A10.2: Detailed Terrestrial and Freshwater Ecology Methods

1 Introduction

1.1.1 This appendix provides the detailed methodologies used to obtain and evaluate baseline information for the terrestrial and freshwater Ecological Impact Assessment (EcIA) of the proposed scheme.

1.1.2 Information was collected through a desk study, consultation and by reference to the results of the DMRB Stage 2 Environmental Assessment Report (Atkins, 2009). Field survey methods are explained below. Section 2 of this appendix covers terrestrial ecology, and Section 3 covers aquatic ecology.

2 Terrestrial Ecology

2.1 Habitats and Plants

2.1.1 Terrestrial habitats information was collected through an Extended Phase 1 habitat survey (Handbook for Phase 1 Habitat Survey – A Technique for Environmental Audit; Joint Nature Conservation Committee (JNCC), 2010). The survey also collected information on other features of potential value for protected species and species of ecological interest. Target notes were made where applicable; the abundance of plant species was noted using the DAFOR scale (D=dominant; A=abundant; F=frequent; O=occasional; R=rare).

2.1.2 Aerial photographs, Ordnance Survey (OS) maps, information such as the Ancient Woodland Inventory (AWI), the Semi Natural Woodland Inventory and lists of designated sites were studied to identify potential habitat areas of nature conservation importance within the study area. Detailed information on these areas was collected via a Phase 2 survey by, for example and where appropriate, using the National Vegetation Classification (NVC) (Rodwell, 2006).

2.1.3 Field surveys were undertaken between June and August 2013 (extended Phase 1 survey) and in July 2013 (Phase 2 survey).

2.2 Terrestrial Invertebrates

2.2.1 This survey methodology was developed by the Joint Committee for the Conservation of British Invertebrates (Dobson, 2011). The methodology is intended to provide an objective assessment of invertebrate habitat quality and identify the potential for notable invertebrate species to be present.

2.2.2 The study area was divided into land parcels, defined by hedgerows, ditches, fences, access tracks and other boundary features. The surveyor walked through each parcel of land examining the habitats present, identifying and categorising those of value to invertebrates. A score was then assigned to each category, based on a set of objective criteria (Table 1). Each category was scored between 0 and 3 with a score of 3 reserved for the best quality examples.

2.2.3 Surveys were undertaken between May and August 2013 during the extended Phase 1 survey.

Table 1: Objective Criteria for Invertebrate Habitat (Source: Dobson, 2011)

Criteria	Description
Decaying timber	In general, early decay on living trees is more valuable than old, long-dead, well decayed timber. Broken or split branches are more valuable than sawn or cut ones and standing dead wood is more valuable than fallen timber. Species composition is less important than the type of decay (e.g. degree of moisture, fungal flora, shade or sun-baked). Signs of decaying timber include rot holes, sap runs, fungal fruiting bodies, signs of beetle exit holes and damage caused by foraging woodpeckers. The quantity of dead wood in a survey parcel is important, but small quantities of high quality decaying timber are equally valuable. The quantities of trees present to replenish decaying timber resources as a measure of the habitats sustainability needs to be considered.

Criteria	Description
Rotational management	This creates and maintains structural diversity. Examples include rotational grass cutting that allows persistence of overwintering patches of un-mown grassland, scrub thinning as opposed to blanket clearance, coppice rotation, staged ditch or pond de-silting. Rotational management can be within the survey parcel or integrated with adjacent parcels.
Spring and Autumn nectar sources	Nectariferous species, i.e. those plants producing nectar, include early spring flowering species such as goat willow (*Salix caprea*), violets species (*Viola* spp.), bluebell (*Hyacinthoides* spp.), daffodil (*Narcissus* spp.), and *Crocus* spp., lungwort (*Pulmonaria officinalis*), bird-cherry (*Prunus padus*), blackthorn (*Prunus spinosus*), ramsons (*Allium ursinum*). It also includes late summer to autumn flowering species such as aster spp., ivy (*Hedera helix*), heather (*Calluna vulgaris*), ragworts (*Senecio* spp.), and buddleia sp.. Other species including ornamental non-natives may be noted if known to be important nectar sources.
Botanically poor wetland	Examples include shaded woodland ponds and wet woodland, wet flushes and seepages, areas of unstable exposed riverine sediments and inundation zones, decaying vegetation and leaf litter in wet situations and other wetlands that are poor botanically but likely or known to support notable invertebrates nevertheless.
Structural patchwork	This refers to the degree of structural complexity within a survey parcel. Whilst survey parcels are normally delineated in part on the extent of a uniform habitat type (such as unimproved grassland), structural complexity operates at a smaller scale. Structural complexity combines physical features e.g. topographical variation, and biological features i.e. variation in vegetation structure. Examples of good structural complexity are grasslands with scattered scrub, ancient woodland with canopy, shrub and herb layers or sites with varied topography, soil type or hydrology.
Still air habitats	Typical features that form recognised important still air habitat for invertebrates include enclosed canopy woodlands (typically with higher than ambient humidity), scrub pockets in grassland (shaped to act as baffles on sites that are otherwise exposed to wind), glades and gaps within woodlands, scalloped edges to woodlands or woodland rides (note that straight woodland rides tend to channel the wind), or topographical features that give shelter from wind.
Connectivity	The degree of connectivity of the survey parcel to adjacent parcels containing similar, or complementary, semi-natural habitat types. Connectivity may not necessarily be via direct contact as many invertebrates are aerial dispersers but the distance between habitat parcels and the barrier effect of habitats between them should be considered. Many invertebrate species require different habitat types for different components of their life history which should be included in the degree of connectivity score.
Ecoclines	These are transitional zones where different habitats grade into one another. Examples include successional sequences (grassland to woodland) and hydrological transition (some coastal habitats or wetland edges). This is more or less linear as opposed to ‘structural patchwork’, with a distinct blend of intermediate habitats across the ecocline.
Bare earth	A wide range of other invertebrates need bare earth for foraging and basking. However, bare earth habitat is particularly important to fossorial (ground nesting or digging) bees, ants and wasps. Not all bare earth is valuable habitat and it should only be scored if it meets the following specifications: (1) largely un-shaded and preferably south facing, (2) free draining, (3) not susceptible to flooding, and (4) no excessive disturbance from vehicles and pedestrian or livestock trampling.
Other habitats	Reserved for use by surveyors with invertebrate experience where a habitat feature is known to potentially support notable or scarce invertebrates and does not fit any of the above categories.

2.2.4 Scores were summed to give an overall score out of a possible maximum of 30. For the purposes of this assessment, it was considered that survey parcels scoring below 10 have low potential to support notable invertebrates, a score between 10 and 20 moderate potential, and those above 20 have high potential to support notable invertebrates.

2.3 Bats

2.3.1 Survey methods followed the recommendations set out in the Design Manual for Roads and Bridges (DMRB) (Highway’s Agency et al., 2001a) and the Bat Conservation Trust’s (BCT) Good Practice Guidelines (2012) and Cowan (2003). Activity and roost inspection surveys were informed by an initial assessment of the suitability of the A9 plus a 500m buffer between Luncarty and the Pass of Birnam. Areas of habitat were classified according to their suitability to support roosting, foraging and commuting bats. This assessment was undertaken in conjunction with the desk study and aimed to identify areas to target more detailed studies, based on daytime visits.

2.3.2 Features which were considered suitable for use by bats include the following: built structures, areas of woodland and single trees especially semi-mature and mature broadleaved trees which are favoured as roosting and foraging sites for bats; underground sites, aquatic habitats including ponds, rivers, burns and other watercourses and linear lfeatures, such as hedgerows and tree lines which could provide commuting cues in the landscape.

2.3.3 The above features were assessed for their importance to bats.

Roost Assessment Surveys

2.3.4 The aim of roost assessment surveys was to identify bat roosts and suitable roosting habitat in built structures, trees and underground sites. Where such features were selected for detailed field study, daytime surveys were conducted to look for signs of bat presence. Signs include droppings, urine staining, insect remains, grease marks, smoothing or a lack of cobwebs at potential access/ egress points and the presence of dead/live bats (Mitchell-Jones, 2004). During these surveys potential access points for bats were assessed, including loose bark, splits, holes, cracks, gaps in masonry, tiles and woodwork, and spaces around doors and windows. To investigate any of these features binoculars and an endoscope were used. Roost emergence/re-entry surveys were undertaken at features which were considered likely to support roosting bats and which were likely to be affected by the proposed work.

2.3.5 In woodlands and built-up areas, where individual roost inspections were not practical, the area was subject to focussed bat activity surveys.

2.3.6 The results of the roost assessment surveys were used in to categorise the roost potential category for trees and buildings along the route Tables 2 and 3.

Table 2: Tree Roost Category. (Source: Cowan, 2003)

Category	Category Description
1*	Capable of supporting large roosts
1	Potential for use by small numbers of bats
2	No obvious potential from ground survey
3	No potential to support bats

Table 3: Roost and Potential Roost Category (adapted from Mitchell-Jones, 2004)

Main Category	Sub Category	Category description (Trees)	Category Description (Structures)	Indicator
1 (Roost)	A	Trees with evidence of current or historic use by bats.	Buildings/man-made structures with evidence of current or historic use by bats.	Sighting/sound of bats (including emergence/swarming). Presence of fresh or old droppings, staining, smoothing and lack of cobwebs.
	B	Trees with anecdotal evidence of current or historical use by bats.	Buildings/man-made structures with anecdotal evidence of current or historical use by bats.	Roosts identified by personal communication from reliable source (e.g. property owner) or unconfirmed roost identified during field surveys.
2 (Potential Roost)	A	Trees with high potential for use as roost.	Buildings/man-made structures with high potential for use as roost.	Presence of cracks, splits, knot holes, loose bark, woodpecker holes, snag ends and other hollows in trees
	B	Trees with some potential for use as roost.	Buildings/man-made structures with some potential for use as roost.	Presence of dense ivy cover, dead wood or other features with lower potential as roost sites.
3 (No potential)		Trees with no or low potential for use as roost.	Buildings/man-made structures with low potential for use as roost.	No such features. Isolated from foraging or commuting routes.

<u>Bat activity surveys</u>

2.3.7 The aim of bat activity transect surveys was to identify areas of important bat foraging and commuting habitat by way of manual night-time surveys focusing on the dusk period. Transect routes were pre-determined according to areas of suitable bat habitat as identified at the desk study and habitat suitability assessment stage.

2.3.8 Transects were undertaken in June and July. The surveys commenced at sunset and continued for 180 minutes after sunset. Where possible, each transect was composed of two 500m survey lines, one on either side of the A9.

2.3.9 Surveyors recorded bat activity for five minutes at least eight recording points along each transect line. Distances between recording points varied between 50m and 200m. Bat detection (Bat Box Duet (STAG Electronics) and Anabat SD1 (Titley electronics)) and recording equipment (MP3 player) were used to record bat activity. The species, number of bats and their behaviour were recorded and mapped.

<u>Roost Emergence/Re-entry Surveys</u>

2.3.10 The aim of roost emergence/re-entry surveys was to confirm the presence of roosts in suitable features and to identify roost type and species of bat. Dusk emergence surveys began 20-30 minutes before sunset and ended around 90 minutes after sunset. Dawn re-entry surveys began 90 minutes before dawn and ended 20-30 minutes after sunrise. Exact survey times were determined by prevailing weather conditions, time of sunset, known activity patterns of the species likely to be encountered and time of year when bats are likely to be active.

2.3.11 Ecologists were positioned around the building(s) and/or structure(s) to be observed and they recorded any bat activity using dual mode bat detectors (Batbox Duet). Details of the time, species and number of bats emerging and/or re-entering the roost were noted. Other information about the roosts, their location and distinguishing bat behaviour observed were also recorded.

2.3.12 A full list of buildings that were subject to emergence/re-entry surveys and the results of these surveys are included in Appendix A10.3 (Section 3).

<u>Commuting Route Surveys</u>

2.3.13 Bat commuting routes potentially affected by the proposed works were surveyed by static remote detector surveys. AnaBat frequency division detectors were deployed for eight days, four consecutive days in June and four in July. They were positioned along linear routes on likely landscape features such as: roads, hedgerows, walls and other boundaries to determine the use of likely commuting routes between habitats. The call files collected were analysed using AnaLookW (3.9c) software to identify species and number of bat passes.

2.4 Badger

2.4.1 The study area was checked for field signs of badger as part of the Extended Phase 1 Habitat Survey in June 2013. The survey corridor was 1km wide centred on the existing road. Surveys were undertaken following DMRB guidance (The Highways Agency et al., 1997) and Harris et al. (1989). The survey corridor was searched for evidence of badgers including their setts, trails, footprints and hair. The survey covered the survey corridor and focused in particular on field boundaries, broadleaf woodland and scrub.

2.5 Breeding Birds

2.5.1 The survey was undertaken following a modified version of the Bird Atlas 2007 - 2011 (British Trust for Ornithology (BTO), 2008) methods. Typically the Bird Atlas method employs quadrats measuring 2km x 2km but due to the presence of the A9 in the middle of the survey area, quadrats were 1km x 0.5km for safety reasons. A total of six survey quadrats were centred on habitat likely to support a high diversity and number of breeding birds. These high value habitats (HVH) included woodlands, marshland, riparian habitats, arable fields and the vegetation along the edge of the A9.

2.5.2 An adapted common bird census technique was employed. A surveyor walked over the quadrat so that all habitats were surveyed and all areas were visited to within 50m. Each quadrat was surveyed for a minimum of one hour and maximum of two. Each quadrat was surveyed twice, once in early June 2013 and again in early July 2013. During each survey the number of individual birds (excluding juveniles) of all species was recorded, ignoring individuals flying over the quadrat with the exception of raptors or hirundines. Colonial nesting species were recorded where present and where possible a count of occupied nests or individual birds was made.

2.5.3 Results for each quadrat (species and number) were collated for the two survey visits. Where identical species were observed during each visit the peak number of observations for that species was presented in the results for that quadrat.

Nocturnal Species

2.5.4 The Bird Atlas survey method used is unlikely to record crepuscular (twilight) or nocturnal species, for example, owls. However, these species were recorded during the bat surveys undertaken at dusk/dawn throughout the study area.

2.6 Reptiles

2.6.1 A walkover of the project footprint and 500m buffer was carried out to identify “potential reptile hotspots” (i.e. locations of suitable reptile habitat) (Edgar et al., 2010). Once suitable areas were identified a combination of artificial refuges and further walkovers were used to assess the presence of reptiles.

2.6.2 The survey methodology followed the Herpetofauna Workers Manual (Gent & Gibson, 2003) and Froglife’s Reptile Survey Advice Sheet 10 (1999).

2.6.3 Artificial refuge surveys consisted of distributing squares of roofing felt or carpet tile (50cm x 50cm) at points around the habitat in areas likely to be inhabited by reptiles (i.e. sunny areas near to cover such as hedgerows, grassy banks and verges). The number and density of refuges were dependent on the size of the area but were based on five and ten refuges per hectare (Froglife, 1999). Refuges were checked at least five times in the season during appropriate weather conditions as described below. If a reptile was located on or under an artificial refuge, the species observed and where possible sex and age were noted (ARC and Holmes, 2013). If refuges were lost or vandalized they were replaced.

2.6.4 Walkover surveys of habitat areas were carried out with visual inspections made in all areas of each site. Particular attention was paid to potential basking sites and care taken to avoid casting a shadow over the survey area.

2.6.5 All surveys were conducted between April and July to maximize the chances of finding reptiles. Surveys were conducted between 08:30-11:00 and 16:00-18:30.

2.6.6 Surveyors noted any species observed, number of reptiles seen and the grid reference of the location. In addition the time, date and weather conditions of the observation were recorded. Reptile field signs such as sloughs were noted and potential hibernacula also recorded. Results for given survey areas were expressed as presence (with peak numbers stated) or likely absence.

2.7 Pine Marten

2.7.1 Habitats along the survey corridor were assessed initially using aerial photography and compared to those preferred by pine marten (*Martes martes*). The suitability of the identified habitat within 500m of the A9 for pine marten was assessed as part of the Phase 1 habitat survey, to note any dens, feeding areas or field signs. Mature trees with cavities, rock faces and other suitable locations including man made features were checked for dens in accordance with Birks et al. (2005).

2.7.2 The survey methodology followed that recommended by Birks et al. (2004), which involved walking transects established along linear features (forest roads, paths, fence lines) in habitat suitable for pine marten dens e.g. forest plantations and woodlands. Transects were walked in early summer as pine martens mark their territories more in summer months due to higher levels of activity (Strachan et al., 1998). Locations typically used to mark territories with scats (tussocks of grass, prominent logs or stones and bridges) were sought out and searched along each transect. Due to the problems of identifying pine marten scats via morphology alone (Davison et al., 2002) any scat suspected as being pine marten and in good condition was collected by surveyors and sent for genetic analysis to confirm identification.

2.8 Red Squirrel

2.8.1 The study area was checked for field signs of red squirrel (*Sciurus vulgaris*) as part of the Extended Phase 1 habitat survey in June 2013. Assessments were made using information in Forestry Commission guidance (Gurnell et al., 2009) on the species composition of suitable habitat, and in SNH guidance (SNH, 2012) which lists tree species suitable for planting to benefit red squirrel. Suitable habitat was walked and surveyed for any feeding signs and potential dreys and any incidental sightings, including road kills, were recorded.

2.9 Wildcat

2.9.1 A desk-based study of the habitat within 500m of the A9 route to consider its potential suitability to support wildcat (*Felis silvestris*) was undertaken, using recent aerial photography. Wildcat favour wooded landscapes with a mosaic of habitat, especially semi-natural woodland, conifer plantation, scrub, moorland and pasture, avoiding high mountain tops, coastal areas and lowland areas with intense agriculture (SNH, 2013a). Wildcat are usually found in habitats below 500m in altitude. The home range of a male wildcat is typically around 4.6km^2, with females occupying a smaller area of around 1.8km^2 (Highland Tiger, 2013). Home range varies with habitat and abundance of food.

2.9.2 Suitable habitat was ground-truthed as part of the Extended Phase 1 Habitat Survey in June 2013 to identify locations suitable for denning and feeding as well as the presence of field signs including scat, footprints and scratching posts.

2.10 Otter and Water Vole

<u>Otter</u>

2.10.1 Otter (*Lutra lutra*) surveys were undertaken in accordance with otter survey guidelines (SNH, 2013b; Chanin, 2003 and Strachan, 2007). Watercourses or other water features, and areas within 50m of these features, were surveyed within the survey corridor. Signs indicative of otter presence were recorded.

2.10.2 Bridges are conspicuous land marks within an otter's territory that and can be regularly used for sprainting. Where no signs of otter were found on the initial survey, spot checks were made on bridges within and adjacent to the study area in accordance with DMRB guidance (The Highways Agency et al., 2001b). In addition, incidental records of otter signs collected during the other species surveys were collected to further inform the results from the dedicated otter surveys.

2.10.3 Where required monitoring was undertaken to determine the level of usage, the importance of the site to otter and in particular whether it was being used as a natal holt or formed a resource as part of a breeding site. Monitoring consisted of a visit to the site fortnightly to record any visible otter signs within the vicinity of the holt and deployment of monitoring techniques. Sand was placed in the entrance of the hole to capture any otter footprints and sprainting activity around the hole was recorded and any spraint removed to enable the assessment of recent activity on the subsequent visit.

Water Vole

2.10.4 Survey methods followed those described in the Water Vole Conservation Handbook (Strachan et al., 2011). This involved searching for evidence of water voles (*Arvicola amphibius*) including evidence of burrows, nests, feeding stations, latrines, runs and foot prints.

2.10.5 Searches for field signs within the 6m zone alongside watercourses and ponds were undertaken as described in Strachan et al. (1998). Searches included checks for any signs of American mink (*Neovison vison*), including footprints and scats, as their presence on a waterbody is known to negatively affect water vole occurrence.

2.11 Great Crested Newt

2.11.1 Pond surveys were conducted between Luncarty and the Pass of Birnam and each pond's Habitat Suitability Index (HSI) assessment calculated. Those identified as suitable for great crested newt (*Triturus cristatus*) (GCN) were targeted for population surveys. Surveys primarily focused on identifying likely presence of this European protected species but also recorded the presence of other amphibian species, e.g. common toad (*Bufo bufo*), common frog (*Rana temporaria*), smooth newt (*Lissotriton vulgaris*) and palmate newt (*Lissotriton helveticus*), in the study areas.

Habitat Suitability Index (HSI) Assessment

2.11.2 Ponds for HSI assessment were identified from existing information, OS maps, aerial photography and a walkover survey. An HSI score was produced for each pond, between 0 and 1.0 using guidance by Oldham et al. (2000). Ten habitat criteria were scored including seven objective habitat measurements (pond area, permanence, shading and density, macrophyte density, number of waterfowl, and terrestrial habitat quality) and three qualitative rule-bases (site geography, water quality and fish occurrence). During the pond survey all sites were clearly identified and marked out using a hand held Global Positioning System (GPS) (Garmin GPS 60).

2.11.3 The results of the HSI assessment were used to inform where targeted surveys would be conducted to further investigate the likely presence of GCN in the study area. Ponds scoring between 0.60 and 1 were targeted for further study; an update to Oldham et al.'s (2000) method (ARG UK, 2010) suggests that scores greater than 0.59 have an average to excellent suitability rating. Ponds with scores <0.6 were also considered for inclusion in targeted surveys on the basis of professional judgement, including their proximity to high scoring ponds or potential for forming a GCN metapopulation habitat. All locations identified in the Stage 2 report (Atkins, 2009) as suitable GCN breeding habitat were also taken forward for further survey.

Presence/Absence Surveys

2.11.4 Field surveys were undertaken according to best practice as set out by the Institute of Ecology and Environmental Management (IEEM, 2006) and were carried out with adherence to standard methods (Langton et. al., 2001). Methods included, where possible, bottle trapping, refuge searching, egg searching, netting and torching. All surveys were conducted under licence from SNH and during suitable weather conditions e.g. temperatures above 5°C.

2.11.5 Each suitable pond was subject to all possible survey methods over the minimum of four visits during the peak survey season (April to June inclusive).

2.12 Deer

2.12.1 Deer were scoped out of an ecological assessment as no conservation status is assigned to the species. However, there is the potential for collisions of deer with vehicles using the A9 and an assessment was undertaken to determine whether there were any health and safety or animal welfare issues.

2.12.2 Deer road collision data for this section of the A9 were reviewed, incidental sightings were noted and the location of existing deer fencing within the study area was also recorded.

3 Aquatic Ecology

3.1 Aquatic Habitat Assessment / River Habitat Survey (RHS)

3.1.1 Assessment entailed a desk based assessment followed by a walkover survey of the watercourses which will be potentially impacted by the proposed scheme taking note of substrate, flow type, depth, ease of access and other general characteristics. The aim of the Aquatic Habitat Assessment was to identify sites on the affected watercourses within the 500m buffer suitable for specific aquatic surveys (detailed below).

3.1.2 Twenty-seven riverine water features were identified within the study area. Of these, three are SEPA classified burns, seven are named burns not classified by SEPA, and the remainder are unnamed field drains, tributaries and drainage ditches.

<u>River Habitat Survey (RHS)</u>

3.1.3 Three watercourses designated by SEPA under the Water Framework Directive (WFD) were surveyed using RHS methodology. The method involved an accredited surveyor surveying a 500m reach of each identified watercourse to provide an audit of its physical characteristics and surrounding land use. Ten spot checks are taken at 50m intervals followed by a sweep up of overall channel, bank and riparian zone characteristics, as well as features of geomorphological, hydromorphological and ecological interest. RHS was used to assess habitat quality and the amount of modification present.

<u>Aquatic Habitat Assessment</u>

3.1.4 Many of the non-SEPA classified and unnamed watercourse within the buffer zone are ephemeral, heavily vegetated or less than 500m long, and therefore unsuitable for standard RHS assessment. Non-classified burns and ditches were visited and professional judgement used to characterise aquatic habitat quality across all the non-designated watercourses. This was undertaken by an experienced ecologist familiar with habitat mapping and aquatic ecology.

3.2 Freshwater Invertebrates

3.2.1 Surveys entailed biannual (spring and summer) sampling of invertebrate communities from watercourses within the 500m proposed scheme buffer, which will be directly impacted by the proposed scheme. Sites were identified during the Aquatic Habitat Assessment as characteristic of the study area, safely accessible, expected to remain wetted year round and appear to provide good ecological habitat. During sampling, standard field sheets were completed detailing the physical characteristics of the sample site (e.g. width, depth, substrate types, channel features, bank features and surrounding land use).

3.2.2 Samples were collected using the standard 3-minute kick sampling technique in the channel and a 1-minute hand search along the water’s edge following standard Environment Agency procedures (Environment Agency, 2012). Samples were preserved using Industrial Methylated Spirits (IMS) and sent for laboratory identification.

<u>Laboratory Analysis</u>

3.2.3 Samples were processed in a laboratory following standard Environment Agency (WFD compliant) procedures (EA, 2008). Samples were identified to species level with the exception of *Oligochaeta*, *Sphaeridae* and Diptera; this provides sufficient resolution for further data analysis including WFD compliant assessment of ecological quality.

3.2.4 Species of conservation importance, based on the JNCC conservation designations, are reported.

3.2.5 The following macro-invertebrate (freshwater invertebrates) metrics were calculated for each site: Biological Monitoring Working Party (BMWP), the number of scoring taxa (NTAXA), Average Score Per Taxon (ASPT), and CCI (Community Conservation Index), descriptions of these metrics are given below.

3.2.6 BMWP: the BWMP score is based on the tolerance of different freshwater invertebrates to organic pollution (Hawkes, 1997). Each invertebrate family is assigned a score from 1 to 10, depending on their tolerance to pollution (low scores are given to pollution-tolerant taxa). Scores are assigned based on the presence of a scoring family in the sample, and abundance within families is not considered. The BMWP score is the total of all the scoring families present in a given sample. The BMWP score are divided by NTAXA to give the ASPT.

3.2.7 The CCI (Chadd & Extence, 2004) represents the national rarity and diversity of species identified within a site and designates a conservation value to the sampled community. A Conservation Score based upon each species national rarity is applied to each species. The CCI is calculated from the sum of Conservation Scores (CS) divided by the number of contributing species to obtain the mean value. This is then multiplied by the community score (CoS), derived either from the rarest taxon present or the BMWP score. CCI scores are assigned into conservation classes, the class boundaries and descriptions are given in Table 4.

Table 4: CCI score classifications (Chadd & Extence 2004)

Conservation class	Score	Description
Low	< 5.0	Site supporting common species and low taxon richness
Moderate	5.0 – 10.0	Site supporting at least one species with limited distribution or moderate taxon richness
Fairly High	10.0 – 15.0	Site supporting at least one uncommon species or several of limited distribution or high taxon richness
High	15.0 – 20.0	Site supporting several uncommon species, one of which may be nationally rare or high taxon richness
Very High	>20.0	Site supporting several rare species or very high taxon richness.

3.2.8 Physical characteristics recorded during the field survey, macro-invertebrate metric data (ASPT and NTAXA) and other site data including water chemistry, distance to source and altitude are used to calculate the ecological quality of each site using the WFD compliant River Invertebrate Classification Tool (RICT). Each site is assigned a status for the macro-invertebrates from Bad to High.

3.3 Freshwater Pearl Mussel

3.3.1 Freshwater pearl mussel (*Margaritifera margaritifera*) surveys were carried out in conjunction with River Habitat Surveys. Surveys followed the methodology set out by SNH (undated). The area to be directly affected by the proposed scheme plus a minimum of 500m downstream and 100m upstream was surveyed for suitable freshwater pearl mussel habitat. If an area of suitable habitat was found a search for mussels using a glass-bottomed bucket was undertaken. The freshwater pearl mussel surveys were conducted by a qualified, licenced surveyor.

3.4 Macrophytes

3.4.1 The number and locations of macrophytes survey sites was determined following the Aquatic Habitat Assessment.

3.4.2 The sites were surveyed for macrophytes in June 2013 following Environment Agency methods (Environment Agency, 2008). A 100m section of watercourse at each site was surveyed; all macrophytes within the 100m were identified to species level. The percentage of the river channel covered by each of the aquatic and semi-aquatic macrophyte taxa listed in the UKTAG WFD guidelines (WFD-UKTAG, 2008) was calculated and Taxon Cover Values (TCVs) from one to nine assigned, as per Table 5:

Table 5: Taxon Cover Vales

TCV	Percentage Cover
1	<0.1%
2	0.1 to 1%
3	1 to 2.5%
4	2.5% to 5%
5	5 to 10%
6	10 to 25%
7	25 to 50%
8	50 to 75%
9	>75%

3.4.3 Any samples that could not be identified with confidence in the field were retained for later identification. Where it was not possible to identify a macrophyte to species level (for example due to the lack of non-flowering or fruiting bodies), it was recorded under its genus or other aggregate taxon level if such was listed in the guidelines.

3.4.4 A sketch map of the survey site was drawn and an overall percentage cover of macrophytes estimated. All non-native invasive species were recorded. During sampling, field data detailing the physical characteristics of the sample site were collected including location, width, depth, substrate, habitats (for example pools and riffles), shading, water clarity and bed stability.

3.4.5 Analysis of plant communities followed the LEAFPACS guidance detailed by WFD-UKTAG (2008). The LEAFPACS classification method uses macrophyte species composition, diversity and abundance (using TCVs) to assess the ecological status of a river. Each macrophyte species is assigned a value according to their tolerance to nutrients and flow. This, together with the species composition, diversity and abundance, is used to calculate a number of metrics. The metrics include nutrients, hydraulics, number of taxa, number of functional groups and alga cover. A comparison of each of the metrics derived from the observed macrophyte community with the metrics that would be expected if the community was in reference condition enables the anthropogenic and natural influences on the macrophyte community to be distinguished.

3.5 Ponds

3.5.1 Following the Aquatic Habitat Survey ponds deemed to be potentially impacted due to their proximity or connectivity to the proposed scheme were selected for PSYM assessment.

3.5.2 PSYM surveys were undertaken following the methodology set out in the PSYM guidance document issued jointly by Environment Agency and Ponds Conservation Trust (2002). PSYM assessments involve a family level macro-invertebrate assessment and species level macrophyte assessment which is undertaken on site. Each pond was surveyed during the summer, within the PSYM survey window. Physical data was recorded from each site including substrate, shading, and presence of inflows/outflow. All data was recorded on standard PSYM field sheets, and photos were taken of each pond.

3.5.3 The PSYM field sheets were entered onto an electronic version and submitted to the Pond Conservation Trust for analysis. The PSYM method compares the results for each pond against expected values based on the physical characteristics. Metrics used to compare with expected values in order to obtain final score are, for macrophytes: number of submerged and marginal plant species, number of uncommon plant species and Trophic Ranking Score (TRS) and for macro-invertebrates: ASPT as described in Section 2.2 (Freshwater Invertebrates) of this document, number of Odonata and Megoloptera Families (OM) and number of Coloeptera families (CO).

3.5.4 Each pond scores an Index of Biotic Integrity (IBI) based on the macrophyte and macro-invertebrate data which is used to determine the PSYM quality category. The following IBI ranges are used: IBI >75%=Good, 51-75%= Moderate, 25-50%=Poor, <25%=Very Poor.

3.5.5 Rare species and incidental observations for each pond are reported.

3.6 Freshwater Fish

3.6.1 The number and location of electrofishing sites was determined following the Aquatic Habitat Assessment. Freshwater fish were surveyed by means of electrofishing using ELBP2 back pack units with single anodes, carried out by suitable trained and qualified surveyors. Guidelines developed by the Scottish Fisheries Co-ordination Centre (SFCC) (2007), were adhered to at all times. Electrofishing was undertaken to the British Standard (BS) EN 14011:2003 (water quality - sampling of fish with electricity).

3.6.2 Where possible a fully quantitative survey (three run catch-depletion method) was carried out. For the purposes of evaluating the importance of freshwater fish within the survey area presence and absence data for each species was deemed sufficient. Catch depletion allowed enumeration of a stock or stock component within a given site, and giving a reasonably accurate estimate of a given population. This method was undertaken to ensure representative sampling, comparable with existing and any future fisheries monitoring.

3.6.3 The sampling site was stop-netted at upstream and downstream limits to stop the movement of fish in and out of the survey site. Prior to the survey water quality readings were taken for conductivity (µS/cm) and temperature (°C) to determine the power output of equipment. Photographs of the site and habitat notes were made prior to the fishing commencing.

3.6.4 All captured fish were retained in oxygenated holding facilities before processing. All fish species were identified to species level and their lengths recorded. Where numbers of any species exceeded 50 at any site, a maximum of 50 individuals were measured, with the remaining fish counted. After processing, all fish were transferred to an oxygenated recovery tank. When all fish had completely recovered they were released back into the sample area.

4 References

Aars, J., Lambin, X., Denny, R. and Griffin, A.C. (2001). Water vole in the Scottish uplands; distribution patterns of disturbed and pristine populations ahead and behind the American Mink invasion front. *Journal of Zoology*, 4: 187-194.

ARC and Holmes, F. (2013). Reptile Identification sheet. ARC.

ARG UK (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

Atkins (2009). A9 Dualling to Pass of Birnam Strategic Planning study – Stage 2 DMRB Environmental Assessment. Report to Transport Scotland.

Bat Conservation Trust (2012). Bat surveys - Good practice guidelines, 2nd Edition. Bat Conservation Trust, London.

Birks, J.D.S., Messenger, J.E. and Messenger, J.E. (2005). Diversity of den sites by pine martins *Martes martes*: a response to the scarcity of arboreal cavities? *Mammal Review.* 35 (3&4): 313-320.

Birks, J.D.S., Messenger, J.E., Braithwaite, A.C., Davison A., Brookes, R.C. and Strachan, C. (2004). Are scat surveys a reliable method for assessing distribution and population status of pine martens? in: Martens and fishers (*Martes*) in human-altered environments: An international perspective, 235-252. Springer, New York.

British Trust for Ornithology (BTO) (2008). Bird Atlas 2007-11, Mapping Britain and Ireland's Birds, Timed Tetrad Visits – Instructions. Norfolk, BTO, SOC and Birdwatch Ireland.

Chadd, R. and Extence, C. (2004). The conservation of freshwater macro-invertebrate populations: a community based classification scheme. *Aquatic Conservation: Marine and Freshwater Ecosystem*s. 14: 597-624

Chanin, P. (2003). Ecology of the European otter. Conserving Natura. (2000). Rivers Ecology Series No 10. Peterborough, English Nature.

Cowan, A. (2003). Trees and Bats. Arboricultural Association Guidance Note 1 (Second Edition). Arboricultural Association, Hanks.

Davison, A., Birks, J.D.S., Brookes, R.C., Braithwaite, A.C. and Messenger, J.E. (2002). On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. *Journal of Zoology*. 257: 141-143.

Dobson, J. (2011). A methodology for rapid preliminary assessment of invertebrate habitat quality potential in the course of extended habitat surveys. Unpublished draft for Field Testing (May 2011).

Edgar, P., Foster, J. and Baker, J. (2010). Reptile Habitat Management Handbook. Amphibian and reptile conservation, Bournemouth.

Environment Agency & Ponds Conservation Trust (2002). A guide to monitoring the ecological quality of and canals using PSYM.

Environment Agency (2003). River Habitat Survey in Britain and Ireland. Field Survey Guidance Manual: 2003 Version.

Environment Agency (2008). Freshwater macro-invertebrate analysis of riverine samples. Document number 024_08.

Environment Agency (2012). Freshwater macro-invertebrate sampling in rivers. Operational instruction. Document no. 018_08

Froglife (1999). Reptile Survey: an introduction to planning, conducting and interpreting surveys for snake and lizard conservation. Froglife Advice Sheet 10, London.

Gent, T. and Gibson, S. (2003). Herpetofauna Workers Manual. JNCC, Peterborough. Joint Nature Conservation Committee (2004).

Gurnell, J., Lurz, P., McDonald, R. and Pepper, H. (2009). Practical techniques for surveying and monitoring squirrels. Forestry Commission Practice Note No. 11, Forestry Commission. http://www.forestry.gov.uk/pdf/fcpn011.pdf/$FILE/fcpn011.pdf

Harris, S., Cresswell, P. and Jefferies, D. (1989) Surveying Badgers. The Mammal Society, Occasional Publication No.9. The Mammal Society.

Hawkes, H.A. (1997). Origin and development of the Biological Monitoring Working Party score system. *Water Research.* 32 (3): 964-968.

Highland Council (2006). Best Practice Guidance – Badgers, the Law and Licensing. Inverness, Highland Council.

Highland Tiger (2013). Wildcat Fact File. Highland Tiger: The Scottish Wildcat http://www.highlandtiger.com/wildcat_fact_file.asp

Highways Agency et al. (1997). DMRB Volume 10 (Environmental Design and Management). Section 4, Part 2 (HA59/92): Nature Conservation - Mitigating Against Effects on Badgers. Highways Agency, Scottish Executive Development Department, The National Assembly for Wales and The Department of Regional Development Northern Ireland.

Highways Agency et al. (2001a). DMRB Volume 10 (Environmental Design and Management). Section 4, Part 3 (HA80/99): Nature Conservation Advice in Relation to Bats. Highways Agency, Scottish Executive Development Department, The National Assembly for Wales and The Department of Regional Development Northern Ireland.

Highways Agency et al. (2001b). DMRB Volume 10 (Environmental Design and Management). Section 4, Part 4 (HA80/99): Nature Conservation Advice in Relation to Otters. Highways Agency, Scottish Executive Development Department, The National Assembly for Wales and The Department of Regional Development Northern Ireland.

Institute of Ecology and Environmental Management (2006). Guidelines for Ecological Impact Assessment in the United Kingdom (Version 7). http://www.cieem.net/data/files/Resource_Library/Technical_Guidance_Series/EcIA_Guidelines/TGSEcIA-EcIA_Guidelines-Terestrial_Freshwater_Coastal.pdf

Joint Nature Conservation Committee (2010). Handbook for Phase 1 habitat survey - a technique for environmental audit. JNCC, Peterborough, UK. http://jncc.defra.gov.uk/page-2468

Kruuk, H. (1978). Spatial organisation and territorial behaviour of the European Badger Meles meles. *Journal of Zoology*. 184 (1): 1-19.

Lambin, X., Telfer, S., and Sah, S.A.M. (unpublished). Estimating Water Vole Population Size With Latrine Counts, Department of Zoology, University of Aberdeen.

Langton, T., Beckett, C. and Foster, J. (2001). Great Crested Newt Conservation Handbook. Froglife, Halesworth.

Mitchell-Jones, AJ (2004). Bat Mitigation Guidelines. English Nature, Peterborough.

Morris, P.A., Morris, M.J., MacPhearson, D., Jefferies, D.J., Strachan, R. and Woodruff, G. L. (1998). Estimating numbers of the Water Vole *Arvicola terrestris*: a correction to the published method. *Journal of Zoolog*y. 246: 61-62.

Neal, E. and Cheeseman, C. (1996). Badgers. Poyser Natural History Series.

Oldham, R.S., Keeble, J., Swan, M.J.S. and Jeffcote, M. (2000). Evaluating the suitability of habitat for the great crested newt (Triturus cristatus). *Herpetological Journal*. 10: 143-155.

Scottish Fisheries Co-ordination Centre (2007). Electrofishing team leader training manual. Scottish Fisheries Co-ordination Centre.

SNH (2012). Squirrels and Development. http://www.snh.gov.uk/publications-data-and-research/publications/search-the-catalogue/publication-detail/?id=1908

SNH (2013a). Wildcats. Scottish Natural Heritage http://www.snh.gov.uk/about-scotlands-nature/species/mammals/land-mammals/wildcats/

SNH (2013b). Assessing the impacts of developments. http://www.snh.gov.uk/about-scotlands-nature/wildlife-and-you/otters/assessing/

SNH (undated). Freshwater pearl mussel survey protocol for use in site-specific projects. http://www.snh.gov.uk/docs/A372955.pdf

Strachan, R. (1998). Water Vole Conservation Handbook. Wildlife Conservation Research Unit. Department of Zoology. Uni. Oxford.

Strachan, R. (2007). National survey of otter *Lutra lutra* distribution in Scotland 2003–04. Scottish Natural Heritage Commissioned Report No. 211.

Strachan, R., Jefferies, D.J. and Chanin, P.R.F. (1998). Pine marten survey of England and Wales 1987-1988. Published by the Joint Nature Conservation Committee, Peterborough.

Strachan, R., Moorhouse, T. and Gelling, M. (2011) Water vole Conservation Handbook (third edition). WildCRu: Oxford.

The Herpetological Conservation Trust (HCT) (2007). NARRS - National Amphibian and Reptile Recording Scheme: Reptile Habitat Guide. http://www.narrs.org.uk/documents/Reptile_habitat_guide.pdf

The Vincent Wildlife Trust (VWT) (2013). Pine Martin Distribution and Status. http://www.vwt.org.uk/species/pine-marten

Water Environment (Controlled Activities) (Scotland) Regulations (2005). ISBN 0110696379 The Stationery Office Limited.

Water Framework Directive (WFD) (2000/60/EC). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32000L0060:EN:HTML

WFD-UKTAG (2008). UKTAG River assessment methods macrophytes and phytobenthos. Macrophytes (River LEAFPACS). SNIFFER.

Woodroffe, G.L. (2000). The Water Vole. The Mammal Society, London.