

14. Biomonitoring protocols for example scenarios

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14.1. Introduction

Currently, assessment of N impacts on condition and long-term integrity of designated sites is not part of Condition Standards Monitoring (CSM) (See Section 15.1). If the conservation agencies are to apply biomonitoring methods at specific sites, identified as being potentially impacted by N inputs, it is necessary to have a set of protocols, which could be applied for a range of N input types and a wide range of habitats. The protocols presented below have been tailored to the N input source (i.e. direct gaseous NH₃ at a point source or diffuse wet N deposition) and not at any specific habitat type.

The objectives of the current study were to produce biomonitoring protocols, which could be applied by conservation and environment agency staff at generic habitats with different N sources. The scenarios needed to reflect different situations to which the agencies are likely to apply nitrogen biomonitoring methods. As each situation will be different, a generic approach is necessary. However, factors will be identified within each scenario, which have specific importance.

Four scenarios were identified by the Project Steering Group for assessment of the applicability of biomonitoring methods:

1. Assessment of N effects on plant communities in the vicinity of a large poultry farm (NH₃ dominated),
2. Assessment of the impacts of a large industrial point source close to a SAC (NO_x dominated),
3. Assessment of the effects on site condition/integrity from long range N deposition inputs on an upland site,
and
4. Assessment of the impacts on a lowland site with critical load exceedance, but no N point source. This scenario is particularly important as a number of the sites used in the UK extensive study (Section 9) would be in this category.

These monitoring protocols could include an integrated approach of using physical/chemical monitoring of nitrogen inputs as well as one or more bio-monitoring methods fit for the particular application.

It should be noted that the scenarios 1 and 2 could be applied in the impact assessment required under the Habitats Regulations/PPC Regulations as part of an application for a PPC permit. Such requirements could include environmental monitoring to assess potential impacts directly associated with the regulated installation.

14.2. Scenarios

14.2.1. Scenario 1. Assessing the impacts of a large poultry farm on a lowland SAC

This scenario covers situations where a SAC is adjacent to a farm unit with a defined N source. The objective of monitoring in such a scenario is to assess current impacts of the farm on the site in relation to site integrity (i.e. effects) and to provide greater confidence in the estimates of concentrations/deposition of NH₃ to the site (i.e. exposure) (Table 14.1).

With a defined N pollution gradient away from an intensive farm unit, a transect (50-100 m upwind to 500-600 m downwind of the farm unit) can be established with defined sampling points that also ideally include physical monitoring of atmospheric NH₃ concentrations. These points should be distributed exponentially at distances (m) from the farm. The length of the transect is dependent on the uniformity of the habitat, the size of the NH₃ source and the land use downwind of the farm but should be at least 250-600 m in length in order to have a control point with background NH₃ concentrations, which are representative of the surrounding area.

An additional scenario with the SAC not adjacent to the farm unit but situated between 200-500 m from the NH₃ source will also be considered in this section. This case is much harder to attribute impacts to the N source, as the gradient in gaseous NH₃ concentration will not be as strongly defined as the situation with the SAC adjacent to the agricultural source.

The results from the current study have established that there are simplified biomonitoring methods that could be applied to assess the current N impacts and also monitor the integrity of a SAC close to a poultry farm. The biomonitoring method applied would be dependent on habitat type and the interest features of the SAC. The advantage of biomonitoring over sole reliance on physical monitoring is that it will identify changes/effects/integrity on the vegetation associated with the SAC vegetation in relation to a known N input, whereas physical monitoring alone can only identify that there is potential for N impacting.

Table 14.1. Preliminary decision tree for selection of nitrogen bioindicator methods for an agricultural point source (poultry farm) at a lowland SAC.

<p>1: To assess effects of poultry farm on lowland SAC.</p> <p>a. Assess the impacts of enhanced N concentrations or deposition? <i>Go to 2.</i></p> <p>b. Assess the magnitude of atmospheric N concentrations or deposition? <i>Go to 7</i></p>
<p>2: Obvious local source present: Visual assessment of the site and odours (e.g. livestock smell) may be sufficient. Use local wind rose data if this is available. In the case of point or line sources, measurements using bioindicators should focus on differences with distance from the source.</p> <p><i>Set up a transect</i> extending from 100 m upwind to 600 m downwind of farm. The downwind distance will be dictated by the uniformity of habitat along the NH₃ gradient. The important criterion is that the furthest point along the gradient represents the background ambient concentration for the surrounding area. Atmospheric monitoring may be necessary depending if site has current monitoring or has had recently. As wind can blow from any direction it is important for the upwind site to be at a sufficient distant to give a background NH₃ concentration which is representative of the surrounding area i.e. at least 100 m.</p> <p>3: Make a species list of higher plants and bryophyte species in the ground flora.</p> <p>Determine <i>mean Ellenberg N Index of higher plants and bryophyte species</i> for at least 5 positions along transect. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history.)</p>

4: Site wooded or trees present? Go to 5

Site treeless. Go to 6.

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophYTE scores.* (Notes: The method gives a broad indication of NH₃ exposure). Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata (See Appendix III for details of macrolichen sampling protocols).

6: Standardised response to nitrogen deposition. *Method: Standardised grass plants (Lolium or Deschampsia)* (see Section 5 and Appendix II). (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants. For long-term application, a series of plants must be exposed over time).

7: Trees absent Go to 8

Trees present Go to 9

Atmospheric monitoring Go to 10

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). A well-studied parameter.

Soluble ammonium concentration of foliage. (Note: A newly identified parameter, which appears to show a very large response to enhanced N). Particularly large responses for pleurocarpous mosses (See section 8 & 10).

9: If trees present you can do simplified macrolichen frequency (See number 5 for details)

10: Atmospheric NH₃ concentrations: Passive NH₃ samplers (ALPHA) available for low cost atmospheric monitoring. The other N pollutants deposition can be obtained from national background maps (See Piddles Wood deposition data for example).

In cases where the SAC is not adjacent to the agricultural point source but in the vicinity (i.e. 200-600 m downwind from the source) the above biomonitoring decision tree could still be applicable. However, due to potentially lower N concentration gradient/deposition the attribution of impacts on the vegetation will be harder to determine, especially in the short term. In such cases air dispersion modelling and physical atmospheric monitoring may be initially more appropriate. Depending on the outcome of these, longer-term biomonitoring could be applied. The single or combined biomonitoring method applied will depend on the NH₃ concentration gradient and the type and uniformity of habitat. The habitat type is important as gaseous NH₃ dispersion and deposition processes are dependent on the habitat structure as well as meteorological conditions i.e. NH₃ is an extremely reactive gas with deposition to surfaces greatest close to the source and when surfaces are wet.

Ellenberg N Index would indicate any longer term changes resulting from the pollutant source. If trees were present the use of the simplified acidophyte/nitrophYTE macrolichen method would indicate any change in species frequency and composition which would be an 'early indication' of impact.

In general, this scenario where the agricultural source is not adjacent to the lowland SAC is much more difficult for the conservation and environment agencies to legislate against as the critical load may not be exceeded at these sites, particularly as the agencies place great emphasis on the critical load values when determining permit conditions. It is important for site officers when assessing a site, to identify the pollutant source (or proportionate sources) and strength, its position in relation to the SAC (i.e. a SAC down-wind of the prevailing wind direction will be at

a greater risk than an upwind site) and the potential short and long term impacts to the interest feature.

Depending on the above, the use of biomonitors to determine short-term change may be inappropriate. However, the use of a combination of physical monitoring and longer-term biomonitoring may be the most appropriate approach to provide an early warning signal of the potential impacts to SACs in the vicinity of agricultural point sources.

14.2.2. Scenario 2. Assessing the impacts of a large industrial point source on a local SAC

This scenario assesses the impacts or potential impacts associated with NO_x emissions from a known point source on a local SAC. Unlike the agricultural point source (Section 14.2.1), emissions of NO_x from point sources generally do not lead to very high local concentrations or deposition. The determination of local NO_x concentrations from a single point source can also be complicated by the type or combination of other local N pollutants emitted and their concentrations i.e. vehicle emissions. NO_x emitted from a stack (depending on the height of the stack) could impact locally or ground at some distance from the stack with regional scale implications. Therefore, a primary assessment to identify the areas most at risk (i.e. position of maximum ground level NO_x concentration) from the source is essential. Depending on the concentration and habitat, this primary assessment will determine if biomonitoring could add to the overall assessment using air dispersion modelling and physical monitoring. It is always important to have physical monitoring or a historical record of the pollutants emitted. It would also be useful for assessment of current status/condition and also for long term integrity to have habitat species composition data, foliar N concentrations, or physical monitoring of the SAC prior to the industrial plant being established.

Table 14.2. Preliminary decision tree for selection of nitrogen bioindicator methods to assess impacts of a large industrial source on a SAC.

1: To assess effects of large industrial point source on local SAC

- a. **Assess the impacts** of enhanced N concentrations or deposition? *Go to 2.*
- b. **Assess the magnitude** of atmospheric N concentrations or deposition? *Go to 7*

2: Obvious local source present: Visual assessment of the site and odours. As the installation is a known emitter of NO_x and other pollutants, obtain emission data from either the installation or through the Pollution Release Inventory (e.g. SEPA are custodians of the Scottish Inventory), any ecological impact assessments done as part of an EIA prior to the plant being established and obtain previous condition assessment of SAC.

Use air dispersion modelling to identify areas that are potentially most at risk from NO_x emission source.

Having established areas at risk, set up transect extending from 100 m upwind to 500 m downwind of and around the perimeter of the source making use of wind rose data etc which should be provided. The length of the transect will depend on the habitat uniformity and the emission size of the installation. Take account of any additional sources in the area such as roads and other industrial plants. If possible find a 'clean' area with a similar habitat for a 'normal' comparison.

3: Make a species list of higher plants and bryophyte *species* present immediately around the source and in the ground flora.

Determine *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 positions along transect. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with a well defined site history) along the transect. Ellenberg Index could indicate enhanced N deposition at the SAC.

4: If site wooded or trees present? Go to 5

Site treeless. Go to 6.

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophyte scores.* This method is more suited to detecting NH₃ impacts but may also provide some evidence of acidification resulting from NO_x exposure and if sufficient time standardised grass response can be included.

6: Standardised response to N deposition. Suitable if area is vandal free. *Method: Standardised grass plants (Lolium or Deschampsia).* (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants. For long-term application, a series of plants must be exposed over time.) The suitability of this longer-term approach will be dependent on the source strength.

7: Trees absent Go to 8

Trees present Go to 9

Atmospheric monitoring Go to 10

8: Foliar responses to additional nitrogen and other pollutants.

Total tissue N of foliage. (Note: Suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna*). Important to identify the interest feature and if possible use the species listed.

Total tissue content of sulphur (S) and heavy metals if presence is suspected.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a very large response to enhanced N). Particularly large responses for pleurocarpous mosses.

Apply low cost methods for physical measurement of NO_x concentrations (e.g. diffusion samplers) in combination with above.

9: If trees are present a simplified macrolichen frequency can be performed.

10: Atmospheric NO_x concentrations

There is no bioindicator method currently available to indicate NO_x concentrations. Methods may yet be developed using lichens, but apart from near NO_x point or line sources responses appear to be stronger to NH₃. The established biomonitoring methods for NH₃ would give an indication of impact on and status of the site (depending on point source strength for NO_x and presence of other sources) but it would be more difficult to directly attribute these to a specific NO_x emission source.

Apply low cost methods for physical measurement of NO_x concentrations (e.g. diffusion samplers)

This scenario is much more complex than the agricultural one for conservation and environment agencies to legislate for (Section 14.2.1.). Biomonitoring methods can easily be applied to the agriculture scenario without the need for air dispersion modelling, as there is a defined localised gradient of NH₃ concentration from a known point source with the NH₃ concentrations being determined by physical monitoring along the concentration gradient. With NO_x it could be difficult to define a gradient of concentration or whether the critical load is being exceeded for the targeted SAC (depending on the distance to maximum ground concentration, other localised sources of the N within the proximity of the SAC and habitat type). These factors make the use of biomonitors much less robust for attributing impacts to NO_x sources.

Short-term NO_x impacts on the interest features of the SAC could be difficult to measure.

However, if biomonitoring was required, the use of the sensitive macrolichen method could be used as an early indication of N impact to the SAC.

The combination of physical monitoring, modelling and selected biomonitoring method/s may be the most appropriate approach for sites with a NO_x source to give an indication of impacts on the site and its long term integrity.

14.2.3. Scenario 3. Assessing the impacts of long-range wet dominated N inputs at an upland SAC

The assessment of N impacts on remote upland areas with a diffuse source of N deposition is more complex than for the SAC's with a defined point source as in scenarios 1 and 2. The N deposition to these sites may be relatively small but continued N accumulation over a longer period could lead to changes in integrity. Upland vegetation is also adapted to low N availability and would be sensitive to large increases in available N through increased wet N deposition (as either reduced or oxidised N). It is important to note that other factors such as management practices and climatic changes could also be impacting on the SAC and causing potential changes in integrity, which are independent of N deposition but could make it difficult to determine if observed changes are due to N deposition or other factors (climatic, management practices, grazing pressures) or a combination of the two.

Table 14.3. Preliminary decision tree for selection of nitrogen bioindicator methods for upland SAC sites.

1: To assess impacts of long-range N inputs at an upland SAC site
<ul style="list-style-type: none">a. Assess the impacts of enhanced N concentrations or deposition? <i>Go to 2.</i>b. Assess the magnitude of atmospheric N concentrations or deposition? <i>Go to 7</i>
2: Obvious local source present: There is unlikely to be any local point sources at this type of site. The source will probably be long-range dominated by diffuse wet N deposition. Enhanced N inputs from occult precipitation may be important depending on the altitude of the site i.e. increasing ionic concentrations in precipitation by at least a factor of two. If livestock are present, check stocking rates for local variation in N deposition. Determine N input for habitat from sources such as APIS. Set up a number of replicate quadrats around the site for long term integrity/ species composition change monitoring.
3: Make a species list of higher plants and bryophyte species in the ground flora. A species list of the site is important for long-term assessment of integrity changes but the use of the Ellenberg N Index is unlikely to give a strong indication of N impact at these diffuse N sites. If Ellenberg Index is applicable then determine the <i>mean Ellenberg N Index of higher plants and bryophyte species</i> for at least 5 quadrats at the site (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history). This survey method would be beneficial as part of a temporal assessment, or wider spatial survey and not as a one off assessment. The use of the simplified acidophyte/nitrophyte method could also be a useful initial assessment.
4: Site wooded or trees present? <i>Go to 5</i> Site treeless. <i>Go to 6.</i>
5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. <i>Method: Survey of key lichen species and calculation of Acidophyte / Nitrophyte scores.</i> (Notes: The method gives a broad indication of NH ₃ exposure. Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata. See also Bark pH.
6: Standardised response to N deposition. <i>Method: Standardised grass plants (Lolium or Deschampsia).</i> (Note: the method is good for visual demonstration and directly records additional N absorption by the plants). For long-term application, a series of plants must be exposed over time. As the N inputs will be low, the use of standardised grass plants will require long-term

exposure (6-12 months) and modified protocols to assess N impacts. The use of other slow growing species such as *Agrostis canina* and *Nardus stricta* could be considered but these would require further field testing. The selection of species is dependent on the habitat type. The ideal situation would be if the selected species was occurring naturally at the selected site. This method is not suitable for use in determining long term integrity of a site.

7: Trees absent Go to 8

Trees present Go to 9

Atmospheric monitoring Go to 10

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). The use of mosses and *C. vulgaris* are well-studied pollution biomonitoring parameters. Tissue N in moss species could be more related to concentration in the precipitation than N deposition. Due to possible seasonal variations in foliar N concentration and the importance of the hydration status of pleurocarpous mosses sampling should be carried out in late spring or early autumn. The regular sampling of pleurocarpous mosses for foliar response will give a long-term indication of whether N is impacting on the site. Tissue N concentrations in *Calluna vulgaris* or other dwarf shrubs may be informative for long-term integrity. However, care should be taken to sample for same age stands as the % N concentration in *Calluna vulgaris* varies with the age of the stand. Younger plants (0-6 years) have a higher % N than older plants.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a large response to enhanced N). As good a method as total tissue N concentration.

9: If trees present can do macrolichen frequency/composition.

10: Atmospheric N monitoring: Physical measurements are necessary to determine actual N inputs to the site so that management practices can be controlled and disturbances, which might contribute along with enhanced N deposition to changes in vegetation composition, are prevented.

The important measurements are wet deposition N (NH_4^+ and NO_3^- concentrations in precipitation. Measurements of NH_3 could be important if in an agricultural area.

14.2.4. Scenario 4. Assessing the impacts on a lowland site, which from the national maps shows an exceedance of the nitrogen critical load but where there is no strong local point source

The objective of this scenario is to assess if biomonitoring methods can be used to help in the judgement of whether nitrogen is affecting site condition presently or as an indicator of this in the long term. As 23 out of the 32 sites in the UK extensive study exceeded their habitat critical load this particular scenario has important implications for possibly a large number of SAC's throughout the UK.

Table 14.4. Preliminary decision tree for selection of nitrogen bioindicator methods for lowland SAC sites

1: To assess impacts of long-range N inputs at an lowland SAC site

a. **Assess the impacts** of enhanced N concentrations or deposition? Go to 2.

b. **Assess the magnitude** of atmospheric N concentrations or deposition? Go to 7

2: Obvious local source present: There is unlikely to be any local point sources at this type of site. The source will probably be long-range diffuse wet dominated N deposition. However, in agricultural areas the dry N deposition (as $\text{NH}_3\text{-N}$) will be as important to the overall total N deposition as wet N inputs from precipitation. If livestock are present, check stocking rates for local variation in N deposition. Determine N input for habitat from sources such as APIS.

Set up a number of replicate quadrats around the site for long term integrity/ species composition change monitoring.

3: Make a species list of higher plants and bryophyte species in the ground flora.

A species list of the site is important for long-term assessment of integrity changes but the use of the Ellenberg N Index is unlikely to give a strong indication of N impact at these diffuse N sites. If Ellenberg Index is applicable then determine the *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 quadrats at the site. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history.) This survey method would be beneficial as part of a temporal assessment, or wider spatial survey and not as a one off assessment. The use of the simplified acidophyte/nitrophyte method could also be a useful initial assessment.

4: Site wooded or trees present? *Go to 5*

Site treeless. *Go to 6.*

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophyte scores.* (Notes: The method gives a broad indication of NH₃ exposure. Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata. See also Bark pH.

6: Standardised response to nitrogen deposition. *Method: Standardised grass plants (Lolium or Deschampsia).* (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants). For long-term application, a series of plants must be exposed over time. As the N inputs will be low, the use of standardised grass plants will require long-term exposure (6-12 months) and modified protocols to assess N impacts.

7: Trees absent *Go to 8*

Trees present *Go to 9*

Atmospheric monitoring *Go to 10*

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). The use of mosses and *C. vulgaris* are well-studied pollution biomonitoring parameters. Tissue N in moss species could be more related to concentration in the precipitation than N deposition. Due to possible seasonal variations in foliar N concentration and the importance of the hydration status of pleurocarpous mosses sampling should be carried out in late spring or early autumn. The regular sampling of pleurocarpous mosses for foliar response will give a long-term indication if N is impacting on the site. Tissue N concentrations in *Calluna vulgaris* or other dwarf shrubs may be informative for long-term integrity. However, care should be taken to sample for same age stands as the % N concentration in *Calluna vulgaris* varies with the age of the stand. Younger plants (0-6 years) have a higher % N than older plants.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a large response to enhanced N). As good a method as total tissue N concentration.

9: Atmospheric N monitoring: Physical measurements are necessary to determine actual N inputs to the site so that management practices can be controlled and disturbances, which might contribute along with enhanced N deposition to changes in vegetation composition, are prevented. Measurements of NO_x concentrations could be important at this type of scenario when localised sources of NO_x are identified. The monitoring of dry gaseous NH₃ and also wet N deposition (NH₄⁺ and NO₃⁻ concentrations in precipitation) are also required.

14.3. Conclusions

- The application of biomonitoring techniques at sites, which fit Scenario 1 (poultry farm on lowland SAC) have been shown in Section 8 to be robust and give an indirect indication of condition, but a direct signal of N impact on the SAC. They also give an 'early warning' signal indicating potential changes and an effect on site integrity.
- A number of different biomonitoring methods could be applied in Scenario 1 depending on the habitat and the designated interest features.
- Biomonitoring methods could be applied to Scenario 2. The method used would depend on the habitat, interest feature and the concentration and type of atmospheric pollutant.
- The availability of a transect with uniform habitat is important for Scenarios 1 & 2 if the epiphytic or ground flora are to be used. Standardised plants give an indication of N impacting on the site but do not indicate the effect on the existing vegetation/interest feature. However, if the habitat is variable and the same indicator species cannot be found along the transect, then standardised plant could be used.
- Scenario 3 is the most difficult situation to apply biomonitoring methods. The diffuse wet N deposition sources together with possible climatic change and management practices make it very difficult to determine if N is impacting. The advantage of using foliar N concentration biomonitors would be to give a long-term record of N concentrations, which could give a reasonably robust indirect 'early warning' of potential impacts of N. As pleurocarpous mosses are prevalent in uplands habitats their use would give added value to CSM.
- As with scenario 3, the upland SAC's, the undefined diffuse source attribution of Scenario 4 makes it more complex to assess current and long-term changes due to N impacts. Other factors such as management practices, climatic variables could also be impacting on the SAC.
- The main difference between Scenario 3 and 4 is the potential level of N deposition. In Scenario 3, N was probably not impacting on the condition of the site, whereas, in Scenario 4 it is recognised as doing so (according to the scenario definition). However, in upland sites occult precipitation could increase NO_3^- and NH_4^+ concentrations by a factor greater than 2 and lead to critical loads exceedance. The use of biomonitors in Scenario 4 would give an indication of the current effect on the habitat and could also be used to determine changes in integrity if applied as part of a regular monitoring programme.