediated through existing pressures and natural drivers. It is known that UK freshwater systems can show detectable responses to annual climate metrics such as the North Atlantic Oscillation (NAO) suggesting a potential for a regression based diagnostic measure of climatic impact to surveillance BQE quality, in effect a correction factor. To use such an approach would require a representative subset of the surveillance network to produce more intensive, annual data that could demonstrate sensitivity to annual climatic processes. More intensive monitoring of pressures at the sites would also be recommended. In particular an emerging concern are climatic event driven changes (e.g., intense summer floods), here the availability of good hydrological and morphological data would be especially helpful in diagnosing their impact.

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Appendices

Appendix 2.1: Priority water bodies for removal from the river surveillance network

Table A1. Prioritisation for removal of water bodies from the existing river surveillance network, based solely on increasing representativeness (1=highest priority to remove).

Removal priority	Water body code	Water body name
1	10919	River Clyde (Mouse Water to Strathclyde Loch outflow)
2	6498	River Tay (R Isla to R Earn Confluences)
3	5200	River Tweed (Coldstream to tidal limit)
4	5202	River Tweed (Ettrick Water to St Boswells Burn confluences)
5	10042	River Clyde (Potrail Water to Mouse Water)
6	6521	River Isla (R Ericht to R Tay Confluences)
7	10642	River Annan (Threewaterfoot to Annan)
8	23065	River Spey - R. Fiddich to tidal limit
9	4700	River Forth (below R. Teith confluence)
10	6800	River Earn
11	23265	River Don - Dyce to tidal limit
12	10610	River Nith (Dumfries - Sanquhar)
13	10918	River Clyde (Strathclyde Loch outflow to North Calder)
14	6499	River Tay (R Tummel to R Isla Confluences)
15	10420	River Ayr (d/s Greenock Water)
16	10391	River Irvine (Cessnock conf to Tidal Weir)
17	10150	River Leven
18	23155	River Deveron - Turriff to tidal limit
19	5799	River South Esk (White Burn Confluence to Estuary)
20	5219	Teviot Water (Kale Water confluence to River Tweed)
21	23066	River Spey - R. Avon to R. Fiddich
22	5203	River Tweed (Scotsmill to Ettrick Water confluence)
23	10758	River Esk (Black Esk to National Boundary)
24	3000	River Almond (Maitland Bridge to Cramond)
25	23165	River Deveron - Huntly to Turriff

Removal priority	Water body code	Water body name
26	23315	River Dee - Peterculter to tidal limit
27	5700	River North Esk (Confluence with Cruick Water to Estuary)
28	5220	Teviot Water (Northhouse Burn to Kale Water confluences)
29	10000	White Cart Water (Kittoch Water to A726 road bridge)
30	5204	River Tweed (Talla Water confluence to Scotsmill)
31	6838	River Earn (Water of Ruchill to Ruthven Water confluences)
32	10130	River Kelvin (Glazert Water to Tidal Limit)
33	23096	River Spey - R. Nethy to R. Avon
34	3800	River Esk
35	23293	River Don - Alford to Inverurie
36	4000	River Tyne (Birns Water confluence to Estuary)
37	10427	Lugar Water
38	23332	River Dee - Ballater to Banchory
39	10924	River Doon (d/s Muck Water)
40	6832	Allan Water d/s of Dunblane
41	6828	River Tummel (L Faskally to R Tay)
42	4500	River Devon (Gairney Burn confluence to Estuary)
43	23231	River Ythan - Methlick to Ellon
44	6834	River Teith
45	3801	River South Esk (Gore Water to N Esk confluences)
46	10545	River Dee (Loch Ken Outlet to Tongland)
47	10152	Endrick Water (d/s Blane Water)
48	6500	River Tay (R Lyon to R Tummel Confluences)
49	4200	River Carron (Bonny Water confluence to Carron Estuary)
50	20339	River Lochy - sea to Spean confluence
51	10076	Avon Water (Powmillon Burn to River Clyde)
52	20165	River Conon - Cromarty Firth to Orrin confluence
53	6200	River Eden (Confluence with Rossie Drain to Estuary)
54	10757	Water of Girvan (d/s Dailly)
55	10747	Black Cart Water
56	6844	Whiteadder Water (Billie Burn confluence to tidal limit)
57	23171	River Isla - d/s Shiel Burn
58	5287	Ettrick Water (Ramseycleuch to River Tweed)
59	23394	River Ness - Inverness Firth to Loch Ness
60	10092	Douglas Water (Poneil to Clyde)

Removal priority	Water body code	Water body name
61	6523	River Isla (Glencally Burn to Dean Water Confluences)
62	23282	River Urie - Lochter Burn to Don
63	23345	River Dee - Braemar to Ballater
64	10379	River Garnock (Caaf Water to Tidal Limit)
65	6506	River Almond (R East Pow to R Tay Confluences)
66	23232	River Ythan - Fyvie to Methlick
67	3100	River Avon (Logie Water confluence to Estuary)
68	6555	Dean Water (Kerbet Water to R Isla Confluences)
69	20637	River Thurso - Loch More to sea
70	6836	River Garry (Errochty Water Confluence to L Faskally)
71	23188	River Bogie - Culdrain to Huntly
72	10465	River Stinchar (d/s Duisk River)
73	23000	River Findhorn - Dorback Burn to sea
74	23215	River Ugie - North/South confl to tidal limit
75	5705	Luther Water (Dowrie Burn to North Esk Confluences)
76	23142	River Spey - Spey Dam to Loch Insh
77	10394	Annick Water
78	23294	River Don - Strathdon to Alford
79	6300	River Leven (Markinch to Estuary)
80	10280	River Awe
81	10030	River Gryfe (d/s Barochan Burn)
82	5266	Leader Water/Kelphope Burn (Cleekhimin Burn confluence to River Tweed)
83	20209	River Beauly - Beauly Firth to Cannich
84	23032	River Lossie - Waukmill to Arthurs Bridge
85	10060	North Calder Water (Luggie Burn to Clyde)
86	6501	River Tay (Loch Tay to R Lyon Confluence)
87	23283	River Urie - Pitcaple to Lochter Burn
88	10153	Endrick Water (u/s Blane Water)
89	10040	River Clyde (North Calder to Tidal Weir)
90	23084	River Avon / River Livet - lower catchment
91	4704	River Forth (Milton to Auchentroig Burn confluence)
92	5701	River North Esk (Water of Effock to Cruick Water Confluences)
93	10001	White Cart Water (above Kittoch conf)
94	10520	River Cree (u/s Newton Stewart)

Removal priority	Water body code	Water body name
95	5105	Blackadder Water (Howe Burn confluence to Whiteadder Water)
96	20305	River Nairn - Moray Firth to River Farnack confluence
97	20036	Wick River - Loch Watten Burn to tidal limit
98	23004	River Findhorn - Tomatin to Dorback Burn
99	6301	River Leven (Loch Leven to Markinch)
100	3806	River North Esk (Elginhaugh to confluence with South Esk)
101	20167	River Conon - Loch Achonachie to Loch Luichart
102	10666	Kirtle Water (d/s Waterbeck)
103	20116	River Oykel - Dornoch Firth to Loch Craggie
104	23264	Bervie Water - lower catchment
105	10491	Water of Luce (d/s Cross Water of Luce)
106	20002	River Helmsdale - Kinbrace Burn to sea
107	10072	South Calder Water (Tillan Burn to Strathclyde Park)
108	5236	Ale Water (Woll Burn confluence to Teviot Water)
109	6502	River Dochart (Confluence Auchlyne West Burn to Loch Tay)
110	10101	Medwin Water
111	23326	Water of Feugh - lower catchment
112	10761	Water of Ken
113	5311	Lyne Water (Tarth Water confluence to River Tweed)
114	20057	River Brora - sea to Loch Brora
115	10285	River Orchy
116	5101	Whiteadder Water (Dye Water to Billie Burn confluences)
117	23224	South Ugie Water - Stuartfield to Longside
118	20093	River Shin - Dornoch Firth to Loch Shin
119	3700	Water of Leith (Murray Burn confluence to Estuary)
120	20595	River Naver - sea to Loch Naver
121	10422	Water of Coyle (d/s Taiglum Burn)
122	6535	R Ericht
123	10583	Urr Water (d/s Drumhumphrey Burn)
124	5222	Kale Water
125	10205	River Eachaig
126	6839	River Earn (Loch Earn to Water of Ruchill confluence)
127	5280	Gala Water (Armet Water confluence to River Tweed)
128	6639	River Lyon
129	5215	Eden Water (Hume Burn confluence to River Tweed)

Removal priority	Water body code	Water body name
130	10156	Blane Water/Ballagan Burn
131	6585	River Tummel (Dunalastair Water to Loch Tummel)
132	10645	Water of Milk (d/s Corrie Water Confluence)
133	20262	River Enrick - Loch Ness to Loch Meiklie
134	20633	Forss Water - Allt Forsiescye to sea
135	6586	River Tummel (Loch Rannoch to Dunalastair Water)
136	10657	Water of Ae (d/s Goukstane Burn)
137	6000	Dighty Water (lower)
138	10927	Cessnock Water
139	20412	River Shiel - sea to Loch Shiel
140	5290	Yarrow Water
141	10080	Nethan Water
142	20010	River Helmsdale - Loch Badanloch to Kinbrace Burn
143	10084	Mouse Water (Dippool Water to Clyde)
144	20483	River Ewe - sea to Loch Maree
145	20156	Alness River - Cromarty Firth to Strone
146	10469	Duisk River (d/s Muck Water Confluence)
147	5900	Lunan Water (Friockheim to Estuary)
148	5231	Jed Water/Raven Burn (Kaim Burn confluence to Teviot Water)
149	20130	River Carron - sea to Alladale Lodge
150	10399	Kingswell Burn/Fenwick Water/Kilmarnock Water
151	20110	River Cassley - Dornoch Firth to Glenmuick
152	23106	River Dulnain - lower catchment
153	5207	Leet Water (Lambden Burn confluence to River Tweed)
154	6576	River Braan
155	23579	River Borgie d/s Loch Craggie
156	10383	Lugton Water
157	10315	River Etive (d/s Allt a Chaorainn)
158	10132	Allander Water
159	20410	River Aline
160	23346	River Dee - White Bridge to Braemar
161	10073	South Calder Water (Auchter Water to Tillan Burn)
162	20614	Halladale River - d/s Forsinain Burn
163	20254	River Garry - Loch Oich to Loch Garry
164	10589	Kirkgunzeon Lane

Removal priority	Water body code	Water body name
165	10612	River Nith (u/s New Cumnock)
166	20733	River Snizort
167	23221	North Ugie Water - lower catchment
168	10521	River Cree (u/s Minnoch conf)
169	4202	River Carron (Carron Valley Reservoir to Avon Burn Confluence)
170	23233	River Ythan - upper catchment above Fyvie
171	20586	River Hope
172	10546	Black Water of Dee (Pullaugh Burn to Loch Ken)
173	5010	Eye Water (Ale Water Confluence to Eyemouth)
174	23371	River Carron - Loch Carron to Loch Dughaill
175	4402	Black Devon (Birkhill Plantation to Forth Estuary)
176	20451	River Ling - sea to Aonach Beag
177	20317	Muckle Burn - Lethen to Speedie Burn
178	10514	Tarf Water (u/s Bladnoch)
179	20446	River Morar - sea to Loch Morar
180	6806	Ruthven Water
181	10562	Water of Deugh (Carsphairn Lane to Water of Ken)
182	3904	Biel Water
183	20558	River Inver - sea to Loch Assynt
184	10356	River Laggan
185	6102	Motray Water
186	23347	River Gairn - lower catchment
187	20330	River Leven - sea to Blackwater Reservoir
188	20099	River Tirry - Loch Shin to Rhian
189	10669	River Sark
190	10321	River Creran
191	23319	Culter Burn
192	20563	River Laxford - d/s Loch Stack
193	5603	Brothock Water
194	20505	Ullapool River
195	20547	River Broom
196	10539	Water of Fleet/Big Water of Fleet/Mid Burn
197	20542	Gruinard River
198	4008	Birns Water/Humbie Water
199	20047	Dunbeath Water - Burn of Houstry to sea

Removal priority	Water body code	Water body name
200	10300	River Nant (d/s Loch Nant)
201	5950	Elliot Water/Rottenraw Burn
202	23129	River Feshie - main stem d/s R. Eidart
203	6830	Bannock Burn (Sauchie Burn confluence to Steuarthall Farm)
204	10527	Water of Minnoch (River Cree to Water of Trool)
205	20111	River Cassley - Glenmuick to Fionn Loch Beag
206	23043	River Lossie - upper catchment
207	20387	River Scaddle / Cona River
208	10484	Piltanton Burn
209	20610	River Strathy - The Uair to sea
210	6108	Kenly Water (Confluence with Kinaldy Burn to Estuary)
211	20329	River Nevis
212	20053	Berriedale Water
213	5953	Barry Burn
214	20211	River Affric - Loch Beinn a Mheadhoin to Loch Affric
215	10493	Cross Water of Luce
216	6320	North Queich River
217	20572	River Dionard
218	23634	River Canaird (lower section) and Allt a' Mhuilinn
219	5228	Oxnam Water (River Teviot to Newbigging Burn)
220	20532	Allt Mor Gisgil
221	10492	Water of Luce (u/s Cross Water of Luce)
222	6107	Kinness Burn
223	23295	River Don - source to Strathdon
224	20823	Abhainn thernaraigh
225	20407	River Ailort
226	4001	River Tyne (Source to Birns Water confluence)
227	10446	Garpel Burn
228	20054	Langwell Water
229	10497	Milton Burn/Dergoals Burn
230	20433	River Elchaig
231	5952	Monikie Burn
232	20430	River Shiel
233	20642	Halkirk Burn
234	23187	River Deveron - source to Black Water confl.

Removal priority	Water body code	Water body name
235	10393	River Irvine (u/s Glen Burn)
236	4734	Drunkie Burn (Loch Drunkie sluice to Loch Venachar)
237	20095	Merkland River - Loch a Ghriama to Loch Merkland
238	10062	North Calder Water (d/s Hillend Reservoir to Shotts Burn)
239	6533	Leddown Burn/Lunan Burn (to Loch of Craiglush)
240	10145	Glazert Water/Finglen Burn
241	23154	River Spey - source to Garva
242	10536	Moneypool Burn
243	10220	River Shira (d/s Lochan Shira)
244	5954	Buddon Burn
245	20496	Allt Bad an Luig
246	5809	Prosen Water (Burn of Lednathie to South Esk Confluences)
247	20801	Abhainn Bharabhais
248	4206	Glencryan Burn
249	23127	River Feshie - Allt a Mharcaidh
254	4720	Calair Burn
254	5011	Eye Water (Source to Ale Water Confluence)
254	10343	Aros River/Ledmore River (d/s Loch Frisa)
254	10457	Water of Girvan (u/s Loch Bradan)
254	23222	North Ugie Water - upper catchment

Appendix 2.2: Priority water bodies for addition to the river surveillance network

Table A2. Priority water bodies for addition to the river surveillance network, after initial reduction to various sizes (1=highest priority to add). The table shows any water body in the top 25 priorities for any one of the simulations. Dashes indicate that the water body was not added at all, that is priority > 250.

Water body code	Water body name	Add to existing network	Add to 200 water bodies	Add to 150 water bodies	Add to 100 water bodies	Add to 50 water bodies
20214	Bruiach Burn - Loch Bruicheach to source	2	1	1	1	-
20598	Meadie Burn	1	2	2	2	-
20123	Abhainn Poiblidh	3	3	4	-	-
20231	Allt Coire Calavie	4	4	3	5	-
6625	River Ericht (Source to Loch Ericht near Dalwhinnie)	7	6	6	3	1
4722	Drunkie Burn (Reoidhte Lochan to Loch Drunkie)	10	7	5	4	2
6656	Lairig an Lochain	5	5	7	-	-
20520	Allt Loch an Tuirc - Loch Crocach to source	6	9	-	-	-
5310	Glenrath Burn	-	-	-	-	8
20497	Allt an Loch Fhada	8	8	9	-	-
20499	Allt a Chladhain	9	10	-	-	-
20604	Mallart River - u/s Loch a Bhealaich	12	12	8	7	-
6658	Allt Breaclaich (Source to Breaclaich Res)	11	11	-	-	-
20366	Allt Feith Thuill	-	-	-	-	12
6816	River Lednock	17	13	10	9	-
10447	Pollcrayvie Burn	18	14	12	8	-
10133	Craigmaddie Burn	-	-	-	-	13
23101	Cromdale Burn	-	-	-	-	14
20024	Allt an Loin Tharsuin - u/s Loch Truderscaig	15	15	13	-	-
4211	Auchenbowie Burn (Source to Loch Coulter Reservoir)	25	19	11	6	-

Water body code	Water body name	Add to existing network	Add to 200 water bodies	Add to 150 water bodies	Add to 100 water bodies	Add to 50 water bodies
20221	Allt Bealach an Sgoltaidh	14	17	15	-	-
20788	Abhainn Giosla - u/s Loch Gruineabhat	13	18	-	-	-
20394	Savary River	-	-	-	-	16
10248	Allt Mor (u/s Loch Ciaran)	19	16	14	-	-
20371	Allt Coire Pitridh	23	24	-	-	3
10344	Allt a Chlogaid (u/s Loch Frisa)	16	21	-	-	-
10552	Cuttiemore Burn	31	26	16	10	-
20763	Abhainn Smuaisibhig	-	-	-	-	21
20829	Tarbert Burn	21	-	-	-	-
23914	Allt Crunachdain	32	25	17	12	-
10548	Dargall Lane	20	20	25	-	-
20284	Allt Phocaichain	34	30	23	16	6
20493	Allt Gleann Tulacha	22	-	-	-	-
4707	Water of Chon (Source to Loch Chon)	28	23	19	-	-
20364	Allt na Caplaich Mor	27	22	21	-	-
10310	Abhainn Dalach	-	-	-	-	24
10329	Abhainn Tir Chonhuill/Allt an Fhir eoin	37	29	20	15	-
3501	Braid Burn (Source to Upstream Dreghorn Barracks)	56	36	22	11	4
10325	Un-named trib of Loch Glashan	39	27	18	-	-
23325	Burn of Corrichie	-	-	42	21	-
20314	Feith Ghlas	42	33	24	-	-
10163	Carn Allt	48	37	33	23	-
20097	Allt na Fearna Mor	58	42	28	20	-
10592	Culloch Burn (u/s Milton Loch)	72	41	27	13	-
5300	Gatehopeknowe Burn	70	46	29	14	-
23134	Allt Na Baranachd	61	50	31	19	-
20704	Lon Coire na h-Airigh	85	-	-	32	10
10095	Parkhall Burn	76	51	32	18	-
6825	Chesterknowes Burn	-	119	-	33	5
23316	River Dee - Banchory to Peterculter	-	-	-	91	20
23356	Crathie Burn	105	77	46	22	-

Water body code	Water body name	Add to existing network	Add to 200 water bodies	Add to 150 water bodies	Add to 100 water bodies	Add to 50 water bodies
4401	Brothie Burn (Source to Gartmorn Reservoir)	111	73	44	24	-
10100	Glade Burn	-	-	-	116	15
5312	Lyne Water (Source to Tarth Water confluence)	-	-	-	124	17
23067	Burn of Fochabers	98	64	37	17	201
20194	Allt Bail a Mhuilinn	153	-	-	-	18
23227	Crichie Burn	245	148	75	37	7
10245	Ballochroy Burn (u/s Loch Garasdale)	24	-	-	-	185
5289	Howden Burn/Hartwood Burn	218	-	-	-	11
10027	Roebank Burn (d/s Barcraigs Reservoir)	-	-	228	-	19
20034	Thrumster Burn - d/s Thrumster STW to Loch Hempriggs	-	-	206	152	23
10603	River Nith (Dumfries)	-	-	245	136	9
6880	Todrig Burn / Langhope Burn	236	167	95	-	22

Appendix 2.3 R code to analyse and improve the representativeness of a monitoring network

This appendix contains R functions and code to test the representativeness of a monitoring network with respect to multiple gradients and find sites to remove or add to the network in order to improve representativeness.

The functions and R commands can be copied directly into R to implement a simplified workflow of the analyses in Sections 1 and 2, using randomly-generated dummy data. Application to real data can be done by formatting the real data to match the formats of the dummy data.

R Functions

```
network_KS_test = function(gradientData, network, numSimulations=1000){
# Function to run two-sample Kolmogorov-Smirnov test on gradient
    distributions inside and outside of the network
  # gradientData = data.frame of gradient values (rows=sites,
    columns=gradients)
  # network = Boolean vector of length equal to the rows in gradientData
    coding whether each site is in the network (TRUE) or not (FALSE)
  # numSimulations = number of simulations for randomisation test

  testResults = t(sapply(1:ncol(gradientData), function(g){ # for each
    gradient, run the test...

    # calculate the observed KS statistic for difference between gradient
      distributions
    # inside and outside the network...
    x_inNetwork = gradientData[network,g] # gradient values in the network
    x_outNetwork = gradientData[!network,g] # gradient values not in the
      network
    KSD = suppressWarnings(ks.test(x_inNetwork, x_outNetwork)$statistic) # KS
      d statistic

    # generate simulated values of the KS statistic under the null hypothesis
    # of no difference between sites inside and outside the network...
    sim_KSD = sapply(1:numSimulations, function(dummy){
      randNumber = runif(nrow(gradientData))
      sim_inNetwork = randNumber < quantile(randNumber, mean(network)) #
      simulated network (random sample of sites)
      x_inNetwork = gradientData[sim_inNetwork,g] # gradient values in the
      simulated network
      x_outNetwork = gradientData[!sim_inNetwork,g] # gradient values not in
      the simulated network
      suppressWarnings(ks.test(x_inNetwork, x_outNetwork)$statistic) # KS d
      statistic

    })

    # calculate the P value...
    P = (1+sum(sim_KSD > KSD)) / (1+length(sim_KSD))
```

```
    # return the result...
    return(c(D=as.numeric(KSD), P=P))
  }))

  # return the results...
  return(data.frame(gradient=names(gradientData), testResults))
}


network_Cramer_test = function(gradientData, network, numSimulations=1000){
# Function to run two-sample Cramer's test on gradient distributions
# inside and outside of the network
  # gradientData = data.frame of gradient values (rows=sites,
      columns=gradients)
  # network = Boolean vector of length equal to the rows in gradientData
      coding whether each site is in the network (TRUE) or not (FALSE)
  # numSimulations = number of simulations for randomisation test

  # transform gradient values to Gaussian distributions with mean=0 and
      standard deviation=1...
  require(GenABEL)
  gradientDataT = apply(gradientData, 2, rntransform)

  # compute pairwise Euclidean distance matrix on transformed gradients...
  distanceMatrix = as.matrix(dist(gradientDataT))

  # function to calculate Cramer's T statistic
  cramersT = function(rep=NULL, DM, gp, permute=FALSE){
    if(permute) gp = sample(gp)
    nIn = sum(gp)
    nOut = sum(!gp)
    (nIn*nOut/(nIn+nOut)) * (sum(DM[!gp,gp])/(nIn*nOut) -
      sum(DM[!gp,!gp])/(2*nOut*nOut) - sum(DM[gp,gp])/(2*nIn*nIn))
  }

  # calculate the observed T statistic for difference between gradient
      distributions inside and outside the network...
  CRT = cramersT(rep=NULL, DM=distanceMatrix, gp=network, permute=FALSE)

  # generate simulated values of the T statistic under the null hypothesis of
      no difference between sites inside and outside the network...
  sim_CRT = sapply(1:numSimulations, cramersT, DM=distanceMatrix, gp=network,
      permute=TRUE)

  # calculate the P value...
  P = (1+sum(sim_CRT > CRT)) / (1+length(sim_CRT))

  # return the result...
  return(c(CramersT=CRT, P=P))
}
```

```
stepwise_removal = function(gradientData, startNetwork, numRemovals=50){
# Function to run stepwise removal of sites from the monitoring network,
# to maximise representativeness (minimise Cramer's T)
  # gradientData = data.frame of gradient values (rows=sites,
      columns=gradients)
  # startNetwork = Boolean vector of length equal to the rows in gradientData
      coding whether each site is in the initial network (TRUE) or not
      (FALSE)
  # numRemovals = number of sites to remove

  # transform gradient values to Gaussian distributions with mean=0 and
      standard deviation=1...
  require(GenABEL)
  gradientDataT = apply(gradientData, 2, rntransform)

  # compute pairwise Euclidean distance matrix on transformed gradients...
  distanceMatrix = as.matrix(dist(gradientDataT))

  # record current network state
  currentNetwork = inNetwork

  # vector for storing order at which sites are removed...
  removalOrder = rep(NA, length(inNetwork))

  # vector for storing values of Cramer's T when sites are removed...
  removal_T = rep(NA, length(inNetwork))

  # function to calculate Cramer's T statistic...
  cramersT = function(DM, gp){
    nIn = sum(gp)
    nOut = sum(!gp)
    (nIn*nOut/(nIn+nOut)) * (sum(DM[!gp,gp])/(nIn*nOut) -
      sum(DM[!gp,!gp])/(2*nOut*nOut) - sum(DM[gp,gp])/(2*nIn*nIn))
  }

  for(removal in 1:numRemovals){ # implement each removal
    message("   Implementing removal step ", removal, " of ", numRemovals)

    # try removing all sites in the current network, and calculate Cramer's
      T...
    removal_CRT = sapply(which(currentNetwork), function(i){
      changedNetwork = currentNetwork
      changedNetwork[i] = FALSE
      cramersT(DM=distanceMatrix, gp=changedNetwork)
    })
    # update the current network by dropping the removal candidate leading to
      minimum Cramers T...
    best_removal = which(currentNetwork)[which.min(removal_CRT)]
    currentNetwork[best_removal] = FALSE
    removalOrder[best_removal] = removal
    removal_T[best_removal] = min(removal_CRT)
    message("      T = ", round(removal_T[best_removal], 4))
  }

  # compile results, plot then return...
  result = data.frame(removed_site=1:nrow(gradientData), removalOrder,
      removal_T)
```

```
  result = result[!is.na(removalOrder),]
  result = result[order(result$removalOrder),]
  par(mfrow=c(1,1))
  plot(result$removal_T, type="l",
       xlab="Number of sites removed",
       ylab="Cramer's T")
  return(result)

}


stepwise_addition = function(gradientData, startNetwork, numAdditions=50){
# Function to run stepwise addition of sites to the monitoring network, to
      maximise representativeness (minimise Cramer's T)
  # gradientData = data.frame of gradient values (rows=sites,
      columns=gradients)
  # startNetwork = Boolean vector of length equal to the rows in gradientData
      coding whether each site is in the initial network (TRUE) or not
      (FALSE)
  # numAdditions = number of sites to add

  # transform gradient values to Gaussian distributions with mean=0 and
      standard deviation=1...
  require(GenABEL)
  gradientDataT = apply(gradientData, 2, rntransform)

  # compute pairwise Euclidean distance matrix on transformed gradients...
  distanceMatrix = as.matrix(dist(gradientDataT))

  # record current network state
  currentNetwork = inNetwork

  # vector for storing order at which sites are removed...
  additionOrder = rep(NA, length(inNetwork))

  # vector for storing values of Cramer's T when sites are removed...
  addition_T = rep(NA, length(inNetwork))

  # function to calculate Cramer's T statistic...
  cramersT = function(DM, gp){
    nIn = sum(gp)
    nOut = sum(!gp)
    (nIn*nOut/(nIn+nOut)) * (sum(DM[!gp,gp])/(nIn*nOut) -
      sum(DM[!gp,!gp])/(2*nOut*nOut) - sum(DM[gp,gp])/(2*nIn*nIn))
  }

  for(addition in 1:numAdditions){ # implement each addition
    message("   Implementing addition step ", addition, " of ", numAdditions)

    # try adding all sites not in the current network, and calculate Cramer's
      T...
    addition_CRT = sapply(which(!currentNetwork), function(i){
      changedNetwork = currentNetwork
      changedNetwork[i] = TRUE
      cramersT(DM=distanceMatrix, gp=changedNetwork)
    })
```

```
    # update the current network by adding the candidate leading to minimum
      Cramers T...
    best_addition = which(!currentNetwork)[which.min(addition_CRT)]
    currentNetwork[best_addition] = TRUE
    additionOrder[best_addition] = addition
    addition_T[best_addition] = min(addition_CRT)
    message("      T = ", round(addition_T[best_addition], 4))
  }

  # compile results, plot then return...
  result = data.frame(added_site=1:nrow(gradientData), additionOrder,
      addition_T)
  result = result[!is.na(additionOrder),]
  result = result[order(result$additionOrder),]
  par(mfrow=c(1,1))
  plot(result$addition_T, type="l",
       xlab="Number of sites added",
       ylab="Cramer's T")
  return(result)

}
```

Example workflow

```
# clear the current workspace
rm(list=ls())

# load libraries (if not installed use install.packages() to install them
      first)
library(MASS)
library(GenABEL)

# now make sure all the above functions are loaded into the workspace!!!


#####################################################################
# Step 1 - create some dummy data for testing...
# (for real applications, read data in to R and format equivalently)

# generate random values of 2 correlated environmental/pressure gradients for
# 1000 sites (i.e. water bodies)
gradients = data.frame(mvrnorm(n=1000, mu=c(0,0),
      Sigma=matrix(c(1,0.25,0.25,1),2,2)))
names(gradients) = paste0("gradient_", 1:ncol(gradients))

# generate a representative monitoring network of 250 sites...
randNumber = runif(nrow(gradients))
inNetwork = randNumber < quantile(randNumber, 0.25)
table(inNetwork) # TRUE/FALSE for in the network
par(mfrow=c(1,2))
boxplot(gradients[,1] ~ inNetwork, xlab="In monitoring network",
      ylab="Gradient 1")
```

```
boxplot(gradients[,2] ~ inNetwork, xlab="In monitoring network",
      ylab="Gradient 2")

# ...or, generate an unrepresentative monitoring network biased to high
      values of gradient 1
inNetwork = 1:nrow(gradients) %in% sample(nrow(gradients), 250,
      prob=exp(gradients[,1]), replace=FALSE)
table(inNetwork) # TRUE/FALSE for in the network
par(mfrow=c(1,2))
boxplot(gradients[,1] ~ inNetwork, xlab="In monitoring network",
      ylab="Gradient 1")
boxplot(gradients[,2] ~ inNetwork, xlab="In monitoring network",
      ylab="Gradient 2")



#############################################################################
# Step 2 - univariate tests for representativeness of the network using
      Kolmogorov-Smirnov test
network_KS_test(gradientData=gradients, network=inNetwork,
      numSimulations=1000)



#############################################################################
# Step 3 - multivariate test for representativeness of the network using
      Cramer's test
network_Cramer_test(gradientData=gradients, network=inNetwork,
      numSimulations=1000)



#############################################################################
# Step 4 - stepwise removal of sites from the network to boost
      representativeness...
site_removal = stepwise_removal(gradientData=gradients,
      startNetwork=inNetwork, numRemovals=50)
print(site_removal)



#############################################################################
# Step 5 - stepwise addition of sites to the network to boost
      representativeness...
site_addition = stepwise_addition(gradientData=gradients,
      startNetwork=inNetwork, numAdditions=50)
print(site_addition)
```

Appendix 3.1 R code to run power analysis

This appendix contains an R function and code to run power analysis on linear mixed effects models for trends in monitoring data. The functions are currently restricted to models with Gaussian error structures, appropriate for continuous, normally-distributed response variables.

The function and R commands can be copied directly into R to implement a simplified workflow of the analyses in Section 3, using randomly-generated dummy data. Application to real data can be done by formatting the real data to match the formats of the dummy data, and by modifying the structure of the trend model to accommodate more complex effects (e.g. random slopes).

R function

```
trend_power = function(trend_model, monitoring_scheme, test_term, test_trend,
      numSimulations){
# Function to run power analysis by simulation on Gaussian mixed effects
      model
  # trend_model = Gaussian mixed effects model fitted with lmerTest
  # monitoring_scheme = data.frame with details of all relevant aspects of
  #       the monitoring scheme used in the trend model (e.g. which sites,
      typologies, years, days)
  # test_term = character name of the model term to be assessed
  # test_trend = numeric value of the trend to be assessed
  # numSimulations = number of power analysis simulations to run

  require(lme4)
  require(lmerTest)

  message("Power analysis for ", test_term)
  message("  Test trend value = ", round(test_trend, 3))
  message("  The monitoring data contains ", nrow(monitoring_scheme), "
      samples from ", length(unique(monitoring_scheme$site)), " sites in ",
      length(unique(monitoring_scheme$year)), " years")

  # get model fixed effects...
  B = fixef(trend_model)

  # set value of annual trend to test...
  B[test_term] = test_trend

  # create parameter object (currently only works for Gaussian, need to
      generalise)
  NP = list("beta"=B, "theta"=getME(trend_model, "theta"),
      "sigma"=attr(VarCorr(trend_model),"sc"))

  # get name of the response variable
  responseTerm = as.character(formula(trend_model))[2] # name of the response
      variable

  # simulate new data and fit the model to it, so power can be estimated...
```

```
  res = lapply(1:numSimulations, function(sim){ # for each replicate
      simulation...
    message(  "     Simulation ", sim, " of ", numSimulations)
    # simulate a new response variable...
    monitoring_scheme[,responseTerm] = simulate(object=trend_model, nsim=1,
      re.form=NA, newparams=NP, newdata=monitoring_scheme,
      allow.new.levels=TRUE)$sim_1

    # try to re-fit the model to the simulated data
    simMod = try(do.call(lmerTest::lmer, list(formula=formula(trend_model),
      data=monitoring_scheme, REML=isREML(trend_model))), silent=TRUE)

    # extract model information (or return NA if model failed)...
    modelTab = if(class(simMod) != "try-error"){
      x = data.frame(summary(simMod)$coefficients)[test_term,]
      if(ncol(x)==5) names(x) = c("estimate", "se", "df", "t", "P")
      if(ncol(x)<5) x = data.frame(estimate=NA, se=NA, df=NA, t=NA, P=1)
      x
    } else data.frame(estimate=NA, se=NA, df=NA, t=NA, P=1)

    return(modelTab)
  })
  res = data.frame(do.call(rbind, res))
  rownames(res) = NULL
  res$model_failed = is.na(res$estimate)

  # estimate the overall power
  power = mean(res$P < 0.05)
  message("Estimated power = ", round(power, 3))

  # return results
  return(res)

}
```

Example workflow

```
# clear the current workspace
rm(list=ls())

# load libraries (if not installed use install.packages() to install them
      first)
library(lme4)
library(lmerTest)

# now make sure the above function is loaded into the workspace!!!


#####################################################################
# Step 1 - create some dummy data for testing...
# (for real applications, read data in to R and format equivalently)

# create a network of 100 sites, sampled every year for 10 years, twice per
      year...
monitoring_data = expand.grid(site=1:100, year=1:10, sample_number=1:2)
```

```
# add 5 dummy typology classes...
monitoring_data$typology = factor(letters[1+(monitoring_data$site %% 5)])

# convert site to factor...
monitoring_data$site = factor(monitoring_data$site)

# centre year on mid point...
monitoring_data$year_c = monitoring_data$year -
      mean(range(monitoring_data$year))

# convert year to a factor...
monitoring_data$year = factor(monitoring_data$year)

# generate sampling days (sample 1 taken from day 50-150, sample 2 taken from
      day 200-300)...
monitoring_data$day = sample(50:150, nrow(monitoring_data), replace=TRUE) +
  100*(monitoring_data$sample_number-1)

# To represent seasonality, add 4 harmonics for day of year, scaled the same
      as year to aid model fitting...
monitoring_data$h1 = cos(2*pi*monitoring_data$day/365) *
      max(monitoring_data$year_c)
monitoring_data$h2 = sin(2*pi*monitoring_data$day/365) *
      max(monitoring_data$year_c)
monitoring_data$h3 = cos(4*pi*monitoring_data$day/365) *
      max(monitoring_data$year_c)
monitoring_data$h4 = sin(4*pi*monitoring_data$day/365) *
      max(monitoring_data$year_c)

# generate a dummy response variable...
beta = c(0, 0.25, 1, -1, 2, -2) # dummy fixed effects
monitoring_data$y = beta[1] + beta[2]*monitoring_data$year_c +
  beta[3]*monitoring_data$h1 + beta[4]*monitoring_data$h2 +
  beta[5]*monitoring_data$h3 + beta[6]*monitoring_data$h4

# add year random intercepts…
year_sd = 0.5 # between-year standard deviation
year_effects = rnorm(n=length(levels(monitoring_data$year)), mean=0,
      sd=year_sd)
monitoring_data$y = monitoring_data$y + year_effects[monitoring_data$year]

# add random intercepts for site nested in typology...
typology_sd = 0.5 # between typology standard deviation
site_sd = 1 # between site, within typology standard deviation
typology_effects = rnorm(n=length(levels(monitoring_data$typology)), mean=0,
      sd=typology_sd)
site_effects = rnorm(n=length(levels(monitoring_data$site)), mean=0,
      sd=site_sd)
monitoring_data$y = monitoring_data$y +
  typology_effects[monitoring_data$typology] +
  site_effects[monitoring_data$site]

# add residual error...
resid_sd = 1 # residual standard deviation
monitoring_data$y = monitoring_data$y +
  rnorm(n=nrow(monitoring_data), mean=0, sd=resid_sd)
```

```
#########################################################################
# Step 2 - fit a trend model...
# (for real applications, specify the appropriate trend model structure)

M = lmer(y ~ year_c + h1 + h2 + h3 + h4 + (1|year) + (1|typology/site),
         data=monitoring_data, REML=TRUE)
summary(M) # note that if using dummy data, estimated fixed and random
       effects should close to the values used above



#########################################################################
# Step 3 - run example power analyses

# Example 1 - estimate power to detect trend of current size, using current
       monitoring strategy...
power_output = trend_power(trend_model=M, monitoring_scheme=monitoring_data,
       test_term="year_c", test_trend=fixef(M)["year_c"], numSimulations=50)
print(power_output)

# Example 2 - estimate power to detect trend of 1/2 of the current size,
       using current monitoring strategy...
power_output = trend_power(trend_model=M, monitoring_scheme=monitoring_data,
       test_term="year_c", test_trend=fixef(M)["year_c"]/2, numSimulations=50)

# Example 3 - estimate power to detect trend of the current size, if only
       took the first sample in each year...
power_output = trend_power(trend_model=M,
       monitoring_scheme=monitoring_data[monitoring_data$sample_number==1,],
       test_term="year_c", test_trend=fixef(M)["year_c"], numSimulations=50)

# Example 4 - estimate power to detect trend of the current size, if only
       monitored half the current number of sites...
selected_sites = sample(levels(monitoring_data$site),
       round(length(levels(monitoring_data$site))/2), replace=FALSE)
power_output = trend_power(trend_model=M,
       monitoring_scheme=monitoring_data[monitoring_data$site %in%
       selected_sites,], test_term="year_c", test_trend=fixef(M)["year_c"],
       numSimulations=50)

# Example 5 - estimate power to detect trend of the current size, if
       monitored twice the current number of sites...

# first, prepare a new monitoring strategy as above but with 200 sites...
new_data = expand.grid(site=1:200, year=1:10, sample_number=1:2)
new_data$typology = factor(letters[1+(new_data$site %% 5)])
new_data$site = factor(new_data$site)
new_data$year_c = new_data$year - mean(range(new_data$year))
new_data$year = factor(new_data$year)
new_data$day = sample(50:150, nrow(new_data), replace=TRUE) +
  100*(new_data$sample_number-1)
new_data$h1 = cos(2*pi*new_data$day/365) * max(new_data$year_c)
new_data$h2 = sin(2*pi*new_data$day/365) * max(new_data$year_c)
new_data$h3 = cos(4*pi*new_data$day/365) * max(new_data$year_c)
```

```
new_data$h4 = sin(4*pi*new_data$day/365) * max(new_data$year_c)

# run power analysis...
power_output = trend_power(trend_model=M, monitoring_scheme=new_data,
      test_term="year_c", test_trend=fixef(M)["year_c"], numSimulations=50)
```

Appendix 5.1: Detailed review of innovative monitoring methods

Rivers

Biological Quality Elements (BQEs)

Phytoplankton

Not applicable for rivers in Scotland.

Macrophytes & phytobenthos

Macrophytes

The current SEPA method, River LEAFPACS2, receives the highest overall score of all the evaluated river macrophyte monitoring methods. It has undergone a lot of development, is well designed for Scottish running waters, produces high quality, reliable data suitable for surveillance monitoring, and is relatively efficient and cost-effective. Although of potential value, alternative intercalibrated and molecular approaches are not yet well developed for ready application in Scottish rivers. Other novel and emerging methodologies, such as drone surveys, although potentially valuable for extending spatial and temporal monitoring coverage of rivers, cannot yet provide the detailed assessments, particularly of submerged plants, for accurately assessing riverine macrophyte communities.

Table A3. A list of monitoring methods for river macrophytes in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (River LEAFPACS2)	4	4	4	4	5	5	4.5
Method 2 Alternative intercalibrated (e.g., DPSI)	3	5	5	4		4	4.5
Method 3	5	5	2	2	5	2	3.5
Novel & emerging (molecular approaches)							
Method 4	5	4	2	3	2	2	3
Novel & emerging (high resolution images)							
Method 5 Novel & emerging (hydraulic methods)	4	3	5	3	3	2	3.5

Method 1. River LEAFPACS2 forms the macrophyte part of SEPA's current WFD compliant method for assessing "macrophytes and phytobenthos" in rivers (WFD-UKTAG 2014a). The method is based on field surveying of macrophytes in representative 100 m stretches of rivers in the summer months and is designed to primarily detect impact of nutrient enrichment although it may be sensitive to other anthropogenic pressures, e.g. changes in river flow and morphological conditions. SEPA's surveillance network typically, as a minimum, only requires macrophytes to be sampled at one river site at least once in every six years although additional sample locations may be added to increase robustness of data. Efficiency and cost is rated 4 as the method usually requires to be carried out by two staff from SEPA's National Monitoring Team with an estimated 0.6 days per macrophyte sample. Species level identification of macrophytes requires a reasonably high degree of expertise and, hence, training. Data quality can vary with the abilities of the surveyors and surveyors require expert training. It is challenging for surveyors to accurately record abundance on the % reach scale. CEH have previously compared detailed measures of reach scale

abundance which illustrate the inherent challenges (Wood et al. 2012). One simple adjustment to the current methodology would be to record the biomass of the dominant macrophyte from small quadrats using the method developed by (O'Hare et al. 2010). This produces a measure of abundance that is highly sensitive to nutrient pollution and takes little additional time in the field.

Method 2. Alternative intercalibrated methods for riverine macrophytes are generally similar to those used in Scotland (e.g., (Birk et al. 2013). The Danish Stream Plant index (DSPI) is a good example of this type of alternative intercalibrated sampling approach. As for SEPA's River LEAFPACS 2 survey method, DSPI involves the collection of macrophyte data from 100m long stretches of lowland streams but also requires assessing the coverage of macrophytes (on a 1-5 scale) within 25 x 25 cm plots located in 10-15 cross-sectional transects across the channel in order to calculate relative species abundances (Baattrup-Pedersen et al. 2013). The use of plots allows for more accurate recording of biomass / abundance and produces data that is better suited to statistical analyses. The DPSI analytical framework, involves the use of an ecological assessment and a classification model based on expert judgement (Baattrup-Pedersen et al. 2013) and the development of a diagnostic tool to disentangle the multiple interacting stressors (Baattrup-Pedersen et al. 2015); (Baattrup-Pedersen et al. 2016). The strengths of such a framework are that it has high transparency (mirroring expert interpretations), it can be used as a general indicator of ecological condition (that is responsive to all stressors and future stressors), and, with a diagnostic tool, it can pinpoint the main stressors and stimulate recovery by efficient mitigation (Baattrup-Pedersen, *pers comm.*; (Baattrup-Pedersen et al. 2017). This type of more field-intensive approach is likely to provide better quality data (rated 5) more suited to investigative analysis than SEPA's current River LEAFPACS2 approach, but it is questionable whether there is any benefit in applying such approaches for surveillance type monitoring in Scotland. Such labour-intensive field sampling methods mean that it is rated 3 for efficiency and but in terms of cost-effectiveness it rates highly, as the additional effort in the field provides data that is more sensitive to change. This type of alternative intercalibrated approach would need little development work to be made ready for use in Scotland. Nevertheless, if such alternative intercalibrated approaches were adopted then the data produced would be potentially suitable and compatible with existing Scottish data and hence are rated 4 for both these criteria. These alternative intercalibrated approaches are given an overall score of 4.5.

Method 3. New approaches for biodiversity monitoring have been developed based on high throughput sequencing in which millions of sequences are generated in a single assay (Read and al. 2017). These high throughput assays are called metabarcoding and come into two forms: **community metabarcoding** in which bulk samples of a whole community are homogenised to form a slurry, from which community DNA is extracted and metabarcoded; **environmental DNA (eDNA) metabarcoding** is based on the detection and description of extracorporeal traces of DNA that are shed into the environment (Read and al. 2017).

Using the eDNA metabarcoding approach, macrophyte DNA can be detected in collected water samples, allowing potentially high throughput, cost-effective descriptions of macrophyte communities, without the need to directly sample them. However, this molecular approach is not currently well developed for river macrophytes and where attempted it has not been possible to identify all the macrophyte species. As a result of the indirect nature of sampling, there is also a greater potential for false negatives compared with more conventional survey methods (Herder et al. 2014). Hence, for these reasons, this method scores only 2 for data quality, stage of development and compatibility with existing data in Table A3. However, the eDNA method does have the potential as a rapid, cost-effective, screening approach to identify rare or invasive macrophytes, particularly submerged species that might otherwise be missed or misidentified by such traditional monitoring methods.

However, compared with some animal species, eDNA techniques, so far, have proven less successful for monitoring invasive non-native species because of problems of production of anemophilous plant species from outwith river catchments and uncertainty over the persistence of pollen in rivers. eDNA techniques may also be of limited use in detecting hybrids (Telford et al. 2011). However, in terms of cost, efficiency and suitability for Scotland, each of these criteria scores 5 in Table A3. A useful development in molecular approaches has been efforts to taxonomically resolve some of the macrophyte groups that are challenging to identify. CEH has in the past developed bar coding techniques for some for the Batrachian *Ranunculus* (Telford et al. 2011). Further development of these approaches could be used to confirm voucher specimens taken in the field. Crucially key visual identification characteristics required to identify macrophytes to species level are present for different groups at different times in the summer. This necessitates either visiting sites multiple times or alternatively compromising on taxonomic resolution. The ability to take samples of material for genetic identification means sites may only have to be sampled once.

Method 4. There is little development of novel field techniques. The use of aerial imaging has been repeatedly trialled since the 1940s initially with balloon's, then aircraft and now drones. High resolution images of rivers, taken with unmanned aircraft systems, such as drones, potentially allows for the identification, mapping and abundance of non-submerged macrophyte species while near-infrared-sensitive DSLR cameras can be used to map the spatial distribution and depth of submerged species. Advantages of satellite remote sensing is that it allows for high spatial and temporal coverage with consistency in measurement. However, for river macrophytes satellite remote sensing is problematic given the relatively narrow nature of Scottish rivers compared to the available spatial resolution. Instream flow characteristics can significantly alter vegetation cover (by either pushing plants over or allowing them to float upward) for a given abundance and all the remote sensing techniques are vulnerable to this error.

Method 5. Hydraulic methods. A simple but novel method for measuring the seasonal growth of macrophytes has been developed as a research tool and is quick to carry out in the field. The method, is used in hydraulic studies and is well developed in some respects but has never been applied to routine macrophyte monitoring. It is most effective on heavily vegetated channels and in this regard has limited application in Scotland to some lowland river systems. It gives an annual measure of standing crop that is especially beneficial in trend analysis and disentangling the effects of climate and eutrophication in particular. It uses velocity and depth sensors that are placed on the river bed and frequently record those two parameters. The data has other applications and telemetry can be applied. An indirect measure of the standing crop is estimated from the seasonal effect the plants have on backing up water in the channel. In summer, water depth is much higher than in winter when the plants are absent or have senesced. By comparing the relationship of depth to velocity / discharge in different seasons, an indirect measure of standing crop is produced.

General comments. Macrophytes are considered sensitive to eutrophication process although they also respond dramatically to hydromorphological alteration. Disentangling whether eutrophication or hydromorphological alteration is the cause of reduced macrophyte status is becoming more practical to do. The supporting environmental data collected as part of the existing SEPA monitoring is useful but it is not sufficient and it would be far better to apply the emerging CEN standard at the same sites.

Macrophyte distribution in Scottish rivers is primarily limited by physical habitat characteristics (O'Hare et al. 2011) and it mediates macrophyte response to eutrophication as one proceeds from steep sloped systems where communities are relatively resilient to slow flowing systems where more lake-like eutrophication processes can proceed. This differential sensitivity to eutrophication has not been fully investigated but it already acknowledged in SEPA's use of macrophytes as indicators of eutrophication for a limited number of sites.

A significant problem with most macrophyte survey methods is the area defined for sampling relates only to the main channel and does not consider associated bodies of water such as oxbows, distributaries or side channels. By not incorporating them the sensitivity of macrophytes surveyed is under represented and where these secondary channels have been lost, which is very common, the absence of macrophytes is not noted and sites 'score' too highly.

Phytobenthos

Of the assessed river phytobenthos methods, eDNA metabarcoding receives the highest overall score. This is primarily because the method offers the promise of much more cost-effective monitoring compared with

SEPA's more traditionally based current River DARLEQ methods while producing compatible data to a high taxonomic resolution. The citizen science RAPPER method and alternative intercalibrated methods, such as pressure independent metrics and algal biomass measurements, also score relatively highly and have potential supplementary value in extending spatial and temporal coverage of river phytobenthos monitoring and improving the understanding of pressures/stressors affecting particular watercourses. New sensors which give daily measures of benthic algal production are patented and prototype are currently being tested by CEH. These are considered most suitable to investigative and operational monitoring and are not discussed in detail.

Method 1. River DARLEQ2 (Diatoms for Assessing River and Lake Ecological Quality) forms the phytobenthos part of SEPA's current WFD compliant method for assessing "macrophytes and phytobenthos" in rivers and focuses on the diatom assemblage composition, based on using the Trophic Diatom Index (TDI) metric (WFD-UKTAG 2014b). This metric is based on the premise that each riverine diatom species has a characteristic response to the predominant pressure, particularly nutrients/organic pollution. The method requires sampling of the diatom biofilms attached to submerged stones and plant stems in rivers. Samples are usually collected by a single member of staff and preserved in the field. Following digestion of the preserved samples, a minimum of 300 undamaged benthic diatoms need to be identified and counted with a high power microscope in the laboratory. Formerly, samples were collected from a site twice a year (spring and autumn) or, alternatively, from a single summer sample. Riverine diatom data is regarded as being more inherently variable than say, invertebrate data, and so, ideally, a total of six samples (over period of three years) is recommended to produce a reliable site classification. However, in practice, riverine diatoms are now monitored by SEPA at sample sites every second year with a concomitant loss in confidence in the resultant WFD classification of the river, which is exacerbated by fact that relationships of diatoms with pressures are largely based on association rather than based on experimental studies. Reliance on this WFD community metric for detecting nutrient/organic pollution, particularly in a multi-stressor environment, may also result in missing the most important pressures that affect a particular river system, e.g. changes in flow regime, pesticides. Method has also limited diagnostic capabilities and may overlook the impacts of stressors on algal biomass and the relative balance of different algal groups leading to poor links to secondary effects/undesirable disturbances. With these uncertainties associated with the River DARLEQ2 WFD method, data quality gets a rating of 4 in Table A4. Efficiency and cost is rated at 3 as staff time per diatom sample is quite high, assessed by SEPA at being, on average, 0.6 days per sample. Species level identification of diatoms is very time consuming and requires a high degree of expertise and, hence, training. In addition, there is a problem with the taxonomy of phytobenthos undergoing constant revision. The River DARLEQ2 WFD method receives an overall score of 4.50.

Table A4. A list of monitoring methods for river phtobenthos in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (River DARLEQ2)	3	3	4	5	5	5	4.5
Method 2 Novel & emerging (eDNA)	5	5	4	4	5	5	4.75
Method 3 Novel & emerging: (Citizen Science: RAPPER)	5	5	3	3	5	3	4.0
Method 4	5	5	3	3	3	3	3.5
Alternative intercalibrated: (e.g., pressure independent metrics; algal biomass measurements)							

Method 2. New molecular approaches, such as eDNA metabarcoding, have been developed to provide more cost-effective monitoring river diatom communities compared with conventional methods that require time consuming species level identification and a high degree of staff expertise as required with the River DARLEQ2 method. The eDNA metabarcoding method for river diatoms is ready to be deployed by the Environment Agency (EA) as part of their routine freshwater monitoring programme in 2018 and SEPA are investing in developing in-house capacity in new eDNA techniques for analysing river diatom samples. However, species-level assignment of sequences is hindered by lack of fully populated reference databases of barcodes although metabarcoding can describe diatom biodiversity to a higher resolution than is usually possible by traditional morphometric methods (Read and al. 2017). For efficiency, cost, suitability for Scotland and comparability with existing data eDNA metabarcoding is rated at 5 in Table A4 but receives reduced scores of 4 for data quality and stage of development. eDNA metabarcoding method receives an overall score of 4.75.

Method 3. Novel and emerging approaches include Citizen Science methods, such as RAPPER (Rapid Assessment of PeriPhyton in Rivers). This is a high level ecological "triage" method designed to complement existing WFD tools based on diatoms for the ecological assessment of rivers (Kelly et al. 2016). The method enables the rapid screening of sites within a waterbody in order to identify sites at risk

from eutrophication. It involves surveying macroscopic algae within 10 m lengths of rivers, collecting samples for subsequent identification and assessing cover. Genus-level identification ensures rapid assessment, comparability and potential use by a wide range of users. The relative percentage cover of "stress tolerant" and "competitive" macroalgal taxa can be used to determine whether a site is at risk from eutrophication, as demonstrated by field trials in Scotland (Kelly et al. 2016). The RAPPER classification categories produced comparable data with that derived by use of the widely used Trophic Diatom Index (TDI) metric. The benefit of the RAPPER is that it, if used as a stand-alone method, it can provide a greater spatial and temporal coverage of rivers, at a lower cost, than the pre-existing WFD methods and help direct where more detailed investigations are required. RAPPER can also be used to complement the established WFD phytobenthos methods by increasing the confidence in their site assessments by incorporating algae other than diatoms. Although there remain a number of challenges with using this method, including a need to increase understanding of the relationship between algae and their chemical/physical environments, timing of surveys, identification training requirements, RAPPER, with its focus on the more visually obvious algae, has the potential to be applicable for 'citizen science" participation. Because of these challenges in successfully applying RAPPER, data quality, stage of development and computability with existing data criteria get ratings of 3 in Table A4. However, for efficiency, cost and suitability for Scotland RAPPER is rated at 5 in Table A4, as it is a method that can be readily deployed to screen Scottish river sites rapidly and efficiently. The RAPPER method receives an overall score of 4.0.

Method 4. For alternative intercalibrated methods, Martyn Kelly (a freelance environmental consultant heavily involved in the development of WFD assessment methods for phytobenthos) suggests, as a potential solution to problem of pressure-specific metrics in multi-pressure situations, is to move towards more pressure-independent metrics: functional groups; diversity; similarity to reference assemblages (but which could have high-level dissimilarity over course of year (including impact of short-term hydrological perturbations) (Kelly, *pers comm.*; (DeNicola and Kelly 2014). With regard to the problem of stressors potentially impacting primarily on biomass and not species composition, he suggests measuring algal biomass by in situ fluorescence ("Benthotorch"), in addition to assessing species composition. Alternatively, one could use "percent algal cover" as a rapid proxy estimate of algal biomass. However, there may be significant interactions with shade and substrate that may make site-level assessments unrepresentative of a whole river. To overcome problems with stressors affecting the relative balance of algal groups non-diatoms could be assessed as well, e.g. Norwegian Periphyton Index of Trophic Status, but would this would require the separate assessment of diatoms and non-diatom groups, thereby, increasing costs and both assessments would still be based on pressure-specific metrics and would results be comparable? (Kelly, *pers comm.*). Most of these alternative intercalibrated methods, although potentially

efficient and low cost (that is rated 5 in Table A4), would need quite a bit of development to make the resultant metrics ready and suitable for assessing the health of Scottish rivers. However, methods have potential to be particularly valuable in more investigative monitoring complementing the more formal WFD assessment methods used in surveillance monitoring. These alternative intercalibrated methods receive an overall score of 3.5.

Benthic invertebrates

The current main SEPA benthic invertebrate surveillance method, WHPT in RICT, receives a high score as it has undergone a long period of development, is well designed for Scottish running waters and produces high quality, reliable data suitable for surveillance monitoring. However, alternative intercalibrated approaches, such as adopted in Finland and Ireland, suggest that the costs and efficiency associated with this method could be improved by reducing the frequency of sampling and the time spent sorting and identifying samples without a significant loss of data quality. Molecular approaches also score highly in terms of efficiency and cost but still require some development work before they will be ready for deployment in Scottish rivers. Citizen Science approaches such as the Anglers' River fly Monitoring Initiative also have the potential to be a cost-effective way to increase the spatial and temporal coverage of SEPA's monitoring network but questions remain over data quality, data comparability and practicality for use throughout the whole of Scotland. Hence, for the reasons outlined above, the alternative intercalibrated methods receive the highest overall score.

Table A5. A list of monitoring methods for river benthic invertebrates in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (WHPT in RICT)	3	3	5	5	5	5	4.5
Method 2 Alternative intercalibrated (e.g., Finland, Ireland, STAR-AQEM)	5	5	5	4	5	4	4.75
Method 3 Novel & emerging (molecular approaches)	5	5	4	3	4	4	4.0
Method 4 Novel & emerging (Citizen Science: Angler's River Fly Monitoring Initiative)	5	5	3	4	3	3	3.5
Method 5 Novel & emerging (Automatic invertebrate identification)	5	3	2	2	3	2	2.5

Method 1. The main existing WFD river benthic invertebrate assessment method used by SEPA is the Whalley, Hawkes, Paisley & Trigg (WHPT) metric in River Invertebrate Classification Tool (RICT) (WFD-UKTAG 2014c). The WHPT metric is primarily designed to detect the impacts of organic pollution but is also used to monitor the general degradation of UK rivers. Site classification derived from using this metric is based on the field collection of two macroinvertebrate samples (in spring and autumn) and associated environmental parameters per year, using well-established standard RIVPACS (River Invertebrate Prediction and Classification System) procedures with analysis of samples to a relatively high taxonomic level. Sampling is typically conducted by one member of staff but efficiency and cost are rated at 3, as staff time per benthic invertebrate sample is quite high. For example, the staff time per invertebrate sample is planned by SEPA based on a Standard Average Time per activity of 0.63 days per sample for family-level identification and 1.9 days for species-level identification. However, the data quality, stage of development,

suitability for Scotland and compatibility with existing data are all rated 5 using this method. Such benthic invertebrate data is considered by SEPA to be robust, rarely giving an unexpected signal that is not explainable. Benthic invertebrate communities have a long history of being used to assess health of Scottish streams and rivers and form an integral part of the surveillance monitoring of SEPA's current monitoring. Because of the ubiquity of organisms and the important roles they play, such river benthic invertebrate communities are an important focus for research into the understanding of environmental change, resistance, resilience and the identification of tipping points in the response of ecosystems to environmental change.

Method 2. For alternative intercalibrated methods the examples of Finland and Ireland are instructive. In Finland and Ireland, unlike in Scotland, benthic invertebrate WFD surveillance monitoring is based on single season sampling (autumn in Finland and summer in Ireland) once every three years (Hellsten & O'Boyle, *pers comm.*). In the Irish example, summer sampling is employed as it is considered to be the time that the benthic invertebrate fauna is most likely to be sensitive to stress and the impacts most detectable. Sampling costs are further reduced by carrying out river bank sorting and identification of collected samples, estimated to take *c.* 1-2.5 hours per sample. Alternative intercalibrated methodical approaches were also investigated in the EU funded research project STAR (Furse et al. 2006) that looked at developing methodologies and tools for assessing the ecological status of European rivers using various WFD biological quality elements, including invertebrates. As part of this project, (Friberg et al. 2006) compared national macroinvertebrate sampling methods with a common standard, the STAR-AQEM sampling method (see http://www.eu.at). This STAR-AQEM method was focussed on sampling multiple habitats within a defined sample reach in proportion to their coverage and involved sub-sampling of the collected samples. (Friberg et al. 2006) showed that the various national methods tested, including the UK RIVPACS assessment system (basis for SEPA's WHPT in RICT method), correlated significantly with the results derived from use of the STAR-AQEM method. The rationale for using sub-sampling was that it reduced the effort required for sorting and identifying, providing an unbiased representation of a large sample and helped provide a more accurate estimate of time expenditure required to process a sample. All these alternative intercalibrated approaches, either by reducing the frequency of sampling or time spent analysing collected benthic invertebrate samples, cut the costs associated with the collection and processing macroinvertebrate samples (and hence are rated 5 in Table A5, a higher score than for costs associated with existing SEPA methods) without a significant loss in data quality. These alternative intercalibrated approaches are given an overall score of 4.75.

Method 3: New molecular approaches have been investigated as a means of overcoming the time consuming and, therefore, costly conventional analyses of lotic benthic invertebrate communities as required in current

SEPA applied WFD sampling methods, outlined above. For example, (Read and al. 2017) state that there is good evidence that metabarcoding of whole homogenised communities is a viable approach, and can outperform existing morphotaxonomic methods on basis of cost, time (and hence both are rated 5 in Table A5) and accuracy (Elbrecht and Leese 2015); (Elbrecht et al. 2016). (Read and al. 2017) also state that other recent studies have demonstrated the potential utility of eDNA metabarcoding for analysing invertebrate communities (e.g., (Deiner and Altermatt 2014); (Deiner et al. 2016); (Elbrecht et al. 2017) although the method requires more validation due to potentially complicating factors of eDNA transport and persistence. (Elbrecht et al. 2017) also found that estimating the abundance of invertebrate species using eDNA metabarcoding was less than successful. Given all these factors of data quality, likely suitability and compatibility with existing data, were all rated 4 in Table A5. However, these new molecular approaches will still need some development before they will be ready for application in Scottish rivers and so were given the rating of 3 in Table A5.

Method 4. A good example of a Citizen Science approach for assessing benthic invertebrate communities in rivers is the Anglers' River Fly Monitoring Initiative – currently hosted by the Freshwater Biological Association (FBA). (http://www.riverflies.org/rp-riverfly-monitoring-initiative) In this scheme, members of fishing clubs and other interested organisations are trained to monitor the health of the rivers they fish, by using a simple standardised sampling protocol to assess the riverfly community (Fitch 2017). In the areas where this initiative is established, it allows these participating groups to detect severe perturbations in river water quality and alert the statutory environmental agencies to investigate further. It potentially is a cost-effective way to extend the spatial and temporal coverage of SEPA's existing water quality monitoring network and can help to act as a potential deterrent to incidental polluters. It also has the additional benefit of getting the general public actively involved in the management of their local rivers. A downside of the initiative is that currently does not cover the whole of Scotland, so far being mainly restricted to the Central Belt. There may also be issues around data quality and bias in recording effort although these should be minimised by the regular workshops run for members. For low cost and high efficiency, this method rates a score of 5 in Table A5 but scores less for the other criteria based on possible issues of data quality, comparability and its applicability for use throughout Scotland.

Method 5. Another potential example of a novel and emerging approach to assessing riverine benthic invertebrate communities is automatic invertebrate identification. This method, which uses automated optical recognition to identify benthic invertebrates (to a minimum of taxa level), has reportedly been in development in Finland with a reported accuracy of *c.* 80-90% (Joutsijoki et al. 2014). However, the method is not yet commercially available, as difficulties with funding have hampered development. If the method did become more widely available, it could potentially be utilised in conjunction with eDNA methods to

provide complementary morphological and biomass data (Helwig et al. 2015). Although theoretically an attractive efficient and possible viable option for deployment in Scotland, it should be stressed that this automatic identification method is still in a relatively early stage of development, is of unknown cost and there are potential issues around data quality that would need to be resolved. Because of these concerns, the method is given an overall rating of 3 in Table A5.

Fish

Compared with more conventional assessment methods, such as electrofishing, molecular approaches, such as eDNA metabarcoding, appear to provide SEPA with the most cost-effective means of monitoring riverine fish populations. These molecular techniques have been validated for UK fish and are ready for deployment, although there remain questions over estimating fish species abundances. Nevertheless, these molecular approaches receive the highest overall score of the evaluated river fish faunal methods.

Table A6. A list of monitoring methods for river fish in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (Fish Classification Scheme 2 (FCS2) Scotland)	3	3	4	5	5	5	4.0
Method 2 Novel & emerging (molecular approaches)	5	5	4	5	5	5	4.75

Method 1. The current SEPA WFD river assessment method for fish fauna is the Fish Classification Scheme 2 (FCS2) Scotland tool. This classification method encompasses the abundance, taxonomic composition and age structure of salmonid fish, and is regarded as being sensitive to water quality and changes in physical habitat conditions prevalent in Scottish rivers (WFD-UKTAG 2014d). The FSC2 Scotland classification tool is based on the predictive statistical models developed by the Environment Agency in their Fisheries Classification Scheme 2 (FSC2) but which has been adapted specifically for Scottish fish species and sites. The FCS2 in Scotland tool relies on accurately estimating the number of salmon and trout

at a survey site, as determined by electric fishing (using CEN standard electric fishing protocols) and relating the number of salmonids caught in a survey to the predicted abundance and prevalence of the species at a specific site. Given all these factors, the stage of development, suitability for Scotland and compatibility with existing data, are all rated 5 in Table A6. However, the FCS2 in Scotland tool is built on using data collected from wadeable sites and cannot be reliably used to provide a classification of sites that are assessed by boat-based electrofishing and requires data from area-delimited surveys. Because of these potential data limitations and SEPA's large reliance for fish data to be supplied by Fisheries Trusts/District Fisheries Boards, data quality is given a rating of 4 in Table A6. On occasion, fish sampling is carried out by SEPA staff to fill gaps in the sampling network. Because of the time consuming staff time required to carry out such fish surveys, both efficiency and cost are given tentative ratings of 3 each. This method is given an overall rating of 4.0.

Method 2. Molecular approaches, such as the analysis of eDNA, have the potential to provide valuable information regarding the presence and absence of fish species, as well as the composition of whole fish communities although estimating fish species abundance is regarded as being more problematic using these techniques. (Civade et al. 2016) showed that eDNA metabarcoding was more efficient than a single traditional sampling campaign for detecting fish species presence in rivers and that the species list they obtained by using this approach was comparable to that obtained by combining the data derived from all the fish sampling sessions since 1988. Fish eDNA metabarcoding has now been developed by funding from the UK environment agencies and, unlike diatoms, fish species in UK already have a complete reference database of fish species barcodes. Molecular approaches have been developed and validated for fish in the UK and are ready to be deployed. Hence, for these reasons all the listed criteria in Table A6 are rated 5 apart from data quality that is rated 4 because of the perceived problems with estimating fish species abundance using these molecular approaches. Hence, this eDNA metabarcoding method receives an overall score of 4.75. It should also be noted that such molecular techniques have the potential additional benefit to be used for the monitoring of invasive non-native fish species in rivers.

Supporting elements

Hydromorphological quality elements

A new CEN standard is being developed. Current SEPA methodologies should fit within the new standard's protocols that are designed to be flexible and allow the use of existing methodologies. In the circumstances the recommendation is for SEPA to maintain a watching brief on the development of the new standard and prepare to adapt existing methods as required.

When diagnosing the impact of hydromorphological degradation on biota some cause – effect relationships are well established; for example, the effect of weirs and other impoundments on fish migration. Other relationships are not as well established or existing metrics are no longer considered diagnostic on their own, e.g. LIFE scores (Extence et al. 1999). Key fundamental information, such as the timing and frequency of routine channel maintenance, which is considered by ecologists to have some of the most dramatic impacts on biota, are not routinely recorded as part of most field surveys. Basic research is required which takes into account the long-term changes to fluvial geomorphological processes that hydromorphological degradation can induce on BQEs. As these relationships become established, existing field methods may need to be adjusted to collect data in a way which is indicative of impact to biota.

Table A7. A list of macrophytes monitoring methods in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (RHS/MiMaS)	4	3	3.5	4	5	5	4.5
Method 2 Alternative intercalibrated	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Method 3 New CEN standard	4	4	5	3	5	4	4.75
Method 4 Novel and emerging (LIDAR)	5	3	5	5	5	4	4
Method 5 Novel & emerging (Citizen Science: MoRPh)	5	5	3	4	5	4	4

Hydromorphology is important in two ways firstly as a WFD supporting element for biota and secondly in its own right. Hydromorphology encompasses both hydrology and fluvial geomorphology. As the science and monitoring of hydrology is well established, it is not addressed here. The fluvial geomorphological component is becoming of increasing interest both in terms of river restoration and as channels are perceived to have become more dynamic in recent years, possibly as a response of changing climatic conditions.

This duel interest is reflected in a distinct two-track approach that has developed in SEPA. River Habitat Survey (RHS), which predates the WFD, remains the standard WFD hydromorphological survey tool. In Scotland, more detailed hydromorphological surveys and audits are also being under taken by SEPA building on the existing MiMAs system. These have a stronger grounding in fluvial geomorphological theory and processes.

On a European level, the new CEN standard under development, takes a hierarchical approach incorporating both field and desks study components to give a holistic overview. It is also strongly grounded in fluvial geomorphological process theory. Scotland has had some influence over its development as SNH has chaired the standards committee and SEPA representatives contributed to the text, as have CEH. With both the development work in Scotland and the new CEN standard there remains an outstanding question as to whether or not they are more or less helpful in explaining the assemblage structure and response to degradation of Biological Quality Elements.

The new CEN standard is likely to be flexible in the detail of approach and it may well be possible to tailor it to Scotland's needs. RHS will need to be reformed if it is to meet the new CEN standard. It is important to understand that when RHS was first developed it was assumed that some of the measurements could be used to describe habitat. Hence, despite being established for over 20 years development is still scored as relatively low. Equally, the survey requires trained surveyors to visit each site; this reduces the efficiency and increases the cost of the method.

A significant component of the new CEN standard involves desk study that can be carried out using traditional techniques such as analysis of maps and aerial photography. An example of analysis which is close to compatible with the CEN standard is the REFORM analysis of the River Tweed, (Blamauer et al. 2014), see **Figure** and Table A7 for example output. There is the potential to automate some of the analysis using GIS tools. These have not been developed yet for Scotland.

Alternative intercalibrated methods – a comprehensive review of 21 methods used by EU member states was undertaken by the REFORM project (Rinaldi et al. 2013). The majority of methods can be considered as physical habitat assessment techniques rather than hydromorphological surveys; for example, only two methods measure stream power, a key fluvial geomorphological metric and one that is increasingly acknowledged as highly ecological relevant. A number of countries use localised versions of RHS (e.g., Poland, Portugal) while others use approaches which are more strongly grounded in fluvial geomorphological processes. No one method stands out with various methods having positive aspects with specific components developed to assess riparian zones and longitudinal connectivity for fish passage. The

new CEN standard aims to incorporate the best of the various techniques. In light of that development, we do not propose an alternative intercalibrated method for hydromorphology.

Figure A1. The River Tweed was delineated preliminary into eight segments based on increases in catchment area caused by major confluences and changes in valley confinement. The study section lies within segments 3 and 4 (Ordnance Survey data © Crown Copyright/database right 2012).

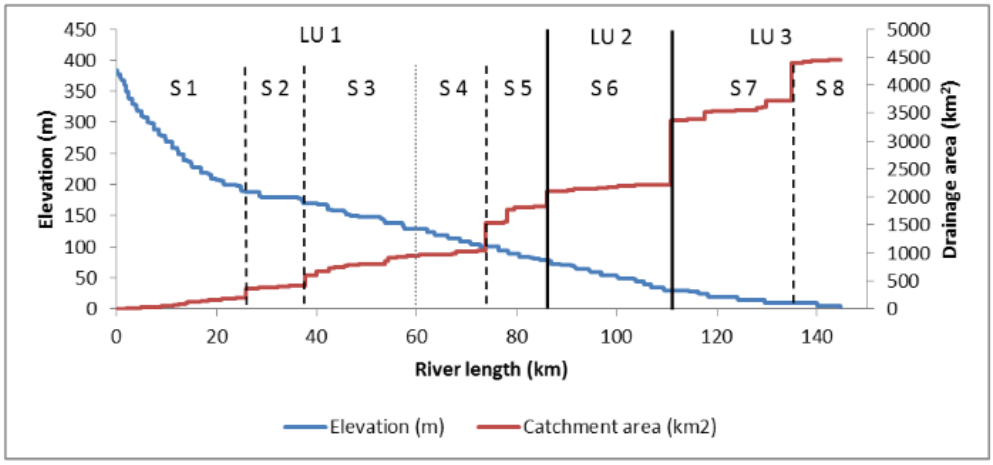


Table A8. Indicators of channel self-maintenance and shaping for study reaches of the River Tweed using the CEN compatible REFORM methodology.

	3a	3b	3c	3d	4
Specific stream power (W m^{-2})					
Qp_2	49	61	69	121	123
Qp_{median}	50	64	74	131	136
Qp_{10}	73	92	105	185	189
Bed sediment size	Gravel / Pebble	Cobble	Cobble	Cobble	Cobble
Bank sediment size	Earth	Earth	Earth	Earth	Earth
Channel gradient (m m^{-1})	0.0017	0.0018	0.0016	0.0023	0.0019
Confinement index	7.42	4.22	12.83	11.05	4.14
Mean bankfull channel width (m)	38	36	39	33	43
Mean bankfull channel depth	1.5	1.2	1.8	1.7	2.2
W:D Ratio	25	31	21	19	20
Sinuosity Index	1.06	1.11	1.07	1.08	1.04
Braiding Index	1.00	1.03	1.01	1.01	1.00
Anabranch Index	1.01	1.03	1.05	1.04	1.07
River Type	13	13	13	13	13

Technical advances – the use of remote sensing has made significant advances in recent years with the application of LiDAR especially powerful. It can provide high spatial resolution bed topography and substrate size data and there is the ability to strip away vegetation from images to reveal channel form. This is especially useful in assessing the history of channels and their interaction with their floodplains. This makes LiDAR a useful research tool and it has some application to investigative monitoring and the understanding of fluvial geomorphological changes processes. Currently it is expensive to undertake. It is becoming increasingly realistic that LiDaR could be deployed using drones. It is not clear yet if this will provide a more cost-effective method of survey than the use of fixed wing aircraft.

MoRPh is a Citizen Science tool developed by Queen Mary University of London and the Environment Agency. The tool is based around a 10 to 40m river reach survey or module that is scaled by channel width. The system is hierarchical so 10 modules contribute to a contiguous 400m MultiMoRPh sub-reach survey and they in turn can field into a HydroMorPh assessment of 10s of kilometres. The system has well developed indices sensitive to differences in hydraulic, sediment, physical and vegetation habitat characteristics. The system has proven popular with circa 1000 surveys undertaken in its first year. It is likely to be highly compatible with the emerging new CEN standard for hydromorphology. Compatibility with existing RHS data has yet to be demonstrated in the peer review literature. The method does use RHS terminology and is designed to complement rather than replace RHS, providing much finer scale and

comprehensive data relevant to, co-located, biological sampling sites. Some development is still required if SEPA were to consider adopting the method; application of MoRPh data to understanding the effects of hydromorphological degradation on BQEs has yet to be developed nor has there been a systematic check on the quality of data collected by volunteers.

Physio-quality elements

Our recommendation is to stay with SEPA's current methods for surveillance monitoring but consider the benfits of reducing the number of samples per season. For rivers the importance of seasonal dynamics is more relevant to detailed investigation rather than surveillance monitoring. For investigative and operational monitoring, other options are more attractive.

Table A9. A list of river water quality monitoring methods in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1: Existing SEPA method (water sampling and laboratory analysis)	5	4	5	5	5	5	5
Method 2: Novel & emerging (Automatic, *in situ*, monitoring stations)	5	3	5	4	5	5	4
Method 3: Novel & emerging (Citizen Science test kits)	5	5	2	3	5	2	3

Method 1: For the determination of physio-chemical river water quality elements, whole water samples are collected in the field and chemical analyses are undertaken in accredited SEPA laboratories. The methods are well established and results have been validated through inter-laboratory campaigns. Limits of detection are also known. This methodical approach is regarded as the "gold standard" in terms of data accuracy, but only if samples are processed rapidly (e.g., by in-field filtering), stored at 4 °C in the dark, and analysed quickly. SRP, chlorophyll and ammonium samples are particularly unstable, and ideally should be analysed within 24 hours of collection.

Samples also need to be taken at an appropriate frequency, to capture the rapid water quality dynamics. Monthly sampling (or three-monthly, as the EA are currently undertaking at many sites) will not capture high-flow events. The impact of sampling frequency on load estimation and nutrient signal is demonstrated in Bowes et al. (2009). See Figure A2 below for an example from the River Frome, Dorset.

Figure A2. Example of annual phosphorus concentration data at different sampling frequencies in the River Frome, Dorset.

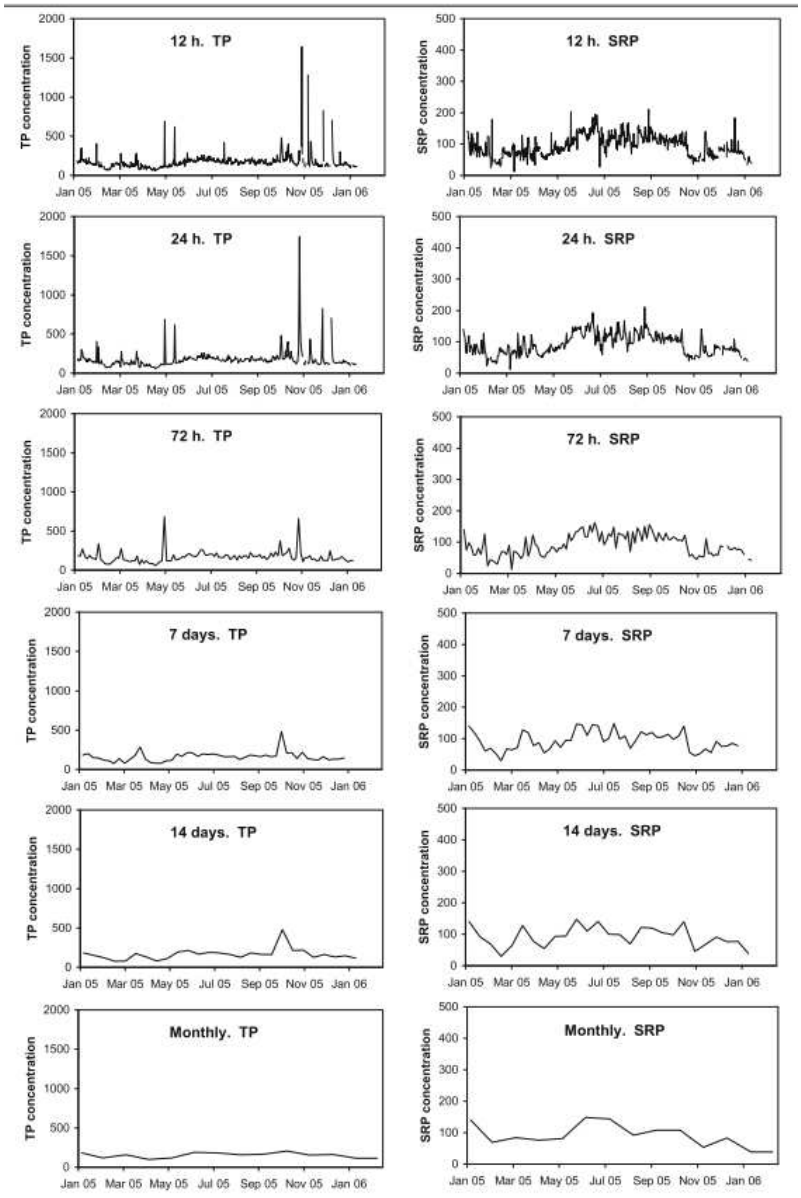


Fig. 4a. Annual phosphorus concentration data at different sampling frequencies. Concentrations are $\mu g\, l^{-1}$.

Method 2: Automatic, *in situ,* monitoring stations. Electronic probes for DO, pH, conductivity, water temperature, ammonium, nitrate, turbidity and total chlorophyll are all well developed and routinely used in EA investigations, Water Company monitoring and as part of academic research. There are also commercially available phosphorus and nitrogen spectrophotometric auto-analysers that can produce excellent quality data, if installed and maintained correctly. A review of the latest high-frequency monitoring systems and how the data can be used is given in (Rode et al. 2016).

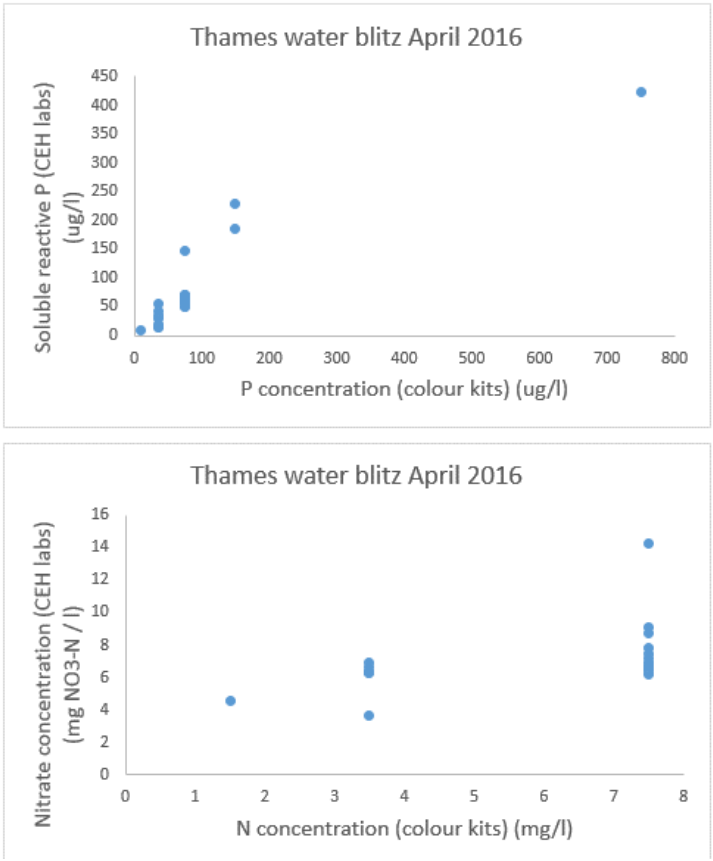
Examples of automated data *vs* laboratory ground-truthing samples are given for the River Thames tributaries in (Halliday et al. 2014) and The Cut (Halliday et al. 2015a).

This high-frequency data produced by automatic monitoring stations is an extremely valuable resource. It can be used to identify the presence of intermittent pollution sources, for example, periodic failures of a sewage treatment works (Bowes et al. 2012), and to develop an understanding of nutrient dynamics (Cohen et al. 2013). It can also be used to determine nutrient pollution sources, using a combination of Hysteresis and Load Apportionment Modelling (Bowes et al. 2015). High-frequency chlorophyll data, alongside supporting physical and water quality data, can be used to identify thresholds for algal growth, leading to an understanding of the timing, magnitude and duration of algal blooms (Bowes et al. 2016).

Method 3: Citizen Science test kits offers the opportunity to get wide spatial coverage of water quality parameters, but quality of data is an issue. There are issues around the choice of sampling site (which could reflect the "agenda" of the sampler); for instance, samples could be taken within the effluent stream of a STW and processed as the concentration of the river as a whole. Samples can also be clustered around locations that are perceived to be a pollution source.

A widely used test kit is the Kyoritsu packtest range http://kyoritsu-lab.co.jp/english/seihin/list/packtest/po4.html. These are used internationally by Earthwatch to monitor phosphate and nitrate concentrations in waterbodies across the world. The six-monthly Thames Water Blitz https://freshwaterwatch.thewaterhub.org/totally-thames-water-blitz results in approximately 700 samples to be taken across the Thames catchment on a single day. The sampling day coincides with CEH's Thames Initiative monitoring schedule that allows the results from the test kits to be directly compared with traditional laboratory analysis (see Figure A3). The phosphorus results from the test kits are robust, although the results are given as coarse concentration categories (<0.02; 0.02-0.05; 0.05 – 0.1; 0.1 – 0.2; 0.2 – 0.5; 0.5 – 1 mg P l^{-1}) that are of little use in academic research. The nitrate test kits are not as robust and less reliable.

Figure A3. Comparison of water quality results from Thames Water Blitz and CEH Thames Initiative monitoring.



Lochs

Biological Quality Elements (BQEs)

Phytoplankton

Methods for loch phytoplankton are reviewed in two parts as new methods for phytoplankton abundance offer very different options compared with methods for phytoplankton composition. Current field and laboratory methods for phytoplankton abundance, measured as chlorophyll-a (chl-a), are standardised across Europe and reliable. We do, however, recommend a potentially more cost-effective alternative of using a hand-held sensor (fluorometer) for measuring chl-a. Almost real-time chl-a products using satellite Earth Observation data will also soon be available and have the potential to dramatically expand the number

of lochs, and monitoring frequency, from current chl-a monitoring by SEPA. These products are, however, currently only validated for larger lochs >10 km^2 area, although data from higher resolution satellites may soon be available for smaller lochs.

Current UK field and laboratory methods for phytoplankton composition using standard microscopy methods are comparable across Europe and SEPA methods are inter-calibrated and reliable. Imaging flow cytometry is a potentially more cost-effective option but would require a "training period" for learning taxa identities and studies to ensure comparability of composition, biovolume and WFD metric results.

Phytoplankton abundance

Current field and laboratory methods for phytoplankton abundance across Europe all adopt a standard approach, chl-a measured spectrophotometrically following an International Standard). Some countries additionally use total biovolume from microscopy counts. The SEPA method and metric is, therefore, comparable, reliable and inter-calibrated. We do, however, recommend a potentially more cost-effective option of using a hand-held sensor (fluorometer) for measuring chl-a. Almost real-time chl-a products using satellite Earth Observation data will also soon be available and have the potential to dramatically expand the number of lochs, and monitoring frequency, from current chl-a monitoring by SEPA. These products are, however, currently only validated for larger lochs >10 km^2 area, although data from higher resolution satellites may soon be available for smaller lochs.

Table A10. A list of monitoring methods for loch phytoplankton abundance in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (Chl-a Spec. method)	4	4	4	5	5	5	4.5
Method 2 Novel & emerging (hand-held sensor (fluorometer))	5	5	5	5	5	5	5
Method 3: Novel & emerging (Earth observation)	5	5	4	4	4	5	4

Method 1. Determination of chlorophyll-a using spectrophotometric method. ISO standard available and recommended for use. Efficiency and cost rated quite high, as most of cost is staff costs in visiting site. Data quality is rated 4 as inter-laboratory differences can be quite large and samples can be affected by contamination or poor storage.

Method 2. A novel and emerging method is the use of hand-held sensors (fluorometers). In vivo fluorometry is the direct measurement of the fluorescence of chlorophyll in living algal cells, using a fluorometer. Examples of fluorometers include: UniLux from Chelsea Instruments (approx. £2000) and the BBE algaetorch (approx. £7000). A study by Pires (2010) reviewed 16 fluorometers from nine manufacturers in terms of cost and ease of use for field purposes. It did not review reliability and detection limit. These systems are generally reliable for chl-a, but can have problems with other algal groups due to similarities in fluorescence between algal groups. The systems have a high capital cost (and maintenance cost) but measurement in the field is quick and easy and requires no laboratory analysis. Several of the cheap hand-held sensors can measures chl-a and Phycocyanin (cyanobacteria abundance) (that is 2 of 3 WFD phytoplankton sub-elements).

Method 3: Earth observation. (Tyler et al. 2016) review suitable satellite algorithms and products that are under development for WFD monitoring. The approach is potentially very efficient and cost-effective at monitoring large Scottish lochs, but currently not applicable to many WFD monitored sites which are smaller than the current recommended applicable size (approx. >10 km^2 but smaller possible dependent on basin shape). An automated monitoring system is being developed in the NERC GloboLakes and EU EOMORES projects using MERIS and Sentinel 3 satellite data (300 m resolution) and should be operational in the near future to increase the frequency of monitoring of large Scottish lochs routinely. The use of higher spatial resolution (10-60 m) data from Sentinel 2 would allow smaller lochs to be monitored. A new operational algorithm selection procedure developed by the Universities of Stirling & Glasgow & PML provides a step-change for surveillance monitoring as it allows automated selection of the optimal chl.-a retrieval algorithms to apply to a broad range of lake types using MERIS & Sentinel data (Spyrakos et al. 2017). Further validation of Sentinel 2 data products, especially for humic and low productivity Scottish loch types, would be advisable.

Phytoplankton composition

Current SEPA field and laboratory methods for phytoplankton composition using standard microscopy methods are inter-calibrated and reliable. Other inter-calibrated methods across Europe are very comparable in approach and metrics used (Carvalho et al. 2013); (Poikane et al. 2015). Imaging flow cytometry is a potentially more cost-effective option but would require a "training period" for learning taxa identities and

validation studies to ensure comparability of composition, biovolume and metric results with existing inter-calibrated methods.

Table A11. A list of monitoring methods for loch phytoplankton composition in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA method (Microscopy)	3	3	4	5	5	5	4
Method 2 Novel & emerging (flow cytometry	5	3	2	4	2	1	2
Method 3 Novel & emerging (imaging flow cytometry	4	3	4	4	5	5	4
Method 4 Novel & emerging (hand-held sensor (fluorometer))	5	3	1	5	5	1	1
Method 5 Novel & emerging (molecular approaches (eDNA))	4	3	1	2	4	1	2

Method 1. Determination of phytoplankton composition is carried out using the Utermohl technique (manual identification and counting using inverted microscope). CEN standards exist for the routine analysis of phytoplankton abundance and composition (CEN 2004); (CEN 2008) and UK (and SEPA) guidance (WFD-UKTAG 2014f) is based on this. Efficiency and cost are rated average, as there are staff costs in visiting site and specialist analysis at the microscope. Data quality is rated 4 as inter-laboratory (analyst) differences are apparent and samples can be affected by contamination or poor storage.

Method 2. Flow cytometry: Flow cytometry is a well-established tool for phytoplankton community analysis, with advantages of rapid, high-throughput analysis and size and fluorescence measurements that allow some discrimination of community composition (e.g., (Read et al. 2014). In spite of these advantages, it is not suitable for replacing conventional microscopy approaches for WFD assessment, mainly due to the lack of taxonomic resolution that it provides (Dashkova et al. 2017); taxa can only be distinguished at the

class level at best. The emerging technology of imaging flow cytometry (IFC) (Method 3) offers much greater potential for WFD monitoring purposes.

Method 3. Imaging flow cytometry (IFC) is a hybrid technology combining the speed and large sample size capabilities of flow cytometry with conventional imaging capabilities of microscopy. The approach is more routinely used in "cleaner" marine phytoplankton studies, as it is potentially much more cost-efficient than routine microscopy, is less biased and less prone to counter identification error (Dashkova *et al.*, 2017). It also captures smaller pico-plankton more effectively. Its main disadvantage over existing conventional microscopy is that it would require a manual training period with some overlap with manual microscopy identification to ensure taxa common in Scottish waters are correctly identified by the machine learning algorithms. Due to the limitations of automated identification it also generally has less taxonomic resolution and greater errors are reported from species-rich phytoplankton samples from natural waters, particularly when more structurally complex colonial or filamentous taxa are present (Jakobsen and Carstensen 2011);(Dashkova et al. 2017). Adoption of this technology would, therefore, require careful comparison to ensure it provided comparable data to existing inter-calibrated methods. In summary, it has the clear potential to be more cost-effective than current methods, but its potential for analysing freshwater phytoplankton composition from Scottish waters is currently untested.

Method 4. Another novel and emerging method is the use of hand-held sensors (fluorometers), for example, the BBE fluoroprobe (approx. £20,000). A study by (Pires 2010) reviewed 16 fluorometers from nine manufacturers in terms of cost and ease of use for field purposes. It did not review reliability and detection limit. From experience, these systems are generally unreliable for distinguishing all algal classes due to similarities in fluorescence between certain algal groups (e.g., overlap between diatoms and dinoflagellates). Cyanobacteria are generally well resolved. Measurement in the field is quick and easy and requires no laboratory analysis but they provide insufficient taxonomic resolution for WFD classification purposes. For these reasons, this method scores very low for data quality and comparability with existing data in Table A11. They also have a high capital and maintenance cost.

Method 5. There is clear potential in developing metabarcoding molecular approaches using whole community analysis of integrated water column or outflow samples. Current activities on metabarcoding approaches in Europe, such as DNAquanet, have not, however, focused on developing DNA libraries of common phytoplankton species, so currently the methods are not close to readiness. For these reasons, this method scores low for data quality, stage of development and comparability with existing data (Table A11). SEPA should maintain a watching brief. These approaches would also not provide the actual abundance of

taxa and so are not comparable with existing inter-calibrated metrics. Future inter-calibration comparison of new metrics based on presence/absence or relative abundance would, therefore, be required.

Macrophytes & phytobenthos

Macrophytes

The current SEPA method, Lake LEAFPACS2, receives the highest overall score of all the evaluated loch macrophyte monitoring methods. This intercalibrated method has undergone a considerable period of development, and is well designed for Scottish standing waters and produces high quality, reliable data suitable for WFD purposes and is relatively efficient and cost-effective. Current SEPA field surveys remain the most suitable method to deliver WFD requirements on composition and abundance. Other alternative intercalibrated and novel approaches, such as the use of macrophyte maximum colonisation depth measurements and hydroacoustics, can provide useful supplementary information on macrophyte coverage and abundance in lochs but still need to be combined with more 'traditional' point-intercept methods to accurately assess macrophyte community composition. Further development of metrics sensitive to hydro-morphological pressures could be developed, as in Finland, but would require elaboration of field survey methods to include emergent communities. Other novel and emerging methodologies, such as satellite remote sensing, although potentially valuable for extending the spatial and temporal monitoring coverage of lochs, cannot yet provide the detailed assessments, particularly of submerged plants, for accurately assessing loch macrophyte communities. Molecular approaches are also not currently well developed enough to assess macrophyte communities in Scottish lochs.

Table A12. A list of monitoring methods for loch macrophytes in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (Lake LEAFPACS2)	3	3	5	5	5	5	4.5
Method 2 Novel & emerging (Hydroacoustics)	5	4	3	5	4	4	4.0
Method 3 Alternative intercalibrated method (e.g., Macrophyte Maximum Colonisation Depth)	4	4	3	4	4	4	4.0
Method 4 Novel & emerging (Remote sensing)	5	4	2	3	3	3	3.5
Method 5 Novel & emerging (Molecular approaches)	5	5	2	2	5	2	3.0

Method 1. The current SEPA WFD loch macrophyte assessment method is Lake LEAFPACS2 (WFD-UKTAG 2014e, 2014g) LEAFPACS2 forms one part of the WFD quality element "macrophytes and phytobenthos". It is primarily designed to detect the impact of nutrient enrichment on UK lake macrophyte communities although it may also be sensitive to other pressures or combination of pressures, for example, shore modification (WFD-UKTAG 2014e, 2014g)The loch macrophyte metrics derived from this method are calculated using field survey data collected in the summer months using a standardised series of transects in a sector sampling approach that conforms to the CEN 15460: 2007 Water quality – guidance standard for surveying of macrophytes in lakes (CEN 2007). Efficiency and cost are rated 3 in Table A12 as the method is labour intensive requiring a minimum of two staff from SEPA's National Monitoring Team to carry it out in each loch macrophyte survey. Ideally, lochs are surveyed annually but it in practice a survey once every six years is regarded as a satisfactory frequency for assessing a loch's macrophyte community. Species level identification of macrophytes requires a reasonably high degree of expertise and, hence, training, but it can largely be done in the field. Other suitability criteria all score 5 in Table A12 as

the method as had long development and the data produced by it is considered reliable and compatible with pre-existing Scottish datasets. The Lake LEAFPACS2 method receives an overall score of 4.5.

Method 2. The use of hydroacoustic techniques has been used for some time to rapidly produce accurate distribution maps of lake macrophytes and associated bathymetry (e.g., (Valley et al. 2005); (Winfield et al. 2007b). Using such techniques, the percent lake volume inhabited by macrophytes along survey transects can be calculated and utilised to provide a rapid assessment of the apparent macrophyte maximum colonisation depth (MCD) in lakes, which can be followed up, if necessary, by a more detailed examination around this point, using traditional sampling methods, e.g. double-headed rake, to determine the actual MCD (Spears et al. 2009). Such hydroacoustic methods can also provide high frequency estimates of percentage volume inhabited (PVI) by which distinct macrophyte community colonisation versus depth relationships can be observed (Spears et al. 2009). As the method is non-destructive, it allows repeatable measures across the same transects annually, or as frequently as needed, allowing unbiased long-term trends in cover, volume-inhabited and maximum growing depth to be reliably recorded. With the recent development of new tools to automate the processing and creation of aquatic habitat maps using "off the shelf" echo-sounder systems with internal GPS and cloud-based software, hydroacoustic methods can now be used to help produce high frequency spatially referenced cover maps of macrophytes present in lakes. For example, the BioBase system (Inc 2014) can be used to produce bathymetries and assessments of the macrophyte communities and bottom characteristics of lakes using hydroacoustic data files recorded by the echosounders. Given their portability, low cost and ability to be effective in relatively shallow water opens up opportunities for such hydroacoustic systems to be more widely used (e.g., citizen science projects using privately-owned boats) to collect repeatable, spatially-referenced macrophyte data in lakes. However, hydroacoustics alone is not a suitable method to deploy if information on species composition of lake macrophytes is required and needs to be combined with other 'traditional' point-intercept survey methods to accurately assess macrophyte species abundance patterns and community composition in lakes (e.g., (Valley et al. 2015). Hydroacoustic techniques receive an overall score of 4.0.

Method 3. Many EU countries have tried to develop WFD compliant lake macrophyte assessment methods, of which 17 have been successfully intercalibrated (Poikane et al. 2015). Nearly all the macrophyte assessment methods (including LEAFPACS2) include sensitivity/tolerance metrics based on species indicators values, e.g. trophic indices, or relative abundance of sensitive or tolerant taxa. Most of the intercalibrated lake macrophyte assessment systems also include some measure of abundance, usually maximum macrophyte colonisation depth (though this is not included in LEAFPACS 2) and the abundance of submerged macrophytes (Poikane et al. 2015). Macrophyte Maximum Colonisation Depth (Macrophyte MCD) can be used as a proxy measure of macrophyte abundance in deeper lakes and is widely recognised

as being sensitive to anthropogenic pressures such as eutrophication, water level fluctuations and climate change, as well as providing a direct measure of lake management activities, for example, if there is a shift from a macrophyte dominated lake to a phytoplankton dominated one. Macrophyte metrics sensitive to hydro-morphological pressures have been developed in Europe, e.g. Finland, and could be highly relevant to Scottish lochs (Hellsten and Riihimäki 1996). Their implementation would, however, require elaboration of field survey methods to include emergent communities. Alternative intercalibrated methods receive an overall score of 4.0.

Method 4. High resolution aerial images of lakes, taken with unmanned drones, allows for the identification, mapping and abundance estimates of non-submerged macrophyte species, e.g. floating and emergent vegetation. Satellite remote sensing also potentially allows for high spatial and temporal coverage with consistency in measurement but normally cannot resolve species within mixed emergent vegetation stands effectively or resolve species identification satisfactorily. Such remote sensing techniques receive an overall score of 3.5.

Method 5. As for rivers, molecular approaches are not currently well developed for lake macrophytes and, where attempted, it has not been possible to identify all the macrophyte species. Hence, for these reasons, this method scores only 2 for data quality, stage of development and computability with existing data in the suitability criteria (Table A12). However, in terms of efficiency, cost and suitability for Scotland, these molecular approaches all score 5 in Table A12. If the methodological problems can be overcome, eDNA could potentially be a very useful tool for monitoring invasive non-native plant species in lakes, particularly for those submerged species that are easily missed or misidentified using traditional monitoring methods, although it may be of limited use in detecting hybrids (Herder *et al.*, 2014). Molecular approaches receive an overall score of 3.0.

Phytobenthos

Current SEPA field and laboratory methods for loch phytobenthos using standard microscopy methods are inter-calibrated and reliable. However, national WFD lake phytobenthos assessment methods, such as Lake DARLEQ2, rely on determining the composition and relative abundance of diatoms, a process that requires time consuming species-level identification and a high degree of staff taxonomic expertise. New metabarcoding approaches are potentially a more cost-effective option but require further development to ensure all common taxa are identifiable and comparability with WFD metric results.

Table A13. A list of monitoring methods for loch phytobenthos in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (Lake DARLEQ2)	3	3	4	5	5	5	4.25
Method 2 Novel & emerging (molecular approaches)	5	5	4	4	5	5	4.5

Method 1. Lake DARLEQ2 (Diatoms for Assessing River and Lake Ecological Quality) forms the phytobenthos part of SEPA's current WFD compliant method for assessing "macrophytes and phytobenthos" in lochs and focuses on the benthic diatom assemblage composition, based on using the Trophic Diatom Index (TDI) metric (WFD-UKTAG 2014g). The DARLEQ2 classification method is designed to primarily detect nutrient enrichment pressures. It is based on the expert-derived riverine TDI metric, which was re-calibrated and applied to lake diatom communities (Bennion et al. 2014).

There are similar uncertainties associated with the Lake DARLEQ WFD method, as outlined earlier for the equivalent River DARLEQ WFD method, so data quality gets a rating of 4 in Table A13. Efficiency and cost is rated at 3 in Table A13 as species-level identifications are time consuming and require a high degree of expertise and training. The Lake DARLEQ2 method receives an overall score of 4.25.

Method 2. As for rivers, new molecular approaches such as metabarcoding have been developed to provide more cost-effective monitoring of lake diatom communities, compared with conventional methods that require time consuming species level identification and a high degree of staff expertise. Method are ready to be deployed by the EA as part of their routine freshwater monitoring programme in 2018 and SEPA are investing in developing in-house capacity in new eDNA techniques for analysing diatom samples. However, species-level assignment of sequences is hindered by a lack of fully populated reference databases of barcodes. Metabarcoding can, however, describe diatom biodiversity to a higher resolution than is usually possible by traditional morphometric methods. Molecular approaches receive an overall score of 4.5.

Benthic invertebrates

The current main SEPA benthic invertebrate surveillance methods, CPET and LAMM, receive the highest scores as they have undergone a lot of development, are well designed and produce high quality, reliable data suitable for surveillance monitoring of the main stressors of nutrient enrichment and acidification prevalent in Scottish lochs. Alternative intercalibrated approaches, such as species richness/diversity metrics and harmonized multimetric ecological assessment tools, also have the potential to be useful additional techniques for assessing other anthropogenic pressures, such as morphological alterations, but would require some development for use in Scotland and would still require labour-intensive sampling and identification of collected samples. In contrast, molecular approaches score highly in terms of efficiency and cost but still require some development work before they will be ready for deployment in Scottish lochs.

Method 1. SEPA currently use two WFD lake methods for assessing the condition of the benthic invertebrate fauna in lochs one of which is the Chironomid Pupal Exuviae Technique (CPET) (WFD-UKTAG 2008a). The CPET metric is designed to be indicative of the impact of nutrient enrichment on the benthic invertebrate biological quality element in UK lakes. The CPET metric is based on the composition of chironomid species or group of chironomid species pupal exuviae collected from lake leeward shores in four monthly samples (two hundred chironomid pupal exuviae in each sample) between April and October. A CPET EQR is calculated based on observed and reference chironomid score values, resulting in an overall EQR representing an ecological status class, as defined by the WFD, ranging from 0 (bad) to 1 (high). As method requires the collection of four monthly samples and requires some taxonomic expertise to identify chironomid pupal exuviae efficiency and cost were rated 4 but all the other criteria were rated 5 to give an overall score of 4.5.

Table A14. A list of monitoring methods for loch benthic invertebrates in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 existing SEPA (CPET)	4	4	5	5	5	5	4.5
Method 2 Existing SEPA (LAMM)	4	4	4	5	5	5	4.5
Method 3 Alternative intercalibrated(species richness/diversity metrics)	4	4	3	3	4	4	4.0
Method 4 Alternative intercalibrated(Harmonized Multimetric Ecological Assessment approach)	4	4	3	3	4	4	4.0
Method 5 Novel & emerging (molecular approaches)	5	5	4	4	4	4	4.0

Method 2. The Lake Acidification Macroinvertebrate Metric (LAMM) is the second WFD compatible method that SEPA use for assessing the condition of the benthic invertebrate fauna of Scottish lochs (WFD-UKTAG 2008b). The LAMM is designed to detect the impact of acidification in UK lakes, based on the benthic invertebrate community. LAMM can be used in lakes that are acid sensitive or in lakes that naturally have a pH lower than 7. Benthic invertebrate samples are collected in the spring, using standardised sampling protocols, from stony-bottomed areas in the shallow littoral of lakes. Method requires only one sample to be collected and analysed in the spring so scores 4 for efficiency and cost in Table A14 but method very specifically targeted for assessing sites sensitive to acidification and thus is not an appropriate monitoring method for all lochs in Scotland. Method given an overall rating of 4.5.

Method 3. Thirteen methods for the lake assessment of benthic invertebrates were successfully intercalibrated ((Poikane et al. 2015) despite macroinvertebrates being considered as one of the most

difficult biological groups for assessing lake quality because of their complex biotic structure, and high spatial and temporal variability (Solimini et al. 2006); (Solimini and Sandin 2012). Sensitivity/tolerance metrics were included in all the national systems reviewed in the intercalibration exercise. These encompassed a range of traditional indices, e.g. ASPT, as well newly developed sensitivity indices, e.g. the LAMM index. Eight intercalibrated methods also utilised species richness/diversity metrics, e.g. EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa richness, while only four methods contained composition metrics and functional metrics were hardly used (Poikane et al. 2015). However, despite the development of all these lake benthic invertebrate assessment systems, it is recognised that there still is a need to reduce the large uncertainty in the metrics used to explain the relationship between lake benthos responses and anthropogenic pressures (Poikane et al. 2015). Method given an overall rating of 4.0 in Table A14.

Method 4. Another alternative intercalibrated method is the new harmonized multimetric ecological assessment approach developed by Miller *et al.* (2013) using benthic invertebrates in relation to a specific stressor in European lakes, in this case, morphological alterations of lake shores. Two biotic multimetric indices were developed based on habitat-specific samples (Littoral Invertebrate based on HAbitat samples, LIMHA) and composite samples (Littoral Invertebrate Multimetric based on Composite samples, LIMCO) which were then correlated with a morphological stressor index to assess the ecological effects of anthropogenic morphological alterations in a range of natural to heavily morphologically degraded lake shores across a number of different geographical regions in Europe. Such stressor-specific assessment tools could allow comparable lake morphology assessments to be made across Europe, as well as complying with WFD standards, and could complement existing benthic invertebrate assessment approaches that are primarily focussed on assessing the impact of lake eutrophication pressures. Method given an overall rating of 4.0 in Table A14.

Method 5. Bista et al. (2017) used a molecular approach by analysing an annual time series of lake eDNA samples in order to describe temporal shifts in the Chironomidae community of a lake in the UK. They were also able to show good correspondence between diversity estimates for this ecologically important group using a variety of sampling techniques and concluded that eDNA metabarcoding can track seasonal diversity at the ecosystem scale. There have also been a number of studies that have used DNA-based methods to monitor macroinvertebrate invasive non-native species in lakes (e.g., (Dougherty et al. 2016). Method is given an overall rating of 4.0 in Table A14.

Fish

Compared with more conventional fish population assessment methods (e.g., gill netting), molecular approaches, such as eDNA metabarcoding, appear to provide SEPA with the most cost-effective means of monitoring lake fish populations. These molecular techniques have been validated for UK fish and are ready for deployment, although there remain questions over estimating fish species abundances. These molecular approaches receive the highest overall score of the evaluated river fish faunal methods. However, novel hydroacoustic techniques are also sufficiently well developed to provide cost-effective, quantitative and non-destructive means of rapidly getting data on fish abundance and distribution within lakes but need to be supplemented by 'traditional' survey methods in order to identify fish species of interest.

Method 1. There are a number of Common Standard Monitoring techniques (e.g., gill netting/hydroacoustics) that are employed for monitoring lake inhabiting fish species of conservation interest, for example, Arctic Charr, whitefish and Vendace (JNCC 2015) but these are not designed for assessing whole lake fish communities and are not carried out by SEPA staff.

Table A15. A list of monitoring methods for loch fish in rank order of suitability. Characteristics are scored on a 1 poor to 5 excellent scale.

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 No existing SEPA method	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Method 2 Novel & emerging (Molecular approaches),	5	5	4	5	5	5	4.5
Method 3 Novel & emerging technologies (hydroacoustics)	5	4	3	4	5	4	4.0
Method 4 Alternative intercalibrated (pressure index based on expert judgement)	3	3	3	3	3	3	3.0

Method 2. Molecular approaches, such as the analysis of eDNA has the potential to provide valuable information regarding the presence and absence of fish species, as well as the composition of whole fish communities. Sampling can be as simple as collecting 0.5-1.0 L water samples across a water body. (Hanfling et al. 2016) successfully used eDNA metabarcoding to monitor UK fish species in a number of large UK lakes, describing both fish diversity and relative abundance. (Hanfling et al. 2016) showed that even for a large lake like Windermere, as few as 10-20 samples were sufficient to capture c. 90% of fish species known to be present – results which were consistent with its known fish community composition, as monitored by extensive netting and other sampling activities such as hydroacoustics. Moreover, the application of the eDNA metabarcoding in Windermere was precise enough to detect the real differences between the fish community composition of the lake's two basins in relation to their different trophic status. (Hanfling et al. 2016) also found that fewer samples needed for smaller standing waters. (Hanfling et al. 2016) were also able to detect sequences of a wide range of non-fish species such as otters and aquatic birds in Windermere. The adoption of such eDNA methodology could allow a significant increase in the number and diversity of waterbodies that are monitored, enabling data on species range shifts to be collected on a national scale (Read and al. 2017). However, estimating fish species abundance using eDNA metabarcoding is regarded as being more problematic although (Hanfling et al. 2016) did show that this approach could produce relative abundance estimates of the fish community in a large lake in line with results accrued from long-term monitoring using more traditional sampling techniques. These molecular techniques also have the potential to be used for the monitoring of invasive non-native species of fish in lakes. As already outlined in the river section of this report, fish species in UK have a complete reference database of fish species barcodes established. Molecular approaches have been developed and validated for sampling UK fish populations and are ready to be deployed. Hence, for these reasons, all the listed criteria in Table A15 are given scores of 5 apart from data quality that is given a rating of 4 because of the perceived problems with estimating fish species abundance using these molecular approaches. This method is given an overall score of 4.5.

Method 3. Hydroacoustic techniques have been used in recent years to show the abundance and distribution of fish in lakes (Jones et al. 2008); (Winfield et al. 2007a) and recent technical advances have allowed hydroacoustic data to be used to assess features such as lake bottom substrates that can be important fish habitats, for example for spawning (Winfield et al. 2015). Advantages of hydroacoustic techniques are that they are cost-effective, quantitative and non-destructive means of rapidly obtaining data on lake fish abundance, demographics and geographical distribution (Winfield et al. 2009); (Winfield et al. 2012); (Winfield et al. 2013). Can combine hydroacoustic methods with limited biological surveys to identify fish species. Hydroacoustic methods alone will not be able to determine age, condition or sex of individual fish

but can be used to complement more 'traditional' fish sampling techniques, such as gill netting, used to assess the populations of fish species of particular interest, for example, Arctic Charr, whitefish species and Vendace (JNCC 2015). Hydroacoustics are rated 5 for efficiency and applicability and 4 for other criteria apart from data quality that is given a score of 3. This method is given an overall score of 4.0.

Method 4. An example of an alternative intercalibrated approach to assess lake fish communities is to use a pressure index, based on expert judgement. Despite fish being regarded as sensitive indicators of environmental stress, the fish community of lakes is an often overlooked aspect of lake monitoring. This is reflected in that only five EU member states have successfully intercalibrated lake fish fauna assessment methods (Poikane et al. 2015). The problems in of using fish communities as indicators of environmental stress are as follows: the diverse range of sampling methods used across Europe; the management of the lakes, e.g. fishing practices, fish stocking, introduction of non-native species have a large impact on natural fish populations; lakes are subject to multiple pressures, and fish indirectly integrate the effects of these on lower trophic levels; high natural variability in fish metrics; fish are mobile and can avoid areas of environmental stress. Hence, there are few significant relationships between fish metrics and specific pressure indicators. One possible way to overcome this problem is to base assessment of pressure on expert judgement; in Austria, Germany and Italy such a response was demonstrated for individual sites. In their overall fish assessments, based on a comparison of all methods employed at a site, a combined pressure index was derived in which all the common pressures were scored and summed up to create an overall pressure index (Poikane et al. 2015). This method not likely to be of wide application in Scotland as there will be a lack of suitable data for many lochs with which expert judgements can be made to relate fish populations with an index of pressure. For this reason, this method is given an overall score of 3.0.

Supporting elements

According to the WFD, the hydromorphological elements that support the biological elements for lochs are:

1. Hydrological regime
    a. Quantity and dynamics of water flow
    b. Residence time
    c. Connection to groundwater body
2. Morphological conditions
    a. Variation in loch depth
    b. Quantity, structure and substrate of the loch bed
    c. Structure of the loch shore

In relation to this, CEN guidance (CEN 2011) indicates that comprehensive data on the hydromorphological condition of a loch contributes to its WFD status classification at high ecological status (HES), only. The

only hydromorphological conditions required for good and moderate status are those that are sufficient to support the WFD biological elements.

Methods for collecting these data and making these assessments are outlined below.

Hydrological regime

Method 1: In terms of assessing the hydrological regime of lochs in relation to WFD, SEPA apply environmental standards to determine the likely impact that disturbances to the loch level regime from abstraction or impoundments will have on loch ecology. These standards stipulate allowable ranges of loss in loch shore habitat (e.g. as a result of loch level drawdown). To determine the likely extent of changes in loch shore habitat, SEPA model the natural (without abstractions) and influenced loch levels and assess the impacts of these using bathymetric data. Where these data are not available, SEPA use expert judgement based upon known drawdown ranges and/or abstraction rates to estimate changes in loch shore habitat. Residence times are estimated by dividing the volume of the loch by the modelled average discharge at the loch outflow.

Method 2: Flushing rates or retention times for lochs can be estimated from catchment characteristsics, loch size and shape, flow data and meteorological records. This has been demonstrated at the European scale using the AGRI4CAST dataset (http://agri4cast.jrc.ec.europa.eu/DataPortal/Index.aspx?o=d) and landcover datasets that are available from from the Centre for Ecology & Hydrology (https://www.ceh.ac.uk/services/land-cover-map-2015). The total amount of water coming into each catchment each day was calculated, and combined with loch volume, and flushing rates and retention times were derived from tehse data. This method was verification lochs across Europe that have flow gauges on their outflows. Although small in number (<100), a good correlation was obtained between the modelled values and gauged values.

Method 3: The Irish EPA use a similar method to the above, but use the bathymetry or volume of the lake combined with measured water levels and rates of inflow, where available, i.e. where there are calibrated flow recorders on lake outflow, or where lakes are used by the ESB for power generation. In other cases, flushing rates and retention times are derived from rainfall and evapotranspiration data.

Table A16. A list of methods for quantifying hydrological regime, ranked in order of suitability. Characteristics of each method are scored form 1 (poor) to 5 (excellent).

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1: Existing SEPA method	4	5	3	4	4	4	4
Method 2: Large scale modelling	4	5	3	4	3	3	3
Method 3: Combination of measured and modelled data	5	4	4	4	4	4	4

Morphological conditions

Method 1: SEPA collect data for some of the elements list above, mainly the structure of the loch shore and the deepest point of the loch, following Loch Habitat Survey (LHS) methodology (SNIFFER 2008b). Principal use of the LHS data is to feed into SEPA's Loch MImAS tool (SNIFFER 2008a), which is used for undertaking WFD classification and regulatory assessments. This tool has been developed to meet SEPA's operational requirements, and for use in Scotland. It is unlikely that SEPA will consider using any other method. So, this is the only method that has been scored for suitability in relation to quantifying loch morphological conditions.

Lake MImAS looks at the extent to which a range of eco-geomorphic attributes are affected by the range of human morphological modifications that are typically encountered on lochs. It does so using expert judgment, built into the tool, of the likelihood of morphological damage arising and of the extent to which this is likely to have an impact on biota (plants, insects, fish), in order to come up with an assessment of WFD status for loch morphology. This also links to biological status. The assessment takes into consideration loch sensitivity through the use of loch typologies.

Table A17. Method for quantifying morphological conditions, ranked in order of suitability. Characteristics of the method are scored from 1 (poor) to 5 (excellent).

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1: Existing SEPA method (Lake-MImAS)	5	5	4	5	5	5	5

Physio-chemical quality elements

Under physical and chemical elements supporting the biological elements, the WFD lists the following elements as being important for monitoring compliance:

1. General physico-chemical elements
    a. Transparency
    b. Thermal conditions
    c. Oxygen conditions
    d. Salinity
    e. Acidification status
    f. Nutrient conditions
2. Specific pollutants
    a. Priority substances being discharged into the waterbody
    b. Other substances being discharged into the waterbody in significant quantities

Table A18. A list of methods for quantifying transparency, thermal conditions, salinity and oxygen conditions, ranked in order of suitability. Characteristics of each method are scored form 1 (poor) to 5 (excellent).

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (e.g., measurement from a boat)	5	3	5	5	5	5	4
Method 2 Novel & emerging (automatic, *in situ*, measurement from a monitoring buoy)	5	1	5	5	5	5	2
Method 3 Novel & emerging (probes fitted to RCV)	5	3	4	1	5	3	2

Method 1. Transparency, thermal and oxygen conditions, and salinity are currently measured from a boat using either a Secchi disk (for transparency), or an appropriate electronic probe system (for temperature, oxygen, salinity). Although the cost of the equipment is relatively low, deployment requires a boat manned by two trained personnel to comply with health and safety requirements. Therefore, overall costs are high.

Method 2. Similar measurements can be made from an *in situ* monitoring buoy that has been fitted with appropriate sensors and a data capture system. A remotely accessible data download facility reduces the need for frequent visits, as does the installation of solar panels to increase battery life and wiper mechanisms to keep probes clean. However, the cost of installation and maintenance of these systems is high.

Method 3. Measurements could be taken with a remotely controlled vehicle (RCV), such as a boat or airborne drone, fitted with appropriate lightweight sensors and a data logging capacity. Efficiency of use is high, because it avoids the need for using a boat and removes the health and safety issues associated with this. However, these methods are unproven and currently still under development (e.g. see http://intcatch.eu/index.php/about-intcatch; https://nimbus.unl.edu/projects/co-aerial-ecologist-robotic-water-sampling-and-sensing-in-the-wild/). The compatibility of the data that they would collect with existing data is unknown, because probes need to be miniaturised to address weight issues and this may affect the quality of data that they can collect.

Table A19. A list of methods for quantifying acidification status, nutrients and specific pollutants, ranked in order of suitability. Characteristics of each method are scored form 1 (poor) to 5 (excellent).

Method	Efficiency	Cost	Data quality	Stage of development	Suitability for Scotland	Compatibility with existing data	Overall score
Method 1 Existing SEPA (water sampling + laboratory analysis)	5	5	5	5	5	5	5
Method 2 Novel & emerging (probes deployed from a boat or monitoring buoy)	5	3	5	3	5	5	4
Method 3 Novel & emerging (Citizen Science test kits)	5	5	2	3	5	2	3

Method 1. For the determination of acidification status, and concentrations of nutrients and specific pollutants, whole water samples are collected in the field and chemical analyses are undertaken in accredited laboratories. The methods are well proven and results have been validated through inter-laboratory comparisons. Limits of detection are also known. However, collection of water samples requires a field visit, preferably using a boat to collect. In some cases, samples are collected from the outflow instead to reduce sampling costs. It is assumed that there is a close relationship between the chemistry of the loch and that of its outflow. Water samples are usually collected at the same time as measurements of transparency, thermal conditions, salinity and oxygen conditions are taken. This reduces sampling costs, to some extent. However, initial investment costs to establish a buoy and/or autosampling system may be high and maintenance costs can be significant.

Method 2. A range of electronic probes have recently become available for determining chemical concentrations in the field. This is especially true for ammonium and nitrate. There are also commercially available phosphorus and nitrogen spectrophotometric auto-analysers that can produce excellent quality data if installed and maintained correctly. Probes or autoanalysers can be permanently installed (e.g. from a monitoring buoy); in this case, the data obtained have a much higher temporal resolution compared to occasional grab sampling from a boat but ground trothing suggests that they are compatible (Halliday et al. 2014);(Halliday et al. 2015b).

Method 3. Citizen Science provides the opportunity to obtain wide spatial coverage of some water quality parameters (especially those that can be recorded using low cost probes, and some nutrient concentrations). However, for many purposes, data quality can be a problem. Issues can include the choice of sampling site (which could reflect the particular interests or "agenda" of the sampler; for example, samples may be clustered around locations that are perceived to be a pollution source. A widely used test kit is the Kyoritsu packtest range http://kyoritsu-lab.co.jp/english/seihin/list/packtest/po4.html, which are used internationally by Earthwatch to monitor phosphate and nitrate concentrations in waterbodies. The 6-monthly Thames Water Blitz (https://freshwaterwatch.thewaterhub.org/totally-thames-water-blitz) collects 700 samples from across the Thames catchment on a single day that coincides with CEH's Thames Initiative monitoring schedule. This allows the results from the test kits to be directly compared with traditional laboratory analysis. The phosphorus results from the test kits have been found to be robust, but the results are given as very coarse concentration categories (that is <0.02; 0.02-0.05; 0.05-0.1; 0.1-0.2; 0.2-0.5; 0.5-1 mg P l^{-1}) rather than as actual concentrations. These may not be accurate enough for WFD monitoring. For nitrate, the test kits are not as robust and the results are less reliable.

Appendix 5.2: Case studies in efficient cost effective monitoring

Current SEPA River Surveillance monitoring

The network is set up to be sampled at a minimum of once every 6 years although some parameters are sampled more frequently, to provide a strong enough signal (diatoms) or because they are also part of the operational network and may require additional sampling if they are on the Good / Moderate boundary.

In general, diatoms and invertebrates are sampled at the same locations and concurrently when sampling year coincides for the two BQEs. Chemistry is sampled by a different team at different times but typically at the same location or in close proximity. Macrophytes will typically be sampled at one site that overlaps with the other BQEs but for sufficient sample robustness up to 5 additional locations may be included to create a strong data signal for a water body. Fish data are usually collected by Fisheries Trusts/DSFB for their own purposes and supplied by some to SEPA, this sampling is, on occasion, augmented by SEPA sampling to fill gaps in the network.

SEPA maintain a National Monitoring Team that carries out routine operational monitoring. SEPA maintain a team of circa 40 ecologists with circa 30 active in the field .They conduct much of the Sentinel Surveillance BQE monitoring. Sampling is typically conducted by 1 staff for invertebrates and diatoms, 2 staff for macrophytes. Hydromorphology is monitored by a separate team, as is hydrology.

Time per sample is planned using a Standard Average Time per activity; 0.63 family level invert identification, 1.9 days per species level identification, 0.63 day for diatom samples, 0.6 day for macrophytes or less (2 persons).

New DNA techniques are attractive to SEPA but they wish to see proven ability. They will decide soon on developing in-house capacity. SEPA have already commissioned DNA analysis for diatom samples from monitoring network (200 samples this year)

The current network is biased towards medium to large rivers and may not be full representative of all stressor – type combinations, although the most common stressor combinations are probably present in some numbers. The ability of a site to represent a reach is something SEPA has considered and clear criteria are documented.

Lessons learned

Invertebrate data is considered to be robust rarely giving an unexpected signal which is not explainable. Diatoms are inherently more variable and thus require more sampling effort to provide a consistent signal.

At the start of full WFD monitoring, diatoms and invertebrates were monitored at river surveillance sites every year – twice per year, spring and autumn. Since then sampling frequency has varied with resources and experience, now for example diatoms are monitored every second year, which allows for a sample integrated over the years which is more reliable.

Sensitivity to pressures is formalised in SEPA with particular BQEs or metrics considered indicative although it is felt this is not always diagnostic and typically requires further resourcing and sampling across groups where a genuine impact needs to be remedied. Some consideration is given to the redundancy in sampling some groups where they are likely to be insensitive to pressures.

The sentinel network is used to some extent to provide evidence for not monitoring some types of rivers as intensively as their frequency in the landscape might suggest. Oligotrophic upland rivers in the highlands are an example. Sites are contained in the network for the purpose of demonstrating these sites are not impacted.

Surveillance monitoring in Finland

Finland is similar to Scotland in having a very large number of lakes and rivers. The river network is between 300-400 sites. Invertebrates and diatoms are sampled approximately every 3rd year while macrophytes are sampled about once every 6 years. Macrophytes are sampled at a sub-set of circa 150 sites. Invertebrates are sampled for 1 season only, in autumn. Where possible samples for different BQEs are taken at the same location or in adjacent locations to facilitate sampling.

Fish are monitored by another government agency and this work is still carried out ‘in-house’. All other groups, water chemistry and hydromorphology are sampled by consultants and the data is passed to open or password protected databases online.

Two years ago, Finland reduced its surveillance network by 20%. The majority of monitoring moved from the state agency SYKE to consultants with additional monitoring carried out by industry as a requirement of discharge licences. The consultants must be certified as having the correct skills before tenders can be awarded. Quality control is not formally carried out thereafter by SYKE. Many of the consultants are ex-

SYKE staff. SYKE retains about 1.5 staff per BQE with specialist expertise and these are supplemented by about 10-15 regional hydrobiologists.

Driving and walking are sufficient to access sites despite the large area of the country.

Surveillance monitoring in the Republic of Ireland

The EPA maintain a 180-site surveillance network for WFD, sampling benthic invertebrates and macrophytes once in 3 years and phytobenthos twice in 3 years. Samples are co-located. Physiochemical parameters are sampled once a month for 12 month for 1 year in 6. For rivers, hydromorphology is recorded using RHAT/MQI. The area covered is about 7/8 that of Scotland and is less geographically complex.

Invertebrate samples are taken once in summer, which is considered to be the time of year the assemblages are most sensitive to stress and impact most detectable. Samples are sorted and identified on the river bank – taking 1-2.5 hrs. Phytobenthos and macrophytes are sampled in a manner comparable to UK monitoring methods.

The EPA has 12 FTE ecologists, 8 of which focus on fresh waters. The team has responsibility for monitoring but is also responsible for River Basin Management Plans. Following a cost benefit analysis 33% of monitoring was outsourced. Time savings are less than expected due to the need to quality control data and samples and time spent on tendering. Movement to framework agreements may reduce the administrative burden. Field teams often combine EPA staff and contractors to help supervise standards and meet health & safety requirements. There are concerns regarding the ongoing ability of the contractors to provide a quality service as experienced skilled staff retire – an issue which is acknowledged in the UK also.

The EPA have adopted the use of ruggedized tablets and electronic versions of data forms. These have had mixed success and require refinement with staff objecting to the additional time required for their use in the field (about 30 minutes per site) and the challenges of using them in adverse conditions.

As in other member, states the EPA are beginning to consider refinements to their surveillance network, to create a data source that can help diagnosis as well as report on system state. This process is refined by an improving understanding of system stresses and responses. This process is being funded through the EPA's grant awarding system and has required substantial investment.

Appendix 5.3: Outcome of UKEOF Workshop 7-8 November 2017

The United Kingdom Environment Observation Framework (UKEOF) held a workshop on the 7-8 November 2017 to discuss the future of Sentinel Monitoring. Representatives from all the UK environment agencies, Natural England and JNCC attended. At our request, the chair of ECOSTAT and a representative from the Irish EPA also attended.

Presentations were give on the interim findings of the statistical analysis and some of the findings regarding emerging methods and sampling strategy. Presentations were also given on the Irish monitoring networks and developments in Europe regarding the sensitivity of BQEs to pressures across member states. This was followed by an interactive workshop that focused on future approaches to the development of sentinel-type monitoring networks and the potential to add value through collaboration. Discussions were broad and wide-ranging; points raised of direct relevance to this review are addressed below:

Attendees reacted positively to the SENTINEL statistical analysis. NRW are intending to carry out their own statistical analysis of the representativeness of their networks. EA are also considering the possibility of doing something similar. It was revealed that only Germany collects more samples than the UK, for WFD reporting.

Attendees are interested in the emerging methods. The EA are already trialling an eDNA method for diatoms and the project is progressing well. The EA suggested that there would be marginal savings in using hand held chlorophyll sensors compared to suing traditional techniques. It was generally acknowledged that new methods would need to provide data compatible with existing methods.

There was an acknowledgement by the chair of ECOSTAT that emerging technologies had potential and he suggested that where application could be demonstrated and a consensus agreed among member states that would generate the potential for change at an EU level.

There were few suggestions for specific questions that the agencies wanted answered by a surveillance network other than the core business of tracking change through time. There was a general feeling that there is potential within the networks and strategic questions could be formulated.

Better means of collaboration and information exchange were considered necessary during the forthcoming period of change.