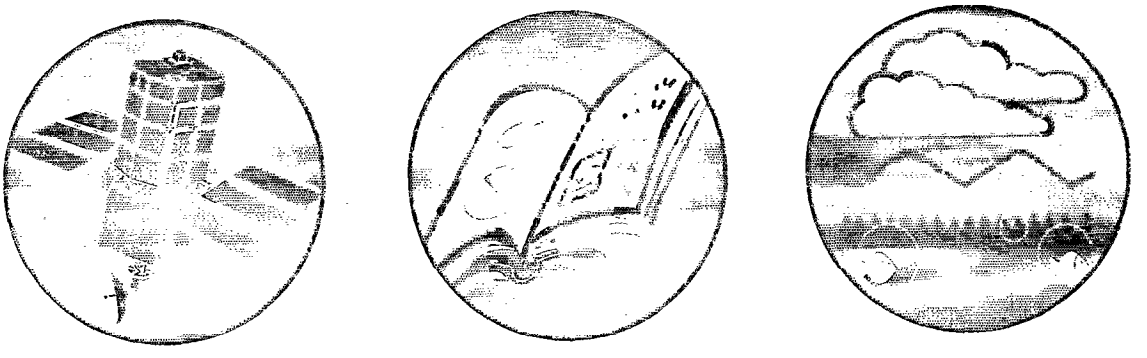
The Use of DNA Fingerprinting to Study the Population Dynamics of Otters (Lutra lutra) in Southern Britain: A Feasibility Study



Research and Development

Technical Report
W202

ENVIRONMENT AGENCY

The Use of DNA Fingerprinting to Study the Population Dynamics of Otters (Lutra lutra) in Southern Britain: A Feasibility Study

R&D Technical Report W202

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Statement of use
This document reports on the findings of R&D Project W1-025. It concludes that the DNA fingerprinting technique applied to otter spraint has great potential for investigation of otter biology but requires development before it can be applied to large-scale projects. Specific recommendations for further development work are made for consideration by the Agency's Conservation Function and by the UK Biodiversity Action Plan Steering Group.

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Tim Sykes, Environment Agency, Southern Region

EXECUTIVE SUMMARY

Many factors have the potential to limit the recovery of otter (*Lutra lutra*) populations including road deaths, resource constraints such as prey, and habitat availability and quality. Current practical conservation measures are based on surveys assessing habitat potential, which is followed up by habitat improvements. There is very little investigation of the requirements of the animals themselves due to lack of suitable survey techniques. The use of DNA fingerprinting of spraint provides a much needed survey tool to address the acknowledged need for research into the conservation needs and population biology of this species. This new approach to surveying otters provides a means of addressing many of the targets of the UK Otter Biodiversity Action Plan.

This was a collaborative project between the Environment Agency, the Universities of Exeter and Aberdeen, the Somerset Otter Group and the Devon and Hampshire Wildlife Trusts together with a large number of volunteers without whom the study would not have been possible.

The Report presents the findings of a one-year feasibility study into the use of DNA fingerprinting to study the otter recovery in southern Britain. Four catchments were surveyed, one in Devon, two in Somerset and one in Hampshire.

The long-term objective of this study is to characterise the population dynamics underlying the otter recovery in the UK over a period of four years, as a contribution to identifying the factors limiting population expansion, to facilitate a more focused, efficient and effective conservation effort.

The objective of the feasibility study was to carry out a field test of the effectiveness of fingerprinting techniques in identifying individual otters and to develop a protocol for applying these techniques to large scale, repeatable projects.

The Feasibility Study was an outstanding success. It answered many of the questions asked, achieved its objectives and identified ways in which the DNA fingerprinting technique needed improving. The Study has provided a unique insight into otter biology in southern England. A brief summary of achievements includes:

- Mobilisation of over 50 volunteers on four river catchments in Devon, Somerset and Hampshire.
- Collection of over 600 spraint for analysis.
- Identification of 57 different otter DNA profiles, including one that was recorded 23 times over a period of 19 months.
- Identification of breeding success on two of the catchments.
- Preliminary findings indicate that the different population level on each catchment affects the distribution and ranges of individual otters.
- 20% of samples analysed were successfully typed; the success rate of analysis of samples ranging from 16 – 43% per month.

Various problems were identified during the feasibility study and were either resolved during the course of the project or recommendations made for solutions to be addressed during a further three year study. The most notable problem was the discovery that two otters on the Itchen, assumed to be closely related, shared the same genetic profile at those loci analysed.

This emphasised the need to check the genetic variability of the population to be surveyed by analysis of tissue samples prior to collection of spraint. DNA profiles of at least 10 otters are required to determine the suitability of a population for applying the technique to spraints.

The duplication of one the DNA profiles within the Itchen population implies that the total number of otters identified, at least on the Itchen, is a minimum. This also means that the home ranges may be over estimated being based perhaps on more than one individual. There was no evidence of similar duplication within the Brue, Tone or Torridge populations.

Continuation of the surveys would confirm the information gained so far on individual otters known home ranges and the estimated total number of otters within each catchment. However, preliminary findings indicate very different distributions between the Brue, Tone and Torridge. The Itchen results are difficult to interpret due to the duplication of DNA profiles. The four catchments are still being surveyed to maintain continuity in the data set with spraint samples stored at the University of Exeter using the protocol developed at the University of Aberdeen.

Improvements are required with the DNA typing, for both the success rate of analysis and the number of loci developed for analysis to ensure individual otter identification. The 6 loci used for spraint analysis were not sufficiently variable to permit identification of individual otters on the Itchen where the genetic diversity of the population is low. The south west population appears to be on the borderline of variability required to successfully identify individuals. The number of loci required will depend on the levels of polymorphism they exhibit but a total of fifteen would be sufficient at the levels found at the loci already used.

The level of genetic variability in the UK otter population is such that it is probably not possible to determine the relatedness of individual otters using existing techniques.

To be cost and resource effective the survey method requires the use of highly committed and motivated volunteers with individual training needs. A sampling protocol and proper equipment is necessary. Health and Safety is of paramount importance. Rapid analysis of spraint is required to enable a continuous review of any survey structure and allow the frequent feedback of results to the volunteers to maintain their support and enthusiasm.

Addressing the problems identified in the Feasibility Study will require new resources and research effort. Improvements to the technique will not only facilitate a longer term study but should also permit its development as a reliable standard tool for monitoring otter populations. Recommendations are made within the report for a further three years study to build on the success of this feasibility study.

ABBREVIATIONS

AONB	Area of Outstanding Natural Beauty
BAP	Biodiversity Action Plan
CCW	Countryside Council for Wales
CTAB	Cetyltrimethyl-ammonium Bromide
DNA	Deoxyribonucleic acid
EA	Environment Agency
EDTA	Ethylene diamine tetra acetic acid
EN	English Nature
FER	Fisheries, Ecology and Recreation
GITC	Guanadium Iso-thio Cyanate
ITE	Institute of Terrestrial Ecology
JNCC	Joint Nature Conservancy Council
LEAP	Local Environment Agency Plan
MGE	Molecular Genetics in Ecology
NERC	Natural Environment Research Council
PCB	Polychlorinated biphenyl
PCR	Polymerase Chain Reaction
R&D	Research and Development
SAC	Special Area for Conservation
SPA	Special Protection Area
SOG	Somerset Otter Group
SRY	Sex Related Chromosome
SSSI	Site of Special Scientific Interest
VIU	Veterinary Investigation Unit

ACKNOWLEDGEMENTS

We are grateful to a large number of people who have contributed to this project and the preparation of the report. We would like to thank all members of the Project Board and Teg Jones and Libby Andrews from the Otter BAP Steering Group, for their guidance and technical input during the project and for their helpful comments on the earlier drafts of the report. Also, at the EA, our thanks go to the Southern Region and South West Regional staff for their help in preparing the maps within the report. At the Wildlife Trusts we thank the Otters and Rivers Project Officers and Somerset Otter Group for their help in providing historic data and co-ordination of volunteers. We would also like to thank Vic Simpson at the Veterinary Investigation Unit for his cooperation with supplying tissue samples and the results of the post mortem of the cub found on the Itchen. We are grateful to Kathy Sykes for preparing a simple introduction to DNA and fingerprinting and to the many riparian owners that gave permission and access to survey sites.

Finally but most importantly a huge thank you goes to all the volunteers who have been out in all conditions collecting spraint. Without their help this project would not have been possible.

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APPENDICES

1. INTRODUCTION

1.1 The Otter in Britain

A detailed study of otter (*Lutra lutra*) hunting records (Chanin and Jefferies, 1978) concluded that there had been a severe decline in the otter population of England, Wales and southern Scotland over a 20 year period which started in 1957/58.

A baseline survey of the distribution and density of sites with evidence of otter activity was carried out between 1977 and 1979 (Lenton *et al.*, 1980). At that time the main stronghold of otters in England was the area covered by the Taw, Torridge and Tamar catchments in the southwest. The survey has been repeated twice since at seven yearly intervals (Strachan *et al.*, 1990; Strachan and Jefferies, 1996). These surveys of England show that the lowest population level was during the period 1977 to 1979, when only 6% of the c3,000 sites searched had signs of otters. In the following 20 years there has been an increase in the number of sites showing signs of otter activity. In southern England, there have been marked increases in the old South West and Wessex Water Authority Regions but very low levels of recovery in the Thames and Southern Regions.

During the third National Survey signs were found in every one of the thirty-two 50 km squares surveyed in England and in every one of the Environment Agency Regions, although two Regions showed very small levels of increase (Strachan and Jefferies, 1996). Surveys of Wales (Andrews and Crawford, 1986; Andrews *et al*, 1993) and Scotland (Green and Green, 1987, 1997) have also shown evidence of recovery. Recovery in England is from the west (ie southwest England and the Welsh borders) towards the east, and from the north towards the south. Population expansion and recolonisation is believed to be occurring both through breeding and by movement. However, calculation of the recovery curve based on the population changes of England, Scotland and Wales to date, shows that recovery to 75% site occupation over all of England is unlikely before 2025 (Strachan and Jefferies, 1996).

Table 1.1 Results of the Otter Surveys of England

Region	1977-79	1984-86	1991-94
South West	24%	44%	67%
Wessex	1%	1%	18%
Southern	2%	3%	4%
Thames	0%	0%	2%
% of sites surveyed which had positive signs of otter activity			

Whereas populations of some species affected by pesticides in the 1950s began to recover in the 1960s, the decline of the otter appeared to continue into the 1970s (Chanin, 1985). There has been much speculation as to the pressures on otter populations that might have caused this long lasting effect. Early authors pointed to the potential importance of disturbance and habitat destruction (eg O'Connor *et al*, 1977, 1979) and there has been much debate about the impact of other toxic chemicals, notably polychlorinated biphenyls (PCBs) (Mason, 1989; Kruuk and Conroy, 1996). More recently, Kruuk (1995) suggested that the availability of a sufficient and suitable food supply is an important factor to consider.

The otter was first afforded legal protection in 1978, when it was added to the list of species protected under the Conservation of Wild Creatures and Wild Plants Act, 1975. Subsequently the otter was given legal protection throughout mainland Britain under the Wildlife and Countryside Act, 1981.

The first practical efforts at otter conservation also date from the 1970s when the Otter Haven Project (of the Vincent Wildlife Trust) and the Otter Trust began to establish otter havens. From the 1980s, some of the County Wildlife Trusts became involved, undertaking a series of county or catchment based projects under the general title of Otters and Rivers Projects. These are joint partnerships between the Wildlife Trusts and various funding organisations including the Environment Agency. Much recent work has been funded or supported with technical expertise by the National Rivers Authority/Environment Agency and the Wildlife Trusts.

1.1.1 Studying and monitoring otter populations: the need for a new approach

The National Surveys provide information on the overall spread of otters, with the assumed increase in otter populations based on changes in the distribution of spraint. However, the extent of the underlying increase in otter numbers and sex ratios are still unknown because these cannot be quantified by counting spraint. Surveys using DNA fingerprinting of spraint overcome this obstacle by identifying and sexing individual otters, allowing the monitoring of individual animals and, in time, trends in the size and structure of the otter population.

This issue has been covered by several reports in recent years:

1. The Otter Survey of England 1991-1994 (Strachan and Jefferies, 1996) identified a need for further research into the habitat usage and behaviour of otters living at low density in the rivers of southern England. The report also highlighted the difficulty in estimating numbers for low-density populations from survey data such as spraint density.

2. The Joint Nature Conservation Committee (JNCC) Framework for Otter Conservation in the UK: 1995-2000 (JNCC, 1996) highlighted the need for the development of agreed methods to allow quantification of population levels, or the production of population indices and suggests that DNA typing of spraint may be a useful technique. The strategy also identified the need to assess the genetic variation within and between otter populations in different parts of the UK, by making greater use of the tissue banks and otter corpses currently available.

3. The UK Otter Biodiversity Action Plan (BAP) identified two key research needs (1) to develop and implement methods to estimate otter numbers and permit population modelling and (2) to monitor populations and distribution of otters to monitor the expansion of fringe populations.

Little is known of the ecology and population dynamics of otters outside Scotland. The Agency currently channels considerable resources into otter conservation through, for example, habitat enhancement schemes, but questions remain as to the long term effectiveness of this effort. More needs to be discovered about the otter populations and the natural recolonisation process in England in order to address these issues.

Factors potentially limiting otter recovery are numerous and include road deaths and resource constraints such as prey and habitat availability and quality. Current practical conservation measures are based on surveys assessing habitat potential, which is followed up by habitat improvements. There is very little investigation of the requirements of the animals themselves due to lack of suitable techniques. The use of DNA fingerprinting of spraint provides a much needed survey tool to address the acknowledged need for research into the conservation needs and population biology of this species.

1.2 The Environment Agency's Responsibilities for Otters

The Agency is Contact Point and Joint Lead Partner with the Wildlife Trusts in delivering the Government's Otter BAP (Biodiversity Steering Group, 1995). The Agency therefore has a responsibility to encourage and promote actions that contribute to meeting the BAP targets. The Agency has also been allocated specific actions in the BAP, including the development and implementation of methods to estimate otter numbers and permit population modelling (Action 5.5.4, Appendix A).

The Framework for Otter Conservation in the UK: 1995 - 2000 (JNCC, 1996) identifies seven objectives for the effective conservation of the otter in the UK:

- survey and monitor populations to determine the UK resource and trends;
- maintain and enhance current populations through good habitat management;
- monitor, assess and reduce (or eliminate where possible) prevalent 'threats';
- promote expansion of populations by the natural recolonisation of areas;
- improve knowledge of ecology and conservation through appropriate research;
- implement and enforce relevant legislation and policy;
- promote education and awareness of the status and needs of otters.

This Framework identifies several other actions for which the Agency has a major role to play. These include contributing to local surveys, monitoring the effectiveness of habitat management schemes and recording of relevant environmental variables to allow the development of predictive modelling of ecosystems.

The Agency achieves these responsibilities by directing resources through its operational, regulatory and advisory activities. The Agency recognises the need to investigate further the otter populations in England, in particular in the south, because there may be external factors limiting the population recovery in southern England. Little is known of otter ecology in southern England. Much of the available knowledge may be inappropriate as it is based on studies of Scottish regions that have not undergone large-scale recolonisation and are largely sea based, not riverine populations.

A better understanding of the ecology of otters in southern England is required for the Agency to ensure that its resources are accurately targeted to meet its responsibilities under the BAP and UK Framework. This study is an important step in achieving this.

1.3 The Feasibility Study

1.3.1 Project Participants

The development of a DNA typing technique for the identification of individual otters from spraint was proposed by Professor Hans Kruuk in 1995, then of the Institute of Terrestrial Ecology (ITE), Banchory Research Station. The project was given as a remit to the Natural Environment Research Council's Molecular Genetics in Ecology Initiative (MGE) based in the Department of Zoology, University of Aberdeen. Dr John Dallas, the senior research fellow in MGE, developed the DNA typing system for otter tissue and spraint. The intention of ITE was to assess whether there were any errors in using DNA typing of otter spraint to estimate population size. This assessment was to be carried out by the estimation of population size by two methods of individual identification, DNA typing of otter spraint and direct observation.

It was intended to carry out this assessment at several sites in Shetland where otters could be identified by direct observation. Mainland sites were not considered suitable as direct observation could not be carried out reliably. However, it was found that the population of otters on Shetland contained so little genetic variation at the loci assessed that DNA profiles would not be specific to individuals (J Dallas, in press).

Prior to the completion of the Shetland study, two independent studies in the south of England became aware of the potential of DNA fingerprinting of spraint and sent samples to Aberdeen for analysis:

1. The Environment Agency and Hampshire Wildlife Trust, through the South East Otters and Rivers Project, were looking at the population on the River Itchen in Hampshire.

2. A PhD study at the University of Exeter, in collaboration with the Somerset Otter Group (part of the Somerset Wildlife Trust) was investigating the otters in Somerset.

Regular otter surveys based on the National Survey method were also being conducted across Devon through the Devon Wildlife Trust's Devon Rivers and Wetlands Project, although spraint was not collected.

These groups became aware of each other's work and agreed to meet and discuss opportunities to collaborate, and to seek Agency support to fully explore the use of DNA fingerprinting in otter surveys.

At a meeting in early 1997 the Environment Agency, the Wildlife Trusts and the two Universities agreed to work together to study a transect of otter populations across southern England, covering Devon, Somerset and Hampshire. The long-term objective of this joint study was to characterise the population dynamics underlying the otter recovery in the UK, as a contribution to identifying the factors limiting population expansion, to facilitate a more focused, efficient and effective conservation effort.

It was decided that the first step in achieving this long term objective was to carry out a one-year feasibility study. Environment Agency R&D funding was secured in mid-1997 and a Project Board set up. The project structure and funding contributions are summarised below.

Through the R&D Project the Agency contracted The University of Aberdeen, The University of Exeter, the Somerset Otter Group, South East Otters and Rivers Project and Devon Rivers and Wetlands Project. Volunteers, co-ordinated by the local otter project officers, collected fresh spraint, which was then sent to the University of Aberdeen for typing. The University of Exeter analysed and interpreted the results in collaboration with the University of Aberdeen and produced the R&D Technical Report. The Agency led the project management and the support of the Project Board.

Table 1.2 Project Organisation

Position	Individuals
Project Executive	Lawrence Talks, Fisheries Ecology and Recreation (FER) Area Manager, Hampshire and IoW Area, Environment Agency
Project Manager	Tim Sykes, Team Leader, Conservation and Recreation, Hampshire and IoW Area, Environment Agency
Project Board	Paul Chanin and Karen Coxon, the University of Exeter John Dallas, the University of Aberdeen Mary-Rose Lane, Devon Rivers and Wetlands Officer James Williams, Somerset Otter Group Chairman Graham Roberts, South East Otters and Rivers Project Officer Tim Holzer and Joe Stevens, Environment Agency Chris Matcham, Surrey Wildlife Trust Otter Project Officer
External Observers	Teg Jones, Environment Agency and Otter BAP Contact Libby Andrews, Technical Advisor to the Otter BAP Steering Group
Corresponding Member	Andrew Crawford, Conservation Officer, Midlands Region

The total project costs were £53K. However, the following contributions were made to reduce the costs.

Table 1.3 Resource Contributions

Organisation	Resources	Contribution
The University of Aberdeen	John Dallas' and a technician's time	£12K
The University of Exeter	Paul Chanin's time to supervise Karen Coxon	£ 7K
The Wildlife Trusts/Otter Projects	Staff time	£ 4K
Environment Agency	Staff resources to plan, initiate and manage the Project	£ 5K
Environment Agency R&D funds		£25K
TOTAL		£53K

The feasibility study was programmed to start in July 1997. The final surveys were to be completed in July 1998 with the R&D report completed in October 1998. Progress meetings

were held every three months for Project Board members. Volunteers were also encouraged to join the meetings and attended on an *ad-hoc* basis.

This Report presents the findings of the feasibility study. It includes recommendations for improvements to the methods used and identifies proposals to achieve the long-term aim.

The feasibility study has close links with an existing Agency R&D Project on Otter Post Mortems (R&D Project Reference W1 – 019) and is potentially a key tool for the UK Otter BAP Steering Group in achieving the above aims and objectives. The Feasibility Study Report will be presented to the UK Otter BAP Steering Group.

1.3.2 Objectives

The feasibility study had five main objectives:

1. The DNA fingerprinting of tissue samples from about 100 carcasses from southern England to provide essential data on levels of genetic diversity in the feasibility study area.

2. The collection and DNA fingerprinting of about 500 spraint from a transect across high to low density otter populations in southern England.

3. To report and review progress regularly during the project.

4. To produce a Research and Development Technical Report to address the above objectives and to provide guidance and recommendations on the feasibility of a long term study into factors limiting otter recovery in the UK.

5. To identify the resource needs, in terms of costs and time, and a robust protocol, which could be repeated by anyone in the future if the method is considered feasible.

To achieve these objectives the feasibility study has looked at the following:

- the practicalities and resource requirements for using volunteers to collect fresh spraint on a regular basis;

- a sampling protocol which maximises the success rate of DNA extraction and typing of individual otters from spraint;

- the number of repeat surveys required to pick up all individuals within a given survey area;

- the length of time over which repeat surveys are needed to identify individual ranges.

2. THE STUDY AREA

2.1 The Catchments Studied

For this project, the three levels of otter populations used for investigation were defined as: 'fragmented', 'intermediate' (sometimes referred to as 'colonising' or 'fringe') and 'established'. Information used to categorise the areas used for the study came from the National Survey results, supplemented by Wildlife Trust/Otter Group records to fill in gaps in the national survey grids.

FRAGMENTED: Environment Agency Southern Region where clusters of positive sites are very scattered and have substantial distances between them.

INTERMEDIATE: North Somerset where there are fewer positive sites but which are spread out over a substantial area.

ESTABLISHED: Much of Devon is covered by the southwest strongholds of the Taw, Torridge and Tamar catchments where the otter population is firmly established/re-established following the population crash of the late 1950s to 1970s.

Although not originally included within the Project Brief, data has been included from spraint collected as part of a PhD research project on the Tone catchment in south Somerset. The population level on this catchment has been variable during the 1980s and early 1990s and was originally included within the PhD study as representative of an intermediate population. However, with the number of otters found and the consistently high level of activity found at all sites surveyed, the population has since been re-categorised as 'established'. The data from the Tone has been included in this Report due to the limited data set obtained from the River Torridge. Map 2.1 shows the locations of the four catchments.

2.2 The River Itchen Catchment

2.2.1 Historical Records

Surveys conducted by the South East Otters and Rivers Project prior to 1996 showed the only resident population of otters in Hampshire to be confined to the Itchen catchment. The nearest potentially viable population is in Dorset. It was therefore assumed to be an isolated or fragmented population. The Itchen population prior to 1993 appeared to be very small and transient. The National Surveys found a low density of otter activity, 4 out of 8 sites positive in 1979, 5 out of 8 in 1986 and 6 out of 8 in 1994. Three captive bred animals were released in August 1993 by the South East Otters and Rivers Project, to establish a breeding stock and boost any natural population recovery. The population has been monitored regularly since the releases.

2.2.2 Catchment Characteristics

The River Itchen is one of the best examples of a chalk river in the UK. It rises at about 75m above sea level on the Upper Chalk of the Hampshire Downs as three spring-fed tributaries; the Candover Stream, the River Alre and the Cheriton Stream. These join to form the River Itchen just west of New Alresford. The Itchen flows west to Winchester and then south through the outskirts of Eastleigh and out into Southampton Water estuary. The course of the

Itchen from source to sea is about 37 km with an average gradient of 2m per km. The River Itchen catchment is 473 km^2 in area. The valley floor is characterised by spring-rich gravels overlain by peat and intersected by numerous channels. Agricultural development has been comparatively slow relative to similar catchments in the south resulting in the survival of extensive areas of semi-natural habitats.

The river is highly braided and for much of its length is divided between two or more separate channels running parallel to each other. The watercourse has many artificial structures for flow and level regulation. Historic management of water meadows and mills has left a legacy of an intricate network of streams and carriers as part of the system. As the river is spring-fed there is only a narrow range of seasonal variation in its physical and chemical characteristics. Fish farming, mainly for trout, is an important local industry (Draft Test and Itchen Local Environment Agency Plan, 1998).

2.2.3 Conservation Status

Large tracts of the Itchen valley basin have been notified as Sites of Special Scientific Interest (SSSI) because of the excellent habitat quality. The river itself is one of only 29 riverine SSSIs in England and Wales. The Itchen is a candidate Special Area of Conservation (cSAC) (under the EU Habitats Directive) on account of its rich aquatic plant communities and populations of Southern Damselfly (*Coenagrion mercuriale*). Parts of the Itchen estuary are also designated as a SSSI, Special Protection Area (SPA) and Ramsar site.

2.3 The River Brue Catchment

2.3.1 Historical Records

The three National Surveys covered only the upper reaches of the River Brue catchment. These surveys found positive evidence of otters at 2 out of 15 sites in 1979, 1 only out of 15 in 1986 and 4 out of 15 in 1994. Data from the Somerset Otter Group (unpublished), covering the rest of the catchment, confirms that the Brue supports a low level of otter activity and it was therefore chosen as representative of an intermediate, potentially colonising, population.

2.3.2 Catchment Characteristics

The River Brue catchment (the Brue and Axe Rivers) covers the most northerly fringe of the southwest otter population. Immediately to the north are the Mendip Hills, which could be a physical constraint on further spread from the southwest population. Much of the catchment is lowland wet grassland, which forms part of the unique flat landscape of the Somerset Levels and Moors.

The River Brue rises in the clay uplands in the east of the catchment, before flowing through the lowlands of the Levels, often in man-made, heavily managed channels before discharging into the sea at Highbridge, within Bridgwater Bay. The River Axe, and its tributaries the Cheddar Yeo and Lox Yeo, rise from the limestone springs on the Mendips before flowing through the Levels and Moors to the sea just north of Brean Down. The three rivers are interconnected in several places by rhynes (ditches) controlled by sluices, forming a very complex artificial drainage system.

2.3.3 Conservation Status

The catchment is of major importance to wildlife conservation. Of over-riding importance is the internationally designated lowland wet grassland resource of the Somerset Levels and Moors, the largest remaining area of this habitat in Britain. Five of the wetland SSSIs within the Levels have recently been designated as a SPA/Ramsar site of international importance. There are a further 51 SSSIs and 33 County Wildlife Sites within the Levels. Significant sites, including Bridgwater Bay, are designated as SPA and Ramsar sites and cSAC. Both the North Drain and South Drain flow through SPA/Ramsar sites. These designations are due to their international importance for over-wintering wildfowl and breeding waders (Brue and Axe Local Environment Agency Plan, 1998).

2.4 The River Torridge Catchment

2.4.1 Historical Records

Within the Torridge catchment the three National Surveys identified otter activity at 15 out of 23 sites in 1979, 17 out of 23 in 1986 and 21 out of 23 in 1994. The catchment was therefore chosen as supporting an established otter population. The catchment has over 320 km of watercourse draining an area of 857 km^2 (Torridge Local Environment Agency Plan, 1998). Due to the very large size of this catchment the main watercourse was chosen for detailed study and collection of spraints, with some of the smaller, less accessible tributaries excluded from the study.

2.4.2 Catchment Characteristics

The River Torridge drains a large area of predominantly agricultural land in northwest Devon. The catchment comprises the main river and the major sub-catchments, the rivers Waldon, Lew and Okement; it drains into the Bristol Channel. The Torridge rises near the north coast northwest of Bradworthy and flows southeast picking up the Waldon and the Lew before turning north flowing towards the estuary at Bideford picking up the Dartmoor tributary, the River Okement, south of Beaford.

2.4.3 Conservation Status

The River Torridge catchment contains areas of regional, national and international importance for wildlife. A range of semi-natural habitats support a variety of species, some of which have very restricted distributions. Several formal designations apply to parts of the catchment, some emphasising its important landscape, heritage and nature conservation importance. Parts of the Torridge surveyed for this project are a designated County Wildlife Site (Torridge Local Environment Agency Plan, 1998).

2.5 The River Tone Catchment

2.5.1 Historical Records

From hunting records (Pring, 1958) and the Somerset Otter Group records, the Tone was always known as a catchment with a strong otter population, but they became extremely scarce by 1979 and were believed absent from 1980 to 1986 inclusive. Regular signs of otter presence reappeared in the spring of 1987, and since then they have increased steadily. Cubs have been recorded annually throughout the 1990s. The River Parrett, into which the Tone flows, was devoid of all otter signs for rather longer, from 1979 to 1989. Between December

1995 and December 1996 the Tone catchment lost 5 otters run over on roads, but this did not reduce the distribution and frequency of signs of otter activity (SOG records).

2.5.2 Catchment Characteristics

The River Tone rises in the Brendon Hills and travels only a short distance before it is impounded to form Clatworthy Reservoir. From here it flows steeply south over slatey bedrock through mainly grazing land. At Greenham it turns east and flows more slowly through arable land past Wellington, and through Taunton, until it reaches the Somerset Levels and becomes tidal at New Bridge. After a short distance it joins the River Parrett, flowing north to discharge into the Bristol Channel near Bridgwater. From its source to the confluence with the River Parrett the Tone is about 33 km long and falls approximately 370 metres.

The main river is protected from low summer flows by augmentation flow from Clatworthy, but the three principal tributaries which flow into it from the north, above Taunton, are subject to low summer flows. The length of statutory main river is 56.5 km draining a total catchment area of 414 km^2

2.5.3 Conservation Status

Within the catchment there are six SSSIs, four of which are water dependent and 35 County Wildlife Sites. Some stretches of the main river course are designated as a County Wildlife Site, partly because of the otter population. More than half the catchment is within an AONB.

2.6 Suitability of the South West Otter Population

Concurrent with the collection of spraint for DNA analysis the otter population across the south west was assessed to check that there was sufficient genetic diversity within the population as a whole for the application of this technique. Ideally this should have been completed in advance of the spraint collection and analyses but this was not possible with only one year available for completion of field and laboratory work.

To establish the genetic diversity of the otter population tissue samples were collected from otter carcasses stored by various organisations (Appendix C) and DNA profiles developed from these samples. Carcasses originating from Cornwall, Devon, Somerset and Hampshire were sampled.

3. LABORATORY METHODS

3.1 Rationale

DNA fingerprinting is based on the finding that in certain places on plant or animal chromosomes there are short sequences of DNA which appear to have no function but are repeated several times. These sequences are known as mini- or micro-satellites (depending on the length of a single segment). If the number of repetitions varies, these may be used for 'fingerprinting'. The sites are known as loci (the singular is 'locus' meaning place) and the different numbers of repeats are known as alleles.

If only a few loci are known and each has few alleles, it is very difficult to distinguish between individuals by DNA fingerprinting because quite often two closely related individuals will have the same fingerprint. Where there are many loci available and each has many alleles it is very easy to distinguish between individuals.

As every animal has two pairs of chromosomes it will have two alleles for each locus. In this report an otter's 'type' or 'fingerprint' will be given in the form of two numbers for each locus (see Appendix D).

DNA is extracted using the relevant method (see below) and PCR (polymerase chain reaction) is used to multiply the extracted DNA to provide sufficient material for typing. The principles behind DNA extraction and typing are described in detail in Appendix B: An Introduction to DNA and Otter DNA Fingerprinting.

3.2 Tissue DNA Extraction

Extraction of DNA from tissue samples was achieved using a standard salt-chloroform method based on Bruford *et al.*, 1992 and Müllenbach *et al.*, 1989 (Appendix C). Nine micro-satellite loci were typed for each individual to generate a DNA profile consisting of 18 numbers: two numbers (for example 03 05) per locus. The PCR primers and conditions for the first eight loci are published in Dallas and Piertney (1998).

The genetic variability was then assessed statistically to determine whether it was suitable to apply the method to DNA extraction and typing from spraint. There were too few carcases available from Hampshire to allow statistical analysis of the DNA profiles found. A technical explanation of the work undertaken on otter carcasses and its implications are presented in Appendix C.

3.3 Spraint DNA Extraction

DNA extraction from otter spraint used a CTAB/GITC/diatom/VectaSpin method. Detailed protocols for both these methods are presented in Appendix C.

4. FIELD METHODS

4.1 Sampling Density

A key aim of the field surveys for the feasibility study was to collect sufficient fresh spraint for analysis from an even distribution of sites across each catchment. Spraint density and frequency at any particular location is highly variable. Therefore site 'surveys' were spot checks, collecting samples from one or two fresh spraint wherever available. Sites were chosen where access was relatively easy, allowing a large number of sites to be covered quickly and early in the day.

The first National Survey in 1977-79 adopted a survey site density of six sites per 10 km^2. This is equivalent to one site for every 5 km to 6 km of watercourse. Various tests of the national survey have shown that this density is reliable for monitoring distribution (Strachan and Jefferies, 1996). However, Lenton et al (1980) found that at low otter densities, where otter activity was low or otters were possibly transient, surveying every 6 km would give a much lower chance of proving presence than when the population was established. The otter populations on the Brue and Itchen were assumed to be low. It was, therefore, decided to increase the survey density to at least one site every 3 km for all the catchments studied to increase the chance of finding fresh spraint and to be able to compare data with the national survey approach. On the Itchen, sampling density was actually much higher with 81 sites over about 50 km of watercourse.

The number and distribution of sites allocated to individual volunteers was based on ease of access and travel distances. Some sites identified at the beginning of the project were changed during the course of the study if they were consistently negative, or access became a problem. It was decided at the beginning of the project to keep the sampling strategy flexible in response to results because of the experimental nature of the feasibility study.

4.2 Sampling Frequency

The programme proposed was for each catchment to be surveyed on the same day at monthly intervals over a 12 month period. Some additional surveys were included on the Brue and Itchen as the total number of spraint collected fell below target.

4.3 Sampling Protocol

Spraint for analysis must be fresh. Early experiments at Aberdeen using spraint from captive otters showed a very rapid reduction in the success of DNA extraction with time (Figure 4.3). To improve the likely success of DNA extraction and subsequent typing, spraint has to be less than 12 hours old. Only very tiny amounts of DNA, if any, will be present in spraint, the DNA coming from cells sloughed off the lining of the otter's gut. Both bacterial and chemical agents can degrade DNA within the spraint. Collecting spraint as early as possible in the day and therefore as fresh as is possible increases the chance of a positive fingerprint from the sample.

From this data it was determined that a reasonable analysis success rate could be achieved from spraint less than 12 hours old. The cut off time for collection was therefore recommended to be 10 am. In the summer months it was possible for an earlier start and

finish to spraint collection to compensate for the higher temperatures which are thought to cause more rapid degradation of the DNA.

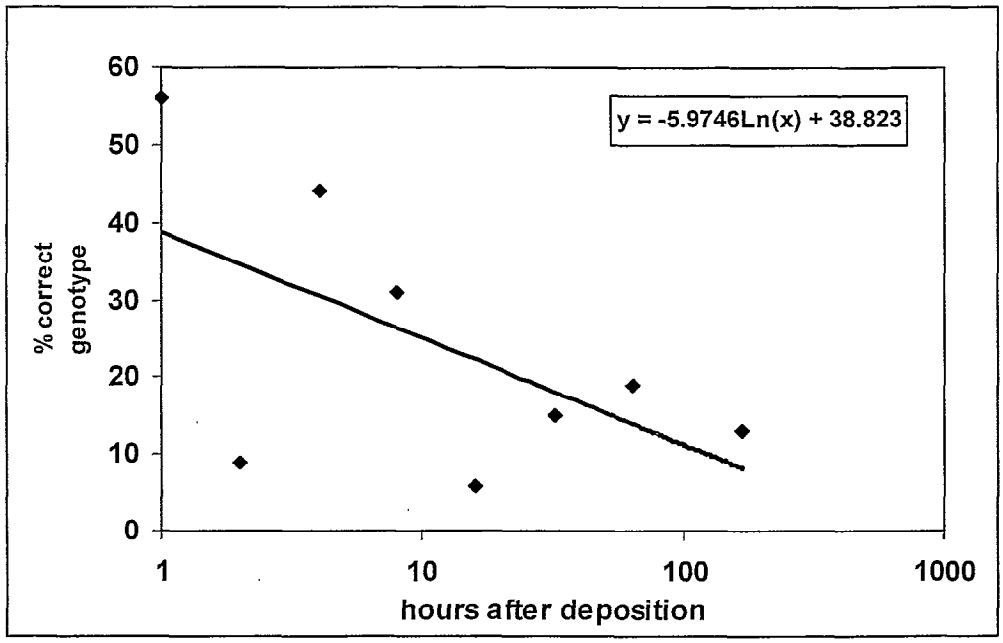


Figure 4.3 Locus 701 genotypes in spraint from 3 captive otters.

Plastic tubes pre-filled with absolute alcohol or industrial ethanol were supplied to the surveyors. These were pre-labelled in pencil (any other marker may get dissolved by any alcohol leaks or spills). The tubes of ethanol were pre-chilled in freezers overnight and transported in cold boxes packed with ice/freezer blocks for the duration of the survey. Ethanol is toxic and safety guidelines were prepared for the surveyors following an Environment Agency Health and Safety review (Appendix E).

The ethanol is used to reduce bacterial degradation of any DNA, and samples are kept as cold as possible to reduce any chemical degradation.

A detailed sampling protocol was prepared for the surveyors together with instructions on storage and transportation of samples once collected. These are presented in Appendix E.

4.4 Use of Volunteer Groups

The catchments chosen were those where networks of volunteers were already surveying the otter populations, although not all were collecting fresh spraint on a regular basis.

Volunteers were asked to all go out on the same day whenever possible. This was to maximise the information gained from any one survey. Samples collected on the same morning provide data on 'daily' movements of individual animals whereas samples collected on different days contribute to the maximum known home range of a particular otter. There are also practical benefits of a coordinated same day survey, as samples can be collected together on the day of survey and sent together to the laboratory for prompt analysis.

In Hampshire the South East Otters and Rivers Project was established in 1986. This is a collaborative project between Hampshire Wildlife Trust and the Environment Agency Southern Region. This project had an existing trained and committed team of volunteers to

undertake practical conservation schemes, surveys and site monitoring for otters. This group had already organised a survey of nearly 70 sites on the River Itchen and had collected spraint for DNA fingerprinting by the University of Aberdeen in January and February 1997.

In Somerset the Somerset Otter Group (SOG) affiliated to the Somerset Wildlife Trust was already surveying the majority of the watercourses in Somerset on a monthly basis. The volunteers record presence and absence of signs of otter activity, noting number and freshness of spraint and anal jelly as well as any otter footprints and evidence of mink (*Mustela vison*) (scats and paw prints). Volunteers are allocated all or part of a river to survey. It is up to individuals which days and times they complete their surveys. There was also an ongoing PhD study using DNA analysis of spraint samples collected from watercourses across Somerset. Volunteers from the SOG also supported the PhD study by collecting spraint. During the SOG annual survey, when all sites were surveyed on the same two days in May, any fresh spraint found was collected for DNA analysis. Therefore a large number of volunteers were potentially available with experience in the collection of fresh spraint.

For the feasibility study sufficient of the SOG volunteers were recruited for the Brue catchment to ensure catchment coverage for coordinated same day spraint collection. Detailed liaison with the SOG was necessary to ensure that survey techniques proposed for the feasibility study were compatible with the SOG's own survey aims and targets. This reorganising took two months because of the large number of individuals involved.

Samples from the Tone catchment were collected by a single volunteer, with sampling sites visited several times each month to ensure samples were collected from as many sites on the catchment at possible each month.

The Devon Wildlife Trust's Rivers and Wetland Project had established a volunteer network to carry out a quarterly otter survey covering the majority of the Devon rivers. For 'Operation Otter' the Trust enlisted and trained volunteers to use a method adapted from the national surveys. These volunteers go out on the same weekend every three months, each surveying 600m stretches of watercourse at a small number of sites. The original intention was to combine the Operation Otter surveys with spraint collection for the feasibility study. This was not possible as the DNA survey required monthly visits to a much larger number of sites, visiting each only briefly. The two survey approaches were therefore incompatible. However, Operation Otter did have a large number of dedicated and committed volunteers to call on to find people willing to take on the additional surveys required for the DNA work. Organising the Torridge surveys also took about two months. There were subsequent resourcing problems for the Torridge spraint collections, which are described in Section 6.2.4.

During the planning stage it was recognised that because of the long time period to be covered by the project it would be important to have 'spare' surveyors. These were used to cover sites when the regular volunteers were not available for any given months survey.

Volunteers preferences for surveying during the week or during weekends was also considered during the planning stage of the project. For example, in Somerset most volunteers were only available at the weekend but in Hampshire the preference was for mid week surveys. It was also found that it was easier for people to fit surveys in with their other commitments if a specific day was chosen each month. For example the Brue catchment was surveyed on the second Sunday each month. The second Sunday avoided most Bank Holidays but coincided

with high tides some months and the possible loss of some spraint in the tidal reaches of the rivers.

4.5 Training Needs

Many of the volunteers involved had previous experience of surveying sites for signs of otter activity. In the established Somerset and Devon surveys spraint were categorised as either fresh, recent or old. With experienced volunteers determining the freshness of samples was not too much of a problem. Where surveyors were not experienced a two-day site visit was recommended until the surveyors felt confident that they were correctly identifying spraint as fresh. The site was visited on day one and all spraint at the site noted. Some individuals used chalk or Tipp-Ex to mark spraint present on day one. The site was then revisited the following morning and new spraint collected. With time surveyors recognised fresh spraint without the need to visit each site on consecutive days.

4.6 Sample Storage and Transport

Samples in alcohol were stored in a spark free domestic deep freeze (at about -20^0C) until sent by special courier to Aberdeen for analysis. All samples were collected from the surveyors either on the day collected or within a couple of days so that they could be sent to Aberdeen as soon as practicable after collection. Samples were again stored at -20^0C with the DNA extracted as quickly as possible after samples were received, typically within a couple of days.

5. RESULTS

5.1 Presentation

This section of the report summarises the results of the DNA analyses of the tissue samples and of the spraint, the results for each catchment are presented in Appendix D. The results of the spraint analyses, including partial fingerprints, and a summary list of all complete DNA profiles are also presented in Appendix D.

5.2 Genetic Variability in the Population

Tissue samples of 162 otter carcasses were obtained from five collections of frozen tissue. These were collected in Cornwall, Devon, Somerset and Hampshire and mainly cover the period from 1986 to 1998. Ninety-five percent of the samples yielded sufficient DNA for reliable typing. Of the samples suitable for DNA analysis, 86% had location details such as OS grid references. Only the 133 samples of known location were used for statistical analyses.

The number of alleles at each locus fell consistently in the range three to five which indicates that otters in the region had low to intermediate levels of variability. One locus had a very high frequency of one allele such that most individuals shared the type 08 08 which meant that it could not be used for individual identification. These results indicate that under some circumstances it may not be possible to discriminate between two closely related otters such as siblings or parent and offspring from DNA fingerprinting. Nor is it possible to recognise first order relatives in these populations using the loci currently available for fingerprinting.

5.3 Subdivision of the Otter Population

No carcasses were received from the area between Bodmin Moor and Dartmoor so the sample was divided into two and a comparison was made between those found to the west of this (from Cornwall) and those to the east (mainly from Devon and Somerset). This revealed significant differences in the two sub-populations, which might result from restricted gene flow between them.

5.4 Identification of Individuals

Analysis of tissue using 9 microsatellite loci indicated that the south west population has sufficient genetic diversity for study using DNA fingerprinting. However, the level of polymorphism (variability) in the otter population corresponds to the borderline of feasibility for individual identification. There were insufficient samples from carcasses originating from Hampshire to allow statistical analysis of the genetic variability of that population.

Furthermore, only six of the nine loci were found suitable for DNA typing of spraint. Initially, it was concluded that, provided all six loci could be typed, it was highly unlikely that two otters would have identical genetic profiles. However, on the Itchen, a juvenile female (assumed no more than six months old) that died in April 1998 had the same genetic profile as another female which was first recorded in September 1997 and last recorded in July 1998. Subsequently, more detailed statistical analyses, using techniques which have only recently been applied to this problem (Appendix C) showed that due to the low genetic polymorphism it may not be possible to distinguish with confidence between same sex siblings, mothers and daughters or fathers and sons, on the Itchen.

In Somerset and Devon the statistical analysis indicated that it is possible to differentiate between parents and offspring but possibly not siblings. In December 1997 two juvenile males were found dead on the same morning a few feet apart on a road adjacent to a watercourse within the Exe catchment which is to the south east of the Torridge. These juveniles were of very similar size, weight and condition and were assumed to be siblings, run over at the same time. Although these were not found on the Torridge catchment they were part of the statistical group covering Devon and Somerset. Tissue analysis generated two very distinct fingerprints, differing at five out of the nine loci analysed showing that the method could distinguish between two probable siblings from the south west population.

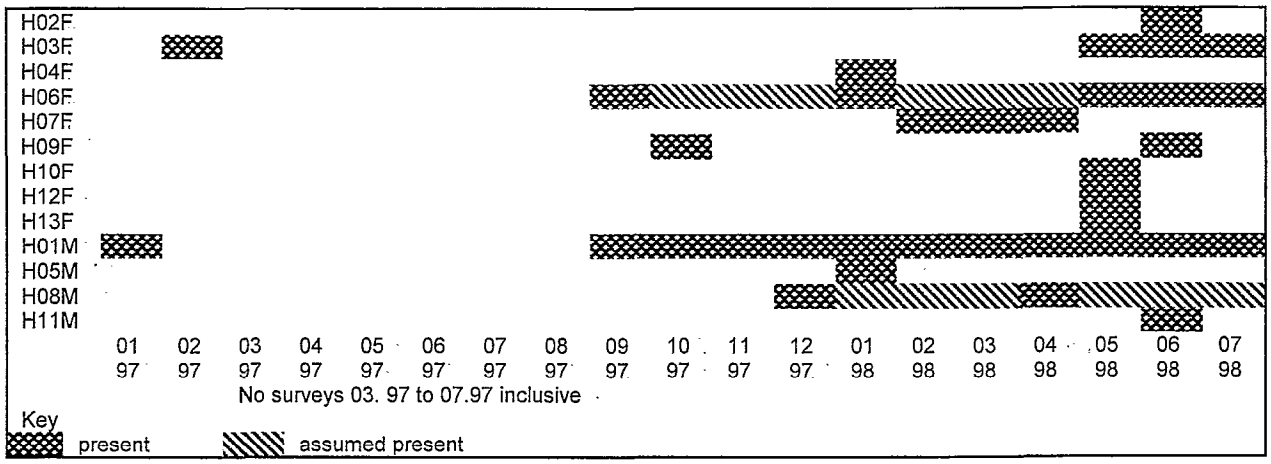
5.5 The Itchen Catchment

Over a nineteen month period 53 out of 282 samples (18.8%) gave positive DNA results which identified 13 different DNA profiles, four males and nine females. Fresh spraint was found on approximately 13% of the site visits (total sampling effort was 1785 site visits). The catch per unit effort for the Itchen was 0.03, where 'catch' is the number of reliable DNA fingerprints and unit effort is the number of sites visited. Tables 5.1 and 5.2 present a summary of the records for each DNA profile.

Table 5.1 Itchen Catchment – Results Summary

DATE	SAMPLING EVENT	SITES CHECKED	SAMPLE TOTAL	OTTER COUNT	NO OF DNA PROFILES	OTTER TOTAL PER EVENT	H02F	H03F	H04F	H06F	H07F	H09F	H10F	H12F	H13F	H01M	H05M	H08M	H11M
1.97	1	68	12	1	1	1										1			
2.97	2	68	9	2	3	1		3											
06.08.97	3	68	8	2	0	0													
20.08.97	4	67	4	2	0	0													
24.09.97	5	67	11	3	5	2				1						4			
21.10.97	6	67	9	4	2	2						1				1			
26.11.97	7	67	14	4	1	1										1			
15-16.12.97	8	67	14	5	3	2										2		1	
19.01.98	9	67	2	6	2	1											2		
21.01.98	10	67	19	6	5	2				1						4			
22.01.98	11	67	5	7	1	1			1										
14.02.98	12	73	4	7	0	0													
16.02.98	13	73	18	8	4	2					1					3			
19.02.98	14	73	9	8	0	0													
09.03.98	15	73	19	8	0	0													
10.03.98	16	73	9	8	3	2					1					2			
10.04.98	17	73	5	8	0	0													
20.04.98	18	73	25	8	3	3					1					1		1	
17.05.98	19	77	6	8	0	0													
18.05.98	20	77	12	11	7	6		1		2			1	1	1	1			
19.05.98	21	77	17	11	0	0													
22.06.98	22	77	14	12	5	4	1			1		2				1			
23.06.98	23	77	7	13	3	3		1								1			1
06.07.98	X	1	1	13	1	1		1											
20.07.98	24	77	17	13	3	2				2						1			
21.07.98	25	71	12	13	1	1				1									
TOTALS	25	1785	282	13	53	AVGE 1.5	1	6	1	8	3	3	1	1	1	23	2	2	1

Table 5.2 Itchen Catchment - Residence Summary



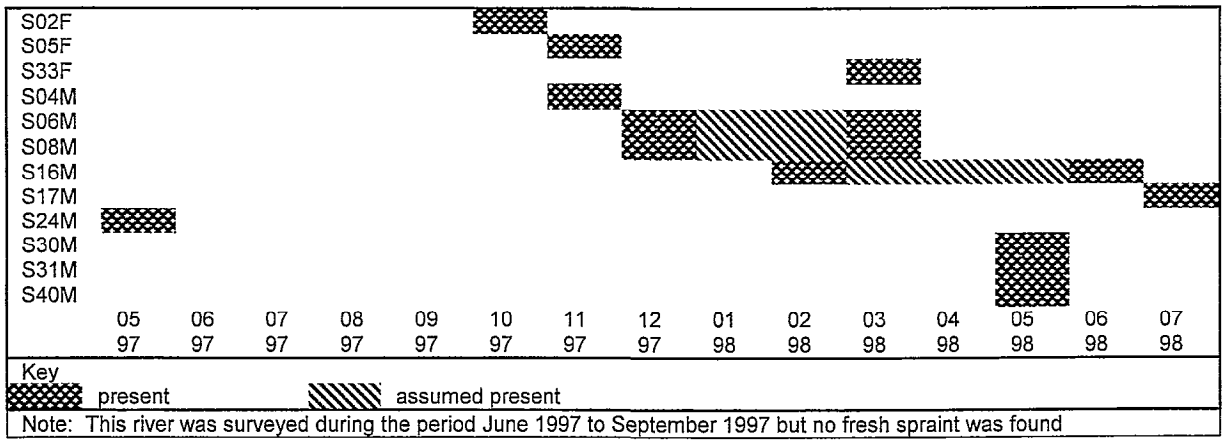
5.6 The Brue Catchment

In the ten month period from October 1997 to July 1998 16 out of 97 samples (16%) gave positive DNA results. These identified 11 DNA profiles and therefore a minimum of 11 otters, eight males and three females. In May 1997 a reduced survey (only 6 sites) identified an additional male which has not been found since. Approximately 28% of all site visits provided fresh spraint. The catch per unit effort for the catchment was 0.05 (including the May 1997 survey). Spraint analysis results are presented in Tables 5.3 and 5.4.

Table 5.3 Brue Catchment - Summary Results

DATE	SAMPLING EVENT	SITES CHECKED	SAMPLE TOTAL	OTTER COUNT	NO OF DNA PROFILES	OTTER TOTAL PER EVENT	S02F	S05F	S33F	S04M	S06M	S08M	S16M	S17M	S24M	S30M	S31M	S40M
11.05.97	1	6	2	1	1	1									1			
11.10.97	2	45	2	2	1	1	1											
23.11.97	3	24	9	4	3	2		2		1								
14.12.97	4	31	11	6	2	2					1	1						
14.01.98	5	32	7	6	0	0												
08.02.98	6	32	12	7	1	2							1					
08.03.98	7	30	8	8	3	3			1		1	1						
05.04.98	8	31	10	8	0	0												
8-10.05.98	9	25	16	11	3	3										1	1	1
23-24.05.98	10	32	4	11	0	0												
7-14.06.98	11	21	7	11	1	1							1					
12.07.98	12	32	9	12	1	1								1				
TOTALS	12	341	97	12	16	AVGE 1.3	1	2	1	1	2	2	2	1	1	1	1	1

Table 5.4 Brue Catchment – Residence Summary

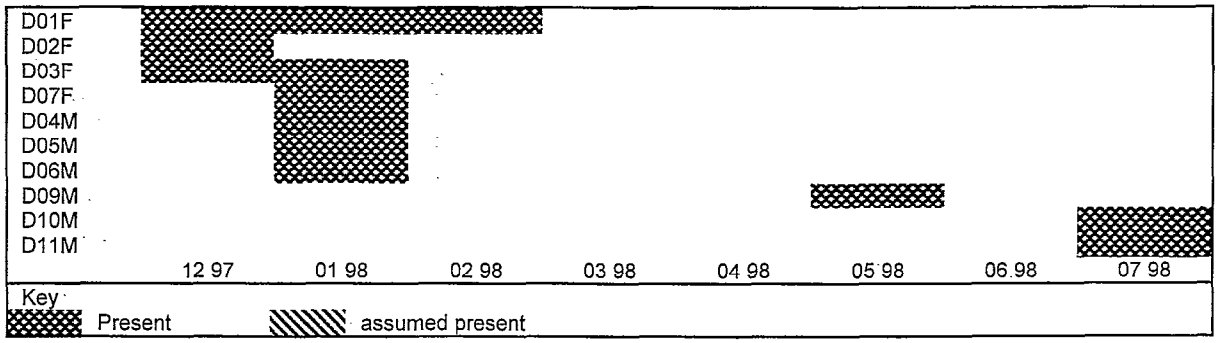


A very high proportion of the sites yielded fresh spraint compared to the other watercourses (66% compared to 13%, 28% and 47% respectively for the Itchen, Brue and Tone). All sites showed evidence of otter activity. The catch per unit effort for the Torridge was 0.1. Tables 5.5 and 5.6 list the results of the spraint analyses. The results are described in more detail in Appendix D.

Table 5.5 Torridge Catchment – Results Summary

DATE	SAMPLING EVENT	SITES CHECKED	SAMPLE TOTAL	OTTER COUNT	NO OF DNA PROFILES	OTTER TOTAL PER EVENT	D01F	D02F	D03F	D07F	D04M	D05M	D06M	D09M	D10M	D11M
10.97		20	12	0	NOT ANALYSED	0										
12.97	1	13	11	3	3	3	1	1	1							
1.98	2	20	24	7	8	6	1		2	1	2	1	1			
2.98	3	4	13	7	1	1	1									
3.98	4	20	16	7	0	0										
4.98	5	20	0	7	0	0										
5.98	6	6	10	8	1	1								1		
6.98	7	11	5	8	0	0										
7.98	8	20	2	10	2	2									1	1
TOTALS	8	134	93	10	15	AVGE 1.6	3	1	3	1	2	1	1	1	1	1

Table 5.6 Torridge Catchment - Residence Summary



5.7 The Tone Catchment

Spraint was collected from the Tone catchment from June 1997 until July 1998. A total of 374 sites was visited with 175 spraint collected. Reliable DNA profiles were typed from 35 samples (20%) and 22 different otter DNA profiles identified, 12 males and 10 females. Four of the female profiles were identified more than once, as were four males. The catch per unit effort for the Tone was 0.09. The results of the analyses are summarised in Tables 5.7 and 5.8.

Table 5.7 Tone Catchment – Results Summary

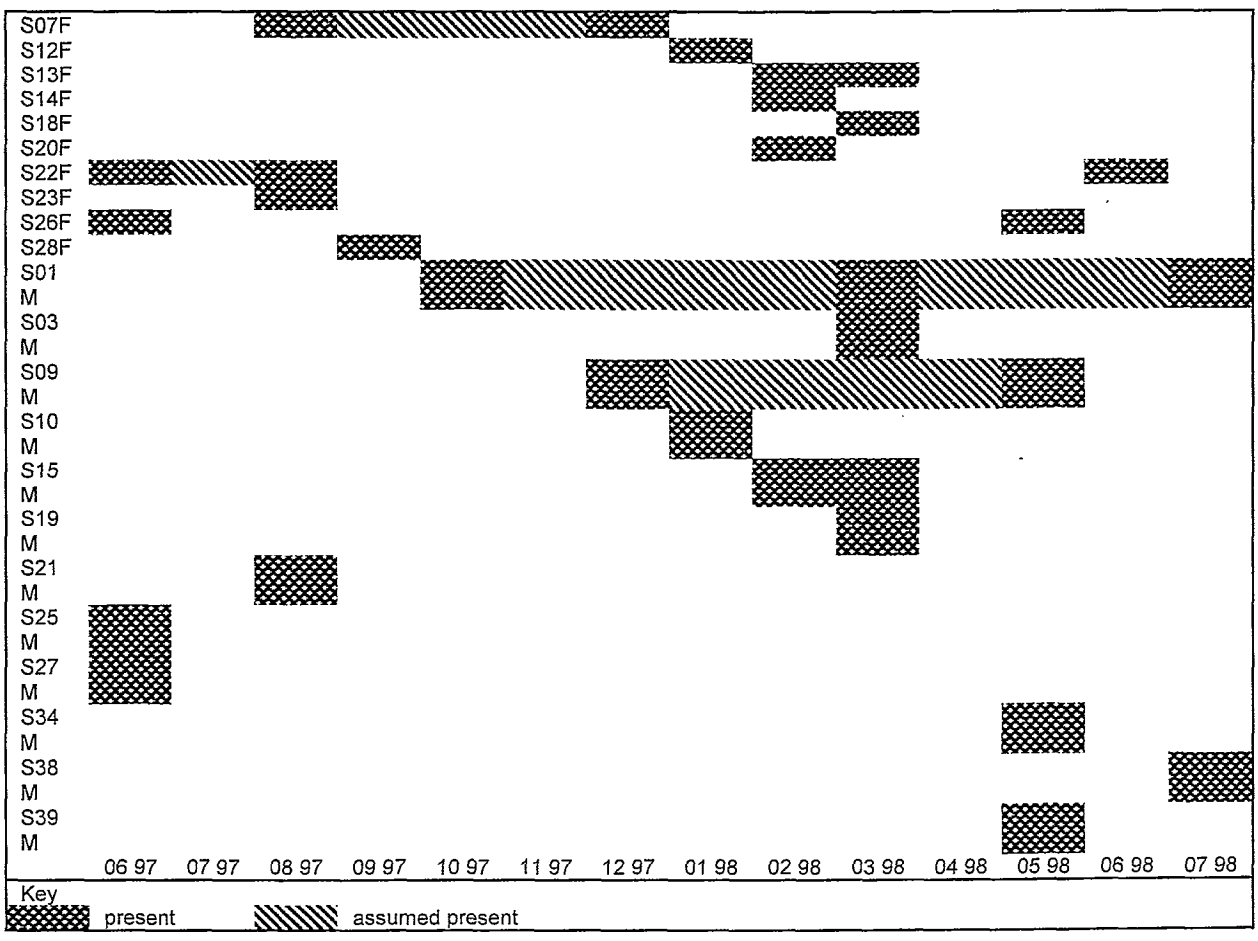
a) Females

DATE	SAMPLING EVENT	SITES CHECKED	SAMPLE TOTAL	OTTER COUNT	NO OF DNA PROFILES	OTTER TOTAL PER EVENT	S07F	S12F	S13F	S14F	S18F	S20F	S22F	S23F	S26F	S28F
6.97	1	36	16	4	4	4							1		1	
7.97	2	6	3	4	0	0										
8.97	3	16	10	7	4	4	1						1	1		
9.97	4	12	5	8	1	1										1
10.97	5	31	3	9	1	1										
11.97	6	6	4	9	0	0										
12.97	7	10	4	10	3	2	2									
1.98	8	48	12	12	3	2		1								
2.98	9	40	16	16	4	4			1	1		1				
3.98	10	55	31	19	7	6			2		1					
4.98	11	8	7	19	0	0										
5.98	12	79	47	21	4	4									1	
6.98	13	14	13	21	1	1							1			
7.98	14	13	4	22	3	2										
TOTALS	14	374	175	22	35	AVGE 2.2	3	1	3	1	1	1	3	1	2	1

b) Males

DATE	SAMPLING EVENT	S01M	S03M	S09M	S10M	S15M	S19M	S21M	S25M	S27M	S34M	S38M	S39M
6.97	1								1	1			
7.97	2												
8.97	3							1					
9.97	4												
10.97	5	1											
11.97	6												
12.97	7			1									
1.98	8				2								
2.98	9					1							
3.98	10	1	1			1	1						
4.98	11												
5.98	12			1							1		1
6.98	13												
7.98	14	2										1	
TOTALS	14	4	1	2	2	2	1	1	1	1	1	1	1

Table 5.8 Tone Catchment - Residence Summary



5.8 Comparisons Between the Different Populations

5.8.1 Results Summary

The results of the survey work on all four catchments are summarised in Table 5.9 below.

5.8.2 Resident Populations

Resident status has been assumed for otters identified in two separate month's surveys. If a profile is found only once, that individual has been assumed a non-resident. However, with only one in five spraint samples analysed giving a DNA profile, it is possible that spraint from these individuals has been collected more than once, but that the samples did not provide sufficient DNA for the development of that individuals profile. Two samples from the same individual on the same night do not indicate resident status.

Where individuals do not show up in the population for long periods it is not possible to know whether that individual had moved out of the area for that period, or was present but had not been found by the survey. For example, otter S22F on the Tone where there is a 9 month gap between positive samples. Individuals identified only once after April 1998 have been omitted from the non-resident category as, if these are new individuals, there is insufficient data to determine which category they belong to.

Figures 5.1 to 5.4 present a comparison of the number of different otter DNA profiles found in any one month's samples compared to the incremental total of profiles identified to date. It is unlikely that all otters have been identified in any of the catchments and new otters continued to be identified up to the end of the project although some of these were undoubtedly only temporarily resident.

Table 5.9 Results Summary

	Itchen	Brue	Torridge	Tone
Otters Identified	14*	12	10	22
Sex ratio (m:f)	1:2.25	1:0.3	1:0.67	1:0.83
Samples collected	282	97	93	175
Total DNA fingerprints	53 (18.8%)	16 (16.5%)	15 (16.1%)	35 (20.0%)
Sampling effort (sites x visits)	1785	341	134	374
Avge number of spraint collected per visit	0.13	0.28	0.69	0.46
Average fingerprints per otter	4	1.3	1.5	1.6
Maximum fingerprints per otter	23	2	3	4
Maximum known residence[1] - male	19 months	6 months	0	10 months
Maximum known residence - female	18 months	0	3 months	14 months
Ratio of resident males to females	1:2	no resident females	no resident males	1:1.3
Ratio of non-resident males to females	1:2	1:1.5	1:0.67	1:1
Maximum known range - male	39 km	12 km	8 km	10 km
Maximum known range - female	17 km	0	13 km	9 km
Catch per Unit Effort	0.03	0.05	0.1	0.09

*A minimum of 14 otters but with 13 different DNA profiles (see Section 6.2.2).
[1] Otter profiles only found once are assumed to indicate non-resident individuals (Section 5.8.2).

The lowest catch per unit effort was identified for the fragmented population on the River Itchen and the highest for the two established populations of the Tone and Torridge. This ratio should increase with increased population density if all animals have an equal chance of 'capture'.

Table 5.10 Percentages of Non-Resident Otters

	Itchen	Brue	Torridge	Tone
Percentage of males non-resident	50	50	100	67
Percentage of females non-resident	25	100	60	60

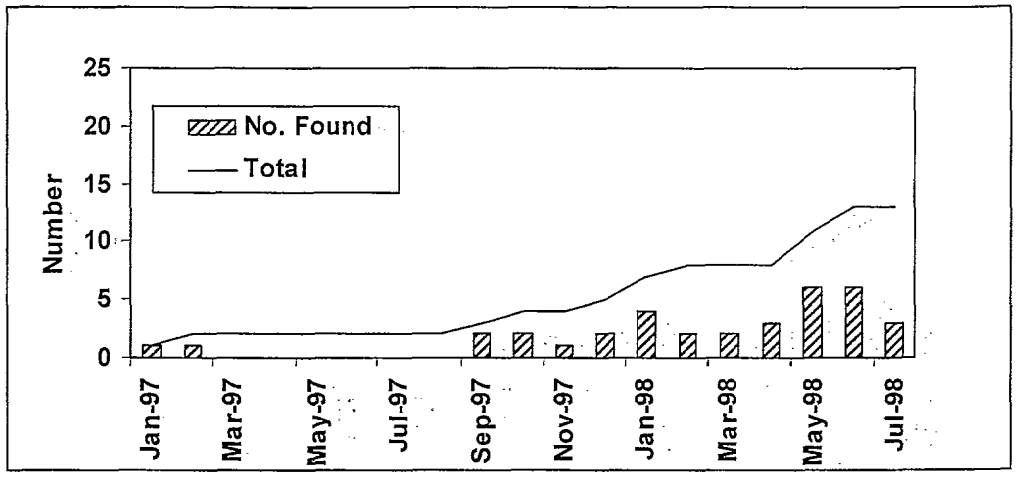


Figure 5.1 Itchen Catchment – Number of Otters Found and Total Identified

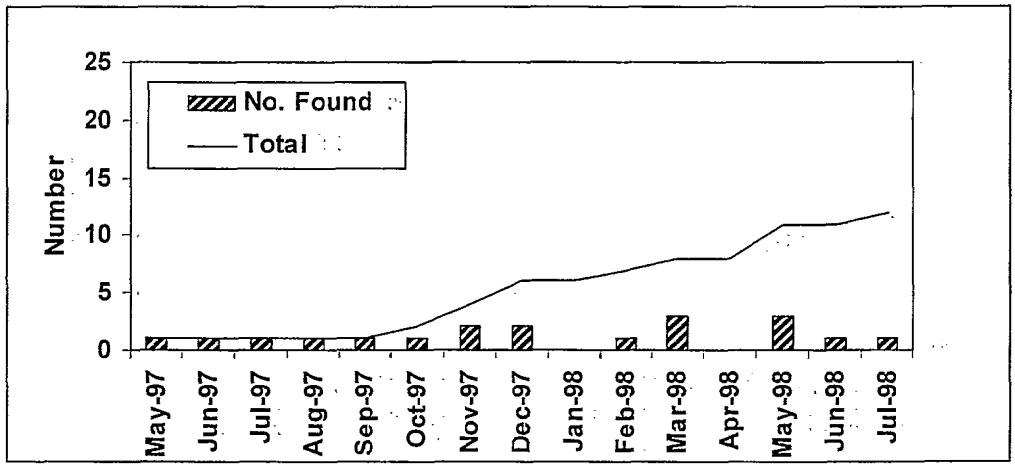


Figure 5.2 Brue Catchment – Number of Otters Found and Total Identified

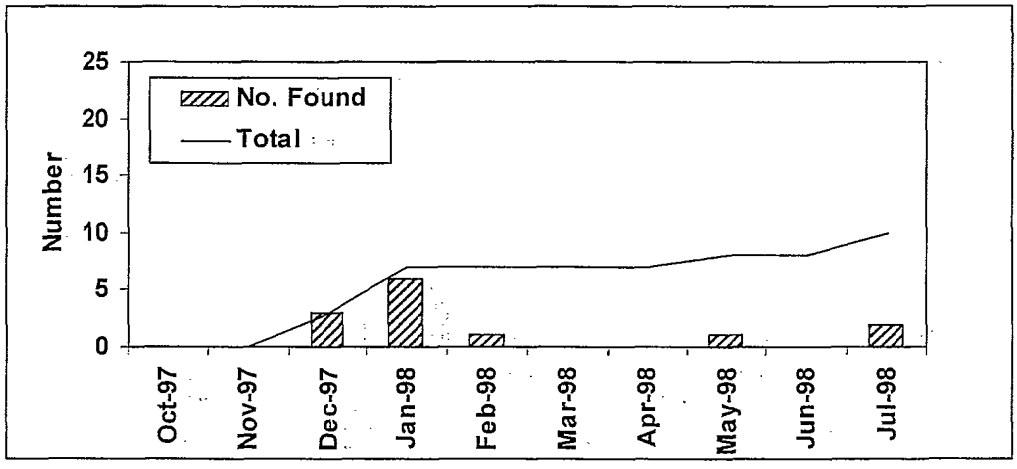


Figure 5.3 Torridge Catchment – Number of Otters Found and Total Identified

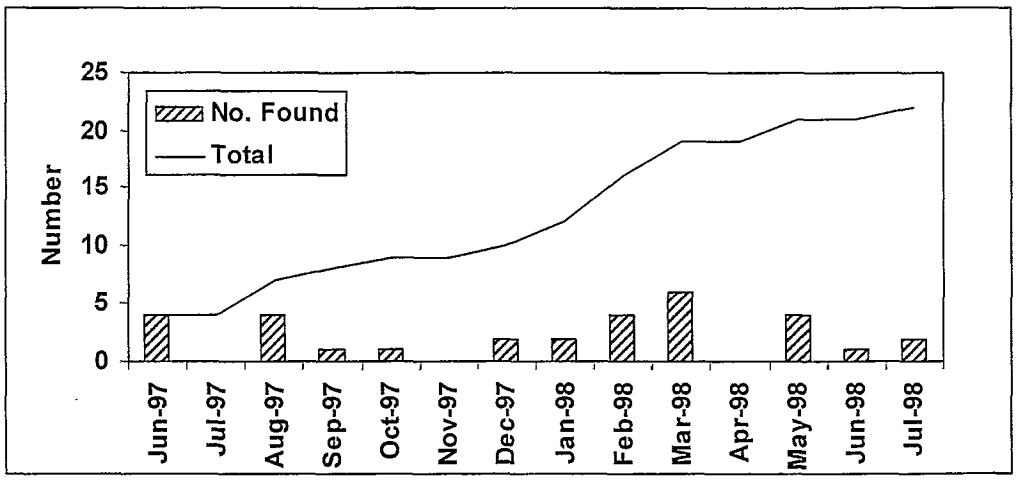


Figure 5.4 Tone Catchment – Number of Otters Found and Total Identified

5.8.3 Sex Ratios

There is no discernible pattern in the sex ratios of the different populations. The apparent absence of resident females in the Brue catchment could limit breeding potential and hence the natural recovery of this population.

5.8.4 Home Ranges

The known or minimum home range is defined as the maximum distance between spraint from an individual, assuming that only one individual is represented by that DNA profile.

Apart from H01M on the Itchen most known home ranges identified so far are between 6 and 17 km. There appears to be little difference between males and females. The home range of H01M overlaps the known ranges of four resident females (see Figure 5.5).

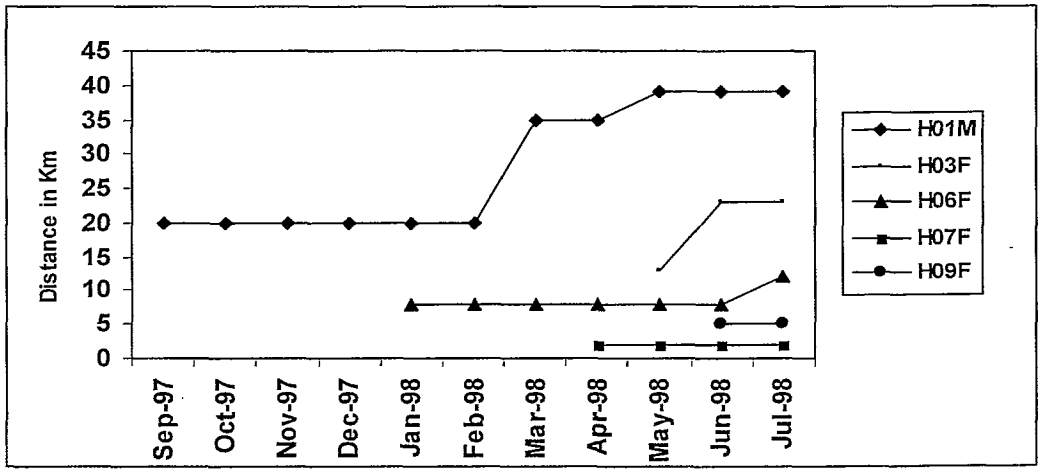


Figure 5.5 River Itchen – Known Home Ranges for Resident Otters

This pattern has not been found in the other catchments although there is a lot of overlap between individuals within those catchments. However, analysis of tissue from a dead juvenile found on the middle Itchen in April 1998 has shown that there were at least two different otters sharing the same DNA profile H06F (see Section 6.2.2) and 'known home ranges' for the Itchen otters could therefore be over estimates.

More data is required before a pattern is identified on the Tone and Torridge. On the Brue the population centres on two areas with a few isolated individuals. Further survey data and an assessment of the resources available is required to understand the patchy distribution of otters within the Brue catchment.

6. DISCUSSION

6.1 The Project Objectives

6.1.1 Analysis of Carcasses

Objective: The collection and DNA fingerprinting of tissue samples from about 100 carcasses from southern England to provide essential data on levels of genetic diversity in the feasibility study area.

The target was exceeded with 168 different tissue samples analysed. Analysis using 9 microsatellite loci indicated that the south west population has sufficient genetic diversity for study using DNA fingerprinting. There were insufficient samples from carcasses originating from Hampshire to allow statistical analysis of the genetic variability of that population. It was concluded that the south west population has sufficient genetic variability for DNA fingerprinting of spraint to be feasible. However, only 6 loci proved reliable for analysis of DNA extracted from spraint. With 6 loci only there is not enough genetic variation to determine relatedness, even with 1st-order relatives.

All individuals from the Itchen population proved to be identical at locus 701 so that it was not possible to differentiate between same sex parent/offspring and full siblings. This is discussed further in Section 6.2.

6.1.2 Spraint Collected

Objective: The collection and DNA fingerprinting of about 500 spraint from a transect across high to low density otter populations in southern England.

The original estimate of 500 spraint was exceeded. However, this estimate was for spraint on the Torridge (200), Brue (200) and Itchen (100). The proportions reflected the estimated density of otters and likely number of spraint to be found. The actual numbers collected from the Torridge and Brue (89 and 97 respectively) fell below the estimated total partly due to the delayed start to surveys. Also, on the Torridge there were several months when spraint sites were washed out. Additional samples were collected on the Itchen (261 in total) as the overall total for all the catchments was below target. An additional 200 samples had been allowed for the PhD study in Somerset. The inclusion of samples collected from the Tone catchment (175) brought the overall total up to well over 600 spraint analysed.

6.1.3 Progress Reviews

Objective: To report and review progress regularly during the project.

Progress was reviewed at regular Project Board meetings, which resulted in some changes to the sampling strategies. These included additional surveys of the Brue and Itchen and splitting the surveys on the Torridge to cover several days to allow for reduced numbers of surveyors. More detailed reviews of survey strategies were hampered as the results of analyses were only received immediately prior to the quarterly Project Board meetings. Ideally samples from each monthly survey should be analysed and reviewed prior to the next survey on the watercourse.

It was recognised that feedback to the surveyors on a frequent basis was important to maintain motivation. Identification of new otters within a volunteers survey area was always received with great excitement and helped to increase surveyors' identification and ownership of the project's objectives. Otters identified became 'their otters', their success. This type of feedback provides very important motivation at 5am on a Sunday morning when it is pouring with rain. Providing feedback on the project as a whole emphasises just how important everyone's role is in the study. Where stretches of river were consistently negative it was found helpful to swap survey areas so that surveyors did not lose interest or become despondent because they had not found any fresh spraint for months.

Minutes of the Project Board Meetings and Progress Reports are available on request to the Environment Agency.

6.1.4 Feasibility of a Long Term Study

Objective: To produce guidance and recommendations on the feasibility of a long term study into factors limiting otter recovery in the UK.

This project has shown that it is possible to survey and monitor otter populations on a catchment basis using DNA fingerprinting where there is sufficient genetic variability within the population. However, in southern England some populations have levels of variability below that needed to reliably discriminate between individuals (River Itchen) whereas others are close to the borderline (in the south west).

Although the proportion of spraints successfully typed in the feasibility study is greater than in some early trials, where only one in seven could be typed, there would be considerable benefit in developing techniques or protocols which would lead to a higher success rate.

A useful baseline data set has been developed which has provided an introduction to the populations of the four catchments. However, there is insufficient information at present to make very reliable comparisons between these populations and published data from other studies. The data have also raised new questions.

Identification of individual DNA profiles in the intermediate and fragmented populations has shown that they contain more otters than expected. These numbers represent minima for the populations since a) in at least one case two otters had the same profile; b) some of the partial fingerprints appeared to differ from the full profiles; c) some otters may not have been recorded, either because their spraints were not collected or their spraint may not have been successfully typed. Further studies are needed to establish sampling procedures that can provide reliable estimates of the numbers of otters present at one time.

The distribution and movements of individuals within the Brue (intermediate) and the Itchen (fragmented) populations are very different. The data for the Itchen identifies at least five resident otters over 40 km of watercourse, higher than expected, while there was no evidence of resident females on the Brue. Longer term studies are needed to determine whether these observations are typical of these rivers or result from different environmental conditions or stages of recolonisation.

For all catchments there is a high percentage of animals found only once. Is there a substantial population of non-residents, which could provide an important source for recolonisation?

The project has demonstrated that surveying and monitoring otter populations using DNA analysis of spraint is feasible and has the potential to answer questions such as these. However, in order to fully exploit this technique, there is a need to identify more polymorphic loci for use in fingerprinting. In addition increasing the proportion of spraints that are successfully typed would greatly enhance its value. Detailed recommendations for a long term study are set out in Section 8.3.

6.1.5 Resource Requirements

Objective: To identify the resource needs, in terms of costs and time, and a robust protocol which could be repeated by anyone in the future if the method is considered feasible.

A protocol for DNA extraction and typing is presented in Appendix D. The field method and sample survey sheets are presented in Appendix E. Possible improvements on the laboratory protocol are recommended in Section 8.2. The costs of the project in terms of resources and mileage are summarised below.

Table 6.1 Resource Requirements per Sampling Event

Catchment	Itchen	Brue	Torridge	Tone
Average number of sites checked	71	28	9	59
Total miles (all surveyors)	167	450	57	120
Total number of hours (all surveyors)	25	28	4	18

The costs, based on mileage and time requirements, are dependent on the catchment characteristics. These may also change with any future improvements to sampling methods and success rate of analysis.

The feasibility study has produced a protocol for field sampling together with Health and Safety guidelines (see Appendix E) and background information on DNA Fingerprinting (see Appendix B).

6.2 Problems Encountered

Several problems were experienced during the feasibility study and these are discussed below. Recommendations to resolve these problems are presented in Section 8.0.

6.2.1 DNA Typing

Many of the spraint samples only gave partial DNA fingerprints. Not all loci developed and those loci that did develop may not have been reliable if the DNA extraction was incomplete. For example, 02 06 could actually have been 04 06. Where four out of the six loci developed it is probable that the results for those four loci were accurate. However, to ensure accuracy only those profiles with five or six loci developed have been included in the analysis of results.

Anal jelly was found to give a significantly higher success rate compared to spraint during analysis (Table 6.2) and should always be collected.

Table 6.2 Comparison of Success of Analysis of Spraint Compared to Anal Jelly

	Sample Size	Percent Identified
Jelly	61	34%
Spraint	269	17%

Table 6.3 Analysis of Effect of Time of Spraint Collection Against Success of Analysis

Time	Sample Size	Percent Identified
Before 07.00	31	13%
07.00-08.00	44	20%
08.00-09.00	74	27%
09.00-10.00	85	21%
10.00-11.00	57	18%
After 11.00	44	14%
Total	335	20%

There appears to be no significant difference between these time periods. It should be noted that there was high variability between the success rate of analysis between each batch of samples. Sample batches were too small to test whether there was any significant differences due to the time spraint was collected in the field within any given batch of samples. Insufficient data was collected to be able to look at the effects of temperature, rainfall and substrate under spraint when collected, on the success rate of analysis.

6.2.2 Identifying Individual Otters

For the Itchen catchment there is uncertainty as to whether any one DNA profile is specific to an individual otter. On 23 April 1998 a juvenile female (2.7 kg and 82cm total length) was found dead on the middle Itchen at SU 4772 2743. There is no reliable database correlating body weight or body length to age for wild animals. The age of this animal is therefore unknown but it is unlikely to have been more than 6 months old. Tissue samples from the dead animal generated the same DNA profile that had been developed from spraint collected during September 1997 and in June and July 1998, after the animal's death. The initial conclusion was that a mother and cub shared an identical genetic profile for the 6 loci analysed.

Following the analysis of the 168 tissue samples it was concluded that there was just enough genetic variability within the south west population for DNA extraction from spraint to provide reliable indications of individual otters based on the 6 loci typed. The Itchen population was not included in this initial analysis as there were too few tissue sample DNA profiles to be included in the statistical analysis. All spraint samples collected from the Itchen catchment have been identical at one locus, leaving only 5 loci for identification of individuals. This would appear to bring the genetic variability below the level at which individual otters can be reliably identified on the Itchen. Statistical analysis of the DNA profiles from spraint collected in Devon and Somerset indicate that individual profiles may be shared by full siblings but not parent/offspring or unrelated individuals using the current

version of the DNA typing system. However, two assumed siblings found in Devon gave two very distinct fingerprints (see Section 5.4).

6.2.3 Project Initiation

A longer than anticipated set up time for two of the volunteer groups was experienced because of the large number of individuals involved. For the River Brue catchment volunteers were asked to reorganise an existing survey programme and routine.

6.2.4 Training Volunteers

Finding and training enough volunteers where there is no existing group to carry out the monthly DNA spraint collections proved difficult in Devon. Loss of volunteers due to job changes and house moves over a long term study will always be a problem. There were also months when some volunteers had other commitments. This was particularly true during school and national holidays.

6.2.5 Fluctuating River Levels

For some rivers, the regular spraint sites were frequently under water or washed-out, preventing collection of spraint samples. For example the Torridge responds rapidly to rainfall with a rise in river levels and high flood peaks which meant that monitoring sites were washed out quite frequently. This resulted in a smaller data set for this catchment than anticipated.

7. CONCLUSIONS

The key conclusions from the project are summarised below:

- The Feasibility Study was an outstanding success. It answered many of the questions asked, achieved its objectives and identified ways in which the DNA Fingerprinting technique needed improving. The Study has provided a unique insight into otter biology in southern England.

- The 6 loci available for spraint analysis were not sufficiently variable to permit identification of individual otters on the Itchen where the genetic diversity of the population is low. The south west population appears to be on the borderline of variability required to successfully identify individuals.

- More loci need to be developed to confidently identify individual otters in populations with low genetic diversity. This will require renewed research effort and resources. The number of loci required will depend on the levels of polymorphism they exhibit but a total of fifteen would be sufficient at the levels found at the loci already used.

- Within the Itchen population two otters, assumed to be closely related, were found to share the same DNA profile for the 6 loci analysed. This implies that the total number of otters identified, at least on the Itchen, is a minimum. This also means that the estimates of home range may be over estimated being based perhaps on more than one individual. There was no evidence of similar duplication within the Brue, Tone or Torridge populations.

- Otter DNA was recovered from 20% of the spraints collected. A consistently higher success rate would greatly improve the effectiveness of this survey method. Staff at the University of Exeter are currently investigating this.

- The level of genetic variability in the UK otter population is such that it is probably not possible to determine the relatedness of individual otters using existing techniques.

- The genetic variability of the population to be surveyed should be checked by analysis of tissue samples prior to collection of spraint. DNA profiles of at least 10 otters are required to determine the suitability of a population for applying the technique to spraints.

- To be cost and resource effective the survey method requires the use of highly committed and motivated volunteers with individual training needs. A sampling protocol and proper equipment is necessary. Health and Safety is of paramount importance.

- The success rate of analysis was greater from anal jelly than spraint. Anal jelly should be collected as well as spraint wherever possible.

- Rapid analysis of spraint is required to enable a continuous review of any survey structure and allow the feedback of results to the volunteers.

- The physical characteristics of the rivers to be surveyed will influence the planning and frequency of surveys.

- The method is appropriate to long term monitoring but could also be used for an intensive survey/sampling programme to assess a population over a short time scale.

- Increasing the density of survey sites, as on the Itchen, does not provide significantly more information on otter movements, known home ranges etc directly. However, the associated increase in the number of spraint found does improve the database. Increasing the number of survey days should have the same effect.

- The period covered by survey should be extended to be able to confirm the information gained so far on individual otters known home ranges and the estimated total number of otters within each catchment. However, preliminary findings indicate very different distributions between the Brue, Tone and Torridge. The Itchen results are difficult to interpret due to the duplication of DNA profiles.

- Addressing the problems identified in the Feasibility Study will require new resources and research effort. Improvements to the technique will not only facilitate a longer term study but should also permit its development as a reliable standard tool for monitoring otter populations.

8. RECOMMENDATIONS

8.1 Project Planning and Management

8.1.1 Project Initiation

Prior to any decision to use DNA fingerprinting of spraint to survey a population it is advisable to establish the DNA baseline of that population through tissue analysis. This will be dependent on the availability of suitable material. DNA profiles from at least 10 carcasses are required to statistically confirm the suitability of the population.

8.1.2 Training Volunteers

Future studies will need to acknowledge the long set up period required for volunteer recruitment, training and deployment and to recognise the organisational time needed for new volunteers throughout the project. Two or three months is a realistic time scale from project start to commencement of fieldwork.

It is recommended to train 'surplus' surveyors, who can stand in, either on a short or long term basis when regular surveyors are unable to cover their usual sites. Where there is a shortage of volunteers, surveys of a river could be completed over several days. However, this puts a lot of pressure on individuals if they need to go out several days a month. Each volunteer's responsibilities should never be so onerous that they lose interest in the project. It is recommended that any individual's programme is readily achievable. The number of sites can always be increased as experience and enthusiasm grows.

In addition to training in field skills to identify and collect fresh otter spraint, volunteers should be provided with adequate health and safety instructions (Appendix E) and offered background information on DNA and the fingerprinting technique (Appendix B).

Surveyors should always be encouraged to work in pairs, for safety reasons and also for support. Covering expenses such as mileage and providing basic equipment such as boots or waders may enable volunteers with spare time but not financial resources to get involved in the project.

8.1.3 Fluctuating River Levels

Programme flexibility will be more important for some rivers than others and should be a consideration in the planning stage of a study. This can be difficult if volunteers are only available at the weekend. It is easier for people to fit surveys in with their other commitments if a specific weekend is chosen each month; for example, the Brue catchment was surveyed the second Sunday each month. The second Sunday avoided most Bank Holidays but coincided with high tides some months and the possible loss of some spraint in the tidal reaches of the Rivers. In some months no samples were collected due to high water levels following heavy rainfall. Reorganising survey dates at short notice to suit weather conditions may not be practical although it may be possible to identify those volunteers with flexibility at the beginning of the study. If monitoring sites are washed out on the specified survey day, these 'reserves' may be willing to go out as soon as water levels drop. These issues should be discussed with individual volunteers as they are introduced to the project.

8.1.4 Feedback of Results

Any survey or project of this type is impossible without highly motivated volunteers. It must be recognised that volunteers are being asked to make a big commitment in terms of time and effort. Early starts, in often far from ideal weather conditions is a lot to ask anyone. Frequent feedback of results is very important in maintaining interest.

The use of a regular newsletter with results and contributions from volunteers is recommended to maintain a regular feedback of results to maintain volunteer interest and involvement. Annual or more frequent meetings of a more social nature will strengthen the team allowing people to share stories and even complaints. These can be very informal social gatherings, a thank you to everyone for their hard work, and can usefully incorporate presentations of the results showing everyone what their hard work has achieved on a wider scale. For example, the Hampshire team had a rounders match followed by a BBQ for the volunteers. The Somerset results were presented to the SOG at their AGM followed by an informal discussion during a buffet supper. One request from the volunteers at these meetings was that they would like the opportunity to go out on the surveys of the other catchments.

8.1.5 Publicity

Publicity was also found very useful in gaining external support for the project and in maintaining the enthusiasm of those involved. A press release prepared and issued by the Project Board in April 1998 raised strong interest in the project with good coverage in local and national papers and several news items on television and radio.

To a certain extent this level of coverage reflects the universal appeal of the otter but it also acknowledges the importance of the application of a new method of surveying otters. Currently there is high level of interest in the use of DNA for surveying different species, and the potential of the application of DNA fingerprinting to otter conservation is no exception. The project was well represented by a feature on BBC TV Tomorrow's World in September 1998. As a result of the publicity, a number of enquiries have been received about the Project from students at various UK universities and from other Government organisations interested in learning more about the DNA fingerprinting technique.

There has generally been good support for the otter surveys from the public. Frequent questions are asked when out surveying. Most landowners approached for permission for access have given enthusiastic support. Any concerns or suspicion as to what you are up to typically changes to support once otters are mentioned, together with requests for information as the project progresses. It may be helpful if a small pamphlet/news sheet on the project was produced for distribution to volunteers, landowners and general public during the next phase of the project. This may also help to encourage sponsorship of the project.

8.2 DNA Typing

8.2.1 Spraint Collection Methods

There is a significant difference between the success rate of DNA extraction and typing from anal jelly compared to spraint and jelly should therefore always be collected for analysis.

It is recommend that, once the success rate of laboratory analysis becomes more consistent, the effect of time of collection of spraint should be assessed. It may be appropriate to record

time spraint collected after sunset previous day (assuming otters only active after dark) to see if there is any correlation with success of analysis. The effects of overnight temperatures and weather conditions should also be assessed.

8.2.2 Success Rates of DNA Profile Development

The following recommendations have been made to improve the success rate of profile development where incomplete DNA Profiles develop.

The detection method used for DNA typing of microsatellites and SRY (sex chromosome) should be changed from radioactivity and ethidium bromide, respectively, to fluorescence detection on an ABI automated DNA sequencer. This should increase the detection rate of low amounts of PCR product undetectable using radioactivity. If this was used in conjunction with multiplex PCR, this change will also lead to labour savings and more efficient use of limited amounts of spraint DNA.

The number of replicate typings per microsatellite locus/sample should be increased to eight in cases where four replicates have given a homozygous genotype. This will increase the confidence in such designations, because they can also arise from the dropout on one allele in a true heterozygote. Four replicates are sufficient for the SRY marker.

Improving the success rate of DNA typing of samples and simplifying the collection and extraction methods would reduce the costs and increase the effectiveness of the technique.

8.2.3 Differentiating Between First Order Relatives

Increasing the number of loci assessed will increase the reliability of identification of individual otters. It has been estimated that, at the levels of polymorphism found in south west England, 15 loci would be required to reach the same level of confidence in identification as has been found for otters in Scotland (J. Dallas, unpublished data). If new loci have a greater degree of polymorphism, fewer will suffice. Fewer loci are required where there is high genetic variability within a population. In inbred populations with very low genetic diversity derived from captive releases it may not prove possible to use this method. Staff at the University of Exeter are addressing this problem by investigating alternative microsatellite sequences.

8.3 Further Research

8.3.1 Proposal

A detailed proposal is presented in Appendix F. To maintain continuity in the data set, in anticipation of the long-term study, the four catchments are still being surveyed, with spraint samples now being stored at the University of Exeter using the protocol developed by John Dallas.

8.3.2 The Otter BAP

The Otter BAP identified two 'Objectives and Targets':

- Maintain and expand existing otter populations.

- By 2010, restore breeding otters to all catchments and coast areas where they have been recorded since 1960.

In order to achieve these, it recommended a series of proposed actions under the headings: Policy and legislation, Site safeguard and management, Species management and protection, Advisory, Future research and monitoring, Communications and publicity.

The proposals for a further three year study mainly addresses issues identified under Future research and monitoring, Site safeguard and management and Species management and protection.

The general aims of the proposed three year study are:

- To develop the technique of DNA fingerprinting otter spraint so that it can become a standard tool for monitoring and studying otter populations.

- To extend the successful pilot project into a further three year study in order to provide information required to ensure that the Otter BAP Objectives are achieved, particularly in terms of successful monitoring and management of otter populations.

- To improve our understanding of otter ecology and provide data for modelling and/or a PHVA (Population and Habitat Viability Analysis) in order to assess the likelihood of the BAP objectives being achieved by natural recolonisation.

- To work closely with other projects where DNA fingerprinting would contribute significantly to the management and conservation of otter populations.

8.3.3 Specific Objectives

Note: the bracketed references (BAP: n.n.n) following each objective below refer to the relevant paragraph(s) in the Biodiversity Action Plan for Otters which is attached as Appendix A.

8.3.3.1 Development of the technique

- To improve the reliability of the technique by increasing the number of genetic loci which can be fingerprinted, thereby reducing the risk of misidentifying otters. A target of 15 polymorphic loci is proposed.

- To improve techniques for collecting and extracting otter DNA from spraints in order to simplify field collection, reduce the costs of extraction and storage and, if possible, increase the number of spraints which can be successfully typed. A target of typing 33% of very fresh spraints is proposed.

8.3.3.2 Monitoring and Modelling

- To determine whether a relationship exists between the standard otter survey method (Strachan *et al.*, 1990) and the results of DNA fingerprinting (monitoring at the national, catchment or county level). (BAP: 5.5.4; 5.5.6)

- To devise a protocol for using DNA fingerprinting as a means of assessing the size and nature of local otter populations (monitoring at the local level). (BAP: 5.5.6)

- To provide information on otter movements, home ranges, population structure for modelling populations. (BAP: 5.5.4)

8.3.3.3 Factors Affecting Recolonisation

- To assess the relationship between habitat quality (including food supply) and otter populations (BAP: 5.2.1, 5.2.2, 5.5.1)
- To assess the impact of disturbance on otter populations (BAP: 5.5.3)
- To assess the impact of riparian management (BAP: 5.2.1, 5.2.2)

8.3.3.4 Relationships with Other Projects

To collaborate with other projects on otters in southern Britain where information on otter identity and genetics would be of value, including:

- the release of otters from captivity;
- proposed studies on breeding;
- the collection of road casualties and other otter corpses.

8.3.4 Approach

It is proposed to develop the technique and extend the programme for a further three years using the same basic approach as in the pilot study but the work will be co-ordinated by the University of Exeter. Work on development of the technique and the extraction and fingerprinting techniques will be undertaken in the laboratory of Professor John Bryant by a full time research assistant and technician. The field work will be undertaken by a second research assistant, also based in the School of Biological Sciences at the University of Exeter, under the supervision of Dr Paul Chanin. The network of volunteers will be maintained and extended and it will be part of the research assistant's responsibility to recruit, train and co-ordinate the efforts of volunteers.

The existing study sites will be maintained if possible to enable us to follow the otter populations in them over a period of at least four years. It is intended to recruit and train more volunteers to work on the Torridge and set up one or more additional sites.

The detailed proposal on how these objectives will be met is set out in Appendix F.

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10. GLOSSARY

Allele	Common shortening of the term allelomorph. One of two or more forms of a gene that arise by mutation and occupy the same locus on homologous chromosomes.
Chromosome	A DNA-histone protein thread occurring in the nucleus of the cell. Chromosomes occur in pairs that associate in a particular way during cell division.
DNA	Deoxyribonucleic acid (see Appendix B)
Fingerprint	Genetic profile specific to individual otter derived from DNA extracted from the spraint or tissue sample
Gene	The fundamental unit of inheritance, comprising a segment of DNA that codes for one or several functions and occupies a fixed position (locus) on a chromosome
Genetic diversity	Differences between genetic material between individuals and within population in general
Habitat	Place with particular kind of environment inhabited by organism(s)
Holt	Enclosed Otter den or resting site
Homologous chromosomes	Chromosomes which contain identical sets of loci. Can be applied to parts of chromosomes
Home range	The area within which an animal normally lives
Locus	The specific place on a chromosome where a gene is located (pl loci)
Microsatellite	Sequence of repeats of base pairs of amino acids
Polymorphism	Having several different forms
Primer	Sequence of DNA on either side of the micro-satellite. Each satellite has its own, characteristic primer sequence
Ramsar	Wetlands designated under the Convention of Wetlands of International Importance, especially as Wildfowl Habitat (Ramsar, Iran) 1971
Resident/non-resident	Otter profiles only found once are assumed to indicate non-resident individuals for the purposes of this study
Spraint	Otter faeces
Transient	Non-resident animal
Type/typing	Specific identification of fingerprint produced by DNA analysis

APPENDIX A: THE BIODIVERSITY ACTION PLAN FOR OTTERS

APPENDIX A: THE BIODIVERSITY ACTION PLAN FOR OTTERS EXTRACTED FROM BIODIVERSITY: THE UK STEERING GROUP REPORT

OTTER (*Lutra lutra*)

1. CURRENT STATUS

1.1 Formerly widespread throughout the UK, the otter underwent a rapid decline in numbers from the 1950s to 1970s and was effectively lost from midland and south-eastern counties of England by the 1980s. Populations remain in Wales, south-west England and much of Scotland, where sea loch and coastal colonies comprise one of the largest populations in Europe. There is also a significant population of otters in Northern Ireland. The decline now appears to have halted and sightings are being reported in former habitats.

1.2 The otter is listed on Appendix I of CITES. Appendix 11 of the Bern Convention and Annexes 11 and IV of the Habitats Directive. It is protected under Schedule 5 of the WCA 1981 and Schedule 2 of the Conservation (Natural Habitats, etc.) Regulations, 1994 (Regulation 38).The European sub-species is also listed as globally threatened on the IUCN/WCMC RDL.

2. CURRENT FACTORS CAUSING LOSS OR DECLINE

2.1 Pollution of watercourses, especially by PCBs.

2.2 Insufficient prey associated with poor water quality.

2.3 Impoverished bankside habitat features needed for breeding and resting.

2.4 Incidental mortality, primarily by road deaths and drowning in eel traps.

3. CURRENT ACTION

3.1 The JNCC has prepared a Framework for Otter Conservation in the UK 1995-2000.

3.2 National surveys have been conducted every five to seven years. Local surveys by Wildlife Trusts and other organisations have established the present distribution and potential for future spread in many areas.

3.3 Research is in progress on the implications of heavy metal and PCB contamination in fish and ecosystems.

3.4 Conservation management (for example creating log piles and artificial holts, and designation of "otter havens") has proved successful in many river catchments.

3.5 The Habitat Scheme Water Fringe Option administered by MAFF is being used to manage waterside habitat in six pilot areas. MAFF also provides advice on creating otter havens on set-aside.

3.6 FA and FE promote sensitive woodland management and expansion to favour otters, through preparation and implementation of their Forest and Water Guidelines, e.g.

managing riparian areas with deciduous trees and shrubs mixed with open grassland and wetland habitat, and the prevention of sediments and other pollution.

3.7 Two SACs have been proposed for this species under the EC Habitats Directive.

4. ACTION PLAN OBJECTIVES AND TARGETS

4.1 Maintain and expand existing otter populations.

4.2 By 2010, restore breeding otters to all catchments and coast areas where they have been recorded since 1960.

5. PROPOSED ACTION WITH LEAD AGENCIES

5. 1 Policy and legislation

5.1.1 Seek to secure agreement on the UK Framework for Otter Conservation. (ACTION: JNCC)

5.1.2 Seek to ensure management agreements and incentive schemes (e.g.: ESAs, Countryside Stewardship and Tir Cymen) take account of the requirements of otters in occupied areas. (ACTION: DANI, MAFF, SOAEFD, WOAD)

5.1.3 Seek to determine by 2000 Statutory Water Quality Objectives for standing and running waters in Britain which will sustain otters. (ACTION: DoE, EA, OFWAT, SEPA, SOAEFD, Water Services Association, WO)

5.1.4 Review the protection afforded to otters by current legislation and investigate the usefulness and appropriateness of licensing to control release of otters. (ACTION: CCW, DoE, EN, JNCC, SNH)

5.1.5 Identify and resolve problems with existing legislation. Seek to clarify the definition of "trap" in the WCA 1981 and resolve inconsistencies over the use of otter guards on fish traps. (ACTION: CCW, DoE, EN, JNCC, SNH)

5.2 Site safeguard and management

5.2.1 Seek to include action for otters in Catchment Management Plans for all rivers containing otter populations by 2000, including "otter havens" in relevant areas. (ACTION: DANI, EA, SEPA, MAFF, WOAD)

5.2.2 Continue to secure appropriate management of riparian habitats and catchments in woodlands to maintain or enhance otter populations. (ACTION: FA)

5.3 Species management and protection

5.3.1 Seek to establish an "Otter Forum" to co-ordinate conservation, information exchange, publicity and research. (ACTION: JNCC)

5.3.2 Ensure otter releases are carried out only under the guidelines set out in the Framework for Otter Conservation. (ACTION: CCW, DoE(NI), EN, SNH, JNCC)

5.3.3 Attempt to limit accidental killing or injury (for example by provision of road underpasses and fyke net guards), particularly on key catchments. (ACTION: DoE(NI), DOT, LAs, EA, SEPA).

5.4 Advisory

5.4.1 Ensure the provision of information on otter requirements to key groups, to include land owners, through the publication of posters and guidelines. (ACTION: CCW, DoE(NI), EN, FA, SNH)

5.5 Future research and monitoring

5.5.1 Collate information on prey productivity, biomass and pollution in occupied and re-colonisation areas (ACTION: DANI, DoE(NI), ITE, JNCC, EA, SEPA, SOAEFD, WOAD)

5.5.2 Develop a standard methodology to analyse the level of pollution accumulation in otters. (ACTION: DANI, DoE(NI), JNCC, EA, SEPA, SOAEFD, WOAD)

5.5.3 Investigate the effects of disturbance on otter populations. (ACTION: DoE(NI), JNCC, EA, SEPA)

5.5.4 Develop and implement means to estimate otter numbers and permit population modelling. (ACTION: DoE(NI), JNCC, EA, SEPA)

5.5.5 Monitor populations and distribution of otters throughout the UK, including local survey to monitor the expansion of fringe populations. (ACTION: JNCC)

5.5.6 Pass information gathered during survey and monitoring of this species to JNCC in order that it can be incorporated in a national database and contribute to the maintenance of an up-to-date Red List. (ACTION: CCW, DoE(NI), EN, SNH)

5.6 Communications and publicity

5.6.1 Use this popular species to publicise the importance of water quality and riparian habitats to biodiversity. (ACTION: CCW, DoE(NI), EN, FA, JNCC, SNH, EA, SEPA)

APPENDIX B: AN INTRODUCTION TO DNA AND TO OTTER DNA FINGERPRINTING

APPENDIX B: INTRODUCTION TO DNA AND OTTER DNA FINGERPRINTING

or 'How a spraint in the hand...' **Dr Kathy Sykes**

B 1.1 The Importance Of Otter Spraints

Spraints are very useful to otters, they help them to 'communicate' with each other. The faecal matter carries chemicals that actually give signals to other otters. For example, an otter might be able to find out whose territory he or she is on just by the smell of a spraint. A frisky male otter may be able to work out whether a female is ready to mate. But otter spraints can be very useful to us too. Indeed, for the last 20 or so years, people have been collecting them to find out more about otter populations. The majority of what we know about otters, their local and national distribution and status, has been gleaned by surveys searching for otter spraint. The finding of a spraint is often the first indication that an otter is around, sometimes after absences of 20 years.

You may ask why use spraint to try to find out about otter populations? Otters are by nature nocturnal and secretive which makes them notoriously hard to study. Smaller animals can easily be trapped and either marked or given a radio tag. But it's expensive doing this with otters, and trapping them is difficult and may change their behaviour. Collecting spraints is cheap and effective, and it doesn't involve interfering with the animals.

However, until recently, a spraint has only been able to tell us a few bits of information: that an otter has been in a particular area; its diet. But in fact, a fresh spraint in your hand holds a whole gold-mine of information about the otter, for example, its gender and clues about its 'identity'. The information is tightly locked up, and needs some clever scientific tricks to unleash it. But we've only learned how to do this recently, with the advent of **'DNA fingerprinting'**. In order to find out how it works, we need to dive into an otter spraint, and take a *much* closer look.

But before we do that, you may be wondering '*why* do we want to know so much about otters?' Well, the more we can learn about otter populations and understand their behaviour, the better position we are in to help them to re-colonise areas and to make sure that otter populations are healthy. In section **B 1.6** there is more explanation about what we can find out by doing otter DNA fingerprinting.

In this appendix, after you've 'dived' into an otter spraint, section **B1.3** will explain the things you found there: the chromosomes, genes and DNA. The following section **B1.4** explains how you can do fingerprinting and how to use it for otters. Section **B1.5** describes the lab and field techniques and how you can interpret the results, so you'll be able to understand all the long lists of numbers in the tables of the report. Finally, the last section explains why we're doing this project and what the feasibility study has shown. It also takes a sneak view at what we'd like to do next.

B 1.2 Taking a dive into an otter spraint

Imagine a fresh otter spraint in your hand. Take a sniff. It has a sweet musky odour, a little reminiscent of jasmine tea or hay, with a dash of fish. Now imagine you're the size of your fingernail. Get ready: you're about to dive into the spraint...

You start your journey by plunging into some soft squelchy stuff. It's hard to tell what it is, but you quickly feel something hard and sharp jutting out. It's like a skeleton. You realise that it *is* part of a skeleton: it's a pile of broken fish bones. You also find some giant shield-like things almost as big as you: they are undigested fish scales. You realise that the soft stuff is actually some mucus from the otter, maybe it's carrying some of those chemicals that aid communication with other otters? You wonder what messages it might be carrying.

Figure 1 An otter spraint

Now it's time to shrink down again. This time you're about the size of the full stop at the end of this sentence. You're surrounded by blobs, like balloons filled with liquid. You grab some and find that they are cells, and that there are a great variety of them. Some are bacterial cells, presumably from the inside of the otter's gut. Others are fish cells, remaining after not being quite fully digested. Otter spraint are a rich broth of fish remains and bacteria. But there are some cells from the otter itself, probably ripped off the otter's gut wall by some of those sharp fish-bones. You also find that the squelchy mucus seems to contain lots of the otter cells.

Now, it's time to zoom into one of those cells, so you'll need to shrink again. You're already the size of a full stop, but imagine having to shrink down by the same amount *again*. Now you're over a million times smaller than you are as you sit reading these words, and you're small enough to plunge into a cell.

You just have to wriggle a little to get through the cell's outer membrane, which feels a little oily as you slide through. Once inside, the most striking thing is the nucleus, right at the centre. The nucleus has its own membrane wall to wriggle through. Once inside, you can feel very fine strands of material, that seem to be in a mad tangle, rather like a bowl of spaghetti. It's too fine to be able to see, but if you could, you'd realise that each is a spiral. Each strand is a *chromosome*, and the stuff in your hand is *DNA*, the 'Spiral of Life'.

In every nucleus of every cell, **all** the information needed to create a particular animal is locked up. The DNA, existing in long tangled strands, holds a 'recipe' with instructions about how to make up the creature. Even a single nucleus from a single cell from the otter's intestine holds information about all the other parts of the body; for instance, it holds the information that an eye cell would have needed to become an eye. It's utterly mind-boggling. This is what makes it possible to 'clone' a new creature from just a single cell of the 'parent'. And all this information is held in these lovely fine strands of material in your hand.

So, by diving into an otter's spraint you have found handfuls of cells from the otter itself which contain nuclei which have these silky spiral strands of DNA. These should enable you to unlock masses of information about the otter. All you'd have to do is:

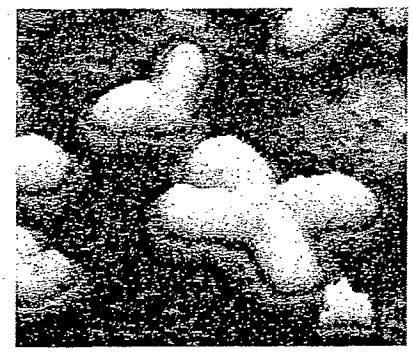
(1) increase the quantity of DNA from the cells (since the DNA from a handful of cells is barely enough to detect);
(2) find some way of unlocking the information in the cells to be able to differentiate between otters. It should be possible, shouldn't it, as every creature's DNA is unique?

The two tasks posed above will be dealt with in the next two sections. But first we'll have a closer look at chromosomes, genes, and DNA.

B 1.3 Chromosomes, Genes, and DNA

Chromosomes

Almost all human cells and otter cells have nuclei which contain chromosomes. Within any species, the nuclei from different animals all contain the same number of chromosomes. It is possible to identify and number each chromosome. So each species of plant and animal has a characteristic number of chromosomes. In humans, there are 23 pairs of chromosomes in each nucleus, making up 46 in total. Each one is a strand of DNA.



When chromosomes are preparing to reproduce themselves, they untangle from each other and ravel up on themselves, spiraling on top of the existing spirals, to form the neat shapes that are usually shown in pictures (e.g. see opposite). When in their natural state, all tangled up, they are very difficult to see under a microscope.

Figure 2 Human chromosomes

Genes

'Genes' are what we usually refer to when we talk about how we inherit traits from our parents. A gene is just a region of DNA that is able to specify the structure of one protein molecule. Each chromosome contains many genes and each gene is found in a characteristic place on a particular chromosome.

DNA

The initials DNA stand for **D**e-oxy-ribo-**N**ucleic **A**cid. Outside the cell, it looks like a tangled mass of string. Our own cells each contain about 2 meters of DNA, specially packaged to fit inside. It can be bent, wrapped, looped, twisted and even tied in knots. But in spite of its apparent flexibility, it has a very rigid way of storing information. The same information content must pass from generation to generation with little change. And it stores enormous amounts of information: all the 'instructions' needed to make an organism.

The incredible thing is that just about **all** life on Earth is built using the information contained in DNA. From lichens in the Arctic tundra to blue whales in the Pacific, from bacteria in the gut of

an elephant to the flowers in your garden: all of it is based on DNA. Us included. And all DNA exists in the same helical structure, based on just four simple building blocks.

Think of DNA as a ladder laid out on the ground. The 'legs' of the ladder consist of a long chain of molecules (including a sugar) which form a 'backbone' which supports the ladder's rungs and spaces them out. The rungs are the important bits and they consist of molecules called *bases*'.

There are just four bases in DNA and they come in pairs: one type of rung consists of the two bases *Adenosine* and *Thymine* linked together, and the other type of rung has the two bases *Cytosine* and *Guanine* linked together. Each rung is called a *base pair.* Usually they are shown as their initials A & T and C & G. It turns out that A can only ever link to T, and C only links to G, but they can link in any order, either C-G or G-C. The ladder is an incredibly long one: millions of base pairs rungs make up a strand of DNA.

- A — T -	
- G — C -	
- C — G -	

Note: A, T, C and G are quite simple molecules, with about 15 atoms (including atoms of carbon, oxygen, nitrogen, and hydrogen).

DNA is normally coiled up and forms a 'Double Helix'. Imagine taking a small flexible ladder in your hands and then twisting the ends in opposite directions. It would coil up into a simple helix like a two stranded piece of rope or string - the double helix:

So, DNA codes the genetic information which makes up our genes and it is usually organised into chromosome structures, described in the sections above. It's just staggering that such a simple system can carry all the information need to make a living thing. Even more staggering is to wonder how it could possibly have formed in the first place. Was it by chance, accidentally forming from the slush of molecules in a primordial soup? Or from outer space, riding in on a meteorite? Scientists speculate, but we really don't know.

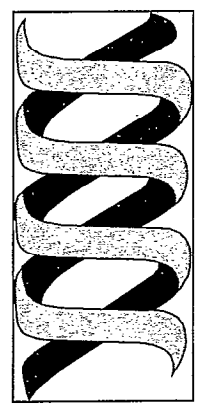


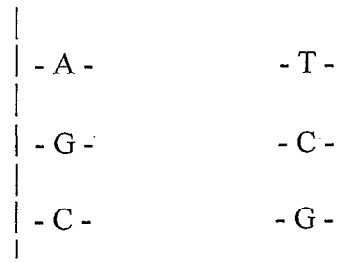
Figure 3 Simplified piece of DNA molecule with two spiralled strands but no 'rungs'

In 1953 in Cambridge James Watson and Francis Crick worked out how the base pairs of DNA could carry instructions. In a marvelous bit of understatement they 'mentioned' in their Nobel prize-winning paper, 'It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material'.

The other crucial thing about DNA, in addition to it being able to store so much information, is that it can copy information with amazing accuracy so that it can be passed on to another generation. The next section describes how this copying happens.

B1.4 Copying DNA - 'How to get lots of otter DNA from the tiny amount in spraints'

We can look at how nature copies DNA and learn how to mimic the process to increase the amounts of DNA we can get from otter spraints. Imagine taking the twisted ladder, standing on the top rung and jumping hard. Imagine that the top rung, and every consecutive rung, breaks in the middle. You would be left with two separate legs of the ladder, each with half rungs sticking out. In fact, this is like the way that DNA splits into two strands. The strands then each consist of the backbone and one half of each base pair sticking out. Each broken rung is attractive to particular bases e.g. T will be attractive to the A rung. So all you need to do to 'grow' more DNA is to add a mixture of all the 4 possible bases (each with a sugar attached), and wait for them to do the work of attaching to the right broken rung of the ladder. The bases will link to the two half strands of DNA, one by one, and rebuild them into two complete double strands. Because A only binds with T, and C with G, the final result is two identical copies of the original DNA. This is how, in nature, one cell divides into two daughter cells, each of which has an identical copy parental DNA.



Incredibly, the DNA copying process that occurs in cells, we are now able to mimic - in test tubes. The ladder can be ripped apart by heating. A mixture of bases, each with a sugar attached, needs to be added along with an enzyme (*DNA polymerase*). This procedure increases the amount of DNA by making copies. It is usually repeated time after time, doubling the amount of DNA every cycle, until there are considerable quantities.

This process is called *'Polymerase Chain Reaction'*, or PCR for short. And it is the perfect tool for getting our tiny amount of nucleic material that we found in the otter spraint into a manageable amount that we can use for other things... such as 'fingerprinting'.

B1.5 How do we use DNA for 'Fingerprinting'? And how does it work for otters?

In the early 1980s Professor Alec Jeffreys at Leicester University discovered a bizarre thing that eventually gave us the key to how to use DNA to distinguish between different animals. While studying a particular gene, Jeffreys found sequences of bases that made sense, but in amongst the sequences, there were lengths that seemed to have no meaning at all.

Imagine having a conversation with someone, and they suddenly started saying 'baaa baaa baaa baaa baaa baaa', then went back to talking to you normally. It was a bit like that.

Alec Jeffreys found that there were sequences of 10-15 base pairs that just repeated several times over: gobble-de-gook, that would not produce anything recognisable. He described these bizarre sequences as *'mini-satellites'*. It soon became clear that other genes also had mini-satellites. The

exact nature of the satellite changed from gene to gene. Later people found that that there are even shorter sequences of repeats, known as *'micro-satellites'*, which have less than ten repeats. Here is an example of a mini satellite:

T C T A C G T ***C A C A C A C A C A C A C A C A C A*** C T C G T A C G
A G A T G C A ***G T G T G T G T G T G T G T G T G T*** G A G C A T G C

Each mini or micro-satellite is found at a different place on a particular chromosome and is described as being at a particular locus (plural - loci).

But why should there be sequences of complete nonsense in DNA? No one yet really understands what they are for, or how they arose. But even though we don't understand mini- and micro-satellites, we're able to make use of them: they have a feature which gives us the key to DNA fingerprinting.

From one person to another, or from one otter to another, the number of repeats of the sequence in any one satellite can vary. In fact, it varies from one chromosome to another. Just as you might have a gene for blood group A from your father and O from your mother, you might have 10 repeats of ***CA*** in the micro-satellite in a particular gene from your mother and 8 repeats in the corresponding gene from your father.

These variants are called *alleles*. You need to have a reasonable quantity of alleles to be able to distinguish individuals. Most satellites have relatively small numbers of alleles and so a single satellite can not be used to distinguish individuals. But if you can examine several satellites, it is possible to discriminate between individuals with more certainty. Even matching up parents and offspring. However, it is generally easier to be certain that an otter is *not* the parent of an offspring, than to be sure that it *is* the parent.

It may seem ironic that, with the huge amounts of information being held in chromosomes, it is the lengths of chromosome that appear to be nonsense that lets us find a way to distinguish between animals. But the lengths of sequence that *do* make sense are very complex and are still being identified (eg you may have heard of the Human Genome project in the media, which is trying to map all the genes for humans). It is the very simplicity of the strings of repeats that makes them easy to identify and 'measure'.

How do we do DNA Fingerprinting on Otter Spraints?

It's necessary to do some detective work with the mini and micro-satellites. Scientists need to find ways of using them to distinguish between animals.

The first step in carrying out DNA fingerprinting is to find suitable satellites for analysis that can help with the detective work. The micro-satellites in the otter DNA need to be quite different from the satellites in any other DNA that may be present (from say the fish cells, or bacterial cells). John Dallas of Aberdeen University has worked with nine micro-satellites found in otter chromosomes that have not been found in any of the other chromosomes. However, only six of these have proved reliable in studies of spraint DNA. These micro-satellites have a number of

alleles. John Dallas has also found a micro-satellite which only occurs in males (it is found on the otter's Y chromosome). This is important: it means that the otter's gender can be established.

Another important factor to check is whether the otters in the population of interest show sufficient diversity in the satellites. If the satellites are similar in a population, the fingerprints of different otters may look too similar to be distinguishable. This was one of the aspects that needed assessing in the feasibility study.

B1.6 Field and Laboratory Techniques

Field Techniques

Spraints need to be collected as fresh as possible. That means, as early in the morning as possible. Ideally they should be less than 12 hours old. On summer mornings, if it starts to get hot, you should try to collect them even earlier. Why? DNA degrades very easily. Many things, including heat, light, enzymes and bacteria, can break it down. DNA in otter spraints has already survived some of the journey through the otter's gut, where it will have been attacked by various enzymes and bacteria. Once deposited in the environment, the degradation continues: it is subjected to atmospheric conditions, including ultra-violet light, more bacteria, fungi, heat and also dehydration.

As time goes on and breakdown proceeds, fewer and fewer micro-satellites can be detected. Sometimes incomplete DNA profiles are still useful, but it's usually only possible to show that an animal is *not* a particular individual, rather than show that it *is* one. Fortunately, the breakdown of DNA can be slowed down once the spraint has been collected, by storing it in a freezer and using chemicals to preserve it.

We have also found that spraints with lots of sticky mucus ('anal jelly') are much more likely to provide useable DNA than those without. The success rate for identifying the otter is 34% when anal jelly is present, compared with 17% when it is not there. This might be because the mucus protects the cells and DNA from breakdown. So collect as much anal jelly as you can!

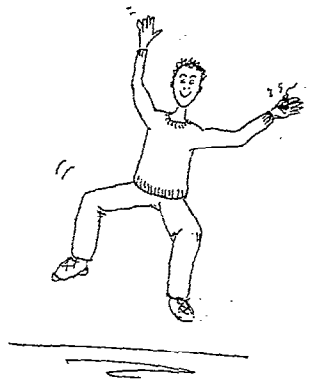


Figure 5 Anal jelly find

Laboratory Techniques.

The first problem back in the laboratory, is that the spraint not only contains otter DNA but also that of other organisms such as prey and gut bacteria as well as a mass of other components. Standard techniques are used to separate DNA from other material and these are used to extract all the DNA from the spraint sample.

It is now necessary to isolate the otter DNA from all the other DNA. This is achieved by gently heating the mixture so that the DNA unwinds and separates into two strands (this is described in **B1.3**), then adding 'primers' to the mixture. Primers are short bits of DNA which have been identified as binding to each end of a particular satellite. They will only bind to appropriate sites on the otter's DNA, not to bacterial or prey DNA. The primers thus mark out the relevant

satellites. Developing these primers specifically suitable for otter DNA is difficult and expensive but it is the key to making the technique viable.

The next stage is to greatly increase the quantity of otter DNA. From section **B1.3** we know that we can just use Polymerase Chain Reaction (PCR) to do this.

At this stage the DNA solution is placed on a jelly-like substance (a gel called 'agar' which is actually made from seaweed) which has an electric current running through it. Because DNA has a negative charge it will tend to move towards the positively charged electrode.
Since larger fragments of DNA move more slowly than small ones, after a period of time, different micro-satellite alleles (which by definition are of different sizes) will move different distances through the gel. The small fragments will cover greater distances than larger ones. By measuring the distance travelled by each fragment and comparing it with distances travelled by known alleles, you can work out which alleles are found in each animal. The method is known as *Electrophoresis*.

Interpretation
The end result of this process is a picture with a series of bands representing the alleles of the micro-satellites being investigated. Three examples are shown. Appropriately, they look a little like bar-codes, and after all, DNA is rather like a 'bar-code' for life.

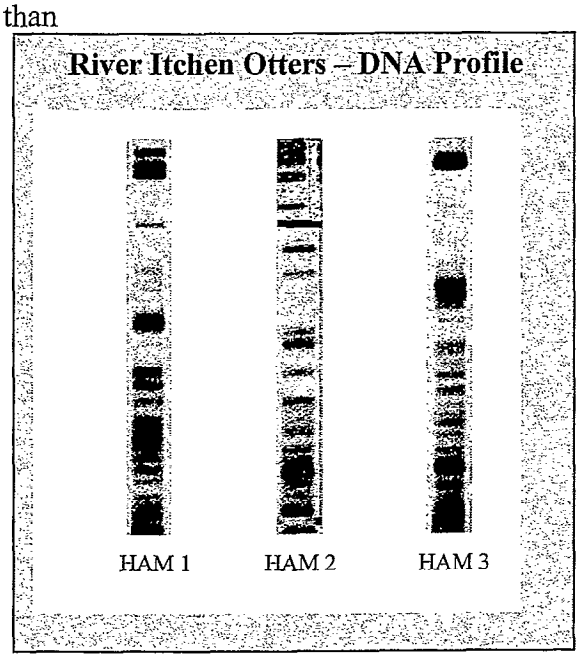


Figure 6 Three electrophoresis gels. Each black bar shows the distance that one of the micro-satellites has travelled.

The following table shows how results are recorded. Results are fed into a computer program which compares new samples with all the previous ones.

Otter No.	701	715	717	832	833	902	Sex
H01M	04 05	04 04	06 06	02 03	02 04	01 07	M
D07F	04 04	04 06	01 05	05 05	02 05	02 02	F
S12F	04 04	06 07	05 05	05 05	05 05	02 06	F
S16M	04 04	04 07	05 05	05 06	02 02	02 02	M
S20F	X	04 06	01 05	05 05	02 05	02 06	F

Most of these are real animals and the codes show that one comes from Hampshire (H01M), one from Devon and three from Somerset. The figures in the top row refer to the loci being investigated and the numbers in the body of the table show which two alleles at each locus were found in each otter. Note that there are always two numbers given as there are two alleles at each

locus (one from each of the otter's parents). Also note that the X for the Somerset female, number 20, at locus 701, means that the bands on the electrophoresis gel were not clear, and it wasn't possible to measure the position. This might be due to the spraint not being quite fresh enough.

From this table we can see that H01M and D07F cannot be closely related (parent and offspring or siblings) because they have no alleles in common for loci 717, 832 or 902. On the other hand, D07F and S20F are very similar, differing in only one allele, at only one locus, 902. They could be sisters or mother and daughter, but with a relatively small number of loci and alleles, and with locus 701 missing completely for one animal, this wouldn't be safe to assume (especially as they come from different counties).

B1.7 So why are we doing otter fingerprinting?

The information that might be gleaned from DNA fingerprinting is of three types:

a) the nature of the population e.g. sex ratios;
b) behaviour e.g. movements, extent of range overlap;
c) genetic e.g. how genetically similar otters in a population are.

As mentioned at the start of this appendix, the more we can learn about otter populations and understand their behaviour (a and b), the better position we are in to help them to re-colonise areas and to make sure that otter populations don't decline again. For example, it is useful to know what sort of otters first recolonise as they move back into river catchments which they formerly occupied. Do males arrive before females and do they behave differently? Do they establish home ranges or travel extensively? If they establish ranges to what extent do they overlap? Where otters do become resident, how long do they stay? We would also like to know how many otters there are in a particular area and hope to obtain information on this by, over a period of time, identifying all the resident otters in each study area. We may be able to find all this information by using DNA fingerprinting.

Surprisingly little is known about otter ecology in southern England. Scottish sea-based populations have been studied, but as they haven't undergone large-scale re-colonisation, much of this information is not relevant.

One reason for studying otters in three different counties is so that we can compare the populations. They are interesting because of their differences: otters in Devon have been well-established for some time; the Somerset population seems to be the current 'front' of recolonisation; and Hampshire has a small population of otters, which seem to be almost in isolation from otters in the south-west.

The genetic information is also useful in its own right. When the otter population declined in the 1960s there was a 'genetic bottleneck' in England. A 'genetic bottleneck' is where a population has been derived from so few individuals that they are all very closely related and genetically very similar. In evolutionary terms this is not ideal: genetic diversity is desirable for the long-term survival of the species. So, it is useful to be able to establish and compare the diversity of the otter populations in England and Scotland. Indeed, various otter conservation strategies in the UK state the need to assess the genetic variation within and between otter populations in different parts of the UK (eg JNCC 1996).

What have we learned from the present study?
The project was a feasibility study, with over fifty volunteers working closely with professional biologists, helping to collect the spraints on a regional basis. The aim was to:

- test whether DNA fingerprinting could be used to identify otters using spraint;
- see if it was practical for volunteers to collect otter spraints of suitable freshness and quantity to make further studies viable;
- set up standard techniques that could be used in future studies.

The project has been a great success. We have demonstrated that we can use the technique in the field, and that although the otter populations are in some cases rather similar, they can in many cases be distinguished. The volunteers have done a fabulous job, and have shown that a larger scale project is viable. Standard techniques have been set up, and some improvements in approach identified. And we have already discovered some useful things about the otter populations; for example, we have learned that the River Brue population seemed to have eight males and only three females during the study period.

As far as we know, there are only a handful of other projects around the World that are using fingerprinting techniques using faeces to study animal populations including: wild baboons; bonobos; sea otters in Alaska; and Brown Bears in Italy, Slovenia, Croatia and Bosnia. The information gained and techniques learned in our study have already been shared with the Alaskan researchers in their studies of otters.

Close to the end of the project, something happened that surprised everyone. The team knew there were problems with some of the populations being so genetically similar it was hard to distinguish the animals (i.e. the 'polymorphism was low'). But they still believed that the technique was sensitive enough, and that individual otters were being identified. The project manager, Tim Sykes, found a dead otter cub on the River Itchen. A post mortem showed that she'd died of pneumonia at less than six months old. The DNA fingerprinting showed that she was H06F, whose spraints had been found seven months before and one month after the discovery of the corpse. Clearly, this wasn't possible! Seven months beforehand she hadn't been born, and one month beforehand, she was already dead.

John Dallas checked the literature, and realised that the technique just wasn't sensitive enough to distinguish between very closely related individuals, in populations with such low polymorphism. The same thing can happen in genetic fingerprinting when used by the police. Twins, for instance, can be hard to distinguish.

While this is a significant drawback, it is important that it has been realised. It is just the kind of thing that the feasibility study was intended to establish. More research is now needed to refine the fingerprinting technique, so that individual otters can be identified with certainty. And it's possible that fingerprinting studies of animals in others countries may have the same problem. Now we have identified it here, we need to share the information.

Another thing to remember is, however sad it may be to find a dead otter, it may show some incredibly useful things about the population, as illustrated by the above example. If you ever find a dead otter, let the Environment Agency know immediately, so that they can collect the corpse whilst it is still fresh.

What next?

Now, thanks to the help of volunteers, we have shown that the technique can work. Protocols have been established that may now be used by anyone wanting to do this kind of work.

Future studies could aim to find out more about the behaviour and ecology of otter populations in southern England. For example establishing otter movements, home ranges and population structure so that populations can be modelled. We are also interested in finding out how the population is affected by the following: habitat quality; disturbance; and management of riverbanks.

However the first priority is to develop DNA fingerprinting further so that we can distinguish between members of the same family. This will involve identifying and producing more primers to refine and improve the technique. We believe that if 15 loci can be developed, it should be possible to differentiate between parents and offspring in populations with low genetic diversity.

And now, while otters swim and play undisturbed at night, we can go ahead by day collecting their spraints. Using our detective work of fingerprinting, we can try to answer some questions that we hope will help to ensure that otter populations continue to recover and eventually recolonise all the UK's wetlands, rivers and coasts.

Further reading

Genetics for Beginners, Steve Jones and Borin Van Loon, Icon Books, 1993

The Fifth Miracle, the search for the Origin of Life, Paul Davies, Penguin, 1998

The Selfish Gene, Richard Dawkins, Oxford University Press, 1976

Otters, Chanin, P.R.F. 1993. Whittet British Natural History Series.

Facts from faeces revisited, Michael H Kohn, Robert K Wayne (1997) Trends Ecol. Evol. vol. 12 no. 6, 223-227, June 6

Wild Otters: predation and populations, Kruuk, H., 1995. Oxford University Press, Oxford.

APPENDIX C1: THE SUITABILITY OF THE SOUTH WEST POPULATION FOR DNA TYPING

APPENDIX C2: DNA EXTRACTION FROM VERTEBRATE TISSUE – SALT-CHLOROFORM METHOD

APPENDIX C3: DNA EXTRACTION FROM OTTER SPRAINTS – CTAB/GITC/DIATOM/ VECTASPIN METHOD

APPENDIX C4: IDENTIFICATION OF INDIVIDUALS

APPENDIX C5: STATISTICAL ANALYSIS

APPENDIX C6: RATIO TESTS FOR INDIVIDUALS

APPENDIX C1 SUITABILITY OF THE SOUTH WEST OTTER POPULATION FOR DNA TYPING

C1.1 Background

The kind of information that DNA typing of wild populations is expected to yield depends on two factors: the levels of genetic polymorphism in the population, and the number of polymorphic loci available to assay. Three kinds of information are typically required: evidence for population subdivision, and the identification of individuals and pairs of relatives. Table C1.1 shows that it is feasible to obtain the first two kinds of information across a wide range of polymorphism levels and numbers of loci, whereas to identify 1st–order relatives requires high values of both polymorphism levels and numbers of loci. It is only feasible to identify pairs of more distant relatives when 40 – 50 highly polymorphic loci are available, and this many loci could not be assayed on the limited amount of DNA in otter spraints.

Fortunately, the levels of genetic polymorphism in the otter population in SW England can be estimated accurately thanks to the availability of a large collection frozen of tissue from carcasses collected in this region over the past 22 years. The number of polymorphic loci available for otter is 14, (13 published, 1 unpublished), and only six of these were found to be both polymorphic in SW England and suitable for DNA typing by the methods employed in this study.

Table C1.1 Factors that influence the yield of information from DNA typing

FACTORS		YIELD OF INFORMATION		
Level of polymorphism	**Number of loci**	**Population subdivision**	**Individual identification**	**1st order relatives**
High – 10 alleles per locus	10 – 15	Yes	Yes	Yes
High	5 – 10	Yes	Yes	Possibly
Intermediate – 5 alleles per locus	10 – 15	Yes	Yes	Possibly
Intermediate	5 – 10	Yes	Yes	No
Low – 2 alleles per locus	10 – 15	Yes	Possibly	No
Low	5 – 10	Yes	Yes	No

C1.2 Methods

C1.2.1 Otter tissue samples

Tissue samples of 162 otter carcasses were obtained from five collections of frozen tissue (Table C1.2). These represent Cornwall, Devon, Somerset and Hampshire, and mainly cover the period from 1986 to 1998. Ninety-five percent of samples yielded sufficient DNA for reliable typing. This is a very high rate considering that the original carcass may have remained at ambient temperature for several days before collection. Of the samples suitable for DNA analysis, 86% had location details such as OS grid references. Only the 133 samples of known location were used for statistical analyses.

Table C1.2 Otter carcass samples from SW England						
	N	**N DNA**	**OS ref.**	**No OS ref.**	**Source**	**Period**
	74	68	60	8	Mr M. Rule, EA Bodmin	1993-97
	37	37	25	12	Mr V. Simpson, VIU Truro	1996-98
	27	27	27		Dr D. Jefferies, JNCC retired	1976, 1986-92
	21	19	19		Dr C. Mason, University of Essex	1988-92
	3	3	2	1	Mrs R. Green, VWT and Mr J. Conroy, ITE-Banchory	1995 and 1997
Total	162	154	133	21		

C1.2.2 DNA typing methods for otter tissue

DNA was extracted from otter tissue using standard methods (Appendix C2). Kidney and gonad appeared to yield more DNA than muscle or liver. Nine microsatellite loci were typed for each individual to generate a DNA profile consisting of 18 numbers: two numbers (03 05) per locus. The loci used were 701, 715, 717, 733, 782, 818, 832, 833 and 902. The PCR primers and conditions for the first eight loci are published (JF Dallas & SB Piertney 1998, Molecular Ecology 7, 1248-1251). The details of locus 902 are not given because these were obtained prior to publication from Professor R.K. Wayne, Department of Biology, UCLA. Please contact Professor John Bryant, University of Exeter, UK, for further details. The remaining five loci published by Dallas & Piertney were not typed because they were considered unsuitable for subsequent typing in spraint DNA, due either to large PCR product size, or to excess production of artefactual stutter bands during the PCR assay.

C1.3 Results

C1.3.1 Levels of polymorphism in the otter population in SW England

The frequencies of the alleles detected at nine microsatellite loci in the sample of 133 carcasses that yielded DNA and had OS references are shown in Table C1.3. The individual genotypes of the 105 carcasses from the Bodmin archive and from VIU Truro that yielded DNA are given in Appendix D4. The samples were separated into two regional groups, ESW and WSE, which correspond roughly to Devon/Somerset and Cornwall, respectively. The justification for doing so is described in the following section. The numbers of alleles detected at each locus fell consistently in the range from 3 to 5, so the otter population in SW England falls between the "Low" and "Intermediate" categories of polymorphism (Table C6.1). Locus 782 has one allele at high frequency, so is of no use for individual identification because most individuals share the genotype 08 08 (Appendix D). These results imply that it will be feasible to study population subdivision and individual identity in SW England, but not to identify pairs of 1st-order relatives.

C1.3.2 Subdivision in the otter population in SW England

It is necessary to identify any subdivision within the study area because many of the calculations involved in the identification of individuals and relatives assume that alleles occur in the same individual by chance, i.e., that there are no statistical associations among alleles either within or between loci. Such associations can arise if two genetically different populations are unknowingly combined. Subdivision of wild populations can arise at the regional scale from barriers to dispersal, and at the local scale from relatives choosing to breed close to their place of birth. As nothing is known about dispersal and breeding patterns

in otter populations in the UK, evidence for subdivision is of great biological interest in its own right, quite apart from its technical relevance to the feasibility of DNA typing.

Evidence for regional subdivision was sought by comparison of allele frequencies between two groups of samples designated WSW and ESW. The WSW samples were all those located west of a straight line running from OS 1 km grid 200 100 to 300 050, and the ESW samples were all those located east of this line. Two samples from Dorset and three samples from the Itchen catchment were excluded as being too spatially separate to be included in the main cluster of samples, and too few to be analysed as separate groups. The rationale for choosing to site the line between WSW and ESW was the correspondance with the areas of Bodmin moor, Dartmoor and Plymouth, which were devoid of samples. These areas may function as barriers to otter dispersal because of lower fish biomass at higher altitudes, and because of the presumed difficulty of crossing an urban area. Allele frequencies were tested for significant differences between groups using the test for genic differentiation in the package GENEPOP 3.1a, option 3.1 (M. Raymond & F. Rousset, 1995, Journal of Heredity, 86, 248-249).

Table C1.4 Genic differentiation between WSW and ESW samples

Locus	All samples	females	males	1986 1992	1993 1995	1996 1998
701	***	***	**	NS	**	**
715	NS	NS	NS	NS	NS	NS
717	NS	NS	NS	NS	NS	NS
733	NS	NS	NS	NS	*	NS
782	NS	NS	NS	NS	NS	NS
818	***	***	***	***	***	***
832	***	*	***	***	***	NS
833	***	***	***	*	**	***
902	***	***	***	**	***	***
All loci	HS	HS	HS	HS	HS	HS

Notes: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, NS $p > 0.05$, HS highly significant.

The results show that there are highly significant differences in allele frequency at five out of the nine loci between the groups WSW and ESW (Table C1.4, column 2). These differences are reasonably consistent when the data were divided into subsets consisting of separate sexes and three different time periods (columns 3-7).

Evidence for further spatial subdivision within the areas WSW and ESW was then sought. The above test of genic differentiation was not used because there were no obvious spatial criteria for division of WSW and ESW into smaller groups. However, if ESW and WSW are further subdivided, there should be detectable associations between alleles: either at the same locus or at different loci. Two types of test were performed to detect such associations. Alleles at the same loci were tested for Hardy-Weinberg equilibrium versus heterozygote deficit (GENEPOP 3.1a, options 1.1 and 1.4). "Equilibrium" means that there are no statistical associations between alleles, whereas heterozygote deficit means that the same allele is more likely to occur in the same individual than expected by chance. WSW shows a deficit of heterozygotes at three loci, which is sufficient to produce a significant overall deficit. ESW shows a deficit of heterozygotes at one locus only, which is not sufficient to give

an overall departure from Hardy-Weinberg equilibrium (Table C1.5). The linkage equilibrium test (GENEPOP 3.1a, option 2.1) test for statistical associations between alleles at different loci was then performed. This showed that alleles at five pairs of loci are significantly associated in WSW and ESW (Table C1.6). The sequential Bonferroni correction for non-independent tests was applied to the probability values generated by the GENEPOP test (a = 0.05, k = 36, cutoff p = 0.0014) because of the large number of tests performed. Only those cases with a p value lower than the cutoff are shown. Finally, tests for genic differentiation within WSW and ESW, between sexes and between the three time periods listed in Table C1.2, provided no evidence for any sexual or temporal differentiation (not shown).

Table C1.5 Hardy-Weinberg disequilibrium in the WSW and ESW samples

Locus	WSW	ESW	WSW females	WSW males	ESW females	ESW males
701	*	NS	NS	NS	NS	NS
715	NS	NS	NS	NS	NS	NS
717	NS	NS	NS	NS	NS	NS
733	NS	NS	*	NS	NS	NS
782	NS	NS	NS	NS	-	NS
818	NS	NS	NS	NS	*	NS
832	**	*	*	NS	NS	*
833	NS	NS	*	NS	NS	NS
902	*	NS	NS	NS	NS	NS
All loci	**	NS	**	NS	NS	NS

Notes: *** p< 0.001, ** 0.001< p < 0.01, * 0.01< p < 0.05, NS p > 0.05, - not possible to test.

Table C1.6 Cases of linkage disequilibrium in the WSW and ESW samples

Locus 1	Locus 2	WSW	ESW
701	902	+	
733	832	+	
701	818		+
717	818		+
832	902		+

The results on subdivision do not pose problems for the feasibility of individual identification in SW England. However, it will be necessary to use separate reference allele frequencies for the areas WSW and ESW, should such references be required for intensive local studies. The study sites in the Torridge and the Somerset levels reported on here are all located within the area ESW. In addition, only one of the cases of linkage disequilibrium involves loci that are also suitable for typing of spraints. Finally, it is only the calculation of relatedness values which makes the assumption of linkage equilibrium, and this is unlikely to be attainable due to a lack of polymorphism.

C1.3.3 Identification of individuals in the otter population in SW England

The level of polymorphism in ESW and WSW corresponds to the borderline of feasibility for individual identification (Tables C1.1 and C1.3). Furthermore, only six of the nine loci were found suitable for DNA typing of spraints. Two types of analysis were therefore performed to

verify whether or not individual identification by spraint DNA typing is likely to be feasible in these regions. In the first analysis, values of the probability of identity (PI) were calculated from the allele frequencies for each locus (Table 6.3, last column). PI is the probability that two individuals chosen at random will match by chance. This analysis assumes the statistical independence of alleles within and between loci. The overall PI values for the six loci used for spraint typing are 10-fold lower than those for the nine loci due to the loss of three loci from the individual genotypes. Nonetheless, the six-locus PI values are sufficiently low that chance matches are expected only when more than 50 otters are present in the same area. This is because there are only 1225 possible pairs of individuals present, so only 1225 opportunities for a chance match to occur, and the inverse of 1225 is greater than the six-locus PI values.

In the second analysis, the number of chance matches between pairs of actual genotypes in the EWS and WSW samples was calculated. Where mismatches occurred, the numbers of mismatched loci was calculated in order to assess the reliability of DNA typing for individual identification. The identification of individuals will be much more reliable if the majority of loci show mismatches than if one or two loci do so. This analysis does not depend on any assumptions about the statistical independence of alleles because the units of comparison are multilocus genotypes. The distributions of mismatched loci are shown for both the nine loci used for tissue typing and the six loci used for spraint DNA typing (Figures C1 and C2). In the case of nine loci, fewer than 5% of all the possible pairs of individuals mismatch at only one or two loci, most pairs mismatch at between five and seven loci, and none show a complete match. In the case of six loci, around 10% of pairs mismatch at only one or two loci, most pairs mismatch at either four or five loci, and five pairs show a complete match (two in ESW and three in WSW).

The two analyses suggest that the majority of DNA profiles of otters in SW England will be individual-specific provided that all six of the loci used for spraint DNA typing are assayed. The loss of any loci due to typing failure would have the effect of removing the left-hand end of the distribution in Figure C2, thus decreasing the numbers of mismatched loci for a given point on the distribution. Thus, 25-30% of five-locus profiles are expected to match completely or to mismatch at only one or two loci, and around 50% of four-locus profiles are expected to do so. In addition, typing failure appears to be nearly random with respect to locus. In consequence, most pairs of profiles lacking one locus each will be comparable at only four loci, not five, because a different locus will be missing from each profile. Lastly, the degree of DNA profile mismatches at the local spatial scale and over short time periods is what will determine the reliability of individual identification. This is the situation in which close relatives are likely to be detected. Such mismatches may have been underestimated by the analysis of carcass samples due to the absence of close relatives, because carcasses were collected from large areas and over long periods of time.

Table C1.3 Allele frequencies and probabilities of identity for each locus in WSW and ESW

		Alleles numbered from 01 to 08					Probability of Identity
		02	03	04	05		
Locus 701-S	WSW	0	0.01	0.50	0.49		0.33
	ESW	0.01	0.03	0.76	0.20		0.43
Locus 715-S		01	04	06	07		
	WSW	0	0.47	0.43	0.10		0.26
	ESW	0.01	0.42	0.41	0.16		0.21
Locus 717-S		01	04	05	06	07	
	WSW	0.17	0.02	0.80	0.01	0	0.49
	ESW	0.22	0	0.76	0.01	0.01	0.45
Locus 733		03	04	05	06		
	WSW	0	0.83	0.06	0.11		0.52
	ESW	0.01	0.76	0.14	0.09		0.40
Locus 782		01	02	07	08		
	WSW	0.03	0.02	0	0.95		0.82
	ESW	0.03	0.01	0.03	0.93		0.75
Locus 818		02	06	07	08		
	WSW	0.22	0.35	0.10	0.33		0.13
	ESW	0.33	0.03	0.45	0.19		0.19
Locus 832-S		02	03	04	05	06	
	WSW	0	0.08	0.02	0.63	0.27	0.29
	ESW	0.01	0.19	0.15	0.61	0.04	0.23
Locus 833-S		02	03	04	05	07	
	WSW	0.26	0.17	0	0.57	0	0.23
	ESW	0.59	0.07	0.01	0.32	0.01	0.29
Locus 902-S		02	03	05	06	07	
	WSW	0.44	0	0.38	0.11	0.07	0.16
	ESW	0.59	0.01	0.03	0.29	0.08	0.24
Nine loci	WSW						2.5×10^{-5}
	ESW						3.8×10^{-5}
Six -S loci	WSW						4.5×10^{-4}
	ESW						6.6×10^{-4}

Note: only the loci marked as –S were used for DNA typing of spraints

The feasibility of distinguishing first-order relatives from unrelated individuals in the otter population in SW England was assessed by simulation. The allele frequencies of the six loci used for spraint typing in ESW were used to simulate the genotypes of 1000 pairs of first-order relatives and 1000 pairs of unrelated individuals. The values of relatedness between pairs were calculated, then the two distributions of relatedness values were superimposed. These calculations were performed using the packages "Kinship" and Excel. If the two distributions do not overlap, i.e., if the first-order relatives have consistently higher R values than the unrelated individuals, then it will be feasible to identify pairs of first-order relatives on the basis of R values. Alternatively, if the two distributions overlap to a large extent, then the identification of relatives will not be feasible. Figure C3 shows that the latter is the case. This confirms the initial conclusion reached above on the basis of the levels of polymorphism. Unpublished data from red grouse suggest that there must be available around 15 loci, each possessing 10 alleles, for the two distributions not to overlap (S.B. Piertney, personal communication). It is most unlikely that such highly polymorphic loci occur in the otter population in SW England, for two reasons. Firstly, the stable otter population in Scotland does not possess such high levels of polymorphism for any of the nine loci analysed here (J. Dallas, unpublished data). Secondly, the population in SW England will have lost some of its original polymorphism as a result of the acute decline that occurred during the late 1950s and 1960s.

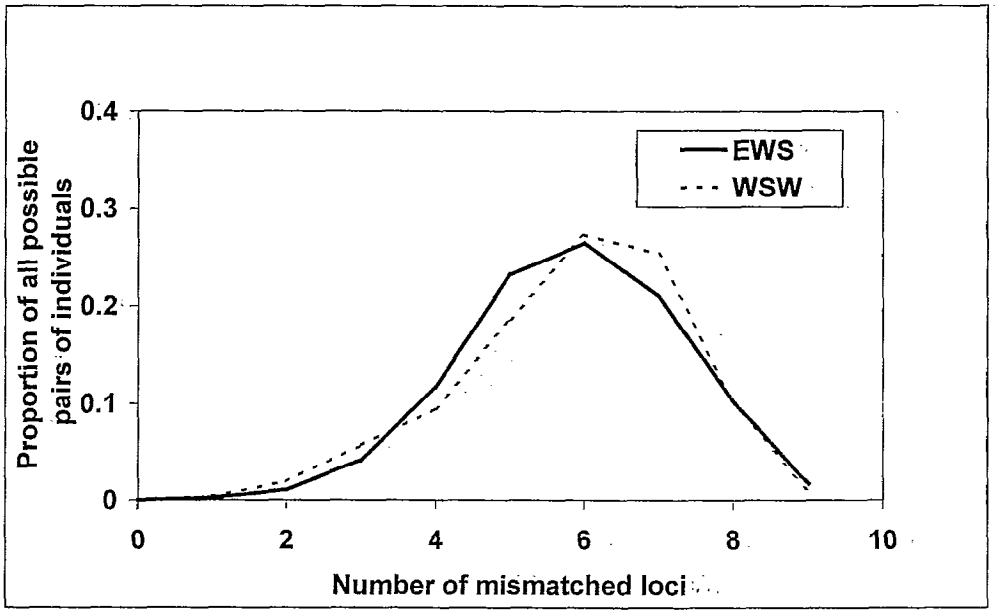


Figure C1 Distribution of Mismatches at the Nine Loci Used for Tissue Typing

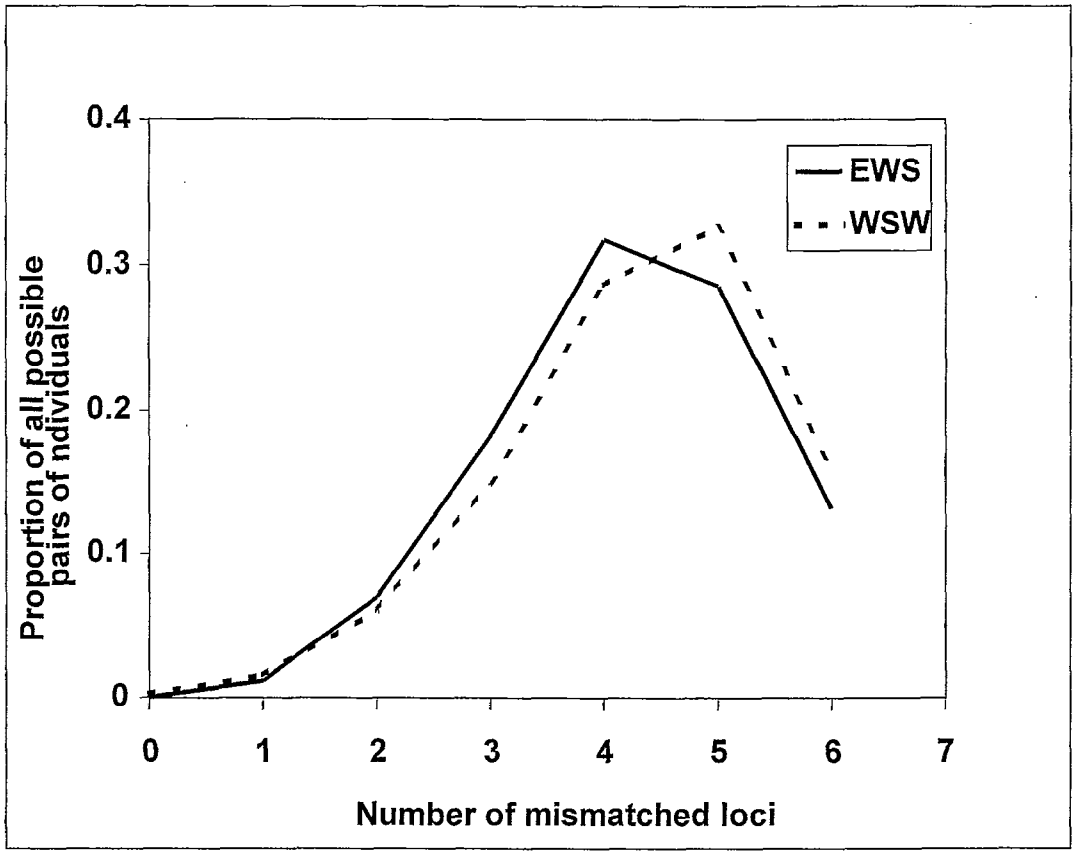


Figure C2 Distribution of Mismatches at the Six Loci Used for Spraint Typing

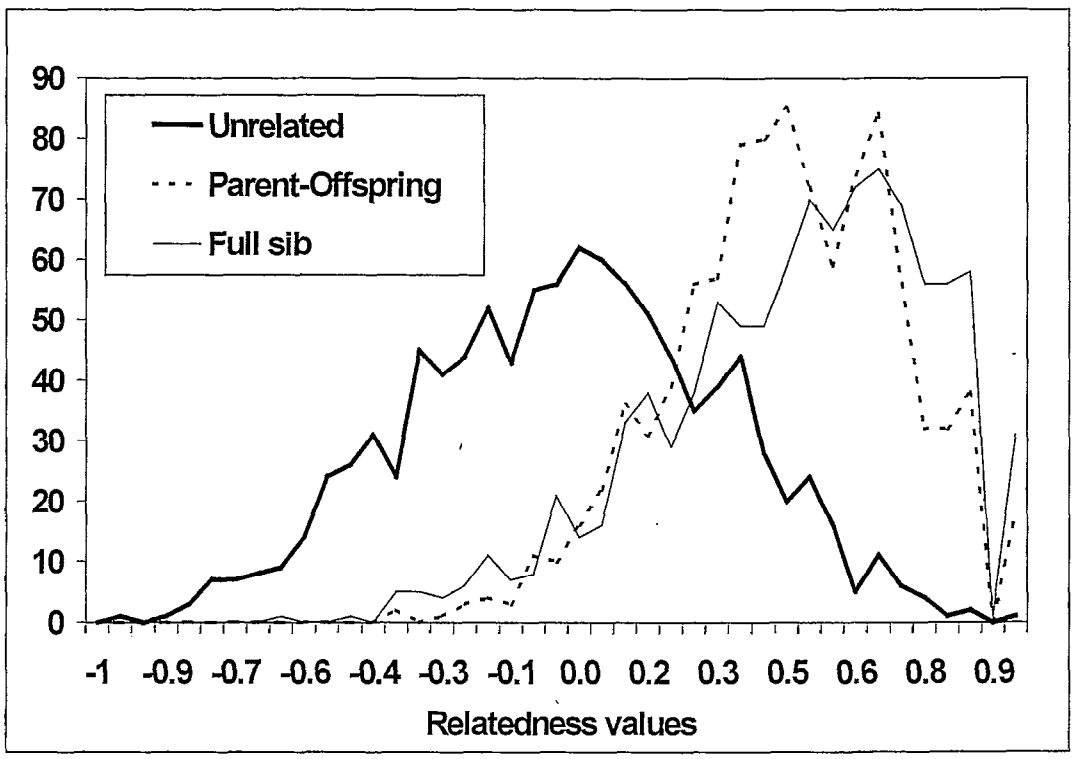


Figure C3 Simulated Values of Pairwise Relatedness Based on the Six Spraint Loci: Unrelated Versus 1st-Order Relatives in ESW

APPENDIX C2 DNA EXTRACTION FROM VERTEBRATE TISSUE: SALT-CHLOROFORM METHOD

Bruford et al. 1992, Molecular Genetic Analysis of Populations, pp. 227-9
Müllenbach et al., 1989, Trends in Genetics 5, 391.

1. Add **800 µl of Extraction Buffer** containing **Proteinase K** and **RNase A** to a 1.5 ml tube. Cut 3 x 1 mm slices (total 50 mm^3 or 50 mg) of tissue using a scalpel and add these to the tube. Place tube in heating block at 55°C immediately. Rinse and wipe with a tissue the scalpel between each sample.

2. Incubate at 55°C overnight, vortexing a few times to disperse the tissue in solution.

3. Spin tube at 13000 rpm / 5 min. to pellet undigested debris, and transfer the supernatant to a **2.0 ml** tube. Be careful not to transfer floating or pelleted debris.

4. Add **350 µl of 5M NaCl**, and **750 µl of chloroform** then vortex 5 sec. until the mixture appears milky. Put the tube on the rotator for 30 min.

5. Spin tube at 13000 rpm / 5 min. The DNA solution is the upper phase, the chloroform is the lower phase, and the precipitated debris is the layer between the two phases. Transfer the upper phase to a new **2.0 ml tube**. Be careful not to transfer debris.

6. Add **750 µl of isopropanol**, and mix by inversion x 25. A clump of DNA fibres may form, if not don't worry.

7. Spin tube 13,000 rpm / 5 min. A pellet should be visible, if not the extraction hasn't worked.

8. Remove supernatant with a 1 ml micropipette. Give the tube a pulse spin to get all remaining liquid to the bottom of the tube, and remove it with a smaller micropipette. Change the tip between each sample for both uses of the micropipettes.

9. Add **1 ml of 70% EtOH**, close and put the tube on the rotator for 15 min. Alternatively, the tube can be left overnight at 4°C at this stage if there is not enough time to finish.

10. Spin tube 13,000 rpm / 1 min. Remove supernatant with a 1 ml micropipette. Give the tube a pulse spin to get all remaining liquid to the bottom of the tube, and remove it with a smaller micropipette. Change the tip between each sample for both uses of the micropipettes. Be very careful that the white DNA pellet remains in the tube.

11. Leave tube open 10 min. at 55°C to dry the DNA pellet. Do not overdry as this makes the pellet difficult to resuspend.

12. Add **200 - 500 µl of TE**, and leave the pellet to resuspend 1 hour at 55°C (or overnight at 4°C). Vortex the tube at low speed to ensure that the DNA solution is homogeneous.

13. Spin tube 13,000 rpm / 5 min. to pellet any debris. Transfer the DNA solution to a 1.5 ml tube, write the sample number on the tube, then store it in a - 20°C freezer.

Plasticware, *etc.*

Always wear gloves, and always include an extraction control in each days-worth of extractions, *i.e.*, a tube containing extraction buffer but no added tissue.
Use only disposable pipettes and blue-cap 50 ml tubes for preparing working solutions.
For large series, it is convenient to work in multiples of 24 tubes, and to do inversions, *etc.* of 24 or 48 tubes simultaneously in an 8 x 12 rack with another rack on top.

Proteinase K solution (20 mg / ml)

Dissolve 100 mg of Proteinase K in 5 ml of AR water. Make 10 x 500 µl aliquots in 1.5 ml tubes and store at -20°C.

DNAse-free RNase A solution (10 mg / ml)

Buy from Sigma, no need to boil before use.

Extraction Buffer

0.1 M Tris.HCl, 0.1 M NaCl, 5 mM EDTA, 0.5% SDS, pH 8.0.
Add Proteinase K to 200 µg/ml and RNase A to 20 µg/ml just before use.

70% EtOH

Mix 35 ml of absolute ethanol and 15 ml of AR water in a 50 ml blue-cap tube.

TE (10 mM Tris.HCl, 0.1 mM EDTA, pH 8)

Mix the following in a 50 ml blue-cap tube:
49.5 ml AR water
0.5 ml 1 M Tris.HCl, pH 8
10 µl 0.5 M EDTA, pH 8

APPENDIX C3 DNA EXTRACTION FROM OTTER SPRAINTS: CTAB / GITC / DIATOM / VECTASPIN METHOD

CAUTION: ALWAYS WORK IN A FUME HOOD, AND WEAR EYE PROTECTION AND GLOVES. GITC PARTICLES CAN BURN YOUR SKIN AND EYES, AND CAN PRODUCE HYDROGEN CYANIDE IF ADDED TO ACIDIC SOLUTIONS. Dispose of all GITC solutions by addition of NaOH pellets, then treat as hazardous waste.

RATIONALE: The first extraction with CTAB removes polysaccharides that otherwise are co-purified with DNA in the second GITC/diatom extraction. Process up to 24 spraints at a time. Use only disposable tubes and pipettes for preparing solutions. Use the same glass bottle from new for any large-volume solutions.

1. Arrange the tubes containing spraints in ascending numerical order. Make a note of the tube numbers in a lab book. Use a temporary extraction code (say 1-24) for labelling during the extraction.

2. Take each tube in turn. First, check that there is no more than 1 ml of spraint in the bottom of the tube. If there is more, remove the excess using a spatula. Retain the fine material and get rid of the excess of solids. Before dealing with the next tube, rinse the spatula in deionised water, wipe it dry, and flame it in a Bunsen.

3. Spin the 24 tubes in a benchtop centrifuge at 4K RPM/24°C for 5 min. to pellet spraint. Tip the ethanol out of the tubes and dispose.

4. Add **2 ml of 2 x CTAB buffer** to each tube, replace the cap, and vortex to resuspend the spraint. Tape the tubes securely in a rack, attach the rack to a rotator, then rotate for 15 minutes.

5. Spin the tubes in a benchtop centrifuge at 4K RPM for 5 min. to pellet spraint debris. Meanwhile, label 24 x 2 ml tubes with the extraction codes in a yellow flipper rack.

6. Transfer 2 ml of CTAB lysis supernatant to a 2 ml tube, taking care not to transfer debris. Spin the 2 ml tube at 13 K RPM for 5 min. in the high-speed microcentrifuge.

7. Transfer 1.5 ml of CTAB lysis supernatant to a new 2 ml tube. Add 0.5 ml of chloroform, vortex at high speed for 10 sec., then put tubes on a rotator for 10 min.

8. Spin the CTAB / chloroform mix at 13 K RPM for 5 min. in the high-speed microcentrifuge. Transfer supernatant to a new 2 ml tube, then repeat the chloroform extraction and spin.

9. Transfer CTAB lysis supernatant to a new 2 ml tube. Add 0.67 ml of isopropanol, mix by inversion, then spin at 13 K RPM for 5 min.

10. Tip out liquid, pulse spin to get all traces of liquid to the bottom of the tube, then remove them with a yellow tip.

11. Add 1.8 ml of GITC buffer to the pellet. Resuspend the DNA pellet by vortexing, and use a blue tip if necessary, then put the 2 ml tube on a rotator for 10 min.

12. Add 100 µl of just-vortexed diatom suspension. Close the tube, and put it on a rotator for 10 min. to bind the DNA. Spin the tubes at 13K RPM for 1 min. to pellet diatoms. Pour off the supernatant and dispose.

13. Label a series of 24 x **10 µm mesh VectaSpin tubes** (Whatman, # 6838 0002) on the caps with the extraction codes: one VS tube per spraint.

14. Add **650 µl of GITC buffer** to each 2 ml tube, resuspend the diatom pellet using a blue tip, then transfer the diatoms plus buffer to the inserts of the VS tubes.

15. Spin VS tubes 10 K RPM for 30 sec. in the high-speed microcentrifuge to spin through the wash buffer. Remove the insert from the VS tube, tip out the wash buffer and replace the insert.

16. Add **700 µl of 70% EtOH** to the insert of the VS tube, close the cap, then vortex the top of the tube at low speed for 5 sec. to resuspend diatoms. DO NOT OVERDO THIS: VORTEX JUST ENOUGH TO RESUSPEND THE DIATOMS.

17. Spin tube 10K rpm / 30 sec. to spin through the EtOH. Remove the insert from the VS tube, tip out the EtOH and replace the insert. Don't worry about the small amount of diatoms that come through the insert.

18. Add **700 µl of absolute EtOH** to the insert of the VS tube, then spin tube 10K rpm / 30 sec. to spin through the EtOH. Remove the insert from the VS tube, tip out the EtOH and replace the insert. Finally, spin the empty VS tube plus insert 10,000 rpm / 30 sec. to spin out residual EtOH.

19. Prepare a series of 2.0 ml tubes labelled with the extraction codes and with the caps cut off, Cut the numbered cap off the VS tube and keep it safe, transfer the inserts of the VS tubes to the same-numbered 2.0 ml tubes. Place inserts + 2.0 ml tubes without VS caps in the 55°C oven for 1 hour to dry off remaining EtOH from the diatoms.

20. Add **130 µl of TE** to the diatoms in the insert and replace the numbered VS cap. Shake the rack containing all the tubes gently back and forth until the diatoms are visibly resuspended in the TE. Place the rack in the 55°C oven for 30 min., shaking intermittently, to complete the elution of DNA from the diatoms.

21. Spin tubes 10,000 rpm / 1 min. to spin through the TE containing the eluted DNA. Remove the VS insert and dispose.

22. Transfer the TE to a 1.5 ml MC tube, spin at 13 K RPM for 1 min., then transfer 100 µl of the TE to a 200 µl PCR tube. Avoid transferring any of the small diatom pellet.

23. Store the TE/DNA tubes in a green 8 x 12 PCR rack labelled with the spraint series and with a taped-on lid in the - 80°C freezer.

SOLUTIONS

2 x CTAB buffer (100 mM Tris.HCl, pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB)
Make up by dilution of stock solutions. From B.G. Milligan, chapter 3 in the IRL Press book "Molecular Genetic Analysis of Populations" (1992, ed. A.R. Hoezel)

Diatom suspension (10 ml)

1. Add 3 g diatomaceous earth and 50 ml AR water to a 50 ml blue-cap tube.

2. Mix 25 times by inversion then spin 500 rpm / 1 min in benchtop centrifuge to pellet the largest particles.

3. Pour off the cloudy liquid above visible solid-liquid interface, then add AR water to 50 ml.

4. Repeat three times the resuspension in water, spin, and removal of cloudy liquid.

5. Add AR water to a final volume of 12 ml.

6. Store suspension in the dark at room temperature.

GITC buffer (5M GITC, 0.1 M Tris.HCl pH 6.4)

Work in a fume hood. This procedure can generate hydrogen cyanide, a highly toxic gas.

1. Add 300 g GITC (Guanidine thiocyanate, Fluka, 50990), 225 ml AR water, 50 ml 1 M Tris.HCl pH 6.4, to a 500 ml blue-cap glass bottle.

2. Place capped tube in hot water in a beaker, and dissolve with intermittent mixing. Store dark at RT.

TE (10 mM Tris.HCl, 0.5 mM EDTA)

Mix in a 50 ml blue-cap tube: 49.5 ml AR water, 0.5 ml 1 M Tris.HCl pH 8, 50 µl 0.5 M EDTA, pH 8

APPENDIX C4 IDENTIFICATION OF INDIVIDUALS IN THE OTTER POPULATION IN SW ENGLAND

The most important concept relevant to this section is that the identification of individuals always involves the comparison of at least two scenarios, or hypotheses. There is no such thing as the simple identification of one individual from one sample. In this study, DNA profiles detected in spraint samples were the potential means to define individual otters. This section describes how useful such profiles appeared to be for this purpose. It was assumed initially that there were no errors of tube mislabelling, and that the multiple PCR assays described above were sufficient to exclude any experimental errors. Any mismatches between profiles were therefore ascribed to genetic differences between individuals, not to experimental artefacts. Only matches within the R. Torridge, Somerset levels, and R. Itchen sites were analysed. Matches between these sites were ignored on the grounds that no individual otter could have travelled between sites during the study period. The profiles obtained were either complete (seven loci consisting of six microsatellites plus SRY) or incomplete (fewer than six microsatellites plus SRY).

Prior to biological interpretation, the profiles of all spraints typed successfully were divided into three categories:

(a) Single (S): a profile from one spraint only that mismatched at one or more of the seven loci with all the other profiles. Single profiles were both complete and incomplete.

(b) Group (P): profiles that formed groups within which profiles matched and between which profiles mismatched. At least one multiple profile in each group was complete. P was used to avoid confusion with G, which is used for another purpose below.

(c) Ambiguities (A): profiles that matched those in more than one of the S or P categories. All A profiles were incomplete, and those for which sufficient DNA remained were set aside for further typing.

The biological interpretation of single and multiple profiles was carried out using two logical steps. The first step was to consider all the possible pairs of profiles, then to consider two alternative hypotheses.

H1.1. that the two profiles in each pair represent the same individual
H1.2. that they represent two different individuals.

No statistical calculations were required for this step. Simple categorisation was sufficient to assign all S profiles to different individuals, and to assign all the P profiles into groups, each of which represents at least one individual. The A profiles were not analysed. The complete list of the S and P profiles is given in Appendix C5.

The second step was to analyse pairs of P profiles within each group by considering the four alternative hypotheses that the profiles represent:
H2.1. the same individual
H2.2. two unrelated individuals
H2.3. parent and offspring
H2.4. two full siblings

It is only when H2.2-4 can be rejected with confidence that P profiles can be attributed to the same individual. The statistical method used for this step was the calculation of likelihood ratios. This method allows two alternative hypotheses to be compared using the value of the statistic "G". For example, the value of G calculated for the comparison of H2.1 versus H2.2 indicates how much more likely it is that the same individual is responsible for a match than two unrelated individuals. The values of G for two pairs of alternative hypotheses (H2.1 vs. H2.2, and H2.1 vs. H2.3) are given in Appendix C5 for "best-case" and "worst-case" groups in each of the three study sites. The best-case groups contain the fewest profiles, most of which are complete. The worst-case groups contain the most profiles, some of which are incomplete. The hypotheses H2.1 vs. H2.4, were not tested except for illustrative purposes for the group S01M because the existing "product-rule" method for calculation of G is know to be biased towards rejection of H2.4 (Donnelly, 1995; P. Taberlet, personal communication). An unpublished computer package that may provide improved calculations has been identified, and has been requested by JD. A description of the calculation and assessment of statistical significance of G values is given in Appendix C6.

There are four factors evident in Appendix C5 that reduce the values of G, and hence reduce the statistical power to exclude the presence of extra individuals within groups. Firstly, it is increasingly difficult to exclude extra individuals in the order: unrelated individuals, parents-offspring, and full siblings (e.g., group S01M). Secondly, incomplete profiles have lower values of G (group D01F, samples 25 vs. 2 vs. 19). Thirdly, the G values tend to be higher in the Somerset levels, where all six microsatellites are polymorphic, that in the R. Torridge (701 monomorphic) and the R. Itchen (715 monomorphic). Lastly, higher values of G are required for larger groups of profiles, due to the application of the Bonferroni correction for multiple tests (Appendix C6). The G values were ranked according to biological inference. Rank 1 has been reserved for the results of improved tests of H2.4.

Rank 3 (rejection of H2.2): unrelated individuals excluded, parents/offspring or full siblings may be present.

Rank 2 (rejection of H2.3): unrelated individuals and parents/offspring excluded, full siblings may be present.

The overall conclusion of this analysis is that when DNA profiles are complete one can be reasonably sure that groups of matching profiles do not arise from the presence of either unrelated individuals or parents and offspring. An important exception is the group H01M, which contains by far the largest number of profiles. In this case, the largest G values are not sufficiently high after Bonferroni correction to reject H2.3. This result strongly suggests that any future DNA typing studies in which large groups are required, e.g., for estimation of individual home ranges, should aim for higher G values. It is worth noting that the analyses employed here do not estimate the number of additional individuals that may be present, but only suggest that their presence cannot be excluded.

It is not clear at this point in time whether DNA typing as performed in this study provides truly individual-specific tags, i.e., has the capacity to exclude full siblings. Two measures should be taken to increase such capacity. Firstly, an improved method for the likelihood ratio test should be employed. Secondly, additional loci should be included in the DNA profile. Simulations using the improved test will indicate how many additional loci having levels of polymorphism typical of the study sites will be required. On a more positive note, DNA typing is clearly able to provide minimum numbers of individuals present in given sites over specified intervals. The utility of such numbers for monitoring population trends remains to

be assessed. This is likely to involve the use of demographically and spatially explicit models, which goes beyond the scope of the present study.

References

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APPENDIX C5: STATISTICAL ANALYSIS

TUBE NO	Otter ID	DATE	OS GRID	701	715	717	832	833	902	SRY	CATEGORY	RANK	pUNR	G UNR vs ID	pPOFF	G POFF vs ID
3	H01M	30-Jan-97	SU 456 161	04 05	04 04	06 06	02 03	X	ND	M	P					
9	H01M	30-Jan-97	SU 445 153	04 05	04 04	06 06	02 03	X	ND		P					
1001	H01M	18-Feb-97	SU 486 293	04 05	04 04	06 06	02 03	02 04	ND	M	P					
4	H01M	24-Sep-97	SU 486 292	04 05	04 04	06 06	02 03	02 04	01 07	M	P					
19	H01M	24-Sep-97	SU 503 325	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
72	H01M	21-Oct-97	SU 503 325	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
109	H01M	16-Feb-98	SU 467 223	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
229	H01M	10-Mar-98	SU 605 322	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
234	H01M	10-Mar-98	SU 605 322	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
230	H01M	20-Apr-98	SU 547 327	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
264	H01M	18-May-98	SU 564 355	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
197	H01M	22-Jun-98	SU 485 228	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
351	H01M	23-Jun-98	SU 584 331	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
314	H01M	20-Jul-98	SU 476 271	04 05	04 04	06 06	02 03	02 04	01 07	M	P	3	2.8E-03	**11.73**	1.1E-02	**9.05**
89	H01M	21-Jan-98	SU 485 228	X	04 04	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
106	H01M	21-Jan-98	SU 467 223	X	04 04	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
113	H01M	21-Jan-98	SU 498 321	X	X	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
124	H01M	21-Jan-98	SU 462 196	X	04 04	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
23	H01M	16-Feb-98	SU 467 214	X	04 04	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
107	H01M	16-Feb-98	SU 467 223	X	04 04	06 06	02 03	02 04	01 07	M	P	3	6.2E-03	**10.17**	2.2E-02	**7.66**
16	H01M	24-Sep-97	SU 498 321	04 05	04 04	06 06	02 03	02 04	ND	M	P	2	9.8E-03	**9.25**	2.7E-02	**7.23**
35	H01M	24-Sep-97	SU 460 169	04 05	04 04	06 06	02 03	02 04	ND	M	P	2	9.8E-03	**9.25**	2.7E-02	**7.23**
92	H01M	26-Nov-97	SU 492 317	04 05	04 04	06 06	02 03	02 04	ND	M	P	2	9.8E-03	**9.25**	2.7E-02	**7.23**
10	H01M	16-Dec-97	SU 485 285	04 05	04 04	06 06	02 03	02 04	ND	M	P	2	9.8E-03	**9.25**	2.7E-02	**7.23**
123	H01M	16-Dec-97	SU 466 192	04 05	04 04	06 06	02 03	02 04	ND	M	P	2	9.8E-03	**9.25**	2.7E-02	**7.23**
15	H01M	24-Sep-97	SU 511 325	X	04 04	06 06	02 03	02 04	ND	M	P	2	2.1E-02	**7.69**	5.4E-02	**5.84**
22	H02F	21-Oct-97	SU 462 192	04 05	04 04	X	02 05	02 04	ND	F	P					
147	H02F	16-Feb-98	SU 462 192	X	04 04	06 06	X	02 04	01 07	F	P					
258	H02F	22-Jun-98	SU 466 192	04 05	04 04	06 06	02 05	02 04	01 07	F	P					
1008	H03F	18-Feb-97	SU 535 327	04 04	04 04	05 06	03 05	X	ND	F	P					
1011	H03F	19-Feb-97	SU 486 293	04 04	X	05 06	03 05	X	ND	F	P					
1013	H03F	17-Feb-97	SU 486 293	04 04	04 04	05 06	03 05	02 03	ND	F	P					
1020	H03F	05-Feb-97	SU 486 293	04 04	04 04	05 06	03 05	02 03	ND	F	P					
1025	H03F	05-Feb-97	SU 486 293	04 04	04 04	05 06	03 05	02 03	ND	F	P					
244	H03F	18-May-98	SU 573 310	04 04	04 04	05 06	03 05	02 03	06 07	F	P					
349	H03F	23-Jun-98	SU 584 331	04 04	04 04	05 06	03 05	02 03	06 07	F	P					
347	H03F	6-Jul-98	SU 579 330	04 04	04 04	05 06	03 05	02 03	06 07	F	P					
65	H04F	22-Jan-98	SU 564 318	04 04	04 04	05 06	02 05	02 02	01 06	F	P					
66	H04F	21-Jan-98	SU 566 317	X	04 04	05 06	X	02 02	01 06	F	P					
82	H05M	19-Jan-98	SU 485 228	04 04	04 04	05 06	03 05	02 04	07 07	M	P	2	1.2E-03	**13.42**	1.2E-02	**8.93**

TUBE NO	Otter ID	DATE	OS GRID	701	715	717	832	833	902	SRY	CATEGORY	RANK	pUNR	G UNR vs ID	pPOFF	G POFF vs ID
25	D01F	27-Jan-98	SS 453 252	04 04	04 04	01 05	03 05	02 05	02 02	F	P	2	5.7E-04	**14.93**	1.2E-02	**8.89**
2	D01F	03-Dec-97	SS 394 221	04 04	04 04	01 05	03 05	02 05	ND	F	P	2	2.3E-03	**12.15**	2.3E-02	**7.50**
19	D01F	03-Dec-97	SS 453 252	04 04	04 04	01 05	X	02 05	ND	F	P	2	1.6E-02	**8.22**	5.9E-02	**5.67**
115	D01F	26-Feb-98	SS 434 202	04 04	04 04	01 05	03 05	02 05	02 02	F	P					
3	D02F	04-Dec-97	SS 426 232	04 04	04 04	04 05	05 05	02 05	ND	F	S					
31	D03F	27-Jan-98	SS 540 063	04 04	04 07	04 05	05 05	02 05	02 06	F	P	2	5.9E-04	**14.86**	9.0E-03	**9.42**
22	D03F	03-Dec-97	SS 540 063	04 04	04 07	04 05	X	02 05	02 06	F	P	2	1.2E-03	**13.43**	1.3E-02	**8.71**
34	D03F	28-Jan-98	SS 506 069	04 04	04 07	04 05	05 05	02 05	02 06	F	P					
10	D04M	27-Jan-98	SS 426 232	X	04 06	01 05	03 05	02 02	02 02	M	P					
15	D04M	27-Jan-98	SS 419 184	X	04 06	01 05	03 05	02 02	02 02	M	P					
133	D04M	19-Jun-98	SS 426 232	X	X	01 05	03 05	02 X	02 X	M	P					
33	D05M	28-Jan-98	SS 540 063	04 04	04 06	05 05	05 06	02 03	06 07	M	S					
27	D06M	27-Jan-98	SS 474 206	04 04	06 07	05 05	05 05	02 05	02 07	M	S					
12	D07F	27-Jan-98	SS 435 202	04 04	04 06	01 05	05 05	02 05	02 02	F	S					
126	D09M	02-May-98	SS 453 252	04 04	04 06	05 05	05 05	05 05	06 06	M	S					
85	D10M	04-Jul-98	SS 533 173	04 04	04 06	05 05	04 04	02 05	06 07	M	S					
84	D11M	04-Jul-98	SS 542 143	X	X	X	04 05	02 02	02 07	M	S					
52	D12F	27-Jan-98	SS 422 095	X	X	04 05	05 05	02 05	02 02	F	S					

TUBE NO	Otter ID	DATE	OS GRID	701	715	717	832	833	902	SRY	CATEGORY	RANK	pUNR	G UNR vs ID	pPOFF	G POFF vs ID	pFSIB	G FSIB vs. ID
409	S01M	06-Mar-98	ST 140 224	04 05	04 04	05 05	05 05	02 05	02 06	M	P	2	4.7E-04	15.3	7.8E-03	9.7	2.0E-02	7.84
629	S01M	07-Jul-98	0790 2020	04 05	04 04	05 05	05 05	02 05	02 06	M	P	2	4.7E-04	15.3	7.8E-03	9.7	2.0E-02	7.84
631	S01M	07-Jul-98	0790 2020	04 05	04 04	05 05	05 05	02 05	02 06	M	P	2	4.7E-04	15.3	7.8E-03	9.7	2.0E-02	7.84
31A	S01M	19-Aug-97	ST 078 202	04 05	04 04	05 05	05 05	X	ND	M	P	2	3.8E-03	11.2	4.0E-02	6.4	6.3E-02	5.53
31B	S01M	19-Aug-97	ST 087 224	04 05	04 04	05 05	X	02 05	ND	M	P	2	3.8E-03	11.1	3.1E-02	6.9	5.7E-02	5.74
112	S01M	22-Jun-97	ST 079 202	04 05	04 04	05 05	X	X	ND	M	P	2	1.3E-02	8.7	7.4E-02	5.2	1.1E-01	4.48
130	S01M	12-Oct-97	ST 137 223	04 05	04 04	05 05	05 05	02 05	02 06	M	P							
127	S02F	04-Oct-97	ST 367 553	04 04	06 06	05 05	03 04	02 05	ND	F	S							
118	S03M	16-Aug-97	ST 565 239	X	04 06	05 05	05 05	02 05	02 03	M	P							
128	S03M	12-Oct-97	ST 467 238	02 05	04 06	05 05	05 05	02 05	ND	M	P							
445	S03M	23-Mar-98	ST 104 216	02 05	04 06	05 05	05 05	02 05	02 03	M	P							
23B	S04M	10-Aug-97	ST 172 230	03 04	04 04	X	05 06	X	ND	M	P							
17B	S04M	23-Nov-97	ST 400 424	X	04 04	01 05	05 06	02 03	02 02	M	P							
11B	S05F	23-Nov-97	ST 400 424	04 04	04 07	05 05	03 05	02 02	02 06	F	P	2	2.7E-04	16.4	7.4E-03	9.8		

	Allele			1	2	3	4	5	6	7	8	9	10	SUM	
Population	sample	locus													
Torridge	DEV-11-M	701					1.000							1.00	
Torridge	DEV-11-M	715					0.556		0.333	0.111				1.00	
Torridge	DEV-11-M	717		0.167			0.111	0.722						1.00	
Torridge	DEV-11-M	832				0.100	0.150	0.700	0.050					1.00	
Torridge	DEV-11-M	833			0.550	0.050		0.400						1.00	
Torridge	DEV-11-M	902			0.500				0.278	0.222				1.00	
Itchen	HAM-14-M	701					0.643	0.357						1.00	
Itchen	HAM-14-M	715					1.000							1.00	
Itchen	HAM-14-M	717						0.192	0.808					1.00	
Itchen	HAM-14-M	832			0.321	0.357		0.321						1.00	
Itchen	HAM-14-M	833			0.500	0.214	0.286							1.00	
Itchen	*HAM-14-M*	*902*		*0.269*					*0.192*	*0.538*				*1.00*	
Somerset	SOM-41-M	701			0.057	0.071	0.714	0.157						1.00	
Somerset	SOM-41-M	715		0.053		0.039	0.447		0.408	0.053				1.00	
Somerset	SOM-41-M	717		0.125		0.025		0.762	0.038	0.038		0.013		1.00	
Somerset	SOM-41-M	832			0.025	0.237	0.125	0.538	0.075					1.00	
Somerset	SOM-41-M	833			0.598	0.061	0.024	0.244	0.049	0.024				1.00	
Somerset	SOM-41-M	902			0.526	0.039			0.408	0.026				1.00	
East SW	VS033 98	701			0.013	0.026	0.756	0.205						1.00	
East SW	VS033 98	715		0.013			0.417		0.410	0.160				1.00	
East SW	VS033 98	717		0.218				0.763	0.013	0.006				1.00	
East SW	VS033 98	832			0.013	0.192	0.147	0.609	0.038					1.00	
East SW	VS033 98	833			0.590	0.077	0.006	0.321		0.006				1.00	
East SW	VS033 98	902			0.591	0.006		0.032	0.286	0.084				1.00	

APPENDIX C6. LIKELIHOOD RATIO TESTS FOR INDIVIDUAL IDENTIFICATION.

It is worth repeating that the identification of individuals using DNA profiles always involves the comparison of at least two hypotheses. The statistical method employed for such comparisons consisted of two stages. The first stage involved the calculation of the probabilities that the DNA profiles from two spraints match for each of the hypotheses (H2.1-4) given below. The probability of a match for H2.1 is always 1, since two spraints from the same individual will always have the same DNA profile, assuming an experimental error rate of zero. The formulae for the calculation of match probabilities for H2.2-4 for a single genetic locus are given below (following Li, 1996). Each locus will be either be heterozygous, i.e., contain two alleles (02 05), or homozygous, i.e., contain the same allele (02 02). The frequencies of the two alleles in the population are denoted by a and b. Estimates of allele frequencies for each site were calculated from the spraint profiles themselves assuming that each different profile represented one individual.

The probability of a match for multi-locus profiles of up to six microsatellite loci was calculated by multiplication of the single-locus probabilities, then this was multiplied by a final factor of 0.5 to account for the match at the SRY locus. Thus, it was assumed that the product rule is valid, i.e., that the single-locus probabilities are independent between loci, and that the sex ratio in the population was 0.5. The first assumption is almost certainly invalid due to statistical associations among loci in the case of full siblings (Donnelly, 1995; P. Taberlet, personal communication). Thus the probability values calculated for H2.4 for S01M are almost certainly too low, and hence may give false rejections of H2.4. However, no improved calculation is available in the scientific literature to our knowledge.

Hypothesis	**Single-locus match probability**	
	Heterozygous locus	**Homozygous locus**
H2.1. same individual	1	1
H2.2. unrelated individuals	2ab	a^2
H2.3. parent and offspring	(a+b)/2	a
H2.4. two full siblings	0.25 + 0.5((a+b)/2) + 0.25(2ab)	0.25 + 0.5(a) + 0.25(a^2)

Whole-profile match probability $p = p_{701} \times p_{715} \times \ldots \times p_{902} \times 0.5$

The second stage involved the calculation of the statistic G from the ratio of the whole-profile match probabilities corresponding to the two hypotheses of interest. For example, to know how much more likely it was that two matching profiles came from the same individual than that they came from two unrelated individuals, the following calculation was performed:

$G = 2 \ln(p(H2.1)/p(H2.2))$

where ln denotes the natural logarithm. Or, more simply:

$G = 2 \ln(1/p(H2.2))$

since p(H2.1) is assumed to be equal to 1.

The values of G were assessed for statistical significance by comparing them to values of χ^2 with one degree of freedom (Sokal & Rolf, 1981, p. 695). For example, a G value of 3.84 was considered to be on the borderline for significance at $p = 0.05$ where one test was carried out. In cases where a group contained several profiles, e.g. S01M, multiple tests were involved in performing all the comparisons. One complete profile detected within each group was designated as the given profile. This profile was then paired in turn with each of the other profiles, and the probabilities corresponding to H2.2-4 were calculated. In such cases, sequential Bonferroni correction for independent tests was employed to lower the threshold for significance (Rice, 1989). For example, there are 6 comparisons between the given and subsequent profiles in the group S01M. The threshold was therefore reduced from $p = 0.05$ to $p = 0.0085$ (independent tests, $k = 6$, $\alpha = 0.05$), and the minimum value of G required for the most significant result raised from 3.84 to 7.00.

APPENDIX D1: MOVEMENTS OF OTTERS BASED ON SPRAINT ANALYSES

APPENDIX D2: SPRAINT GENOTYPES

APPENDIX D3: OTTER CARCASS GENOTYPES

APPENDIX D4: RESULTS OF SPRAINT ANALYSES

APPENDIX D1 MOVEMENTS OF OTTERS BASED ON SPRAINT ANALYSES

D1.1 Important Note

The sampling sites and distribution of otters identified from spraint analysis are presented in Maps D1 to D9.

For this discussion DNA profiles have been assumed to represent individual otters. However, it is possible that some profiles may be shared by more than one otter. This is only known to be true of H06F.

D1.2 Itchen Catchment

One male profile, H01M, was identified during the first survey in January 1997, again in September 1997 and then most months up to July 1998 (the last samples analysed) and is assumed to have been present over the 19 month period. Between December 1997 and May 1998 his range extended over 39 km. Samples collected on the same morning have been 4.5 km, 14 km, 18 km, 18 km and 20 km apart. Over the night 23/24 September 1997 H01M moved from north west of Winchester at Martyr Worthy to just north of the Itchen Valley Country Park, covering at least 20 km in one night. One of these spraints was at the City Mill in Winchester, close to a spraint from female H06F left on the same night.

Spraint from H01M and H07F were found at the same location on 16 February, to the south of the known range of H06F (about 10 km). In March 1998 spraint from H01M and H07F were found 22 km apart but their known ranges for April overlapped. H07F has only been found over 2 km of the Itchen.

Spraint from H01M and H03F were found together on 23 June 1998, to the north east of Winchester and within the known range of H06F, which extends to the south of Winchester. Other possible spraint 'associations' were found for H03F and H10F and also for H06F and H12F. Eight other otters, six females and two males, were recorded within the known range for H03F which is about 17 km in length.

H08M was found in December 1997 and April 1998 in the south of H01M's range, although there is very little overlap between their ranges.

Six individuals were only 'found' only once, H02F, H04F, H10F, H12F, H13F and H11M. Two spraint have been identified for H05M but at the same location on the same day in the middle of Winchester, two days before spraint from H01M was picked up at the same site.

The data suggest that there is one resident male, H01M, whose known home range extends over most of the length of watercourse surveyed. There have been four resident females, H03F, H06F, H07F and H09F whose individual home ranges overlap with each other, to varying degrees, but have all been found within H01M's known home range. H03F was recorded from February 1997 onwards. H06F was recorded from September 1997 onwards and H09F was recorded from October 1997 onwards. All four females were recorded from February to April 1998 inclusive.

A high concentration of individuals was found to the north east of Winchester and also to the west of Alresford. These are both areas where breeding evidence has been found.

Unfortunately the genetic diversity is insufficient to determine relatedness from the spraint DNA profiles, although it may be possible to confirm which otters are not related. From the data there may be associations between H03F and H10F and also between H06F and H12F, although the evidence is not sufficient to determine whether they are related or not.

There is a confluence of two significant tributaries to the Itchen to the west of Alresford, which may increase sprainting effort in this area and hence the number of individuals identified in this area.

D1.3 Brue Catchment

The majority of otters identified have been found within a 6 km^2 area centred on Burtle, with four otters (one female) on the North Drain and five otters (one female) on the South Drain. All of the South Drain otters have been found at Catcott Bridge, ST 400 424, between May 1997 and May 1998. This site is within 3 km of repeated sightings of a bitch and two cubs in late August 1997. This is the only confirmed evidence of breeding within the catchment. S08M was also picked up 5 km to the east of Catcott Bridge, but not on the same night as he was found at Catcott.

On the River Axe one female (S02F) was identified in October 1997 and, although spraint was been picked up at the same site in July 1998 and older spraint found in the area on two further occasions, no other spraint have been typed. One male, S17M, has been picked up on the Axe, about 12 km to the east of S02F. A very low level of otter activity was found along the Axe during the survey period. Between May 1996 and April 1997 no signs of otter activity were found during monthly surveys upstream of Bleadney along the 6 km stretch to the headwaters at Wookey. There is no data for the Axe downstream of Bleadney for this period (SOG records).

Otter S06M was identified from spraint from Tealham Moor in December 1997 and again in March 1998. During the Study period there was nearly always fresh or recent spraint at this site. In May 1998 a new male, S31M, was identified at the same location. The fingerprints for S06M and S31M only differ at one locus where they have one allele in common.

Fingerprints with a partial match (4 loci only) to S06M first occurred on the South Drain at Catcott Bridge in May 1997. These could be the same individual but we can not be certain. It is feasible for an otter to move between these locations, about 7 km, as there are many interconnecting watercourses between both the North and South Drains and the River Brue which flows on a parallel course between them. Given the high number of otters at Catcott Bridge area, and low level of recorded activity in the adjacent watercourses, such movement or dispersal could be expected. This is one of several examples where the development of additional primers for other loci could greatly improve the information gained from the results.

One other male, S40M, was identified at the B3151 road bridge over the Panborough Drain. This runs 800m to the north and parallel to North Drain with frequent interconnecting drains and ditches. The B3151 site is between the Tealham Moor and Dags Lane sites. Female S33F was identified at Dags Lane, 6 km from Tealham Moor, in March 1998.

Male S16M does not overlap with any other otters identified so far. His known range covered about 12 km of the upper reaches of the River Brue to the west of Castle Cary between February and June 1998. S16M has the longest known residence time having been present in

the population for at least 6 months. S06M and S08M have both been present for at least four months.

D1.4 Torridge Catchment

Only one male was identified more than once, D04M, and his known range overlaps with two females (D01F and D07F) but none of the other males. Otter D01F is using two tributaries of the Torridge as well as the Torridge itself. She has been found about 7 km up the River Yeo and 3 km up the River Duntz as well as on the Torridge itself. A total known range of 13 km. D07F was found at the same site as D01F on the River Duntz, one month earlier.

D03F was the nearest female to these and was found over 25 km upstream on the Torridge at Hele Bridge and also 4 km further west at Hele Barton. Otters D03F and D05M have both been found at the Hele Bridge site, during December 1997 and January 1998 respectively.

The only fresh spraint found upstream of Hele Barton (approximately 10 km) yielded a partial fingerprint (4 loci developed) which could match D02F or be a new individual D08F. If this was D02F she would have had to travel about 5 km down the Yeo and 40 km upstream along the Torridge to get to Gidcott Mill. Alternatively, because the Torridge is 'U' shaped, the direct distance to the nearest positive fix on D02F is 15 km, if she crossed over on to the Torridge catchment from the River Duntz. This is another example of where the development of primer for more loci would greatly increase the information gained from the typing. There is too little data on the Torridge population to comment usefully on residence times.

D1.5 Tone Catchment

One male, S01M, has been present in the population for at least 10 months. Two other partial fingerprints match S01M at four loci and, if from this individual, would increase its residence time to 14 months. S01M has a minimum home range of about 10 km on the Tone in the Greenham area.

Otters S27M, S39M, S19M, S18F, S03M and S23F have all been found within that 10 km range but on only one occasion each. S27M, S39M and S01M have all been recorded at Greenham Bridge. S18F and S19M were at the same site in Wellisford and S03M 400m away on the same night in March 1998. A bitch and cubs were seen in this area the previous evening. Otters S28F, S14F and S38M have all been identified once, 3 km, 6 km and 10 km respectively upstream of S01M's range on the Tone.

There is a partial fingerprint matching S14F at 4 loci from a sample taken on the River Batherm in October 1997 (data from PhD study). S14F may have crossed over from the Batherm onto the Tone. There is evidence in the hunting records of this occurring in the past in the Coombe Park area (J Williams, pers com).

Two females, S22F and S26F have been present in the population for at least 14 months.

Female S22F has a known range of at least 6 km of the Hillfarrance Brook in the Milverton area. Her range is almost identical to that found for S07F. S34M has also been found on this stretch.

S26F has a slightly larger known home range, of at least 8 km, on the Halse Water between its confluence with Hillfarrance Brook at Taunton and Halse. Otters S25M and S26F were

identified at Halse at the same time in August 1997 and S20F was identified at the same site in February 1998.

Male S15M was found in both February and March 1998, about 3 km downstream of S26F on the Tone. In February S15M was found in Creech St Michael, 7 km to the east of Taunton and in March 1998 he was picked up in the centre of Taunton. A different male, S21M, was found on this stretch in August 1997 immediately east of Taunton.

In January 1998 spraint from S10M and S12F were found 200m apart near Fitsroy on the Back Stream. S10M was also found the same date 3 km further upstream at Cedar Falls.

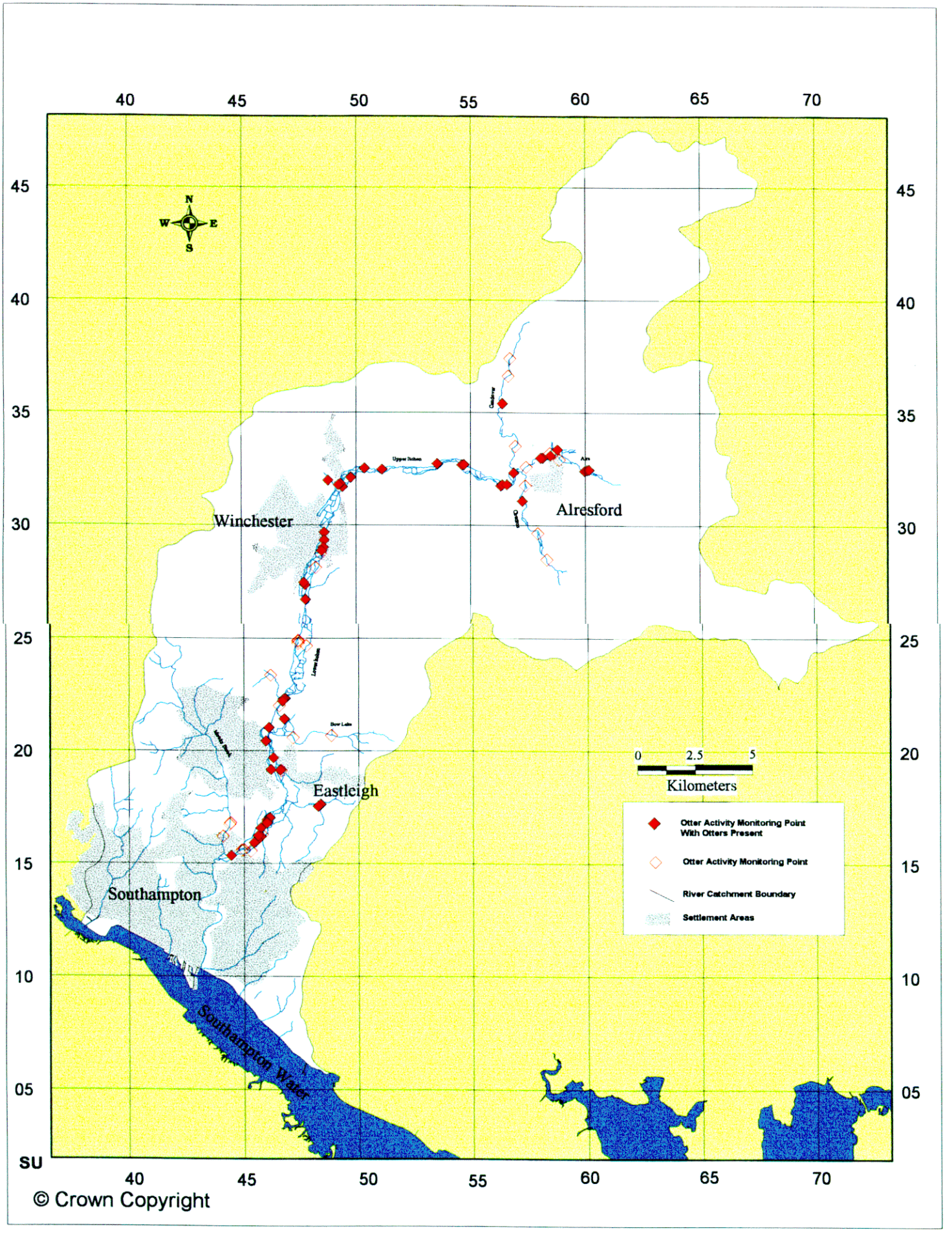
Three Bridges Stream flows north from Luxhay Reservoir, under the M5 to the Tone at its confluence with Hillfarrance Brook. Two otters have been found on this watercourse, S13F and S09M. S09M has been identified twice from spraint collected at Stoford, in December 1997 and May 1998. In February 1998 S13F was also recorded at Stoford and then 4 km away at Bradford-on-Tone in March 1998.

The recorded ranges of four females on the Tone catchment are all 6 - 9 km. This is in the middles of the ranges identified for the three resident females on the Itchen which were 2 to 17 km. There are no males with anything near the 39 km range recorded for H01M.

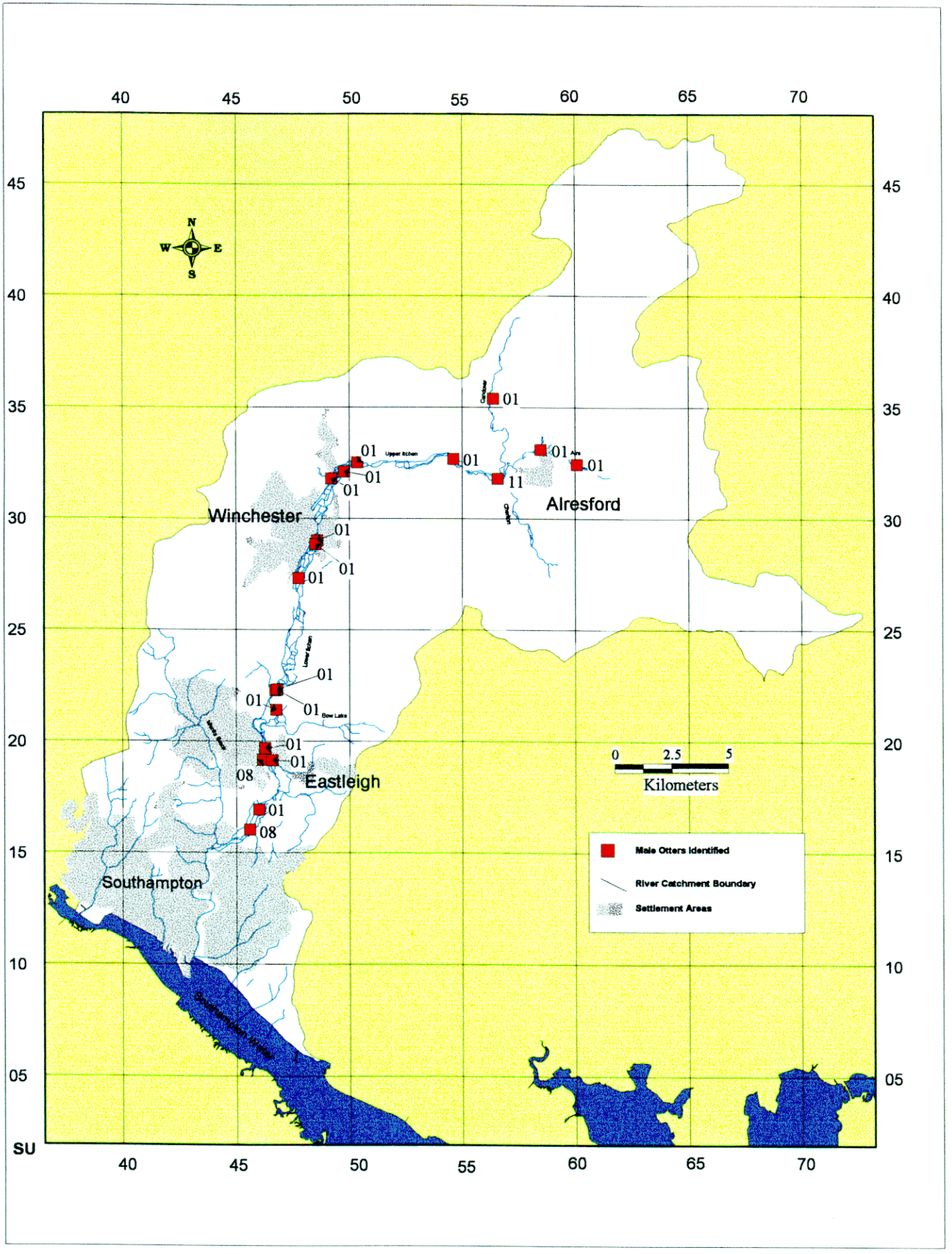
No individual DNA profiles/otters have been recorded on more than one tributary.

Breeding evidence on the Tone is patchy. There were several recorded sightings of a bitch and two cubs in the Nynhead/Westford stretch of the Tone. There were also sightings of a bitch and one cub on the Hillfarrance Brook. A female with three cubs was seen at Wellisford on 22 March 1998. Spraint from S19M and S18F were found at the same site the next day within 400m of spraint from S03M. The cubs were thought to be too small to be the same litter as seen in the Nynhead/Westford area 7-8 km away. Foot prints from much older cubs were found at Stoford on the Three Bridges Stream in April 1998.

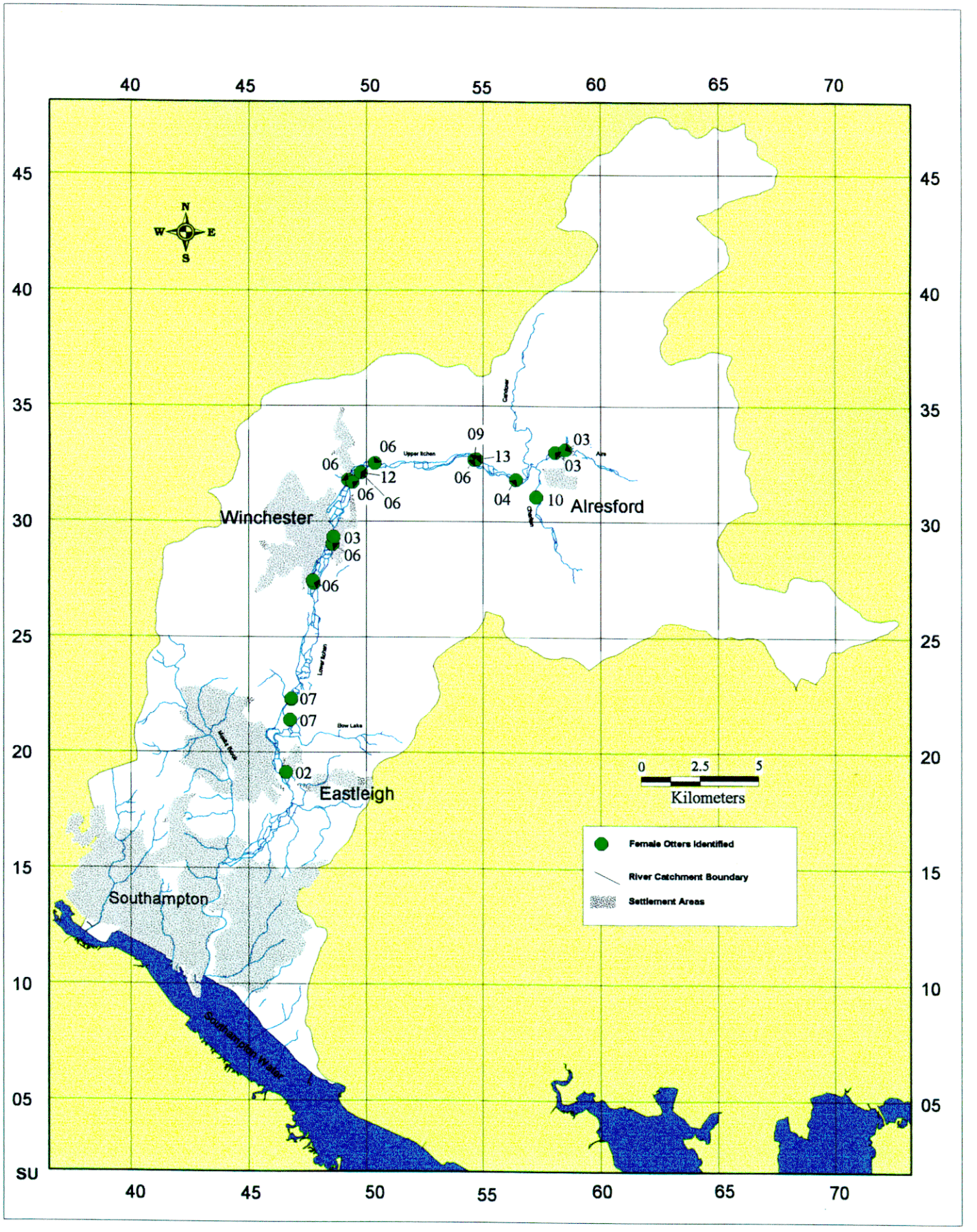
Map D1 - River Itchen Catchment: Monitoring Sites



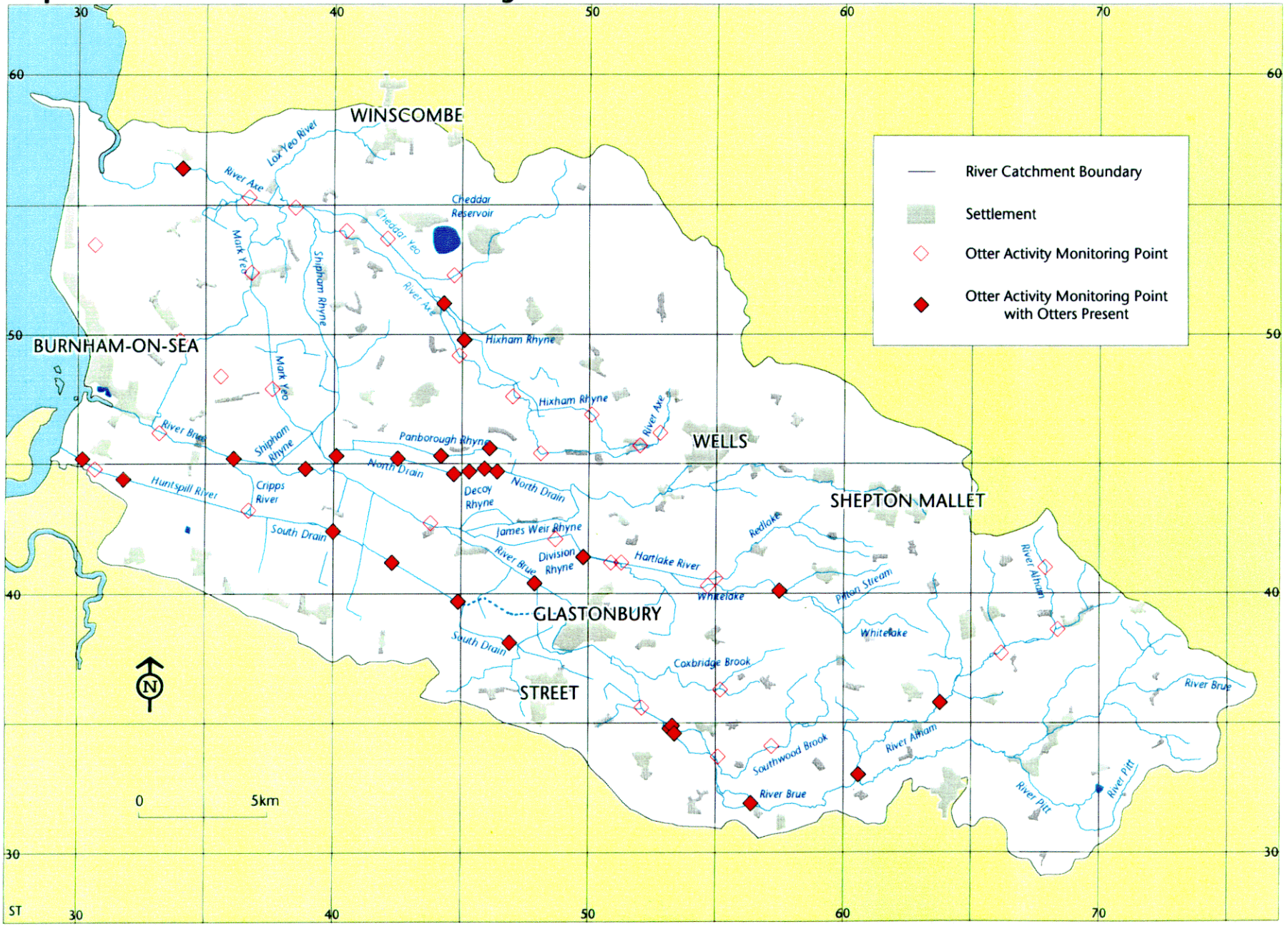
Map D2 - River Itchen Catchment: Male Otters Identified



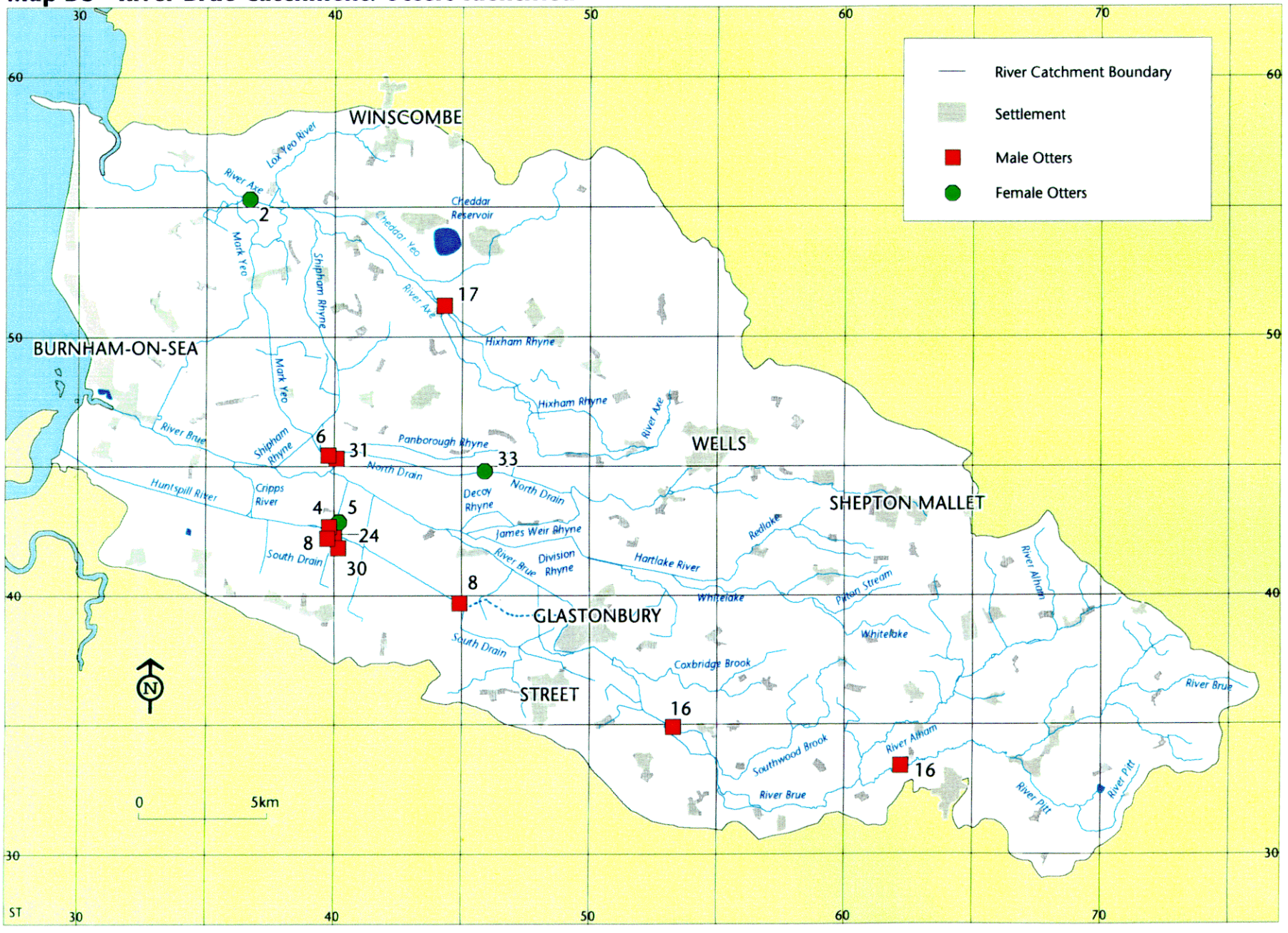
Map D3 - River Itchen Catchment: Female Otters Identified



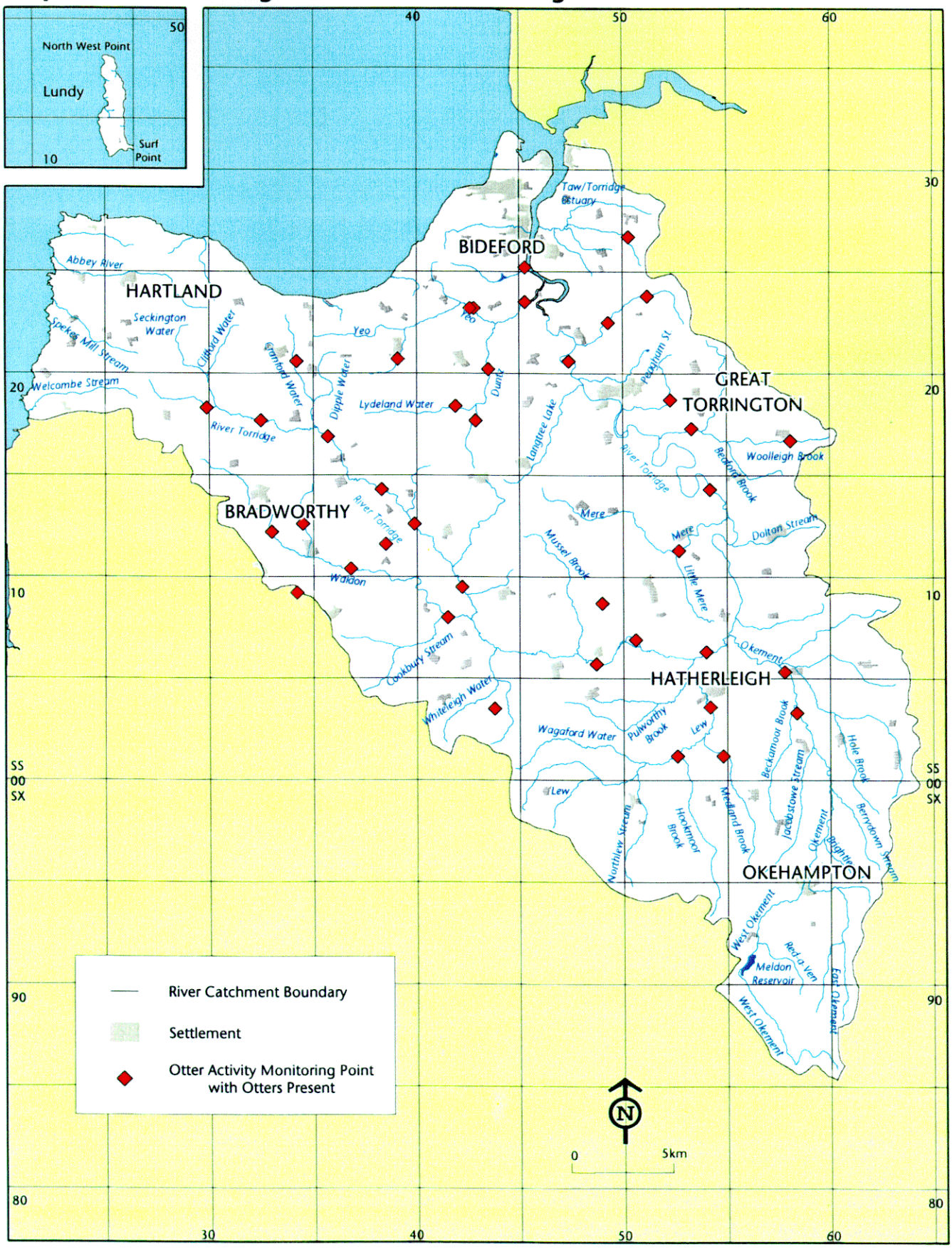
Map D4 - River Brue Catchment: Monitoring Sites



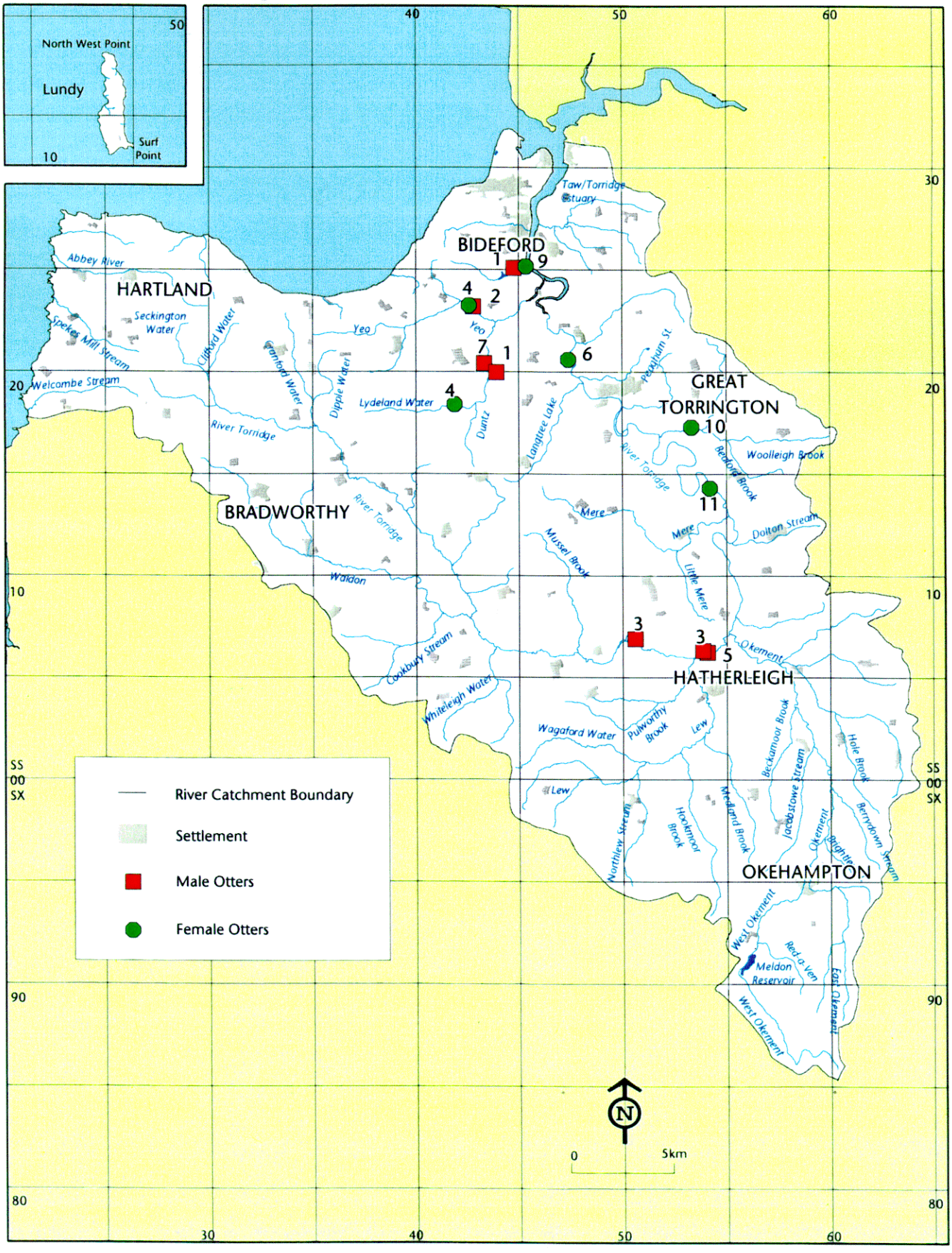
Map D5 - River Brue Catchment: Otters Identified



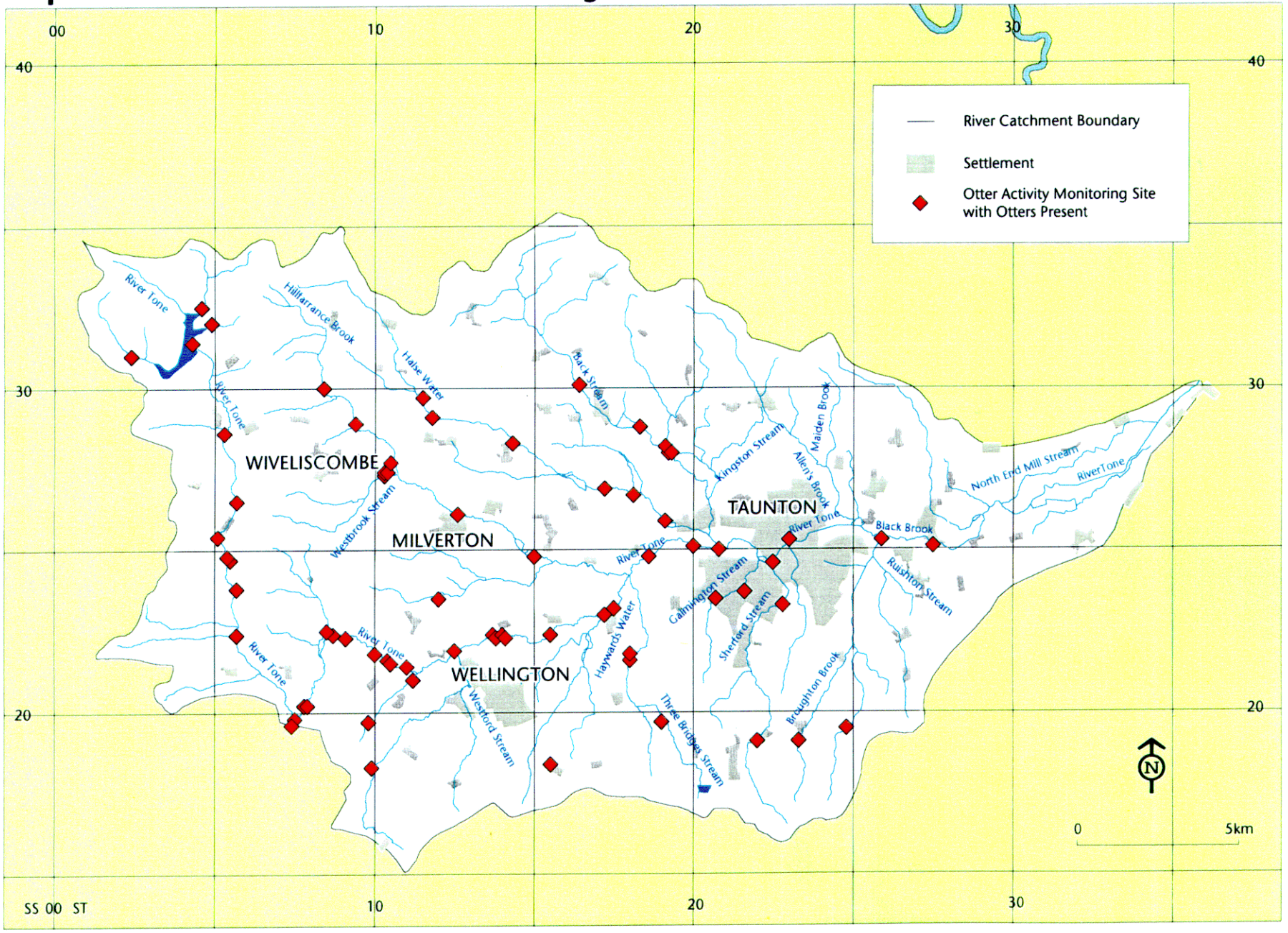
Map D6 - River Torridge Catchment: Monitoring Sites



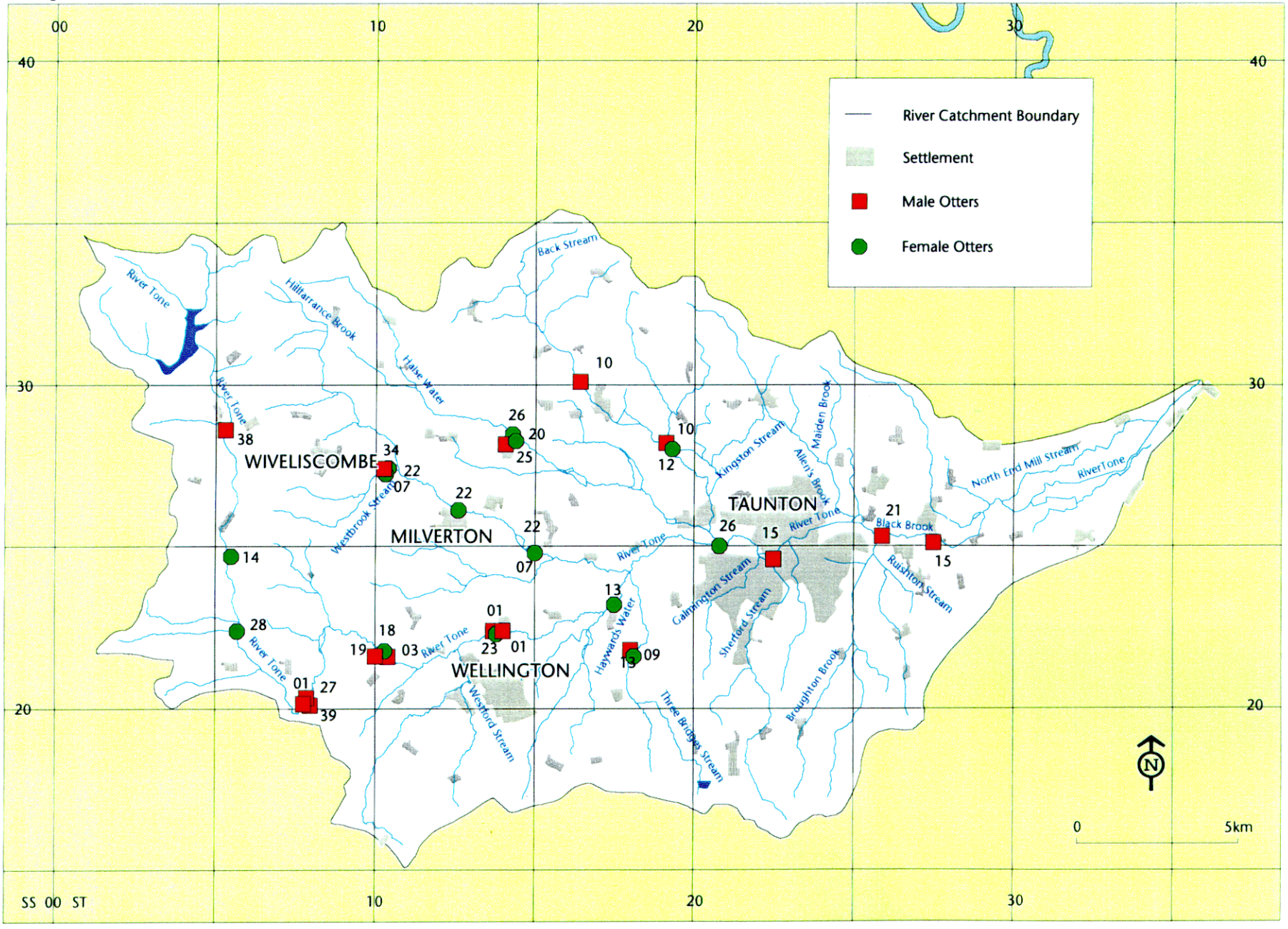
Map D7 - River Torridge Catchment: Otters Identified



Map D8 - River Tone Catchment: Monitoring Sites



Map D9 - River Tone Catchment: Otters Identified



APPENDIX D2 SUMMARY OF SPRAINT GENOTYPES

RIVER ITCHEN CATCHMENT							
OTTER ID	701	715	717	832	833	902	SRY
H01M	04 05	04 04	06 06	02 03	02 04	01 07	M
H05M	04 04	04 04	05 06	03 05	02 04	07 07	M
H08M	04 05	04 04	06 06	02 05	02 04	01 07	M
H11M	04 05	04 04	05 06	02 03	02 04	01 07	M
H02F	04 05	04 04	06 06	02 05	02 04	01 07	F
H03F	04 04	04 04	05 06	03 05	02 03	06 07	F
H04F	04 04	04 04	05 06	02 05	02 02	01 06	F
H06F	04 05	04 04	06 06	03 03	02 02	01 07	F
H07F	04 05	04 04	06 06	02 05	03 04	06 07	F
H09F	04 05	04 04	06 06	03 05	02 03	01 06	F
H10F	04 05	04 04	05 06	03 05	02 03	07 07	F
H12F	04 05	04 04	06 06	02 03	02 04	07 07	F
H13F	04 04	04 04	X	02 03	02 03	X	F
RIVER BRUE CATCHMENT							
S04M	X	04 04	01 05	05 06	02 03	02 02	M
S06M	04 04	06 06	05 05	03 04	02 02	02 06	M
S08M	03 04	01 04	05 07	05 05	02 02	02 06	M
S16M	04 04	04 07	05 05	05 06	02 02	02 02	M
S17M	X	04 06	05 05	03 05	02 02	06 06	M
S24M	04 04	06 06	03 05	03 05	02 05	X	M
S30M	03 04	01 04	X	05 05	02 06	02 06	M
S31M	04 04	06 06	05 05	03 04	02 06	02 06	M
S40M	04 04	X	05 09	X	02 06	06 06	M
S02F	04 04	06 06	05 05	03 04	02 05	ND	F
S05F	04 04	04 07	05 05	03 05	02 02	02 06	F
S33F	04 04	04 06	05 05	03 05	02 05	06 06	F
RIVER TONE CATCHMENT							
S01M	04 05	04 04	05 05	05 05	02 05	02 06	M
S03M	02 05	04 06	05 05	05 05	02 05	02 03	M
S09M	04 04	06 06	05 05	03 05	02 02	02 02	M
S10M	04 04	06 06	05 05	03 05	02 03	02 02	M
S15M	03 04	03 04	01 05	03 05	04 05	06 06	M
S19M	04 05	04 06	05 05	04 05	02 02	02 02	M
S21M	04 05	04 06	03 05	05 05	02 05	X	M
S25M	04 05	04 06	05 05	05 05	02 05	02 06	M
S27M	04 05	04 04	05 06	03 05	02 02	02 06	M
S34M	04 04	04 06	01 05	05 06	02 03	02 02	M
S38M	04 04	04 04	05 05	05 06	02 03	06 06	M
S39M	X	X	01 05	04 05	02 05	02 06	M
S07F	04 04	04 06	05 05	03 06	02 02	02 02	F
S12F	04 04	06 07	05 05	05 05	05 05	02 06	F
S13F	04 05	06 06	05 05	03 03	02 05	02 07	F
S14F	X	04 06	01 05	03 05	02 05	02 06	F
S18F	04 04	06 06	05 05	04 06	02 02	02 02	F
S20F	X	04 06	01 05	05 05	02 05	02 06	F
S22F	04 05	04 06	05 05	03 05	02 02	02 02	F
S23F	04 05	04 06	01 05	04 05	02 05	02 07	F
S26F	04 04	01 04	05 06	03 05	02 03	02 06	F
S28F	04 05	04 04	01 05	05 05	05 05	02 06	F
RIVER TORRIDGE CATCHMENT							
D04M	X	04 06	01 05	03 05	02 02	02 02	M
D05M	04 04	04 06	05 05	05 06	02 03	06 07	M
D06M	04 04	06 07	05 05	05 05	02 05	02 07	M
D09M	04 04	04 06	05 05	05 05	05 05	06 06	M
D10M	04 04	04 06	05 05	04 04	02 05	06 07	M
D11M	X	X	X	04 05	02 02	02 07	M
D01F	04 04	04 04	01 05	03 05	02 05	02 02	F
D02F	04 04	04 04	04 05	05 05	02 05	ND	F
D03F	04 04	04 07	04 05	05 05	02 05	02 06	F
D07F	04 04	04 06	01 05	05 05	02 05	02 02	F

APPENDIX D3 GENOTYPES OF NINE MICROSATELLITE LOCI DETECTED IN OTTER CARCASSES FROM SW ENGLAND

EMG REF	PM REF	701	715	717	733	782	818	832	833	902
BD 001	M 089 /08/95	04 04	06 06	05 05	04 04	08 08	07 08	05 05	02 02	02 07
BD 006	M 016 /10/93	02 04	04 04	01 07	X	01 08	02 07	03 03	02 04	01 03
BD 008	M 024 /10/96	04 04	04 06	05 05	04 05	08 08	02 07	05 05	02 05	02 02
BD 010	M 030 /03/93	04 04	04 06	05 05	04 04	08 08	08 08	04 04	02 03	06 07
BD 011	M 031 /09/93	04 04	04 04	05 05	04 04	08 08	07 07	05 05	02 02	02 02
BD 014	M 043 /11/94	04 05	04 04	01 05	04 05	08 08	07 07	05 05	02 02	02 02
BD 015	M 070 /11/95	04 05	06 06	05 05	04 06	08 08	06 07	05 05	05 05	05 07
BD 016	M 071 /10/95	04 04	04 07	05 05	04 04	08 08	02 07	03 04	02 05	02 07
BD 017	M 071 /02/96	04 05	04 04	05 05	04 04	08 08	06 08	05 05	05 05	05 05
BD 018	M 077 /08/94	05 05	04 06	05 05	04 04	08 08	02 06	05 06	03 05	05 06
BD 019	M 082 /11/95	04 04	04 06	05 05	04 05	07 08	02 02	03 05	02 05	06 06
BD 022	M 110 /02/95	05 05	06 06	05 05	04 04	08 08	06 08	06 06	02 05	05 05
BD 024	M 111 /03/96	04 04	06 06	05 05	04 04	08 08	07 07	03 03	02 02	02 06
BD 025	M 112 /03/95	02 04	04 04	05 06	04 04	02 08	06 07	05 05	02 02	02 03
BD 026	M 113 /09/96	04 05	04 06	05 05	04 04	08 08	02 08	05 05	02 02	02 02
BD 029	M 131 /10/93	04 05	04 06	05 05	04 06	08 08	06 07	03 06	02 05	02 05
BD 030	M 135 /07/93	05 05	04 04	05 05	04 04	08 08	06 08	05 06	02 05	05 05
BD 031	M 139 /12/94	04 05	04 06	05 05	04 04	08 08	02 08	05 05	05 05	06 07
BD 034	M 153 /11/95	04 04	04 07	01 05	04 04	08 08	07 08	05 05	02 05	02 02
BD 035	M 153 /04/96	04 04	04 06	05 05	04 06	08 08	02 07	03 05	02 02	02 02
BD 037	M 154 /12/93	04 04	06 06	05 05	04 04	08 08	02 07	04 05	02 02	02 02
BD 038	M 157 /05/95	04 05	06 06	01 05	04 05	08 08	02 06	03 06	02 05	05 06
BD 039	M 161 /04/94	04 04	06 07	01 05	04 04	08 08	02 07	03 05	02 03	02 06
BD 040	M 161 /04/96	04 04	04 06	05 05	04 06	08 08	07 07	05 06	02 05	02 07
BD 042	M 180 /11/94	04 05	04 04	06 06	04 04	02 08	02 07	02 05	02 03	06 06
BD 044	M 190 /01/96	04 04	04 04	01 05	04 04	08 08	02 02	05 05	03 05	02 02
BD 045	M 191 /01/96	04 04	04 06	01 05	04 05	08 08	02 07	05 05	03 05	02 02
BD 046	M 191 /04/96	04 05	04 04	05 05	04 04	08 08	02 08	04 05	02 05	02 06
BD 049	M 206 /12/93	04 04	06 06	05 05	04 05	08 08	07 07	03 03	02 02	06 06
BD 051	M 210 /07/94	04 05	04 06	05 05	04 04	01 08	02 07	03 03	02 02	02 07
BD 052	M 211 /10/94	04 05	06 07	05 05	04 04	08 08	07 07	04 06	02 05	02 06
BD 055	M 219 /01/96	04 04	04 04	01 05	04 06	08 08	06 08	03 05	02 05	05 06
BD 056	M 219 /11/94	04 04	04 07	05 05	04 04	01 07	02 07	03 05	02 05	02 06
BD 058	M 224 /03/93	04 04	04 04	01 05	04 05	08 08	02 07	03 05	02 05	02 02
BD 059	M 225 /03/93	04 05	04 04	05 05	04 05	08 08	02 07	05 05	03 05	02 02
BD 061	M 281 /12/93	04 04	04 06	05 05	04 06	08 08	07 08	03 05	02 02	02 07
BD 062	M 290 /06/94	04 05	06 07	05 05	05 05	08 08	02 07	03 05	02 05	02 06
BD 063	M 290 /09/94	04 04	04 06	05 05	04 05	07 08	02 07	05 05	02 05	02 02
BD 064	M 301 /02/94	04 04	06 06	01 05	04 06	08 08	06 07	05 05	05 05	02 07
BD 066	M 311 /09/94	04 05	04 06	05 05	04 04	08 08	06 06	06 06	05 05	05 07
BD 067	M 340 /10/94	04 05	04 06	05 05	04 04	08 08	06 08	05 06	02 05	02 05
BD 068	M 348 /02/94	04 05	04 06	01 05	04 04	08 08	02 08	05 05	05 05	02 02
BD 069	M 364 /03/94	04 04	04 06	05 05	04 06	01 08	08 08	03 05	05 05	02 02
BD 082	M 090 /08/95	04 05	04 06	01 05	04 05	08 08	07 07	04 05	02 02	02 02
BD 083	M 332 /02/95	04 04	06 07	05 05	04 04	08 08	07 07	05 05	02 03	02 02
BD 087	M 115 /04/93	04 04	04 06	01 05	04 04	08 08	02 08	05 06	02 05	02 06
BD 088	22/M 048 /03/97	04 05	06 07	05 05	04 05	08 08	02 07	05 05	02 02	02 06
BD 089	M 061 /03/97	05 05	04 06	05 05	04 04	08 08	06 08	05 06	05 05	05 05
BD 090	M 080 /04/97	04 04	04 06	01 01	04 05	08 08	08 08	04 05	02 05	02 02
BD 091	22/M 042 /01/97	04 04	06 06	05 05	04 04	08 08	02 07	05 05	03 05	02 06
BD 092	22/M 077 /04/97	05 05	04 06	01 01	04 04	08 08	02 07	05 05	02 02	06 06
BD 093	22/M 078 /04/97	04 05	06 06	01 05	04 06	08 08	07 07	05 05	05 05	02 06
BD 094	22/M 113 /03/97	04 05	04 07	05 05	04 06	08 08	07 08	04 05	02 02	02 06
BD 095	22/M 041 /01/97	04 04	04 04	01 01	04 04	08 08	02 07	05 06	02 05	02 06
BD 096	22/M 164 /04/97	05 05	04 06	05 05	04 04	08 08	02 06	05 06	02 05	05 05

BD 097	22/M 086 /04/97	03 04	04 04	01 06	04 04	08 08	02 02	05 05	02 04	02 02
BD 098	22/M 125 /03/97	04 05	04 04	05 05	04 04	08 08	02 07	03 06	05 05	02 06
BD 099	M 222 /01/97	05 05	04 06	05 05	04 05	02 08	06 06	03 05	05 05	05 07
BD 100	M 131 /04/97	04 04	04 06	01 01	05 06	08 08	06 08	03 05	02 03	02 06
BD 101	M 289 /09/94	04 05	04 06	05 05	04 04	01 08	02 07	03 05	03 03	02 02
BD 102	M 204 /10/94	04 04	04 04	05 07	02 04	08 09	05 07	03 04	02 04	07 07
BD 103	M 173 /12/94	04 04	06 07	01 05	04 04	08 08	02 08	04 05	02 05	06 06
BD 105	M 203 /10/94	04 05	03 04	05 07	04 05	08 09	02 07	03 04	02 02	07 07
BD 106	M 090 /12/94	05 05	04 06	05 05	04 04	08 08	02 08	03 03	02 05	07 07
BD 107	M 134 /01/96	04 04	06 07	05 05	04 04	08 08	07 08	05 05	05 05	02 06
BD 109	M 050 /03/96	04 04	04 06	05 05	04 04	08 08	07 07	05 05	05 05	02 05
BD 110	M 062 /12/95	04 05	07 07	05 05	04 04	08 08	07 08	05 05	02 03	02 02
BD 111	M 044 /01/95	04 04	06 07	01 01	04 05	08 08	02 07	05 05	02 03	02 02
BD 112	M 153 /03/95	04 04	04 06	01 01	04 04	08 08	08 08	05 05	02 05	X
VS 001	M 020 /10/96	04 04	06 07	01 05	04 05	08 08	07 07	03 05	02 05	02 07
VS 002	M 108 /10/96	04 04	04 06	05 05	04 04	08 08	06 08	05 06	05 05	02 06
VS 003	M 161 /10/96	04 05	04 04	05 06	04 04	01 08	02 02	02 05	02 04	06 06
VS 004	M 027 /11/96	04 05	04 06	01 01	04 04	08 08	02 08	05 05	02 03	02 02
VS 005	M 104 /11/96	04 04	06 07	05 05	04 05	08 08	07 07	05 05	05 05	05 06
VS 006	M 154 /11/96	05 05	04 04	05 05	04 04	08 08	06 08	05 05	02 05	05 05
VS 007	M 158 /11/96	05 05	04 06	04 05	04 04	08 08	02 08	05 06	03 05	02 06
VS 008	M 217 /11/96	04 05	04 04	05 05	04 04	08 08	06 08	05 05	03 05	02 05
VS 009	M 009 /01/97	04 05	04 04	05 05	04 04	08 08	08 08	05 06	03 03	02 05
VS 010	M 140 /01/97	05 05	04 06	05 05	04 04	02 08	06 08	05 06	05 05	02 05
VS 011	M 061/03/97	05 05	04 06	05 05	04 04	08 08	06 08	05 06	05 05	05 05
VS 012	M 113/03/97	04 05	04 07	05 05	04 06	08 08	07 08	04 05	02 02	02 06
VS 013	M 164/04/97	05 05	04 06	05 05	04 04	08 08	02 06	05 06	02 05	05 05
VS 015	M 053/05/97	04 04	06 06	05 05	04 04	08 08	02 07	03 05	02 02	02 06
VS 016	M 007/06/97	04 04	04 07	01 05	04 04	08 08	02 08	05 05	02 03	02 02
VS 018	M 012/09/97	04 04	04 07	05 05	04 04	08 08	02 07	04 05	02 02	06 07
VS 019	M 111/09/97	02 03	01 04	01 05	03 04	08 08	06 07	05 05	02 02	06 06
VS 020	M 193/10/97	03 04	01 06	05 07	03 04	08 08	02 08	02 05	05 07	02 06
VS 021	M 194/10/97	04 05	06 07	01 05	04 04	08 08	02 07	03 05	02 05	06 06
VS 022	M 208/10/97	04 04	06 07	01 05	04 04	08 08	06 08	05 05	02 03	02 02
VS 023	M 115/12/97	02 04	02 02	01 06	04 04	08 08	02 07	03 05	04 04	03 06
VS 024	M 120/12/97	04 05	06 07	01 05	04 05	08 08	02 02	03 05	02 05	02 06
VS 025	M 126/12/97	04 04	06 06	01 05	04 04	08 08	02 02	03 05	02 02	02 02
VS 026	M 144/01/98	03 04	04 06	05 05	04 04	08 08	07 08	05 05	03 05	02 06
VS 027	M 015/02/98	04 05	04 04	01 05	04 04	08 08	06 08	05 06	02 03	02 06
VS 028	M 049/03/98	04 04	04 06	01 05	04 04	08 08	02 02	03 05	02 05	02 06
VS 029	M 131/03/98	04 05	04 04	05 05	04 04	08 08	06 06	06 06	02 05	05 07
VS 030	M 202/03/98	04 04	04 04	01 05	04 05	08 08	06 08	03 03	03 03	02 05
VS 031	M 017/04/98	05 05	04 06	05 05	04 04	08 08	02 08	05 05	03 05	02 05
VS 032	M 050/04/98	04 04	06 06	05 05	04 04	08 08	07 08	03 06	02 03	02 07
VS 033	22/M45/5/98	04 05	04 06	05 05	04 04	08 08	02 08	05 05	02 02	02 06
VS 034	22/M46/5/98	04 04	04 04	05 05	04 04	08 08	07 07	05 05	02 05	02 05
VS 035	22/M47/5/98	04 05	06 06	05 05	04 05	08 08	07 08	05 05	02 02	02 06
VS 036	22/M48/5/98	04 04	04 04	05 05	04 04	08 08	08 08	05 06	02 03	02 06
VS 037	22/M49/5/98	04 05	04 04	05 05	04 04	08 08	07 08	05 05	02 02	02 02
VS 038	22/M50/5/98	04 05	06 06	05 06	04 06	08 08	02 06	05 05	02 05	05 06
VS 039	22/M70/5/98	04 04	04 07	01 05	04 06	08 08	02 06	04 05	05 05	02 02
VS 040	22/M123/6/98	04 05	04 07	05 05	05 05	08 08	02 02	05 05	02 02	02 06
VS 041	22/M138/4/98	04 05	04 04	06 06	04 04	08 08	02 02	03 03	02 02	01 07

APPENDIX D4: RESULTS OF SPRAINT ANALYSES

Key to Appendix D4

701, 715, 717, 832, 833, and 902 are the locus specific primers
SRY is the sex chromosome

DNA profiles in bold have been assessed as reliable fingerprints and used within the data analysis

BRUE CATCHMENT

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
71	**S24M**	**11-May-97**	**12.20**	**Catcott Bridge**	**ST 400 426**	**AJ**	**04 04**	**06 06**	**03 05**	**03 05**	**02 05**	**X**	**M**
72	S06M /37M	11-May-97	12.20	Catcott Bridge	ST 400 426	S	04 04	X	05 05	X	02 02	02 06	M
127	**S02F**	**04-Oct-97**	**10.20**	**White House Fm**	**ST 367 553**	**AJ**	**04 04**	**06 06**	**05 05**	**03 04**	**02 05**	**ND**	**F**
133	S14F	11-Oct-97	7.00	Huckley Br	SS 970 235	S	X	04 06	01 05	03 05	X	02 06	F
17B	**S04M**	**23-Nov-97**	**09.05**	**Catcott Stn**	**ST 400 424**	**S**	**X**	**04 04**	**01 05**	**05 06**	**02 03**	**02 02**	**M**
11B	**S05F**	**23-Nov-97**	**09.05**	**Catcott Stn**	**ST 400 424**	**S**	**04 04**	**04 07**	**05 05**	**03 05**	**02 02**	**02 06**	**F**
15B	**S05F**	**23-Nov-97**	**09.05**	**Catcott Stn**	**ST 400 424**	**S**	**04 04**	**04 07**	**05 05**	**03 05**	**02 02**	**02 06**	**F**
46B	**S06M**	**14-Dec-97**	**10.40**	**Tealham Moor Br**	**ST 401 453**	**S**	**04 04**	**06 06**	**05 05**	**03 04**	**02 02**	**02 06**	**M**
47B(A)	**S06M**	**14-Dec-97**	**10.45**	**Tealham Moor Br**	**ST 401 453**	**S**	**04 04**	**06 06**	**05 05**	**03 04**	**02 02**	**02 06**	**M**
255	**S08M**	**14-Dec-97**	**09.40**	**Ashcott Corner**	**ST 449 397**	**S**	**03 04**	**01 04**	**05 07**	**05 05**	**02 02**	**02 06**	**M**
319	**S16M**	**08-Feb-98**	**09.00**	**Twinkham Br**	**ST 622 334**	**AJ**	**04 04**	**04 07**	**05 05**	**05 06**	**02 02**	**02 02**	**M**
361	S06M/37M	08-Feb-98	09.30	Tealham Moor Br	ST 401 453	S	X	X	05 05	X	02 02	02 06	M
364	S06M/37M	08-Feb-98	10.18	Dags Br	ST 459 448	S	X	X	05 05	X	02 02	02 06	M
505	**S06M**	**08-Mar-98**	**7:04**	**Tealham Moor Br**	**ST 401 453**	**AJ**	**04 04**	**06 06**	**05 05**	**03 04**	**02 02**	**02 06**	**M**
264	**S08M**	**08-Mar-98**	**4:48**	**Catcott Bridge**	**ST 400 424**	**S**	**03 04**	**01 04**	**05 07**	**05 05**	**02 02**	**02 06**	**M**
403	**S33F**	**08-Mar-98**	**2:24**	**Dags Lane**	**ST 459 448**	**S**	**04 04**	**04 06**	**05 05**	**03 05**	**02 05**	**06 06**	**F**
214	**S40M**	**08-May-98**	**07:00**	**B3151 Bridge**	**ST 442 453**	**S**	**04 04**	**X**	**05 09**	**X**	**02 06**	**06 06**	**M**
213	**S31M**	**10-May-98**	**07:30**	**Tealham Moor Br**	**ST 401 453**	**S**	**04 04**	**06 06**	**05 05**	**03 04**	**02 06**	**02 06**	**M**
208	**S30M**	**10-May-98**	**08:10**	**Catcott Bridge**	**ST 400 424**	**S**	**03 04**	**01 04**	**X**	**05 05**	**02 06**	**02 06**	**M**
304	**S16M**	**07-Jun-98**	**8.30**	**Farm Bridge 2**	**ST 533 349**	**S**	**04 04**	**04 07**	**05 05**	**05 06**	**02 02**	**02 02**	**M**
287	**S17M**	**12-Jul-98**	**9.20**	**Clewer Bridge**	**ST 443 512**	**AJ**	**X**	**04 06**	**05 05**	**03 05**	**02 02**	**06 06**	**M**
645	S16M?	11-Jul-98	10.15	Lower Farm	ST 623 334	S	X	04 07	05 05	05 06	02 X	02 X	M

Otter DNA Fingerprint
Results 1997 to 1998

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
2	D01F	03-Dec-97	09.00	Venton	SS 394 221	S	04 04	04 04	01 05	03 05	02 05	ND	F
19	D01F	03-Dec-97	08.15	Jennets underpass	SS 453 252	J	04 04	04 04	01 05	X	02 05	ND	F
3	D02F	04-Dec-97	08.20	Hoopers	SS 426 232	S	04 04	04 04	04 05	05 05	02 05	ND	F
22	D03F	03-Dec-97	11.25	Hele bridge	SS 540 063	S	04 04	04 07	04 05	X	02 05	02 06	F
10	D04M	27-Jan-98	08.00	Hooper's	SS 426 232	S	X	04 06	01 05	03 05	02 02	02 02	M
15	D04M	27-Jan-98	08.40	Bableigh Barton	SS 419 184	S	X	04 06	01 05	03 05	02 02	02 02	M
33	D05M	28-Jan-98	08.25	Hele Bridge	SS 540 063	S	04 04	04 06	05 05	05 06	02 03	06 07	M
27	D06M	27-Jan-98	09.00	Beam College	SS 474 206	S	04 04	06 07	05 05	05 05	02 05	02 07	M
12	D07F	27-Jan-98	08.20	Venton	SS 435 202	S	04 04	04 06	01 05	05 05	02 05	02 02	F
25	D01F	27-Jan-98	08.20	Jennets Pipe	SS 453 252	J	04 04	04 04	01 05	03 05	02 05	02 02	F
31	D03F	27-Jan-98	08.55	Hele Bridge	SS 540 063	S	04 04	04 07	04 05	05 05	02 05	02 06	F
34	D03F	28-Jan-98	08.45	Hele Barton	SS 506 069	S	04 04	04 07	04 05	05 05	02 05	02 06	F
52	D08F/02F	27-Jan-98		Gidcott Mill	SS 422 095	S	X	X	04 05	05 05	02 05	02 02	F
115	D01F	26-Feb-98	8.45	Venton, Duntz	SS 434 202	S	04 04	04 04	01 05	03 05	02 05	02 02	F
126	D09M	02-May-98	9.00	Jennetts pipe	SS 453 252	S	04 04	04 06	05 05	05 05	05 05	06 06	M
133	D04M	19-Jun-98	7.40	Hoopers	SS 426 232	S	X	X	01 05	03 05	02 X	02 X	M
84	D11M	04-Jul-98	7.25	Beaford	SS 542 143	S	X	X	X	04 05	02 02	02 07	M
85	D10M	04-Jul-98	8.00	Woolleigh Barton	SS 533 173	AJ	04 04	04 06	05 05	04 04	02 05	06 07	M

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
112	S01M	22-Jun-97	14.30	Greenham Br	ST 079 202	J	04 05	04 04	05 05	X	X	ND	M
108	S22F	22-Jun-97	13.10	Slape Moor	ST 104 274	S	04 05	04 06	05 05	03 05	02 02	02 02	F
106	S25M	22-Jun-97	11.35	Halse	ST 143 283	J	04 05	04 06	05 05	05 05	02 05	02 06	M
107	S26F	22-Jun-97	11.35	Halse	ST 143 283	S	04 04	01 04	05 06	03 05	02 03	02 06	F
113	S27M	22-Jun-97	14.30	Greenham Br	ST 079 202	S	04 05	04 04	05 06	03 05	02 02	02 06	M
23B	S04M	10-Aug-97	9.40	Bradford on Tone	ST 172 230	S	03 04	04 04	X	05 06	X	ND	M
31B	S01M	19-Aug-97	8.00	Lutley Weir	ST 087 224	AJ	04 05	04 04	05 05	X	02 05	ND	M
31A	S01M	19-Aug-97	7.30	Greenham Weir	ST 078 202	S	04 05	04 04	05 05	05 05	X	ND	M
33A	S21M	22-Aug-97	7.55	Black Brook Br	ST 259 253	S	04 05	04 06	03 05	05 05	02 05	X	M
34B	S07F	25-Aug-97	9.06	Hillacre	ST 103 273	AJ	04 04	04 06	05 05	X	02 02	02 02	F
35A	S22F	25-Aug-97	9.19	Milverton	ST 126 261	S	04 05	04 06	05 05	03 05	02 02	02 02	F
35B	S23F	26-Aug-97	7.21	Nine Head Park	ST 138 223	AJ	04 05	04 06	01 05	04 05	02 05	02 07	F
120	S28F	28-Sep-97	8.50	Stawley	ST 057 224		04 05	04 04	01 05	05 05	05 05	02 06	F
130	S01M	12-Oct-97	8.55	Nynehead	ST 137 223	S	04 05	04 04	05 05	05 05	02 05	02 06	M
1 (X 3)	S09M	04-Dec-97	08.15	Stoford 'otter loo'	ST 180 218	S	04 04	06 06	05 05	03 05	02 02	02 02	M
162	S07F	13-Dec-97	09.30	Oake Golf Course -top s	ST 150 248	S	04 04	04 06	05 05	03 06	02 02	02 02	F
168	S07F	13-Dec-97	09.20	Oake Golf Course - bott	ST 150 248	S	04 04	04 06	05 05	03 06	02 02	02 02	F
28A	S10M	27-Jan-98	10.10	Cedar Falls	ST 164 301	AJ	04 04	06 06	05 05	03 05	02 03	02 02	M
28B	S10M	27-Jan-98	10.30	Cedar Falls Drain	ST 191 282	S	04 04	06 06	05 05	03 05	02 03	02 02	M
29A	S12F	27-Jan-98	10.45	Fitzroy Crossing	ST 193 280	S	04 04	06 07	05 05	05 05	05 05	02 06	F
45A	S13F	03-Feb-98		Stoford	ST 180 216	S	04 05	06 06	05 05	03 03	02 05	02 07	F
48B	S14F	08-Feb-98	09.15	Viaduct Br	ST 055 247	S	X	04 06	01 05	03 05	02 05	02 06	F
93J	S20F	09-Feb-98	9:25	Halse	ST 144 283	AJ	X	04 06	01 05	05 05	02 05	02 06	F

Otter DNA Fingerprint
Results 1997 to 1998

42A	S18F	01-Feb-98	10.40	Nynhead Weir	ST 137 224	S	X	X	05 05	04 06	02 02	02 02	F
43A	S18F	01-Feb-98	10.50	Nynhead Weir	ST 137 224	S	X	X	X	04 06	02 02	02 02	F
261	**S15M**	**08-Feb-98**	**08.45**	**Creech St Michael**	**ST 275 251**	**S**	**X**	**03 04**	**01 05**	**03 05**	**04 05**	**06 06**	**M**
409	**S01M**	**06-Mar-98**	**10:15**	**Nynhead Park**	**ST 140 224**	**AJ**	**04 05**	**04 04**	**05 05**	**05 05**	**02 05**	**02 06**	**M**
455	S01Mor25M	06-Mar-98	10:25	Nynhead Pk Br	ST 137 224	AJ	X	X	05 05	X	02 05	02 06	M
445	**S03M**	**23-Mar-98**	**3:36**	**W Rewe Mead**	**ST 104 216**	**S**	**02 05**	**04 06**	**05 05**	**05 05**	**02 05**	**02 03**	**M**
462	**S13F**	**05-Mar-98**	**8:02**	**Bradford Slide**	**ST 175 232**	**S**	**04 05**	**06 06**	**05 05**	**03 03**	**02 05**	**02 07**	**F**
492	**S13F**	**05-Mar-98**	**8:02**	**Bradford Slide**	**ST 175 232**	**S**	**04 05**	**06 06**	**05 05**	**03 03**	**02 05**	**02 07**	**F**
416	**S15M**	**05-Mar-98**	**9:03**	**Goodland Gdns**	**ST 225 246**	**S**	**03 04**	**03 04**	**01 05**	**03 05**	**04 05**	**06 06**	**M**
414	**S18F**	**23-Mar-98**	**7:03**	**Wellisford**	**ST 100 218**	**AJ**	**04 04**	**06 06**	**05 05**	**04 06**	**02 02**	**02 02**	**F**
506	**S19M**	**23-Mar-98**	**7:03**	**Wellisford**	**ST 100 218**	**AJ**	**04 05**	**04 06**	**05 05**	**04 05**	**02 02**	**02 02**	**M**
407	S6/25/01M	05-Mar-98	6:00	Nynhead Pk	ST 140 224	S	X	X	05 05	05 05	02 05	02 06	M
567	**S09M**	**03-May-98**	**06:50**	**Stoford**	**1800 2180**	**AJ**	**04 04**	**06 06**	**05 05**	**03 05**	**02 02**	**02 02**	**M**
571	**S26F**	**06-May-98**	**0.31**	**Silk Mills**	**2080 2500**	**AJ**	**04 04**	**01 04**	**05 06**	**03 05**	**02 03**	**02 06**	**M**
561	**S34M**	**03-May-98**	**09:05**	**Crowford Main Road**	**1030 2740**	**AJ**	**04 04**	**04 06**	**01 05**	**05 06**	**02 03**	**02 02**	**M**
575	**S39M**	05-May-98	0.34	Greenham Bridge	0800 2020	AJ	X	X	01 05	04 05	02 05	02 06	M
568	S9M	03-May-98	0.28	Stoford	1800 2180	AJ	X	X	X	03 05	02 02	02 02	M
400	S14orS20F	10-May-98	09:00	Hurston Farm	0540 2480	AJ	X	X	01 05	X	02 05	02 06	F
402	S43?	10-May-98	09:30	Hagley Bridge	0560 2370	S	04 05	X	X	X	05 05	06 06	X
553	**S22F**	**13-Jun-98**	**06.30-7.1**	**Oake Golf Course**	**1500 2480**	**S**	**04 05**	**04 06**	**05 05**	**03 05**	**02 02**	**02 02**	**F**
554	S42F	13-Jun-98	06.30-7.1	Oake Golf Course	1500 2480	S	X	X	01 05			02 02	F
629	**S01M**	**7-Jul-98**	**9.30**	**Greenham Br**	**0790 2020**	**S**	**04 05**	**04 04**	**05 05**	**05 05**	**02 05**	**02 06**	**M**
631	**S01M**	**7-Jul-98**	**9.30**	**Greenham Br**	**0790 2020**	**AJ**	**04 05**	**04 04**	**05 05**	**05 05**	**02 05**	**02 06**	**M**
625	**S38M**	**14-Jul-98**	**8.30**	**Washbattle Br**	**0530 2860**	**S**	**04 04**	**04 04**	**05 05**	**05 06**	**02 03**	**06 06**	**M**

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
1001	**H01M**	**27-Jan-97**	**09.40**	**G2 Win. city mill**	**SU 486 293**	**AJ**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
3	H01M	30-Jan-97	10.00	K10 Itchen valley CP	SU 456 161	S	04 05	04 04	06 06	02 03	X	ND	M
9	H01M	30-Jan-97	13.00	L1 Woodmill	SU 444 153	AJ	04 05	04 04	06 06	02 03	X	ND	
1020	**H03F**	**05-Feb-97**	**15.40**	**G2 Win. city mill**	**SU 486 293**	**X**	**04 04**	**04 04**	**05 06**	**03 05**	**02 03**	**ND**	**F**
1025	**H03F**	**05-Feb-97**	**15.40**	**G2 Win. city mill**	**SU 486 293**	**X**	**04 04**	**04 04**	**05 06**	**03 05**	**02 03**	**ND**	**F**
1013	**H03F**	**17-Feb-97**	**15.15**	**G2 Win. city mill**	**SU 486 293**	**AJ**	**04 04**	**04 04**	**05 06**	**03 05**	**02 03**	**ND**	**F**
1008	H03F	18-Feb-97	09.15	E4 Itchen Abbas	SU 535 327	AJ	04 04	04 04	05 06	03 05	X	ND	F
1011	H03F	19-Feb-97		**G2 Win. city mill**	**SU 486 293**	X	04 04	X	05 06	03 05	X	ND	F
4	**H01M**	**24-Sep-97**	**10.45**	**G3 Wharf Milll**	**SU 486 292**	**AJ**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
19	**H01M**	**24-Sep-97**	**10.15**	**F2 M3 (N)**	**SU 503 325**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
9	**H06F**	**24-Sep-97**	**07.30**	**G3 Wharf Milll**	**SU 486 292**	**AJ/S**	**04 05**	**04 04**	**06 06**	**03 03**	**02 02**	**01 07**	**F**
15	H01M	24-Sep-97	08.10	F1 Easton Bridge	SU 511 325	JS	X	04 04	06 06	02 03	02 04	ND	M
16	**H01M**	**24-Sep-97**	**09.45**	**F3 Fulling Mill**	**SU 498 321**	**AJ**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
35	**H01M**	**24-Sep-97**	**08.30**	**K6 Decoy Wood (N)**	**SU 460 169**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
72	**H01M**	**21-Oct-97**	**10.05**	**F2 M3 (N)**	**SU 503 325**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
71	**H09F**	**21-Oct-97**	**09.50**	**E2 Nature Reserve**	**SU 546 327**	**S**	**04 05**	**04 04**	**06 06**	**03 05**	**02 03**	**01 06**	**F**
22	H02F	21-Oct-97	09.45	J7 Sparshatts G'ge	SU 462 192	S	04 05	04 04	X	02 05	02 04	ND	F
92	**H01M**	**26-Nov-97**	**09.20**	**F6 Link Rd Br**	**SU 492 317**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
13	H01M	26-Nov-97	07.00	H6 Confluence	SU 467 223	S	04 05	04 04	06 06	02 05	X	ND	M
42	H01/08M	15-Dec-97	10.25	L1 Woodmill	SU 442 153	S	04 05	04 04	06 06	X	02 04	ND	M
10	**H01M**	**16-Dec-97**	**07.40**	**G4 Blockbridge**	**SU 485 285**	**J**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
123	**H01M**	**16-Dec-97**	**08.05**	**J8 Bishopstoke Rd**	**SU 466 192**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**ND**	**M**
79	**H08M**	**16-Dec-97**	**09.30**	**K1O Itchen valley**	**SU 456 160**	**S**	**04 05**	**04 04**	**06 06**	**02 05**	**02 04**	**ND**	**M**

Otter DNA Fingerprint
Results 1997 to 1998

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
82	**H05M**	**19-Jan-98**	**09.25**	**G4 Blackbridge**	**SU 485 228**	**J**	**04 04**	**04 04**	**05 06**	**03 05**	**02 04**	**07 07**	**M**
85	**H05M**	**19-Jan-98**	**09.25**	**G4 Blackbridge**	**SU 485 228**	**J**	**04 04**	**04 04**	**05 06**	**03 05**	**02 04**	**07 07**	**M**
66		21-Jan-98	08.20	D7 Ovington meadows	SU 566 317	S	X	04 04	05 06	X	02 02	01 06	F
114	**H06F**	**21-Jan-98**	**10.00**	**E1 Nature Reserve**	**SU 547 327**	**S**	**04 05**	**04 04**	**06 06**	**03 03**	**02 02**	**X**	**F**
89	**H01M**	**21-Jan-98**	**08.50**	**G4 Blackbridge**	**SU 485 228**	**S**	**X**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
106	**H01M**	**21-Jan-98**	**06.15**	**H6 confluence**	**SU 467 223**	**J**	**X**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
113	**H01M**	**21-Jan-98**	**07.45**	**F3 Fulling Mill**	**SU 498 321**	**S**	**X**	**X**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
124	**H01M**	**21-Jan-98**	**08.55**	**J6 Barton Junction**	**SU 462 196**	**S**	**X**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
65	**H04F**	**22-Jan-98**	**09.10**	**D6 Itchen Stoke Mill**	**SU 564 318**	**S**	**04 04**	**04 04**	**05 06**	**02 05**	**02 02**	**01 06**	**F**
23	**H01M**	**16-Feb-98**	**09.10**	**J1 Highbridge**	**SU 467 214**	**S**	**X**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
107	**H01M**	**16-Feb-98**	**05.50**	**H4 Bambridge House**	**SU 467 223**	**J/S**	**X**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
109	**H01M**	**16-Feb-98**	**06.00**	**H5 Bambridge**	**SU 467 223**	**J/S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
133	H01M	16-Feb-98	09.15	J1 Highbridge	SU 467 214	S	X	04 04	06 06	X	02 04	01 07	M
108	**H07F**	**16-Feb-98**	**06.00**	**H5 Bambridge**	**SU 467 223**	**J/S**	**04 05**	**04 04**	**06 06**	**02 05**	**03 04**	**06 07**	**F**
147	H02F	16-Feb-98	07.27	K7 Sparsathe Grg	SU 462 192	S	X	04 04	06 06	X	02 04	01 07	F
166	H01M	10-Mar-98	08.10	B5 Carp Lake	SU 581 331	AJ	X	04 04	06 06	X	02 04	01 07	M
229	**H01M**	**10-Mar-98**	**09.55**	**C2 Western Court**	**SU 605 322**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
234	**H01M**	**10-Mar-98**	**09.55**	**C2 Western Court**	**SU 605 322**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
17	**H07F**	**10-Mar-98**	**06.15**	**H4 Bambridge House**	**SU 468 223**	**S**	**04 05**	**04 04**	**06 06**	**02 05**	**03 04**	**06 07**	**F**
230	**H01M**	**20-Apr-98**	**09.00**	**E1 Nature reserve**	**SU 547 327**	**S**	**04 05**	**04 04**	**06 06**	**02 03**	**02 04**	**01 07**	**M**
138	**H07F**	**20-Apr-98**	**09.25**	**J1 High bridge**	**SU 467 214**	**S**	**04 05**	**04 04**	**06 06**	**02 05**	**03 04**	**06 07**	**M**
152	**H08M**	**20-Apr-98**	**07.40**	**J7 Sparshafts garage**	**SU 462 192**	**S**	**04 05**	**04 04**	**06 06**	**02 05**	**02 04**	**01 07**	**M**
132	H01M/O8M	20-Apr-98	09.25	J1 High bridge	SU 467 214	S	X	X	X	02 03	02 04	01 07	M

Otter DNA Fingerprint
Results 1997 to 1998

TUBE NO	OTTER REFERENCE CODE	DATE	TIME	SITE	OS GRID	TYPE	701	715	717	832	833	902	SRY
264	H01M	18-May-98	09.15	A3 The Tower	SU 564 355	S	04 05	04 04	06 06	02 03	02 04	01 07	M
244	H03F	18-May-98	06.45	D3 Vernal farm	SU 573 310	S	04 04	04 04	05 06	03 05	02 03	06 07	F
282	H06F	18-May-98	07.00	F6 Link road bridge	SU 492 317	AJ	04 05	04 04	06 06	03 03	02 02	01 07	F
283	H06F	18-May-98	07.20	F4 Pipe bridge	SU 494 317	S	04 05	04 04	06 06	03 03	02 02	01 07	F
248	H10F	18-May-98	06.45	D3 Vernal farm	SU 573 310	S	04 05	04 04	05 06	03 05	02 03	07 07	F
285	H12F	18-May-98	09.15	F3 Fulling Mill	SU 498 321	S	04 05	04 04	06 06	02 03	02 04	07 07	F
284	H13F	18-May-98	08.25	E2 Nature reserve	SU 546 327	AJ	04 04	04 04	X	02 03	02 03	X	F
278	H9F?	19-May-98	06.30	B6 Fish farm feeder	SU 579 330	S	X	X	X	03 05	02 03	01 06	F
295	H6F?	19-May-98	08.05	F3 Fulling Mill	SU 498 321	S	04 05	X	06 06	X	02 02	X	F
197	H01M	22-Jun-98	06.40	G4 Blackbridge	SU 485 228	S	04 05	04 04	06 06	02 03	02 04	01 07	M
258	H02F	22-Jun-98	07.05	J8 Bishopstoke road	SU 466 192	S	04 05	04 04	06 06	02 05	02 04	01 07	F
304	H06F	22-Jun-98	07.40	F4 Pipe bridge	SU 494 317	AJ	04 05	04 04	06 06	03 03	02 02	01 07	F
305	H09F	22-Jun-98	08.10	E1 Nature reserve	SU 547 327	AJ/S	04 05	04 04	06 06	03 05	02 03	01 06	F
306	H09F	22-Jun-98	08.15	E2 Nature reserve	SU 546 327	S	04 05	04 04	06 06	03 05	02 03	01 06	F
316	H06F?	22-Jun-98	09.10	F3 Fulling Mill	SU 498 321	S	X	X	X	03 03	02 02	01 07	F
351	H01M	23-Jun-98	07.50	B4 Memorial gardens	SU 584 331	S	04 05	04 04	06 06	02 03	02 04	01 07	M
349	H03F	23-Jun-98	07.50	B4 Memorial gardens	SU 584 331	S	04 04	04 04	05 06	03 05	02 03	06 07	F
342	H11M	23-Jun-98	06.45	D7 Footbridge	SU 566 317	S	04 05	04 04	05 06	02 03	02 04	01 07	M
347	H03F	6-Jul-98	10.30	B6 Fish farm feeder	SU 579 330	AJ	04 04	04 04	05 06	03 05	02 03	06 07	F
314	H01M	20-Jul-98	06.25	G6 St. Cross bridge	SU 476 271	AJ	04 05	04 04	06 06	02 03	02 04	01 07	M
330	H06F	20-Jul-98	06.10	F3 Fulling Mill	SU 498 321	S	04 05	04 04	06 06	03 03	02 02	01 07	F
331	H06F	20-Jul-98	06.30	F2 M3 (north)	SU 503 235	S	04 05	04 04	06 06	03 03	02 02	01 07	F
207	H06F	21-Jul-98	06.10	G6 St. Cross bridge	SU 476 271	AJ	04 05	04 04	06 06	03 03	02 02	01 07	F

APPENDIX E: FIELD SURVEY PROTOTCOL AND HEALTH AND SAFETY RECOMMENDATIONS

APPENDIX E DNA TYPING OF OTTER SPRAINT - SAMPLING PROTOCOL EXAMPLE

1 Check site for spraint on day one, revisit on day two **AS EARLY AS POSSIBLE.** Samples should be collected as soon after day light as possible, and not after 10.00 am.

2 Each tube supplied holds approximately 9ml of ethanol (90% alcohol - see attached 'Safety Procedures for Ethanol). Use a clean knife or spoon to put about 1cm of spraint into the tube of ethanol and ensure fully tightened then shake to mix contents thoroughly. Avoid contamination with any vegetation if you can. The knife/spoon should be thoroughly cleaned between samples.

Take only one sample from each fresh spraint and put only one sample in each tube. Please take a sample from every fresh spraint found[1].

3 Fill in the data sheet for each spraint sample collected as it is collected. The form supplied has an example of the information required filled in for you. The 'time' refers to the time the spraint is put into the alcohol.

4 Bring all samples to the agreed meeting point when you have visited all your sites. If you cannot deliver your samples on the day collected please store your spraint sample in the ethanol tube in a freezer. Phone your survey coordinator to arrange a time for collection as soon as possible. Samples need to be sent for analysis within a day or so of collection.

SAFETY NOTE: THE CONTENTS OF THE TUBE IS ABSOLUTE ALCOHOL (ETHANOL) WHICH IS TOXIC IF SWALLOWED AND HIGHLY INFLAMMABLE. PLEASE READ ATTACHED SAFETY SHEET AND STORE SAFELY.

[1] This may vary depending on the resources available for analysis.

SAFETY PROCEDURES FOR ETHANOL
(FROM HEALTH AND SAFETY DATA FOR BDH PRODUCTS, 1988)

TRANSPORT CLASS 3, UN 1170, PGII

FIRST AID

EYES	Irrigate thoroughly with water for at least 10 minutes. OBTAIN MEDICAL ATTENTION.
LUNGS	Remove from exposure, rest and keep warm. In severe cases, or if exposure has been great, OBTAIN MEDICAL ATTENTION.
MOUTH	Wash out mouth thoroughly and give water to drink. OBTAIN MEDICAL ATTENTION. DO NOT INDUCE VOMITING.

FIRE-FIGHTING

Extinguishing media: Water spray, dry powder, carbon dioxide or vaporising liquids.

SPILLAGE

If local regulations permit, mop up with plenty of water and run to waste, diluting greatly with running water. Otherwise absorb on inert absorbent, transfer to container and transport to safe open area for atmospheric evaporation. Ventilate area to dispel residual vapour.

SURVEYORS NAME:

<table>
<tr><td colspan="2">SAMPLE TYPE COLLECTED
Spraint / Anal Jelly / None</td><td>SITE NAME</td><td>WATERCOURSE</td></tr>
<tr><td>TUBE NO</td><td>DATE

TIME</td><td>GRID REF</td><td>TRACKS SIZE (mm)</td></tr>
<tr><td colspan="2">SUBSTRATE UNDER SAMPLE
Rock / Gravel / Sand /Mud/ Other (describe):</td><td colspan="2">DEGREE OF EXPOSURE OF SAMPLE
e.g. Under Bridge / Trees / Open ALSO was it in Sun / Shade</td></tr>
<tr><td colspan="2">WEATHER CONDITIONS ON DAY OF SURVEY</td><td colspan="2">WEATHER CONDITIONS DURING PREVIOUS WEEK</td></tr>
<tr><td colspan="4">COMMENTS - please add any information that may be relevant to the condition of the sample collected or other signs of otter activity</td></tr>
</table>

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<tr><td colspan="2">SAMPLE TYPE COLLECTED
Spraint / Anal Jelly / None</td><td>SITE NAME</td><td>WATERCOURSE</td></tr>
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<tr><td colspan="2">SAMPLE TYPE COLLECTED
Spraint / Anal Jelly / None</td><td>SITE NAME</td><td>WATERCOURSE</td></tr>
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Rock / Gravel / Sand /Mud/ Other (describe):</td><td colspan="2">DEGREE OF EXPOSURE OF SAMPLE
e.g. Under Bridge / Trees / Open ALSO was it in Sun / Shade</td></tr>
<tr><td colspan="2">WEATHER CONDITIONS ON DAY OF SURVEY</td><td colspan="2">WEATHER CONDITIONS DURING PREVIOUS WEEK</td></tr>
<tr><td colspan="4">COMMENTS - please add any information that may be relevant to the condition of the sample collected or other signs of otter activity</td></tr>
</table>

THE USE OF DNA FINGERPRINTING TO STUDY THE POPULATION DYNAMICS OF OTTERS (*LUTRA LUTRA*) IN SOUTHERN BRITAIN: A FEASIBILITY STUDY

VOLUNTEERS HEALTH & SAFETY AND ACCESS INFORMATION PACK

ENVIRONMENT AGENCY R&D PROJECT W1 – 025

The health and safety of people, volunteers and employees alike, who are undertaking and supporting our work, is paramount to the Environment Agency. This document sets out guidance on Health and Safety issues for volunteers of this R&D project. It is in four simple sections dealing with:

1. General guidance on being near water, highlighting hazards such as Weil's disease and Blue-Green Algae when collecting otter spraint.

2. The hazards and risks of handling, storing and transporting Industrial Menthylated Spirits (IMS), the preservative in which the spraint samples are stored. The pack includes copies of the Agency's CoSHH Assessment for IMS and the IMS Safety Data Sheet.

3. An assessment of the potential hazards and risks of handling otter spraint.

4. General guidance on Access is also covered.

Reference to "volunteers" means everyone involved in collecting otter spraint as part of this R&D Project, including staff from the Hampshire and Devon Wildlife Trusts and the Environment Agency as well as local unpaid volunteers of those organisations and the Somerset Otter Group. These groups may also have their own H&S protocol for volunteers and these too must be taken into account at the local level.

The guidance presented here is based upon common sense. If you wish to obtain further information or believe that there is a health and safety problem not adequately covered by this document please contact the R&D Project Manger, Tim Sykes.

Tim Sykes, Conservation & Recreation Team Leader
Environment Agency, Hampshire & Isle of Wight Area
Colvedene Court
Wessex Business Park
Wessex Way
Colden Common
Winchester
Hampshire
SO21 1WP

Tel: 01962 713267
Fax: 01962 841573
e-mail: tim.sykes@environment-agency.gov.uk

1. GENERAL HEALTH AND SAFETY

Being near rivers, streams or any other body of water, either for work or recreation, is potentially dangerous. Working over, on or near water presents a number of potential problems, in particular the ever present risk of persons falling into water. Safety should be an integral part of any survey, conservation or management activity. Knowledge about Weil's Disease is essential.

Every effort should be made to minimise risks in the field by following common-sense behaviour such as:

- wearing a life jacket where necessary;
- avoiding steep or unstable banks;
- avoiding rivers during spate conditions;
- not entering the water if the river-bed is not visible;
- working in pairs if river channels need to be crossed;
- watching out for hazards, especially in urban rivers, such as broken glass, sharp metal or decomposing waste;
- taking care to avoid contact with the water, soil or low vegetation before eating or drinking during field work;
- wearing the right clothes for the job and weather conditions;
- carrying a basic first-aid kit;
- establishing a system of action in case of emergency;

It is the responsibility of the volunteers of this R&D Project to ensure they are properly equipped with adequate clothing for the job and weather conditions. Life-jackets/waders/first aid kits etc are not provided by the Agency. The local volunteer co-ordinators are asked to arrange appropriate emergency and lone-working procedures.

Working Alone

Whenever possible, working alone should be avoided. If it is unavoidable, the following precautions should be considered:

- If possible, carry a mobile phone and maintain regular contact with base or home. Women working alone may wish to consider carrying a rape alarm.

- Inform someone of your plans; where you are going, how long you expect to be. Try to give an indication of the time at which you expect to return.

- Do not take risks.

The local volunteer co-ordinators are asked to arrange appropriate emergency and lone-working procedures.

Working Near Deep Water

In addition to the standard safety precautions already outlined, special consideration needs to be given to deep water.

- Avoid working near deep water if you are unable to swim.

- Ensure life jackets are available for all persons required to work near deep water.

- Be aware of the likelihood of flooding, e.g. tidal river, periods of prolonged heavy rainfall, and avoid working in areas with a high risk.

- Avoid steep or unstable banks adjacent to deep water.

Blue-Green Algae

Blue - green algae are natural inhabitants of many inland waters, estuaries and the sea.

In fresh waters, they are found in suspension and attached to rocks and other surfaces at the bottom of shallow waters and along edges of lakes and rivers.

For reasons that are not fully understood, bloom and scum forming blue-green algae in fresh water, brackish water and sea water are capable of producing toxins. These toxins have caused the death of wild animals, farm livestock and domestic pets in many countries, including farm animals and dogs in the UK in 1989. In humans, rashes have occurred when blue-green algae have been swallowed.

- Minimise risks by always washing hands after direct contact with blue-green algae, water or with clothes/equipment that has been in contact with water.

For the purposes of this R&D Project, all volunteers will be issued with a copy of the Environment Agency informative leaflet on Blue-Green Algae.

Leptospirosis (Weil's Disease)

Weil's disease is the most serious form of an illness called Leptospirosis. In the UK it is most commonly associated with rats, which excrete the bacteria in their urine. The bacteria can survive

in fresh water for about four weeks and people can become infected through contact with water or muddy soil contaminated by infected rat urine. The leptospira bacteria can enter the human body through cuts, grazes and sores and mucous membranes of the eyes, nose and mouth.

Simple precautions to reduce the chances of contracting the disease are:

- Ensure cuts, scratches and skin abrasions are thoroughly cleansed and covered with a waterproof plaster.
- Avoid submerging hands or other parts of the body with cuts or abrasions in water.
- Avoid rubbing eyes, nose or mouth during work.
- Wear protective clothing where appropriate, e.g. waders and rubber gloves, and ensure these and other protective equipment are cleaned after use.
- After work and particularly before taking food or drink, wash hands thoroughly.
- For more information contact the Health and Safety Executive for a leaflet *Leptospirosis - are you at risk?*

For the purposes of this R&D Project, all volunteers will be issued with an informative Environment Agency card on Weil's Disease, and a letter which should be presented to their GP if they should have cause to seek medical attention for any illness.

Personal safety precautions which will address the risk of infection from Weil's Disease are detailed in 2 below.

2. CoSHH ASSESSMENT OF WORKING WITH IMS

Attached is the Environment Agency's CoSHH Assessment of using IMS as part of this R&D Project. Also attached is the IMS Manufacturers Safety Data Sheet. Both these papers provide simple information on:

- the hazards of using IMS in the way proposed in the R&D Project;
- the risks to users in handling, storing and transporting the samples;
- guidance on safety precautions when using the IMS;

and first aid should an accident occur.

Please ensure that you read and understand this information. If having done so you have any questions or wish to seek further information please call the R&D Project Manger, Tim Sykes on Tel: 01962 713267.

To address the risks identified above the Agency will, for the purposes of this R&D Project issue the following advice and undertake the following actions:

Handling: Everyone involved in collecting spraint samples/preserving them in test tubes full of IMS will be issued with plastic, re-usable gloves and eye-goggles which should be worn at all times when handling the IMS. Additionally each volunteer will be issued with an eye-irrigation bottle. Volunteers will also be issued with copies of this paper including CoSHH Assessment and IMS Safety Data Sheet. It is also recommended that volunteers carry with them and use packs of antiseptic handi-wipes (not provided by the Agency).

IMS is very flammable so all users are required not to smoke when handling the test tubes and to wash hands thoroughly after handling test tubes.

Storage: Volunteers will be asked to store test tubes in sealable plastic bags within Tupperware containers (not provided by the Agency) and to keep them away from sources of ignition.

Volunteer co-ordinators who will store the bulk of the test tubes at any one time must ensure that the tubes are stored in sealable plastic bags (not provided by the Agency), within sealable, hard containers - one each provided to the co-ordinators by the Agency.

Test tubes containing spraint samples should be kept cold in proper freezer facilities e.g. Environment Agency laboratories. Samples should not be stored in fridges as these are not spark proof.

Transportation: The co-ordinators will collect all test tubes containing spraint samples and send these via courier to Aberdeen University in sealable plastic bags within the sealable, hard containers. The boxes should be properly labelled as hazardous (flammable liquid) using official labels supplied by Aberdeen University and appropriate paperwork completed to standard for the benefit of the courier service.

3. ASSESSMENT OF WORKING WITH OTTER SPRAINT

Otter spraint is the faecal matter of a wild animal. Consultation with colleagues at the Environment Agency can identify no known hazard associated with handling, storing or transporting otter spraint itself, although common sense would dictate that basic personal hygiene is essential when dealing with otter spraint. However, collecting samples of otter spraint may potentially bring volunteers into contact with Weil's Disease.

For the purpose of this R&D Project volunteers are asked to use inorganic tools/equipment e.g. plastic spoon (not provided by the Agency), to collect the spraint samples in order to avoid

contamination of the otter DNA by human DNA material. Volunteers are also supplied with plastic gloves in order to address the risk of infection from Weil's Disease from water (see 2 above).

These two precautions in themselves minimise the potential risk of a volunteer ingesting or otherwise coming into direct contact with otter spraint or Weil's Disease carried on otter spraint.

4. GUIDANCE ON ACCESS

Although not always possible, every effort should be made to obtain prior permission for access to private land. Indeed, presume that unless otherwise indicated, riparian land is privately-owned.

If not obtained in advance, surveyors should always attempt to obtain permission by approaching nearby houses or farms or asking people working in nearby fields or other appropriate land.

If a surveyor is working without permission and is challenged by an owner or tenant, he or she should:

- provide proof of identity;
- apologise for not obtaining prior permission;
- describe the work in progress;
- explain exactly what the survey involves and how long it will take;
- offer the owner, or tenant an extract of the R&D Report, when available;
- leave the site without fuss if the person becomes aggressive or distressed;
- report the incident (s) to the survey co-ordinator.

Otter Project surveyors should at all times be courteous and helpful to landowners and fishermen, and must abide by the Country Code.

Produced by:

Tim Sykes
Environment Agency
as part of R&D Project W1 - 025
16th March 1998

APPENDIX F: PROPOSAL FOR FUTURE RESEARCH

APPENDIX F DNA FINGERPRINTING: A PRACTICAL APPROACH TO THE STUDY AND MANAGEMENT OF OTTER POPULATIONS

Background

Historical Perspective

The otter population of Britain underwent a serious decline over a period of approximately 20 years from the late 1950s. The initial cause of this decline was probably the introduction of cyclodiene pesticides in the mid 1950s (Chanin and Jefferies; 1978) but while other species which had been affected by these compounds started to recover, otter populations continued to decline.

The first signs of recovery were observed in the results of the Otter Survey of England 1984 - 86 (Strachan, Birks, Chanin and Jefferies; 1990) when otter populations in western England appeared to be increasing while those of eastern England were not. There has been much speculation as to the pressures on otter populations which might have caused this long lasting effect. Early authors pointed to the potential importance of disturbance and habitat destruction (eg O'Connor et al; 1977, 1979) and there has been much debate about the impact of other toxic chemicals, notably PCBs (Mason; 1989; Kruuk and Conroy 1996). More recently, Kruuk (1995) has suggested that the availability of a sufficient and suitable food supply is an important factor to consider.

The earliest conservation measures involved legal protection for the otter when, in 1978, it was added to the list of species protected under the Conservation of Wild Creatures and Wild Plants Act, 1975. Subsequently the otter was given legal protection throughout Britain under the Wildlife and Countryside Act, 1981. The first otter havens were set up by the Otter Haven Project and the Otter Trust in the late 1970s and from the 1980s, the County Wildlife Trusts undertook a series of county or catchment based projects under the general title of Rivers and Otters Projects. Much recent work has been directed, funded or supported with technical expertise by the National Rivers Authority/Environment Agency. Independently of this, the Environment Agency focused on the otter as a flagship species in its efforts to enhance the quality of freshwater and riparian habitats.

Practical conservation work on otters has focused on 'habitat improvement' by planting trees and scrub on river banks for cover, restoring ponds, lakes and wetlands, managing riparian land and habitats in a sympathetic way, reducing disturbance by various means and providing artificial dens. Despite nearly twenty years of conservation work, there is no clear evidence to show which measures have been beneficial to the species. Otter populations have recovered in some areas where little practical conservation work has been done but not in others where much has been carried out. Nevertheless, there is no doubt that the riparian environment has been greatly improved in the name of otter conservation.

Current Approaches to Otter Conservation.

In 1996 the Joint Nature Conservation Committee published a Framework for Otter Conservation in the UK for the period 1995 to 2000 (JNCC, 1996). This was subsequently used as a basis for the Biodiversity Action Plan for otters.

The UK Framework identified seven objectives for effective conservation of the otter in the UK:

- survey and monitor populations to determine the UK resource and trends;
- maintain and enhance current populations through good habitat management;
- monitor, assess and reduce (or eliminate where possible) prevalent 'threats';
- promote expansion of populations by the natural recolonisation of areas;
- implement and enforce relevant legislation and policy;
- improve knowledge of ecology and conservation through appropriate research;
- promote education and awareness of the status and needs of otters.

The Otter Biodiversity Action Plan (Otter BAP) identified two 'Objectives and Targets':

- Maintain and expand existing otter populations.
- By 2010, restore breeding otters to all catchments and coast areas where they have been recorded since 1960.

In order to achieve these, it recommended a series of proposed actions under the headings: Policy and legislation, Site safeguard and management, Species management and protection, Advisory, Future research and monitoring, Communications and publicity.

Our proposals (below) mainly address issues identified under *Future research and monitoring* but they are also directly relevant to *Site safeguard and management* and *Species management and protection.*

Requirements for Achieving the Otter BAP Targets

Otter populations have been expanding in England for the past 15 to 20 years and, so far, there is no evidence to suggest that they will not continue to do so. The requirements for achievement of the first objective are therefore:

1 Methods of monitoring populations to detect changes (whether continuing expansion, a decline in numbers or a contraction of range)
2 Maintenance of habitat quality at its current levels, in particular:
   i) food supply
   ii) water quality, including pollutants and other parameters which might affect food supply
   iii)suitable breeding sites.

Ensuring that breeding otters are restored to the areas where they have occurred since 1960 is a much more demanding target since otter hunting records indicate that although numbers of otters began to decline in the late 1950s, declines in the areas occupied by otters occurred later than this. To achieve the objective it will probably be necessary to demonstrate that otters are breeding on all major river systems in England and Wales. In order to ensure that this target is achieved (or even achievable) a number of questions must be answered in the near future. These include:

1 Can natural recolonisation achieve the objective without intervention?
   i) Will it be necessary to enhance the rate of expansion by, for example, translocation or the release of captive reared or bred animals?

2 How does the natural process of recolonisation occur?
   i) What animals are involved?
   ii) How far do they travel?
   iii)What time scales are involved?

3 Are there any factors which inhibit or prevent the natural expansion of otters?
   i) Food supply?
   ii) Pollutants?
   iii)Physical barriers to otter movements?
   iv)Availability of suitable breeding sites?
   v) Excessive disturbance or other human activity?

4 If there are inhibiting factors can these be reduced or removed?
   i) What are the minimum requirements for otters to breed?
   ii) How should limited resources be used most effectively?

5 How can we tell that a population is not expanding or capable of expansion?
   i) Are there demographic or behavioural characteristics of populations which are not expanding or capable of expansion, which can be used to identify areas where conservation management may need to be undertaken?

The difficulty of answering many of these questions is that we have very little knowledge of the habits of otters in southern Britain and no baseline data from established populations against which to measure differences in recolonising areas. In particular while it is fairly easy to obtain firm evidence of the presence of otters, we have no firm basis for discriminating between an established breeding population and one in which otters are present but are not resident and/or not breeding. The proposed project will provide direct answers to some of the questions, provide data which may be used to answer others and to enable comparisons to be made between otter populations in different circumstances.

A Pilot Project Using DNA Fingerprinting

In 1997 the Environment Agency funded a one year collaborative project to carry out a feasibility study for investigation of the Otter Recovery in Southern Britain (Environment Agency R&D Project W1-025: The Use of DNA Fingerprinting to Study the Population Dynamics of Otters (*Lutra lutra*) in Southern Britain: A Feasibility Study). Agency staff and biologists from Exeter and Aberdeen Universities initiated the project and worked with groups of volunteers from the Hampshire, Somerset and Devon Wildlife Trusts in an effort to determine whether the use of DNA fingerprinting on otter spraints could be successfully used as a field technique to monitor otter populations.

In addition, an investigation of the natural genetic variation of otters in Southern England was carried out at the University of Aberdeen using DNA from the tissues of otters which had been killed on the roads.

Results of the field study:

The field study has been an undoubted success. A brief summary of achievements includes:

- Mobilisation of over 50 volunteers on four river catchments in Devon, Somerset and Hampshire.
- Collection of over 600 fresh spraints for DNA fingerprinting
- Identification of 57 different otter fingerprints on these rivers including one which was recorded 23 times over a period of 19 months.
- 20% of samples analysed were successfully typed, ranging from 16 - 43% per month
- Considerable press coverage promoting the cause of otter and wetland conservation.

A number of interesting points emerge from the data collected during this study. For example:

1. The density of otters using relatively small rivers was higher than expected. The Itchen is only 40 km long with no large tributaries and yet at least 13 otters were recorded there over a period of 18 months. In their *Review of British Mammals* Harris et al.(1995) used an estimate of the density of otters in England of one adult per 27 km (1 per 24 km in the 'high density' area of Scotland), considerably lower than that observed on the Itchen. Our data show that 4 animals (one male and three females) were resident on the Itchen during the period September 1997 to June 1998 and all would have been adult by the end of this period.

2. It is notable that many otters were only recorded once or for very short periods during the study while others were found on the same river over periods of several months. Sixty percent of the otters identified up to April 1998 were only recorded during one month's sampling. This suggests that there may be a substantial proportion of non-residents in some populations (such as has been found, for example, in mink).

   This may have significant implications for otter conservation since catchments with resident otters and a high proportion of transients could be important sources for recolonisation. On the other hand the presence of few or no resident animals but a high proportion of transients might indicate poor habitat. These differences could be

discriminated by DNA fingerprinting while spraint surveys might suggest that both had 'good populations'.

3. The sex ratio of otters overall is close to unity (31 males and 26 females) but on the river Brue, which is considered to be a recolonisation zone, there were 8 males and only 3 females.

Further studies are needed to determine whether differences in degrees of residency (or turnover) and sex ratio can be used to characterise recolonising populations compared to those in established areas or as indicators of habitat quality. The feasibility study has clearly demonstrated that these and other important parameters can be readily obtained from DNA data. We have some preliminary data on population structure, home ranges and movements but not enough to make any comparisons between areas, or show whether home ranges in lowland areas differ in size from those reported from the Shetland Isles and the Highlands of Scotland.

A notable feature of this project was the successful involvement of a considerable number of volunteers, mainly from the County Wildlife Trusts, who worked closely with professional biologists to collect samples for analysis and made a substantial contribution to a project which could not have succeeded without them.

Genetic variability of otters in Southern Britain
The study of road casualties showed that there was considerably less genetic variability in Southwest England than in Scotland. It was concluded that 'the majority of DNA profiles of otters in SW England will be individual-specific provided that all six of the loci used for spraint DNA typing are assayed'. However these samples came from a wide area and therefore probably involved very few close relatives, difficulties might occur when studying small populations with close relatives present.

Limitations of the technique:
The problems associated with small populations with low genetic diversity were clearly demonstrated when it was found that two different otters on the River Itchen had the same genetic profiles. Although the Itchen population is thought to be much less genetically diverse than other populations following the release of a very small number of captive reared animals, more confidence in the ability to discriminate between individuals is needed and this requires the identifying of further loci for typing.

It would also be very beneficial to increase the proportion of spraints which can be successfully typed in order to maximise the rate of return in terms of volunteers' time. In preliminary trials in Scotland a success rate of 1 in 7 was achieved (Hans Kruuk, pers. comm.) and in the feasibility study this had been increased to 1 in 5 on average though with considerable variation about this figure. A further increase in the success rate would considerably enhance the value of the technique.

General Aims of Proposed Project

- To develop the technique of DNA fingerprinting otter spraint so that it can become a standard tool for monitoring and studying otter populations.
- To extend the successful pilot project into a further three year study in order to provide information required to ensure that the Otter BAP Objectives are achieved, particularly in terms of successful monitoring and management of otter populations.
- To improve our understanding of otter ecology and provide data for modelling and/or a PHVA (Population and Habitat Viability Analysis) in order to assess the likelihood of the BAP objectives being achieved by natural recolonisation.

- To work closely with other projects where DNA fingerprinting would contribute significantly to the management and conservation of otter populations.

Specific Objectives and how they will be Achieved

Note: the bracketed references (BAP: n.n.n) following each objective below refer to the relevant paragraph(s) in the Biodiversity Action Plan for Otters (Appendix A).

1. Development of the technique

Objectives:

- To improve the reliability of the technique by increasing the number of genetic loci which can be fingerprinted, thereby reducing the risk of misidentifying otters.
- To improve techniques for collecting and extracting otter DNA from spraints in order to simplify field collection, reduce the costs of extraction and storage and, if possible, increase the number of spraints which can be successfully typed.

a) Reliability

Of nine loci available for typing otter corpses only six were suitable for spraint analysis in the Southwest and it was concluded that under these circumstances problems of misidentification might occur in populations containing more than 50 individuals. However, in spraint samples from the Itchen and the Torridge the variability was considerably less than this with one locus being monomorphic in each case. The variability recorded from Somerset was similar to that found in the corpses. In order to improve the reliability of the technique in areas other than Somerset, more polymorphic loci are needed.

Preliminary studies at the University of Exeter have demonstrated that new loci can be detected using other microsatellite sequences and a small number of candidate loci have already been identified. Further studies are required to increase the number of polymorphic loci to a level at which we can be confident that the probability of two otters having the same profile will be very small.

If sufficient polymorphic loci can be identified, it may be possible to estimate differences in relatedness in some otter populations, though recognition of first order relatives requires very large numbers of loci when the variability at each locus is low (as in otters from the Southwest).

Target:

J. Dallas (unpubl.) has calculated that at the levels of variability shown in south western populations, a total of 15 loci would be required to achieve the same confidence in identification of individuals as is currently possible in mainland Scottish populations. On this basis increasing the number of polymorphic loci to 15 is proposed as a target for the project.

b) Collection, Extraction and Storage

A major problem in using spraint samples for fingerprinting is the large amount of faecal material from which otter DNA has to be extracted and the wide range of contaminating and potentially destructive materials and organisms within it. Fresh spraint samples have to be placed in absolute alcohol and stored at extremely low temperatures to reduce the rate of degradation of otter DNA. Extraction is extremely time consuming and expensive and spraints have to be stored in expensive and specialised equipment.

A novel approach to sampling has been developed at Exeter by which means otter cells are separated from faecal material at the time of collection. Initial trials reveal that otter DNA can be successfully recovered and suggest that long term storage conditions may be much less demanding. The technique needs to be developed further and fully field tested but offers a

potential route to making the collecting of samples a much simpler and easier procedure and to considerably reducing the cost of extraction and storage.

Target:
The successful extraction of DNA from one third of very fresh spraint is proposed as a target.

2. Monitoring and Modelling

Objectives:

- To determine whether a relationship exists between the standard otter survey method (Strachan et al 1990) and the results of DNA fingerprinting (monitoring at the national, catchment or county level). (BAP: 5.5.4; 5.5.6)
- To devise a protocol for using DNA fingerprinting as a means of assessing the size and nature of local otter populations (monitoring at the local level). (BAP: 5.5.6)
- To provide information on otter movements, home ranges, population structure for modelling populations. (BAP: 5.5.4)

a) Standard Survey Method
The standard otter survey technique has been in use for over 20 years in Britain and has been widely used abroad. It evolved from recommendations made by Dr Sam Erlinge for the monitoring of otters by the Mammal Society during the 1970s. It is based on the principle of surveying (usually) 600m of stream every 5-8km and is thus a sample survey covering ca 10% of riparian habitat. It can be used to survey an area such as a catchment, a county, a country or an arbitrary unit such as an Ordnance Survey grid square. The data recorded are the presence or absence of signs (particularly spraints) and in some cases the density of such signs.

There has been considerable argument about the interpretation of such surveys. While it is generally accepted that they can demonstrate gross patterns of distribution there is some doubt as to whether the density of positive sites or density of spraints within a site can be useful as a means of extrapolation to the density of otters in a population (Mason and Macdonald, 1986; Conroy and French, 1987; Strachan and Jefferies, 1996). Kruuk et al. (1986) were unable to demonstrate any relationship between the density of otter spraint and the numbers of otters seen in parts of Shetland. They, and others, have also shown that there are seasonal variations in the intensity of spraint marking which could not be related to changes in otter density. There have been no other attempts to relate the results of spraint surveys to otter populations, simply because there are no places where the otter population is sufficiently well known.

Method:
Otter surveys using the standard method will be carried out in parallel with DNA fingerprinting to determine whether there is a relationship between the two survey techniques. Surveys will be repeated at intervals during the project to determine whether any relationship is stable over a period of time and between seasons.

b) Use of Fingerprinting to Assess Populations on a Local Scale
While otter surveys for signs can provide information on the presence or probable absence of otters at the local scale (river or small catchment) they do not provide information on the size, structure or density of a population. Such demographic information is essential for guiding conservation management and assessing the impact of major development such as road construction or river diversion. DNA fingerprinting can undoubtedly help to establish demographic parameters but more work is needed to determine sampling protocols including frequency, intensity and minimum period of sampling and the best season.

Method:
The data obtained from the project will be used as a basis for devising appropriate protocols for sampling intensity, frequency and timing.

c) Use of Fingerprinting in Modelling Populations
In order to answer the question as to whether natural recolonisation can result in otters recolonising areas occupied in the 1960s, it will be necessary to carry out population modelling and this is not possible without knowledge of a number of demographic and social parameters. The following parameters will be obtained from this study:

- Minimum numbers of otters present
- Proportion resident
- Turnover of residents
- Home range
- Territoriality (inferred from home range distribution)
- Movements of non-residents
- Sex differences in the above parameters
- Sex ratio
- Differences between areas where otters are established and recolonising

In addition, data on breeding will be obtained by combining sightings and field signs (small footprints etc.) with genetic information from DNA sampling.

3. Factors Affecting Recolonisation: Habitat Quality and Conservation Management

Objectives:
- To assess the relationship between habitat quality (including food supply) and otter populations (BAP: 5.2.1, 5.2.2, 5.5.1)
- To assess the impact of disturbance on otter populations (BAP: 5.5.3)
- To assess the impact of riparian management (BAP: 5.2.1, 5.2.2)

a) Habitat Quality
Efforts to assess the impact of habitat quality on otter populations have two central problems, how to define quality and how to measure its impact. Kruuk has pointed out that there is a significant difference between habitat *preferences* and habitat *requirements* (he *prefers* malt whisky to blended but does not *require* either). Attempts to use spraint distribution to determine habitat use are fraught with difficulties. Radio-tracking of otters is very difficult and usually only involves very small numbers of animals.

In recent years it has become clear that otters will use waterways which would once have seemed inimical to their well being (otters are regularly found within large cities in the UK and breed within oil terminals in Shetland) . There is still however a widely held view that female otters, particularly when breeding, might have more stringent habitat requirements than males. This concerns both food supply, since lactating females face very high metabolic demands, and the availability of suitable den sites which many people believe should be in some form of cover and free from disturbance. We therefore predict that if there are significant effects of habitat quality on otter populations this will be manifest most clearly in differences in the behaviour between male and female otters.

A further potential indicator of habitat quality is the rate of turnover of otters. In good quality habitat one would predict that resident otters would be found throughout a catchment whereas in poorer quality habitat a much higher turnover of animals would be expected, with few or no resident animals or short residence times.

Method:
DNA fingerprinting makes it possible to determine the locations of home ranges of a number of animals, to determine their sex and to determine the proportions of resident and transient animals using a catchment.. The Environment Agency and WRc have considerable amounts of information on a number of parameters relating to habitat quality, particularly fish stocks and water quality and these will be collated in a desk study. Field data on other parameters (cover, potential dens sites, conservation management activities) will also be collected. Field signs will be used to provide evidence of breeding. Data from these sources will be collated to determine whether there are associations between the various measures of habitat quality and otter behaviour.

In the pilot project, some intriguing differences in sex ratio, residency and the proportion of transients between areas were observed but there is insufficient data as yet to demonstrate statistically significant differences or to establish baseline data on these parameters and further work is needed.

b) Disturbance
A similar approach will be taken to assessing the impact of disturbance. If otters are found to be using sites with high levels of disturbance the sex ratio of animals doing so will be used to determine whether or not there are differences in behaviour between males and females.

To a certain extent the sites available to test this will be dependent on the areas available within our chosen study areas (one of which includes the City of Winchester and another the town of Taunton). We will however attempt to select a small number of sites which will be greatly disturbed during the period of the project in order to assess use before, during, and if possible after major disturbance events.

c) Riparian Management
In addition to recording general features of riparian habitat and management we will seek to carry out comparative studies in areas where conservation management has been undertaken with a view to assessing its impact on otters. This is possible in the Hampshire and the Somerset study areas, currently being used.

4. Relationships with Other Projects

Objective:
To collaborate with other projects on otters in Britain where information on otter identity and genetics would be of value, including:

- the release of otters from captivity
- proposed studies on breeding
- the collection of road casualties and other otter corpses.

a) Release of Captive Otters
While there is a general antipathy to releasing captive bred animals in areas where otters are well established or colonising, it is possible that this may occur, possibly under some form of regulation (BAP: 5.3.2) or that 'rescue' otters may be released back into the wild. It is essential that where this happens the genetic identities of the animals should be known and ideally the animals should be permanently marked. We would encourage responsible organisations in doing this by carrying out DNA fingerprinting on samples collected from animals before release, by advising on the collection of spraint samples as part of a release programme and by typing spraints collected. We have already advised staff from the Department of Zoology at Oxford University who are monitoring the release of otters on the upper Thames catchment.

b) Otter Breeding

As part of a project to safeguard Natura 2000 rivers in the UK, a study of otter breeding sites on the rivers Teifi, Wye and Camel has been proposed. This will be used as a basis for the enhancement of a site on the river Wye to determine whether habitat modification can lead to successful breeding. The project would be greatly facilitated by the ability to determine the social context of otters near the site by using DNA fingerprinting to confirm the presence of resident males and females. In addition, the high level of observation that will be undertaken as part of this project would make it possible to determine the stage at which young otters can first be confirmed as part of the 'identifiable' population, a useful parameter for DNA studies. Furthermore data from our own study sites and breeding records will be made available to the Natura 2000 project in order to maximise the dataset on which decisions will be made.

We are keen to collaborate in the proposed project by carrying out DNA fingerprinting of spraint collected in the project and providing supplementary data on breeding. The co-ordinator for the Wye project is also keen to collaborate and we understand that confirmation of funding for this project (from the European Community LIFE Program) is due in July 1999. (BAP: 5.5.4)

c) Road Casualties

Preliminary studies of the genetics of otter corpses collected in southern Britain prior to 1998 have already been undertaken by John Dallas. We would offer the facility to type any future otter casualties and, if appropriate, will co-ordinate their collection and dispatch for *post mortem* analysis and ensure that data are disseminated as necessary and summarised at intervals. (BAP: 5.1.3, 5.5.1, 5.5.6)

Approach

Overview

We propose to extend the programme for a further three years using the same basic approach as in the pilot study but the work will be co-ordinated by Exeter University. John Dallas has provided information on the extraction and fingerprinting techniques and this work will be undertaken in the laboratory of Professor John Bryant. A laboratory research assistant and technician will be employed to carry out analyses and assist in the development of the field and laboratory techniques. The field work will be overseen by a full time field Research Assistant, also based in the School of Biological Sciences at Exeter University, under the supervision of Dr Paul Chanin. The network of volunteers will be maintained and extended and it will be part of the research assistant's responsibility to recruit, train and co-ordinate the efforts of volunteers.

The existing study sites will be maintained if possible to enable us to follow the otter populations in them over a period of at least four years but we hope to recruit and train more volunteers to work on the Torridge and set up additional study sites, one in the recolonising area, either in Dorset or Somerset and another, possibly, on the River Camel in Cornwall. In addition a number of sites may be selected to specifically test hypotheses about the effects of riparian management and disturbance.

We have collected and analysed ca 600 spraints during the pilot project yielding 120 identifications. By employing full time staff we plan to increase the annual throughput to 2,000 spraints per annum. Assuming 33% of samples are successfully typed, this would yield ca 2000 otter identifications.

Roles of the Research Assistants

Laboratory Staff
Based in the School of Biological Sciences at Exeter University and supervised by Professors John Bryant and Steven Hughes.

Tasks:
- to assist Professors Bryant and Hughes in developing the technique for extracting and typing otter DNA
- to extract and type up to 2,000 samples per annum
- to extract and type otter tissues provided from road casualties, otters to be released etc.

Field Research Assistant
Based in the School of Biological Sciences at Exeter University and supervised by Paul Chanin.

Tasks:
- Co-ordinate current volunteers
- Recruit and train new volunteers
- Carry out spraint collections
- Monitor incoming data and feed back to field workers and supervisors
- Produce quarterly, annual and final reports to the project board
- Carry out 'Standard' otter surveys
- Collate additional data from field and from desk study on:
  - Habitat
  - Riparian Management
  - Food supply
  - Water quality
  - Pollutants
- Liaison with other projects on otters where DNA fingerprinting is being used:
  - proposed study of otter breeding in the Welsh Borders
  - any releases of otters
- Liaison with organisations (Wildlife Trusts for example) carrying out otter conservation work within or close to the study areas.
- Ensure that otter road casualties are collected, samples obtained for DNA typing and corpses are sent for *post mortem* analysis.

Timing
A scheme has been put into place to ensure that sampling continues in the period between the ending of the pilot project and the end of March. Efforts will be made to continue this until the start of the three year project. In order to maintain the interest of the volunteer groups, it is important to ensure continuity and an early start to the project is essential.

Cost

We estimate that the project will cost between £250,000 and £300,000 over a period of three years, depending on the sources and nature of funding.

Principal Personnel

Professor John Bryant is a Plant Molecular Biologist in the School of Biological Sciences at the University of Exeter. His research interests include DNA replication in plants, gene activity during the development of plants and animals and the cloning and expression of genes

for biotechnology. The author of over 50 scientific papers, author/editor of eight books and on the editorial boards of five journals he is also firmly committed to the Public Understanding of Science. To this end, he regularly gives talks to schools and societies and appears on television and the radio. He is the Chairman and Executive Director of Biotechnology SouthWest.

Dr Paul Chanin is a part time Lecturer at the University of Exeter and a Consultant Mammal Ecologist. He is internationally known for his work on otters and has been involved in research and conservation of otters for over 25 years. He was a member of the Joint Otter Group and co-author of its two reports; supervised the first two Otter Surveys of England; discovered the cause of the otters decline; was a principal investigator for the pilot project and has written two books on otters. He has supervised research on a range of other mammal species including badgers, pine marten, deer and spider monkeys.

Professor Steven Hughes joined the School of Biological Sciences in 1997 as Professorial Research Fellow working in applied molecular biology. His early career at Edinburgh University was in microbial genetics and the development of methods for the analysis and manipulation of DNA (subsequently known as genetic engineering). He then turned to plant genetics and plant molecular biology and more recently became closely engaged with the plant breeding industry (PBI-Cambridge) and the application of modern genetic techniques as tools of breeding strategy and progeny selection. Through this he developed familiarity with a range of genomic fingerprinting techniques.

Project Management

A Project Board consisting of the Academic Supervisors with representatives from key partners and collaborating volunteer groups will meet twice a year to receive reports and review progress. The board will report annually to the autumn meeting of the Environment Agency's Conservation Technical Group.

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