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

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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

Karen Coxon, Paul Chanin, John Dallas and Tim Sykes

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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.

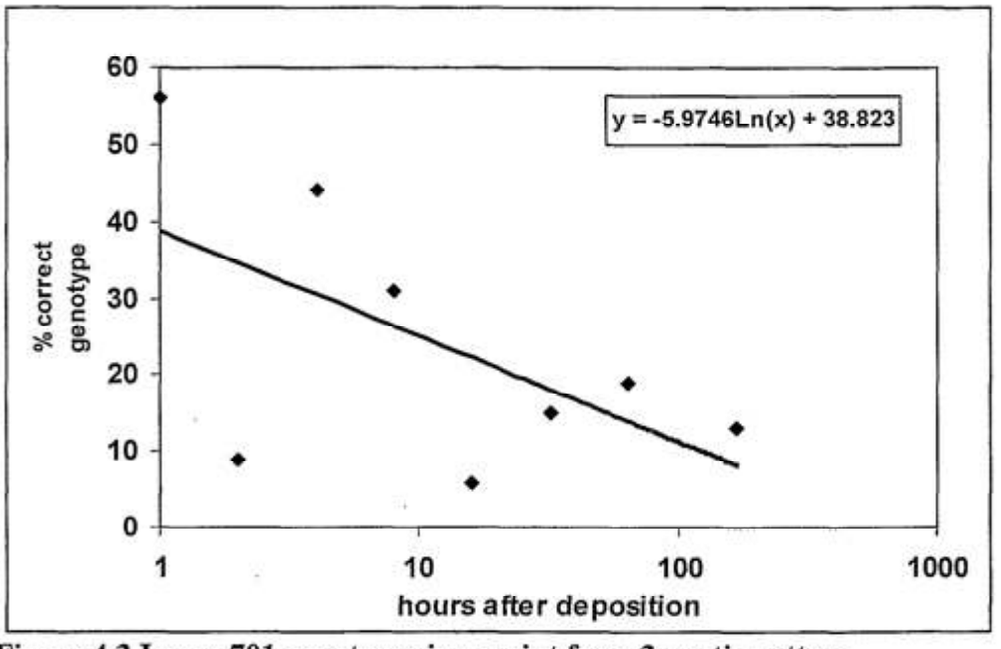


Figure 4.3 Locus 701 genotypes in spraint from 3 captive otters.

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.

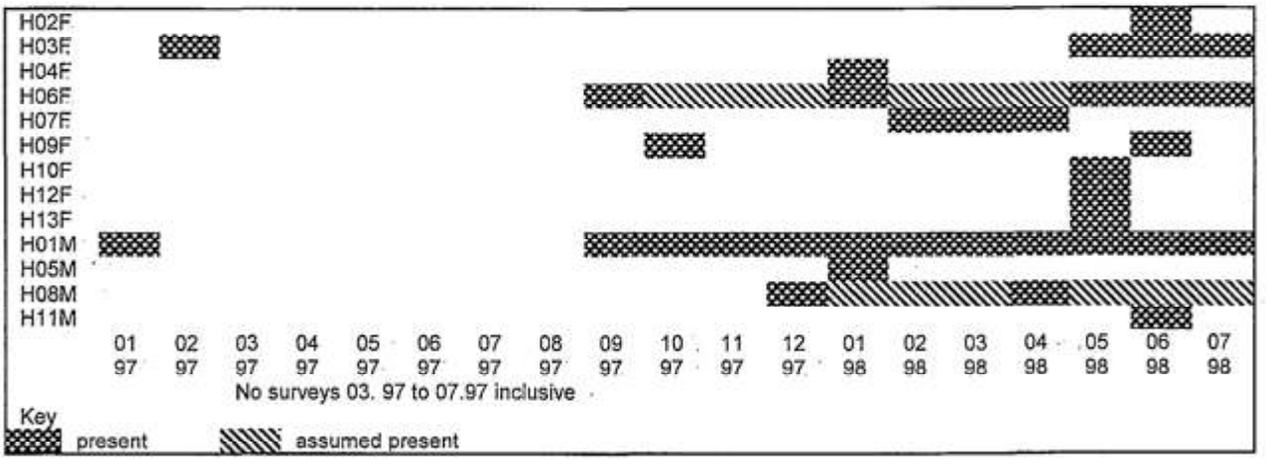
5.5 The Itchen Catchment

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



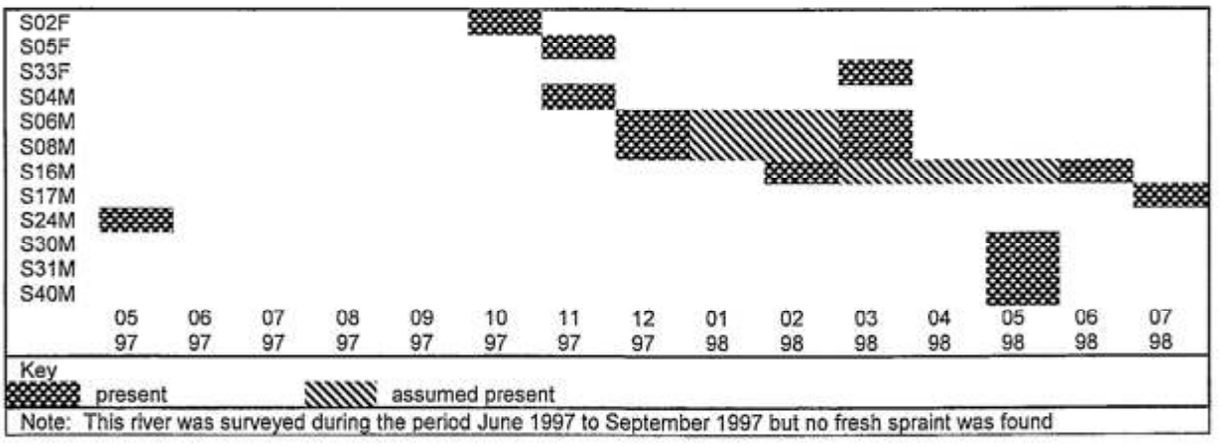
5.6 The Brue Catchment

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

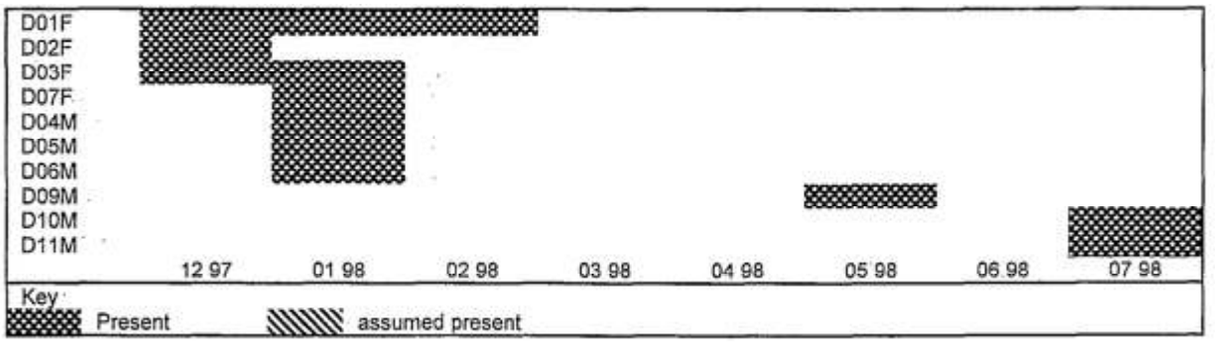


A very high proportion of the sites yielded fresh spraint compared to the other watercourses (66% compared to 13%, 28% and 47% respectively for the Itchen, Brue and Tone). All sites showed evidence of otter activity. The catch per unit effort for the Torridge was 0.1. Tables 5.5 and 5.6 list the results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

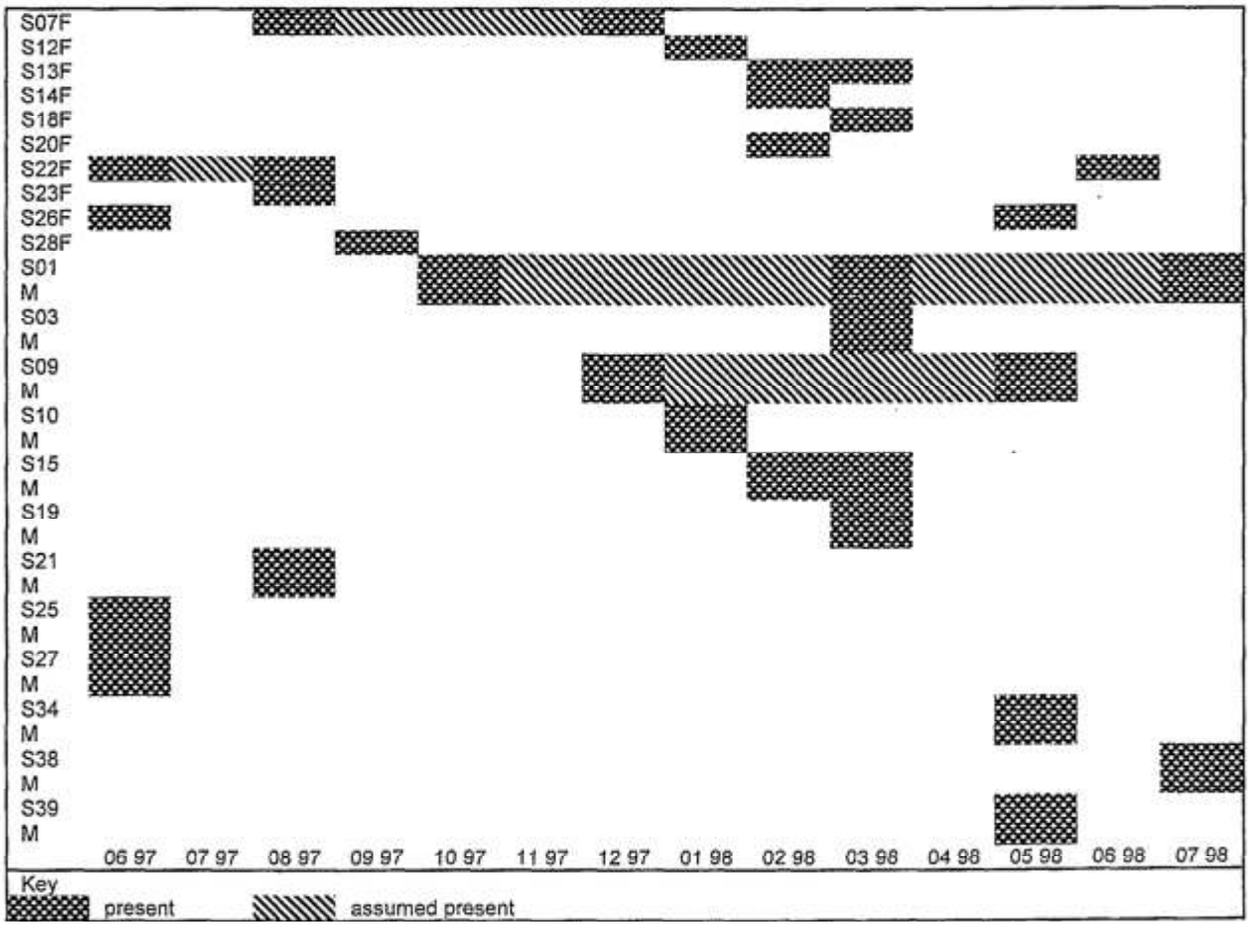
a) Females

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

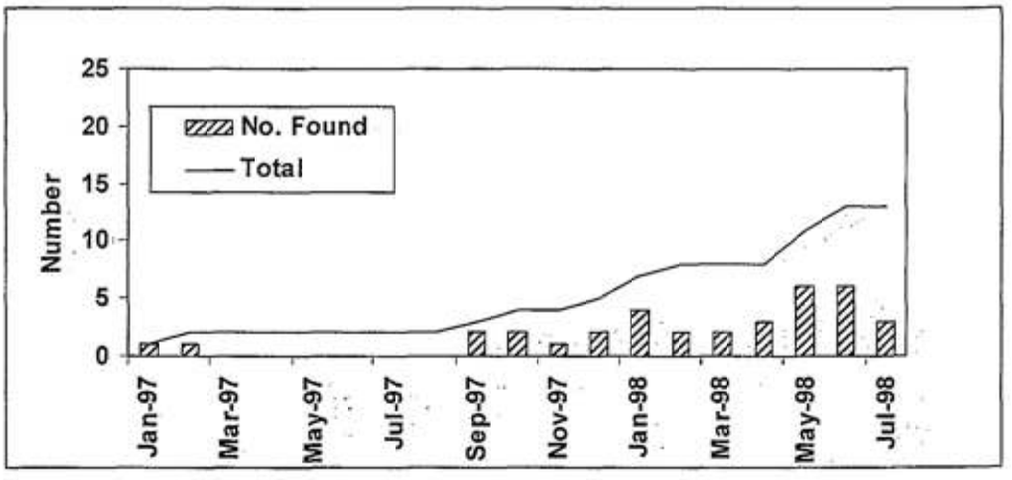


Figure 5.1 Itchen Catchment – Number of Otters Found and Total Identified

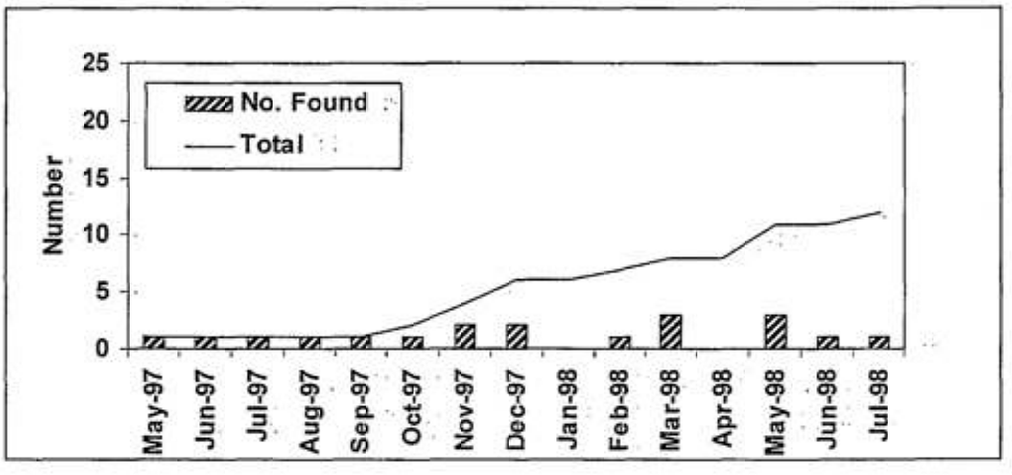


Figure 5.2 Brue Catchment – Number of Otters Found and Total Identified

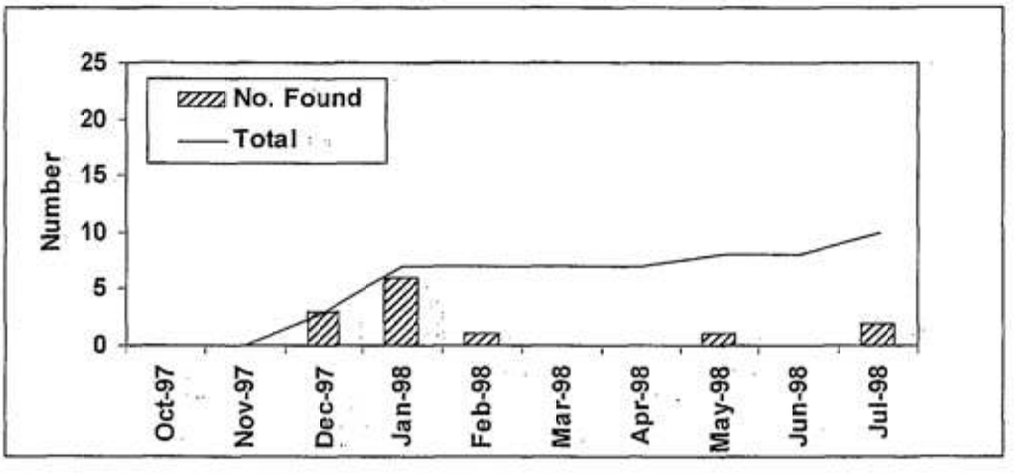


Figure 5.3 Torridge Catchment – Number of Otters Found and Total Identified

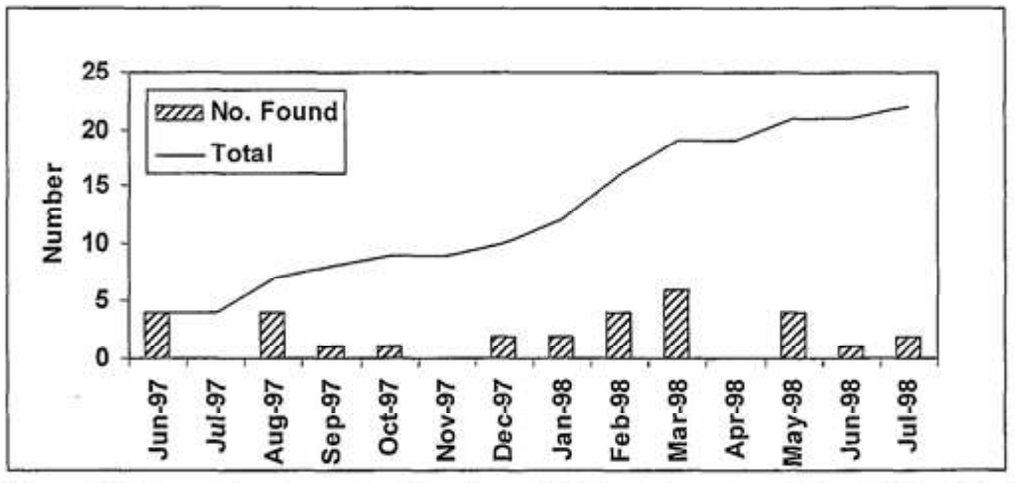


Figure 5.4 Tone Catchment – Number of Otters Found and Total Identified

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).

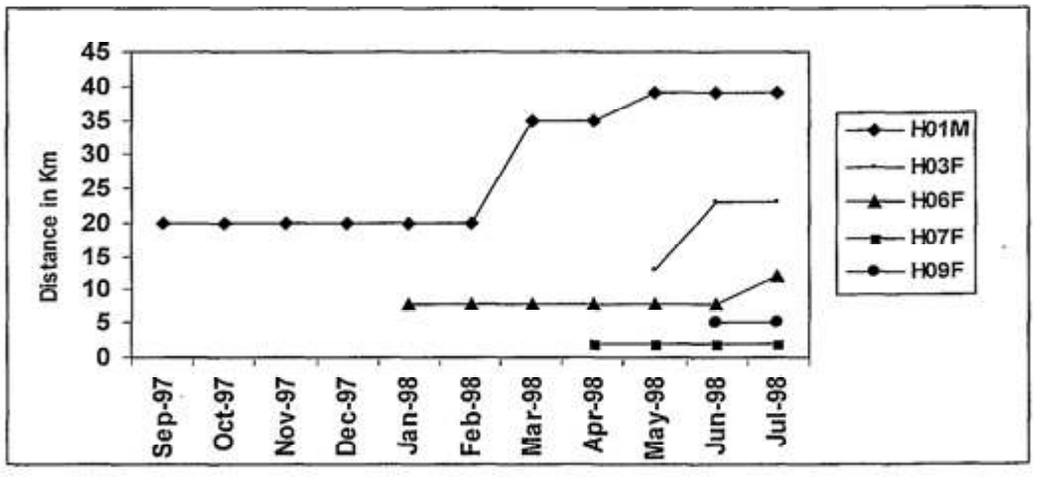


Figure 5.5 River Itchen – Known Home Ranges for Resident Otters

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.