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A seasonal survey of surface water habitats within the River Spey basin, Scotland: major nutrient properties

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ABSTRACT

1. Current monitoring strategies of governmental organizations tend to be focused on relatively large flowing and standing waters, and until recently those polluted by point sources. Consequently areas of high conservation interest tend to be understudied, and defining reference conditions, as required by current legislation, is difficult to achieve.

2. In order to address this imbalance, water samples have been collected and analysed once in each of four seasons during 2003 from 72 locations within a 100 km^2 area of the oligotrophic River Spey catchment in NE Scotland. The sampling design included examples of running water (headwater streams and the main rivers) and standing water (lochs, lochans, pools, ditches, backwaters, bogs). Altitude ranged from 220 to 980 m and incorporated a climatic regime from cool temperate to sub-alpine. Each sampling campaign targeted low-flow conditions to evaluate steady-state nutrient concentrations.

3. Concentrations of the major soluble nutrients nitrogen and phosphorus demonstrated high spatial and temporal variability, with soluble organic and molybdate unreactive forms generally being dominant. Concentrations of ammonium-N, nitrate-N and soluble reactive phosphorus were extremely small, with 50% of samples falling below 8, 5 and 1 μg L^{-1}, respectively, during spring and summer.

4. Sampling sites were grouped either by water-body type or by the properties of their immediate biophysical zone. Together these two groupings explained 33–38% of the variance in water chemistry. Certain changes were detectable across most habitats and biophysical zones.

5. A decline in the concentration of nitrate that occurred in reaches downstream from certain headwater streams draining the mountain areas indicated the potential for its within-stream utilization. Inorganic N dynamics differed between small streams and large rivers.

6. Landscape-scale patterns were recorded in spring and summer nutrient availability with inorganic N and P thresholds (arbitrarily defined) of 10 and 1 μg L^{-1}, respectively.

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KEY WORDS: biophysical zone; aquatic habitats; nutrient thresholds; N:P ratio; reference conditions; carbon; nitrogen; phosphorus

INTRODUCTION

The chemical composition of individual water bodies is sensitive to various landscape attributes that include geology, geomorphology, soil type and land cover. Catchment-based studies, often nested within biophysical zones, either on lakes (e.g. Maberly *et al.*, 2003) or streams (e.g. Edwards *et al.*, 2000) have had some success in predicting water chemistry using landscape attributes. However, the situation becomes more complicated in floodplains where spatio-temporal connectivity plays a central role (e.g. Grieve *et al.*, 1995). Moreover, the internal processes occurring within water bodies such as water turbulence, food-web interactions or sediment water column physico-chemical exchange may also be significant (e.g. Scheffer *et al.*, 1993). Floodplains and wetland systems have undergone extensive modification as a result of drainage and canalization (e.g. Krause, 1971) and their restoration is an important feature of current European legislation. Therefore, an approach based on habitat types and biophysical zones, in addition to defining the catchment, would allow a more inclusive sampling of all water-body types.

There has been a growing requirement to define and predict nutrient reference conditions and critical nutrient thresholds, and to identify nutrient limiting conditions (e.g. USEPA, 2000; Moss *et al.*, 2003). Atmospheric pollution and changes to land cover caused by human activities (past and present) have had an impact even on some of the most remote locations (e.g. Jones *et al.*, 1993; Kopacek *et al.*, 1995). Establishing reference conditions may therefore be difficult. However, there is a need to assess the state of aquatic ecosystems, particularly in relation to their nutrient dynamics, under the European Water Framework Directive 2000/60/EC (WFD).

In Scotland, field surveys and monitoring of aquatic chemistry have been undertaken for a variety of purposes (Harriman and Pugh, 1994). Historical background data in the Cairngorm Mountains collected by Gorham (1957) have been subsequently re-analysed and used in acidification studies (e.g. Helliwell *et al.*, 2002). Scottish Natural Heritage (and its predecessors), a national agency for nature conservation, determined the physico-chemistry of many lochs during macrophyte surveys as initiated by Spence (1967), although generally on a one-off basis during the period of vegetation growth (Palmer *et al.*, 1992). Typically, one of two strategies have been adopted for the routine chemical sampling of surface waters. This has involved either a network of monitoring sites, sampled on a regular timescale (e.g. Benzie *et al.*, 1991), or a more targeted but less frequent sampling of specific water-body types, such as those thought to be sensitive to acidification (UK Critical Loads Advisory Group, 1995). Much less common are systematic surveys designed to provide quantitative information across a diverse range of aquatic habitat types, especially where these also include a component of seasonality. Information of this type is becoming increasingly significant owing to legislation such as the WFD, particularly where a high diversity of habitats can constitute an important natural resource possibly supporting a high biological diversity.

This paper describes the results of such a systematic survey of a mid-section of the River Spey (NE Scotland) that has a high intrinsic ecological diversity and value at both local and national scales. We hypothesize that biophysical zones and habitat types are useful and complementary categorical descriptors of water chemistry. More specifically, (i) the spatial and temporal ranges in major nutrient concentrations and the relative proportions of organic and inorganic forms are quantified; (ii) the probability of exceedance of arbitrarily defined threshold concentrations and nutrient availability during the biologically active growing period is illustrated; and (iii) the implications for conservation and management are explored.

RIVER SPEY CATCHMENT

The River Spey rises from the Monadhliath Mountains and flows 157 km north-eastwards to the North Sea (Figure 1(a)). The catchment area (*ca* 3000 km^2) is underlain by crystalline rocks (schists and gneisses) of Cambrian age or older, and intruded in places by granitic rocks (Maizels, 1988). Much of the current

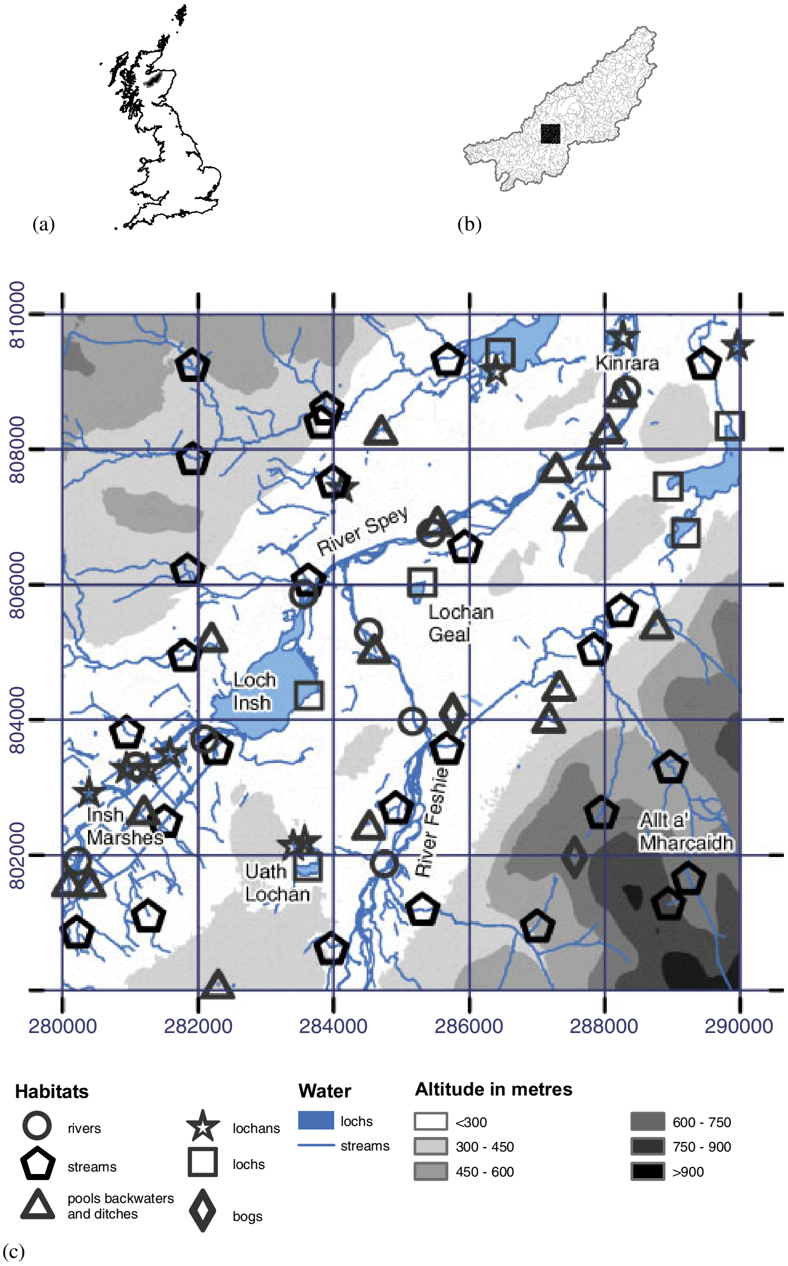

Figure 1. Location ((a), (b)) and sampling sites within the 10 × 10 km grid square (c). © Crown copyright 2006. All rights reserved Macaulay Institute GD27237X 2006.

landscape reflects the action of successive glaciations. The River Spey has a generally alpine flow regime characterized as having maximum discharges during spring snow-melt periods and minimum flow during summer months. Its longitudinal profile differs markedly from other upland Scottish rivers owing to a relatively flat middle section (slope 1:1200 over 60 km; Goody, 1988). Part of this middle section of the Spey represented the focus of the present study.

The main stem of the River Spey is a Site of Special Scientific Interest (SSSI) and a candidate Special Areas of Conservation (SAC), part of the Natura 2000 network, mostly on the basis of its large populations of Atlantic salmon (*Salmo salar*), sea lamprey (*Petromyzon marinus*), otter (*Lutra lutra*) and freshwater pearl mussel (*Margaritifera margaritifera*). The study area is part of the Cairngorms National Park, an area of great conservation value (Gordon *et al*., 1998; RCAHMS and Historic Scotland, 2001; Gimingham, 2002). Further information can be found in Jenkins (1988) and NERPB (1995).

Recently, the River Spey catchment management plan (SCSG, 2003) stated that limestone outcrops have no substantial chemical effect on the watercourses. This is a valid comment when it refers to the main tributaries and rivers, because of the thick glacial drift deposits at the bottom and the sides of the valleys (Benzie *et al*., 1991); however, local heterogeneity has been reported before (Gorham, 1957). Hence the catchment management plan may underestimate the potential for freshwater life associated with higher pH conditions. Water quality as assessed by SEPA uses macroinvertebrates and certain chemical parameters, primarily designed to monitor impacts of point-source pollution. The catchment review highlighted that diffuse pollution indices would be more relevant for the Spey catchment, and that the WFD will require a more comprehensive classification system than is currently in place. The review also stressed that ecological and health-related issues, such as toxic cyanobacterial blooms, have occurred in lochs around Aviemore and are probably attributable to P enrichment. Scottish guidelines have been produced for P concentrations in lochs (SEPA, 2002) and are part of a SEPA river water quality classification scheme; however, the guidelines differ dramatically between standing and flowing systems.

MATERIAL AND METHODS

Sampling area

The study area (Figure 1), a 10 × 10 km grid square (national grid reference NH80), has an altitude range between 220 and 980 m. The averaged (1961–1990) annual precipitation of the Spey basin down to Kinrara was about 1300 mm (National River Flow Archive, http: //www.nwl.ac.uk/ih/nrfa/). The landscape is characterized by contrasting geology, soil and land cover with the SW–NE floodplain axis separating the Monadhliath (NW) from the Cairngorm Mountains (SE). The River Spey flows diagonally (SW–NE) across the area and receives various tributary flows from high areas either side. A major tributary, the River Feshie (*ca* 230 km^2) drains land from the Cairngorm Mountains. The alluvial fan of the Feshie partially constricted the Spey and therefore played an important role in the formation of Loch Insh and the Insh Marshes (Gordon *et al*., 1994) and still contributes to the annual flooding of the Insh Marshes (largest Scottish floodplain). Flood protection embankments were installed between 1750 and 1850 (Inglis *et al*., 1988) and are still in place today, but are now breached in places and the sluice of the main drain has been removed. This allows the Insh Marshes to flood 'naturally', although mostly from Loch Insh backflow, through the lateral drain. There is therefore no significant natural scouring occurring in the floodplain and much of the sediment transported by the River Spey is deposited in Loch Insh.

The low population density (<5 persons km^{-2}; Potter, 1988) of the River Spey catchment draining the study area and little influence of summer tourists mean that direct human pressure on water quality should be minimal. The land use is farming (sheep and beef cattle), hunting (grouse shooting and red deer

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stalking), forestry (conifers) and non-motorized watersports at Loch Insh. A few consented and unidentified point sources of effluent discharge were present in the hectad.

Sampling sites

The sampling design consisted of a stratified random survey. The hectad NH80 was divided into 25 tetrads (Figure 1(c)) and 1/25 000 Ordnance Survey maps used to identify the various aquatic habitats (Figure 1(c)). A representative sampling plan was devised (based on water-body types) which resulted in a different number of sites being sampled per tetrad (Figure 1(c)). The number of sampling sites was constrained for logistical (sampling and laboratory) reasons to an average of three sites per tetrad to represent the whole hectad.

Collection and analysis of water samples

Water samples were collected during four periods in 2003 – 22 February–1 March; 1–7 June; 6–9 and 13–16 September; 23–27 November and 1–4 December. These are referred to subsequently as winter, spring, summer and autumn seasons. Every attempt was taken to ensure that for each occasion sites were sampled under uniform hydrological conditions. All the samples were filtered (Whatman GF/C) immediately after collection, kept cool with ice-packs during transportation, and stored at 4°C until analyses started within four days of collection. A subsample was digested using a persulphate oxidation procedure (Williams *et al.*, 1995) to quantify the concentrations of total dissolved nitrogen (TDN) and phosphorus (TDP). Soluble reactive phosphorus (SRP), nitrate plus nitrite (hereafter NO_3) and ammonium (NH_4) were determined colorimetrically on filtered samples using a Skalar SAN^{++} autoanalyser and standard procedures. A detection limit of $1\,\mu g L^{-1}$ was achieved for all N and P analyses (Y. Cook, unpublished data). Ammonium-N and NO_3-N together represented the total inorganic nitrogen (TIN) while the organic nitrogen fraction (N_{org}) was calculated as TDN − TIN. Soluble unreactive phosphorus (SUP) was calculated as TDP − SRP. Dissolved organic carbon (DOC), major cations, and chloride and sulphate were analysed using catalytic combustion, inductively coupled plasma optical emission spectrometry, and ion chromatography, respectively. Acid neutralizing capacity (ANC) was calculated as the difference between the base cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and acid anions (NO_3^-, SO_4^{2-}, Cl^-) (Sigg *et al.*, 1994; cf. Sullivan *et al.*, 1989). Temperature, dissolved oxygen (DO), pH and conductivity were determined in the field at the time of sampling using a multi-parameter sonde (YSI600XLM, Yellow Springs, Ohio).

Data processing

Seventy-two sites were sampled on four occasions, except for two sites that were not sampled during the winter and two additional sites that had dried up completely during the summer. Two further sampling sites required slight relocation in the summer owing to water shortages.

Individual sampling sites were grouped and statistically analysed on the basis either of their habitat (water-body type) or of their surrounding biophysical zone. The delimitation of the six individual water habitats was rather pragmatic but two broad categories — flowing and standing water bodies — were distinguished that allowed the distinction between a short and longer residence time, respectively. Five of the habitat groupings were located mostly within the 100-km^2 tetrad. The remaining sampling points on the large river systems (Spey and Feshie) received their drainage water from a combination of local sources (within the hectad) and 'external' sources (outside the hectad). Flowing water habitats consisted of two groups, the larger rivers (catchment area $\approx 200–1000\,km^2$) and the remaining smaller streams (first to fourth order, catchment area $\approx 0.08–21\,km^2$) which also included the lateral drain of the Insh Marshes. Standing waters were subdivided into three habitats using area and depth relationships. These consisted of 'pools' (area $< 10\,000\,m^2$, depth $< 1\,m$) comprising ditches, isolated pools and river backwaters; 'lochans'

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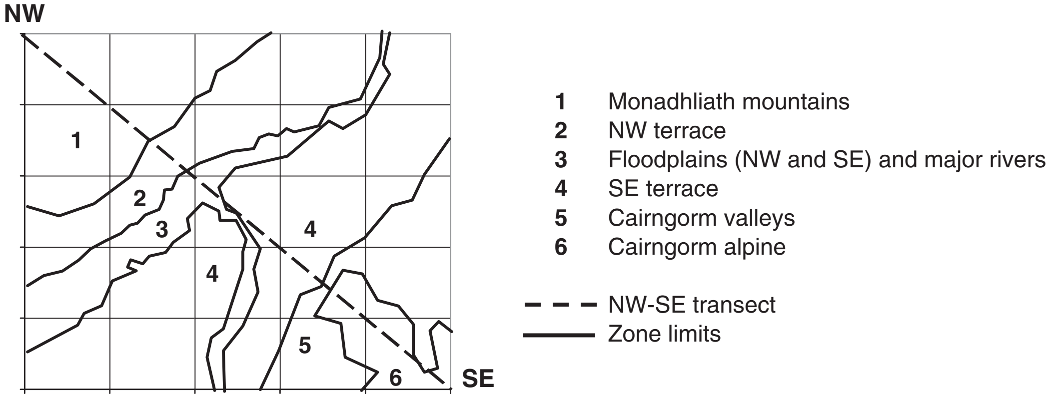

Figure 2. Biophysical zones based on geology, soils and land cover.

– shallow lakes with an area between 5000 and 80 000 m^2 and depth < 2 m; and 'lochs' — larger ($>40\,000\,m^2$) and deeper (>2 m) lakes. Two sites were classified as bogs because of the abundance of *Sphagnum* moss.

The solid geology (Maizels, 1988), soils (Walker *et al.*, 1982) and land-cover characteristics (Macaulay Land Use Research Institute, 1993) formed the basis upon which eight biophysical zones were distinguished along the NW–SE transect highlighted in Figure 2. These included the Monadhliath Mountains, NW terraces, NW (Spey left bank) floodplain, main stem of the River Feshie and Spey (including Loch Insh), SE floodplain (Spey right bank and Feshie floodplain), SE terraces (including east and west of the Feshie), and the valleys and the tops of the Cairngorm Mountains.

Redundancy analyses (RDAs) were performed to investigate the predictive power of the categorical variables 'habitats' and 'biophysical zones'. Unrestricted Monte Carlo random permutations were run for the test of significance of all canonical axes. These multivariate analyses were based on correlation matrices, performed with CANOCO 4 on centred and standardized data (ter Braak and Šmilauer, 1998). For this purpose missing winter and summer data were replaced by autumn and spring data, respectively.

The data were also divided into 25 tetrads (Figure 1(c)), after which, the probability of nutrient concentration exceedance threshold, $P[E]$, was calculated as follows:

$$P[E] = \frac{\text{number of sites exceeding the nutrient threshold}}{\text{total number of sites}} \tag{1}$$

The probability of nutrient availability, $P[A]$, was calculated as follows:

$$P[A] = P[E_{\text{nitrogen}} \cap E_{\text{phosphorus}}] \tag{2}$$

with

$$P[E_{\text{nitrogen}}] = P[E_{\text{nitrate}}] \cup P[E_{\text{ammonium}}] \tag{3}$$

For the purpose of this study, the TDN and TDP thresholds were arbitrarily set to 100 and 10 $\mu g\,L^{-1}$, respectively, while inorganic nitrogen and SRP thresholds were set to 10 and 1 $\mu g\,L^{-1}$, respectively. Finally, since this research underpinned a parallel study on aquatic plants, a N:P ratio for optimum growth was based on Gerloff and Krombholz (1966), rather than on the more traditional Redfield ratio which is primarily for algae.

RESULTS

Hydrology

Comparison of the 2003 flow duration curve for the River Spey at Kinrara with the long-term (1980–2003) trend (Figure 3(a)) indicates that the flow conditions for most of the sampling year were below average. Low stable flows were associated with the summer period (Figure 3(b)). Despite being such a dry year, only two sampling sites dried-up completely, although water levels dropped in smaller standing water bodies (particularly on the fluvio-glacial terraces).

Overview of the chemical properties

Water samples from the 10 × 10 km square were characterized by circumneutral pH, a low conductivity and moderate ANC under the comparatively stable flow conditions that were sampled (Table 1). Averaged water temperatures were <4°C for winter and autumn samples compared with >13°C during the summer and autumn. Other seasonal differences included a higher DO saturation and lower Si concentrations during the biologically active periods. DOC concentrations were higher in the autumn. Generally N_{org} and SUP were the dominant fractions and this was especially the situation during spring and summer, when

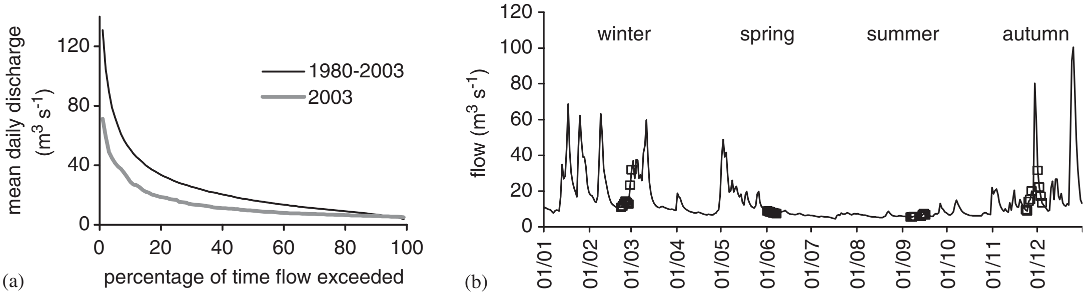

Figure 3. Flow duration curves at Kinrara, River Spey (a) and hydrograph with sampling periods (b).

Table 1. Comparison of the median physico-chemical determinants for 72 sites on each sampling occasion during 2003. Note that the concentrations of NO_3 and SRP were sometimes under the limit of detection ($<0.001\,mg\,L^{-1}$). Unless otherwise stated, all determinants are reported in mg L^{-1}. The range of temperature is reported in brackets

Season	Winter	Spring	Summer	Autumn
Temperature (°C)	3.3 (0.1–6.5)	15.6 (7.1–21.9)	13.3 (8.0–18.7)	3.9 (1.2–6.5)
pH (unit)	6.7	6.9	7.0	6.7
Conductivity (μS cm^{-1})	60	60	63	58
ANC (μeq L^{-1})	141	258	292	223
dissolved O_2 (%)	96	105	102	97
DOC	2.814	3.325	3.255	5.429
NH_4-N	0.022	0.006	0.008	0.013
NO_3-N	0.047	<0.001	0.005	0.055
N_{org}	0.108	0.171	0.118	0.150
SRP	<0.001	<0.001	<0.001	0.006
SUP	0.016	0.011	0.011	0.015
Si	2.906	2.238	2.302	2.622

they accounted for $>90\%$ of the TDN and TDP respectively. Inorganic nitrogen and SRP were so low that more than half of the sites had concentrations below the $1\,\mu g\,L^{-1}$ limit of detection, which was particularly true during spring and summer (Table 1). Interestingly, NH_4 was the dominant form of inorganic-N during the spring and summer samplings.

Grouping of sampling sites

There was a certain amount of complementarity between the two groupings of habitat and biophysical zone used, although the combined amount of explained variance was only moderate (33–38%, Table 2). The following sections consider various chemical attributes after sampling sites were designated on the basis of their habitat and biophysical zone.

Chemical attributes using the habitat approach

Water pH ranged over four orders of magnitude with samples collected from the two bog sites generally having the lowest values (*ca* pH = 4) while some stream sites approached a pH of 8 (Figure 4). As a habitat type the lochans demonstrated the widest range in pH values. ANC showed a similar variability. Some streams and one loch had low ANC ($<50\,\mu eq\,L^{-1}$) although none was negative under the low flow conditions being sampled. Both parameters tended to be higher under the reduced spring/summer flow conditions in most habitats. The ANC for stream samples collected during the summer appeared to be bimodal.

The median N_{org} ($0.1–1\,mg\,L^{-1}$) and SUP ($4–40\,\mu g\,L^{-1}$) concentrations were similar across habitats and seasons, although variability of two orders of magnitude occurred within stream and pool habitats for each sampling (Figure 5(a),(b)). In general N_{org} dominated the TDN fraction, particularly during the spring and summer across all habitats except rivers (Figure 5(c)). The proportion of SUP was well over 75% of the

Table 2. Percentage of explained variance in water chemistry (as in Table 1, without SRP and temperature) by habitats and biophysical zones, singly and all-together, based on redundancy analyses

Seasons	Habitats		Biophysical zones		All-together	
	%	*P*	%	*P*	%	*P*
Winter	18.0	0.006	21.9	0.003	32.7	0.010
Spring	20.9	<0.001	22.2	<0.001	37.7	<0.001
Summer	22.3	<0.001	22.8	<0.001	37.7	<0.001
Autumn	20.7	0.002	20.7	0.004	34.9	0.003

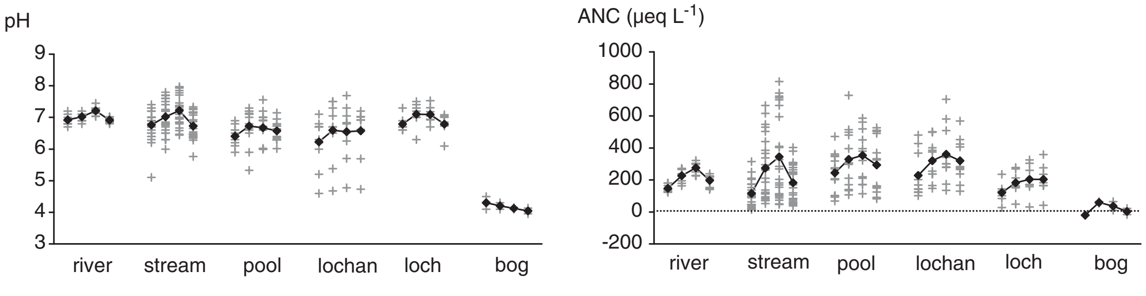

Figure 4. Habitat seasonal variability in buffering capacity, with individual samples (grey cross) and seasonal medians (linked filled diamonds).

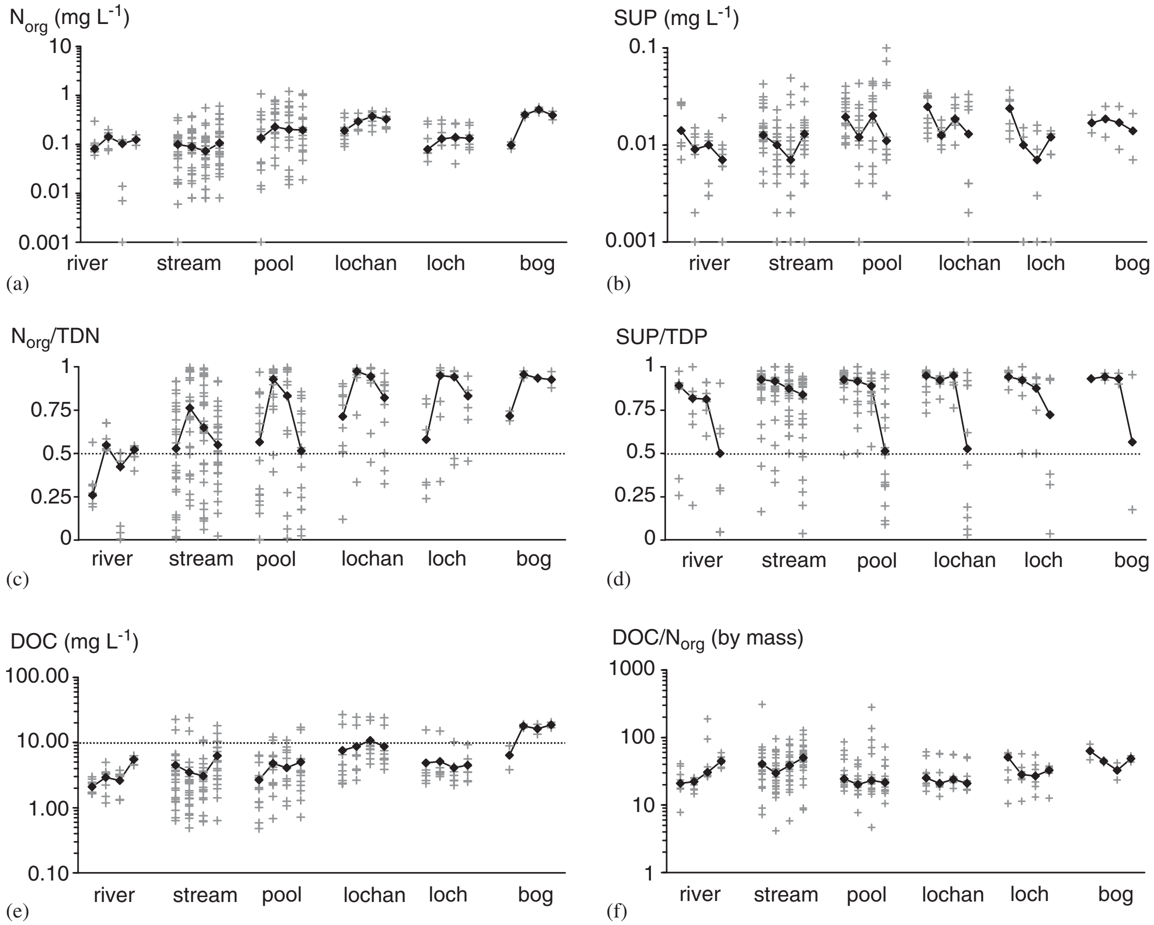

Figure 5. Habitat seasonal variability in organic properties, with individual samples (grey cross) and seasonal medians (linked filled diamonds).

TDP in all habitats and seasons, except in the autumn when this declined to 50% for most habitats (Figure 5(d)). Concentrations of DOC mainly fell within the 2–10 mg L^{-1} range (Figure 5(e)) although some streams and pools had concentrations that were below 1 mg L^{-1} and a few sites from each habitat (except rivers) had DOC concentrations greater than 10 mg L^{-1}. There was no clear seasonal trend in the DOC signal. The DOC:N_{org} ratio by mass was between 10 and 100 (Figure 5(f)).

Dissolved oxygen concentrations in river, stream and loch habitats were generally $>95\%$ and peaked during the spring–summer (Figure 6(a)) while oxygen depletion occurred in a few pools and lochans. The Si concentrations were generally greater than 1 mg L^{-1}, except during the spring/summer period in pool and lochan habitats (Figure 6(b)) when concentrations declined sometimes being less than detection limit and at one loch where it was always undetectable ($<30\,\mu$g L^{-1}). The Si:Ca molar ratio highlighted stronger depletion in the standing water systems with a greater water residence time (Figure 6(c)). Concentrations of SRP were generally very small for all habitats and seasons except during the autumn which was marked by a uniform increase (Figure 6(d)). Concentrations of NO_3-N and NH_4-N ranged over more than three orders of magnitude (Figure 6(e),(f)) although median values were similar in the winter and autumn sampling

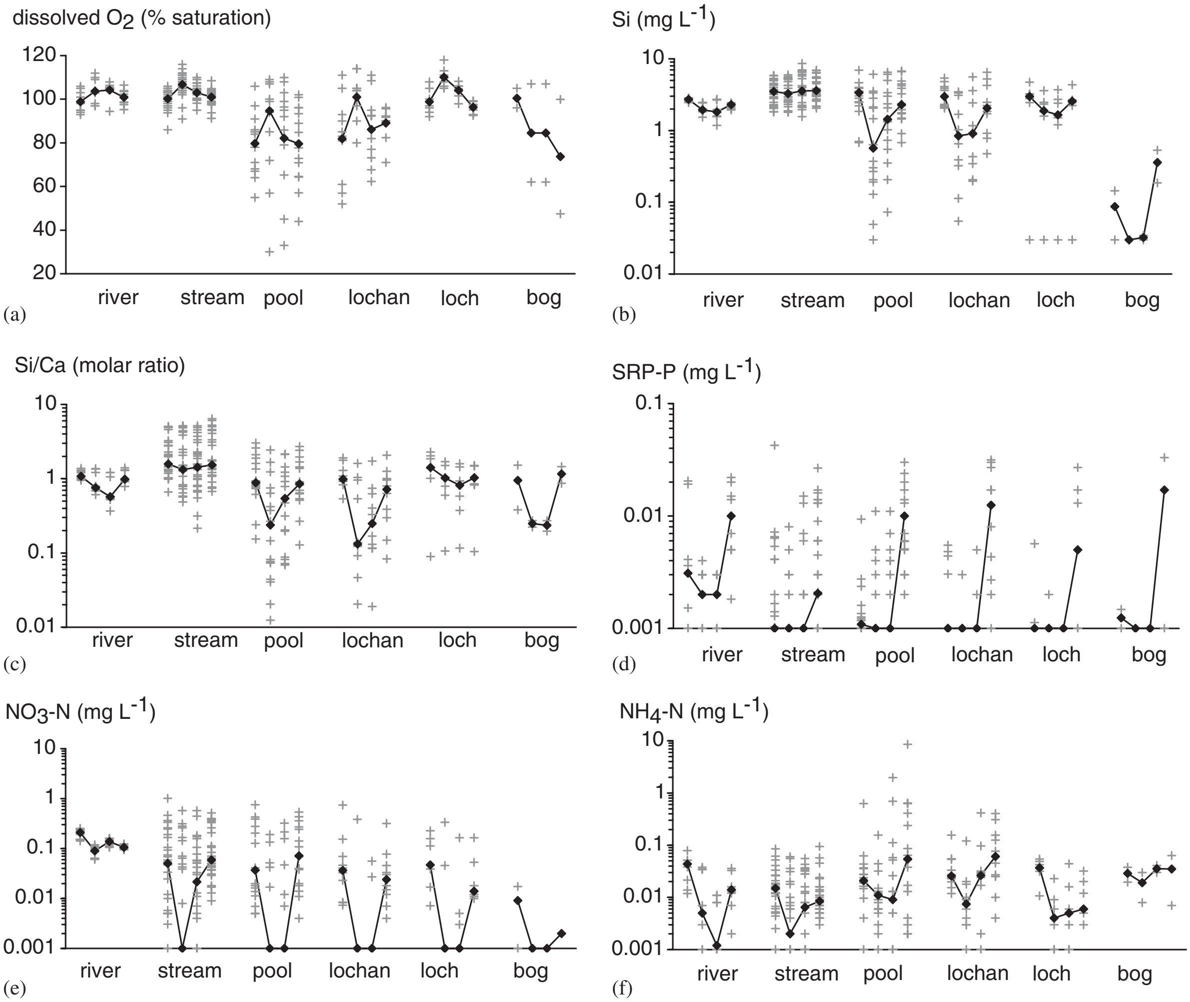

Figure 6. Habitat seasonal variability in inorganic properties, with individual samples (grey cross) and seasonal medians (linked filled diamonds).

periods. Smaller NO_3 concentrations occurred during the spring/summer period for all habitats except rivers where they remained continuously high (90–$210\,\mu g\,N\,L^{-1}$). Ammonium concentrations showed a similar seasonal response although median concentrations typically remained greater than the detection limit for all habitats except rivers.

Biophysical zone approach

A degree of additional spatial variability in water chemistry became apparent when sites were grouped according to their biophysical properties (Figure 7). The seasonal variability in ANC was very pronounced in the NW mountains and glacial terraces, and to a lesser extent in the Spey and SE floodplain. ANC was very variable between sites in the SE glacial floodplains and terraces but remained low at all sites situated in the Cairngorm Mountains. The seasonal variability in Si/Ca ratio was noticeable in most biophysical zones, but was strong only in the floodplain. The spring/summer depletion in NH_4 was remarkable in all zones

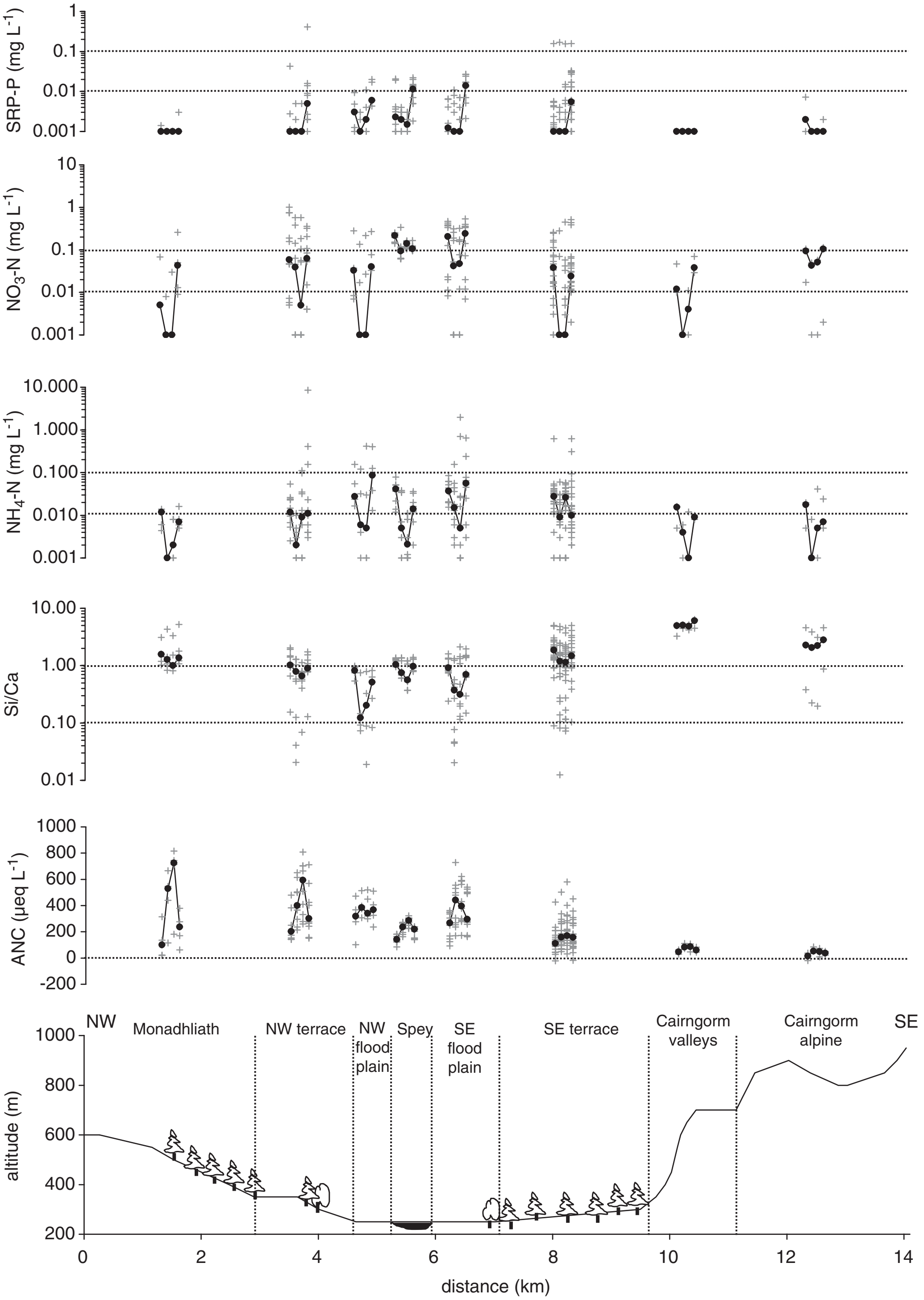

Figure 7. Biophysical zone seasonal variability in buffering capacity and inorganic nutrient properties, with individual samples (grey cross) and seasonal medians (linked filled circles).

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except in the SE terraces (Figure 7). In the mountains, NH_4 was mostly below the limit of detection for at least one season. Nitrate concentrations spanned over a range of three orders of magnitude (<1–$1000\,\mu g\,N\,L^{-1}$). One of the most striking features was that NO_3 concentrations remained comparatively high (50–$100\,\mu g\,N\,L^{-1}$) in first-order streams draining the mountain tops of the Cairngorms, even during spring and summer (Figure 7). This contrasted with downstream locations which were often below detection limit during spring and remained very low during the summer ($<10\,\mu g\,NO_3\text{-}N\,L^{-1}$). Nitrate depletion was also significant in the SE terraces and the NW floodplain. The SE floodplain had similar NO_3 concentrations to the River Spey and differed from those of the NW floodplain. SRP concentrations were predominantly below the limit of detection at most localities (Figure 7). The autumnal release of SRP was not apparent in samples collected from the mountain regions.

Nutrient availability

The N:P ratio, 10:1 by mass, was plotted using either the TDN–TDP or TIN–SRP averaged data for the spring/summer period (Figure 8(a)). These were considered most likely to reflect conditions during the active growth period. Using the two concentration values indicative of N or P limitation, sites were partitioned into those that suggested either P, N or co-limited. When TDP and TDN were plotted approximately two-thirds of sites suggested no-limitation, while the remainder were either P or co-limited. Only when TIN and SRP were used was there a suggestion of N limitation. The seasonal variability of the TDP/TDN ratio showed that the spring/summer depletion in P is proportionally higher in all habitats (Figure 8(b)). It also shows that there is a wide variability between sites within the same habitat type. The

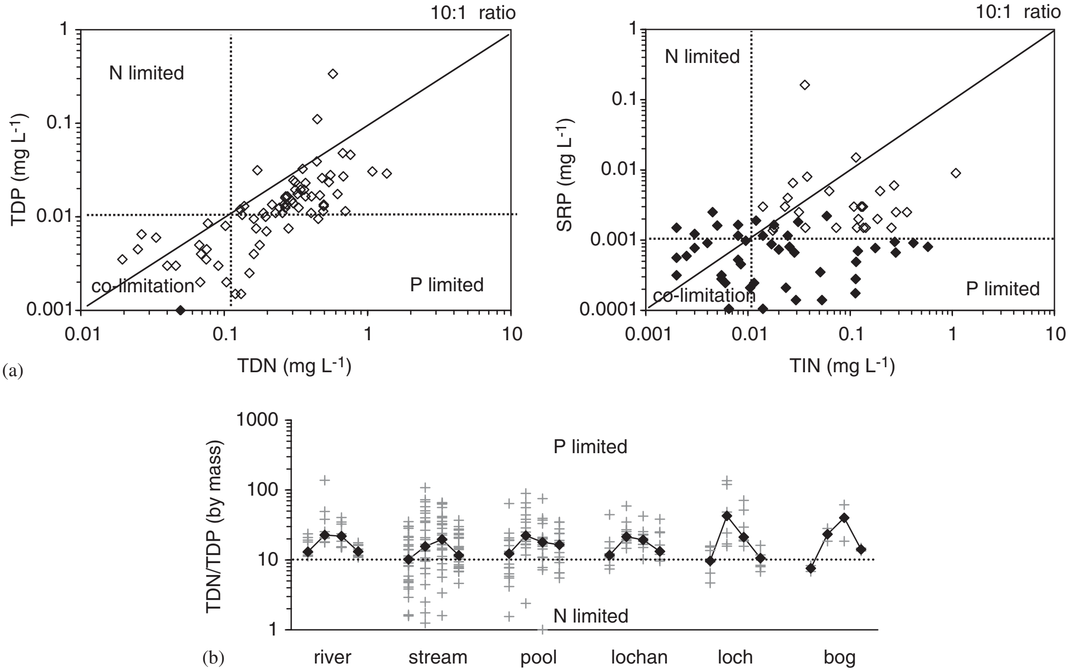

Figure 8. N:P ratios. The 10:1 ratio is the Gerloff and Krombholz ratio by mass, a potentially useful indicator for aquatic plants. Samples with analytical concentrations below detection limits are represented by filled diamonds. (a) TDN:TDP and TIN:SRP ratios for all sites based on averaged spring/summer data. SRP of samples under detection limit were calculated as 7% of TDP. (b) Seasonal variability in TDN/TDP across habitats, with individual samples (grey cross) and seasonal medians (linked filled diamonds).

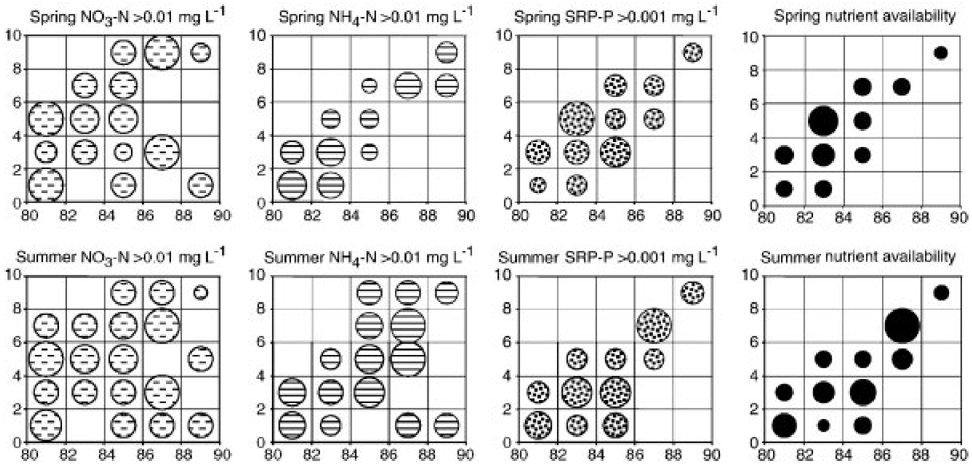

Figure 9. Grid square approach: probability of exceedance of critical nutrient threshold and nutrient availability. The area of the discs represents the probability, from zero (empty square) to 1 (square size).

N:P ratio across biophysical zones showed that the sites situated in the Cairngorm valleys appeared more likely to be deficient in N compared with P in winter and spring (data not shown).

The probability of exceedance in NO_3, NH_4 and SRP differed between biophysical zone–habitat combinations sampled (data not shown). Small standing waters (pools and lochans) and SE terraces and floodplain tended to exceed the $10\,\mu g\,N\,L^{-1}$ threshold in NH_4. Large standing waters and flowing systems were likely to exceed the $10\,\mu g\,N\,L^{-1}$ threshold in NO_3. Exceedance in SRP occurred mostly in the main rivers and connected water bodies. The overall nutrient availability was mostly constrained by SRP probability of exceedance. Nutrient depletion seemed more severe in spring than in summer.

When the probability of exceedance is calculated for each tetrad, some spatial patterns emerged (Figure 9). While NH_4 and SRP probability of exceedance was higher along the floodplain (SW–NE orientation), it was not necessarily so for NO_3 (Figure 9). The probability of nutrient availability was rather low and this was due mostly to P availability as described earlier.

DISCUSSION

Hydrology

Hydrology, and particularly the routing drainage water takes through soil, can directly influence the chemical composition of surface water and groundwater. A research emphasis has been placed in many other studies upon understanding the relationships and changes in composition that take place during individual storm events. This reflects their importance with regard to specific issues, such as freshwater acidification and where estimates of total substance fluxes are required. By contrast it is more likely that stable, low-flow conditions might provide better indicators for the evaluation of impacts arising from nutrient enrichment in flowing systems. For this reason we have specifically targeted low, stable flow periods as this more appropriately meets the longer-term objective of establishing relationships between water composition, substrate properties and aquatic plant chemical composition and diversity. Specifically targeting low-flow periods will have increased the significance of the groundwater component. Despite the exceptional summer drought experienced during 2003, only two sites dried up completely. A significant summer groundwater contribution to surface flows was also supported by the geochemical composition of water samples. The increase in ANC during spring and summer occurred in all habitats (Figure 4), and was most obvious in the Monadhliath Mountains reflecting geological differences (Figures 7). The water circumneutrality for pH and the relatively low DOC (cf. Harriman *et al.*, 1990) with little variability between sampling seasons (Figure 5) suggest that the water bodies were mostly fed by a common subsurface source of water.

Spatial connectivity and heterogeneity

One overwhelming feature of the survey is the tremendous range in composition of water bodies located within the hectad. Two prominent features were apparent in the data when expressed on the basis of habitat and season. The first is the variability (up to three–four orders of magnitude) shown within single habitat groups (stream, pool, lochan and loch) for certain measured parameters during individual sampling periods. Second, a marked seasonality underlies changing concentrations of N and P which, importantly, occurs simultaneously across habitat types. Two habitats, rivers and bogs, generally displayed a small overall spatial or temporal variability.

For stream and river habitats the immediate environment around the point of sampling was used to define its biophysical zone classification. An important aspect of subsequent interpretation of results is the recognition that, for the larger streams and all rivers, water samples will reflect a mixed origin, integrating attributes from all biophysical zones located upstream. While most standing waters are solely located within a single biophysical zone, the larger water bodies are also likely to receive drainage from upstream and different biophysical zones. This may be responsible for part of the remaining unexplained variance for each sampling period. There is also the potential for considerable overlap between certain habitat types; for example, there was a degree of variability in water residence times between the standing water habitats (e.g. Loch Insh crossed by the River Spey compared with Lochan Geal, a glacial kettle hole with no obvious surface inflow) and site location in the pool habitat (e.g. floodplain ditches, river backwaters along the hillslope, isolated pools on glacial terraces). Outliers often reflected local point-source impacts (e.g. STW effluent, road overflow, agricultural livestock). Other unidentified potential nutrient sources (e.g. septic tank leakage, impact of wild animals, past land use) may exist within the hectad.

pH and ANC

The spring–summer peak in ANC was very strong in the Monadhliath while being much less obvious elsewhere (Figure 7). This was probably the result of the underlying solid geology and explained the bimodal stream summer ANC described previously. Hence, although most of the valleys are covered by thick mixed glacial drifts, the underlying geology can have local influences (Gorham, 1957) and can provide a very different habitat type for the freshwater fauna and flora within the catchment. These areas, not recognized in the Spey catchment management plan (SCSG, 2003) should receive more attention, perhaps through local biodiversity action plans.

Soluble organic components

The limited seasonal DOC signal was perhaps not surprising since sampling was biased to low stable flow conditions. The spatial heterogeneity in DOC was, however, large mostly reflecting land-cover/soil-type heterogeneity and complex local hydrological pathways: for example, the closed Uath Lochan (SE terrace) had DOC concentrations of 24–27 mg L^{-1}, while the groundwater-fed backwater at the bottom of Spey-Bank hillslope (NW floodplain) only had 0.5–1.1 mg L^{-1} all year round. Light absorption and attenuation by DOC is likely to be of great ecological importance in the studied area, particularly for submerged aquatic macrophytes (e.g. Wetzel, 2001: 549–552). At the moment, the shallow peaty standing waters of the

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floodplain in the Insh Marshes generally support macrophyte-dominated ecosystems, but this would be sensitive to changes in patterns of DOC loss.

Organic nitrogen and DOC showed little variability across habitats. The DOC:N_{org} ratio (by mass) was mostly within 10–100, so the quality of the organic matter differed widely and for some situations was relatively poor in N. Extreme values were generally associated with N_{org} being near detection limit. A significant summer depletion in SUP concentrations in stream and loch habitats was probably due to their utilization by internal processes. Nitrogen loss was primarily in the form of dissolved organic compounds similar to that reported for the adjacent River Dee system (Edwards *et al.*, 2000). Seasonal variability in the proportion of N_{org} to TDN probably reflected the summer depletion in inorganic fractions. Mineralization was suggested by the autumnal change in SUP:TDP which occurred simultaneously across all water-body types, except mountain streams, and was associated with release of SRP.

Inorganic properties

The near-complete depletion of N and P in the majority of sites across nearly all water-body types during the spring/summer period may be due to reduced leaching from the terrestrial system (Cresser and Edwards, 1987: 87–88), in-stream retention (Newbold *et al.*, 1982) or more likely a combination of the two (Mulholland and Hill, 1997).

The streams of the mountain tops of the Cairngorms had nitrate in their groundwater and this source of N was not totally utilized by the in-stream biota during spring and summer (Figure 7). Similar observations, although with higher concentrations, were previously reported for Scottish upland moorland catchments (Black *et al.*, 1993) and Alpine streams (Lepori *et al.*, 2003). This might result from differences in anthropogenic atmospheric inputs (Gordon *et al.*, 1998; Lepori *et al.*, 2003). In the Allt a' Mharcaidh catchment, atmospheric inputs of nitrate were only partly retained by the terrestrial alpine vegetation (Ferrier *et al.*, 1990). In-stream biological activity of the mountain tops may be seriously limited by other elements (such as P; Figure 7) and therefore nitrate fluxes from the headwater regions may represent a very important source of N in the downstream mid-slope reaches. The small nitrate concentrations detected in the valleys of the Cairngorms, particularly during spring (Figure 7) are indicative of in-stream biological activity and/or dilution and denitrification (e.g. Mulholland *et al.*, 2004; Pribyl *et al.*, 2005). The beds of streams draining the Cairngorms were carpeted by mosses and liverworts. The other set of streams that did not show spring NO_3 depletion were all nutrient-sufficient being affected by a combination of farming activities and STWs.

One important contrast in behaviour occurred for N between the main rivers and the remaining habitats: while the concentrations of NO_3 remained relatively constant for the rivers, they were consistently reduced to below detection limits for the other habitats during the spring and summer. The opposite situation existed for NH_4 which showed a greater overall depletion within the larger rivers Spey and Feshie. An explanation for this difference in response may be found in the processes that control sources and sinks of the two inorganic N species in surface waters. Nitrate depletion is thought to occur in the downstream reaches of certain well-supplied headwater tributaries. This suggests that while NO_3 is present in the local groundwater, it has the potential for rapid localized utilization within this essentially N-poor environment. Sustained spring/summer NH_4 concentrations (although low) in all habitats except rivers could well result from the continued release and mineralization of primary biomass. In the larger river habitats that are continually supplied by N from upstream sources, the local within-stream demand for N appears to be completely overwhelmed, even under these oligotrophic conditions. Importantly, this is indicative of a different response to nutrient enrichment between larger rivers compared with local stream habitats. This observation has implications regarding the extrapolation of findings across geographic scales, even with flowing waters.

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Chemical indicators for conservation and management

Assuming that Si and Ca were weathered in the same proportion throughout the year, the decrease in Si/Ca ratio, as well as dissolved oxygen depletion, during the spring/summer period was probably indicating biological uptake of Si by diatoms. It was strongest where the water residence time was the longest (small standing waters of the NE floodplain, (Figures 6 and 7)). During the spring/summer period Si concentrations were generally above $100\,\mu g\,L^{-1}$, showing no sign for potential diatom growth limitation. Winter depletion of oxygen was caused by ice cover preventing dissolved gas exchange. Concentrations of Si at Lochan Geal, a glacial kettle hole, were undetectable ($<30\,\mu g\,L^{-1}$) on all occasions (Figure 6) and requires further investigation.

N and P critical thresholds, arbitrarily defined in the present study, were lower than some recommended reference conditions already published for streams and shallow lakes (e.g. USEPA, 2000; but see Ice and Binkley, 2003; Moss *et al.*, 2003; Dodds and Oakes, 2004) but similar to those based on deep lakes (OECD, 1982; SEPA, 2002). It should be remembered that since the chemical analyses of the present study were carried out on filtered water samples, it did not include particulate fractions (cf. Edwards *et al.*, 2000). Moreover, interesting spatial patterns emerged from the grid-square approach (Figure 9). It showed that more than half of the tetrads did not exceed these critical thresholds during the spring/summer period indicating that the concentrations were relevant at least for the area studied. This is also in line with other studies where biological response was obtained under extremely low nutrient concentrations (e.g. Borchardt, 1996; Mulholland and Hill, 1997). Lakes have sometimes been perceived as more sensitive than running waters and prescribed lower nutrient threshold concentrations (e.g. EA, 2000). However, concentrations do not take into account the cycling rates of the elements which can be similar in lakes and streams (Borchardt, 1996; Dodds, 2003). Models based on nutrient mass balance (rather than concentration) have had some success in predicting chlorophyll *a* concentration in standing waters (e.g. OECD, 1982). This is not to say that concentration values are not useful (e.g. Dodds *et al.*, 1998), particularly under low stable flows. Indeed, application of nutrient kinetic theory, based on steady-state concentrations of the limiting nutrient, was found to be a robust approach (Borchardt, 1996). Therefore concentrations should be supported by evidence of biological response. The difference in nutrient critical threshold between running and standing waters probably stems from the view that river spate flows can scour the river bed and limit its biological activity therefore reducing eutrophication risks (e.g. Biggs, 2000). However, this may not apply as well for regulated or lowland rivers and during summer droughts.

The tentative DIN:SRP ratio during the period of vegetation growth should only be seen as an indicator to set up hypotheses and direct future experimental work. The N:P ratio should also be interpreted alongside the concentration values. Here N and P concentrations were so low that the seasonal increase in N:P ratio was likely to indicate true P limitation under most circumstances during the vegetation growth period. During winter and autumn, N:P ratio was generally much closer to the Gerloff and Krombholz ratio (10:1 by mass). The narrow N:P ratios in the mountain streams of the Cairngorms during spring may explain the presence of N_2 fixers (Holmes, 1985), although there may also be co-limitation of P. The actual wide N:P ratio found in Loch Insh should not favour the development of toxic benthic cyanobacteria which caused the death of dogs at Loch Insh in 1990–91 (Edwards *et al.*, 1992).

Implications for conservation and management

This study has explored a range of chemical determinants that proved to be useful descriptors of the water quality for their potential ecological role in an area more closely reflective of reference conditions. However, this is only the first step. Specific thresholds for key chemical indicators linked to specific ecological responses would be beneficial. The response of aquatic plant C:N:P stoichiometry to nutrient availability does not seem to be promising (Demars and Edwards, in preparation). Other ongoing projects, such as LEAFPACS (a predictive system to assess the ecological status of rivers and lakes using macrophytes), are testing alternative ways to develop these thresholds.

Comparison between aquatic systems is possible if samples are collected during steady flow conditions when subsurface flow and soil-type influences are minimum. Under these hydrological conditions, and if samples are collected seasonally, then some inferences can be drawn on the potential biological role in nutrient cycling. While it would be prohibitively expensive to monitor all these sites in the long term, or more frequently, it may be possible to repeat the survey in the future to monitor any potential changes. The inter-annual variability could be studied at a subset of sites.

This study introduced arbitrary nutrient thresholds because the ecology of these systems, and particularly their sensitivity to nutrient enrichment, remain to be elucidated. The rationale for having distinct nutrient thresholds between standing and flowing aquatic systems does not recognize habitat connectivity and the fact that nutrient cycling rates under steady-state concentration may be similar. Hence there may be a case for greater harmonizing of nutrient thresholds between standing and running water systems. The nutrient criteria reported by the SEPA river water quality classification scheme (http: //www.sepa.org.uk/pdf/data/classification/annex1.pdf), class A1 (excellent) SRP $< 20\,\mu g\,L^{-1}$, NH_4-N $< 250\,\mu g\,L^{-1}$; class A2 (good) SRP $< 100\,\mu g\,L^{-1}$, NH_4-N $< 600\,\mu g\,L^{-1}$; appear to be one or two orders of magnitude higher than the reference conditions established in this study. The use of existing data to develop a wider range of chemical indicators (such as total N, nitrate, N:P ratio) should be considered, even if these, at the moment, are not backed up by ecological response.

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