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

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.

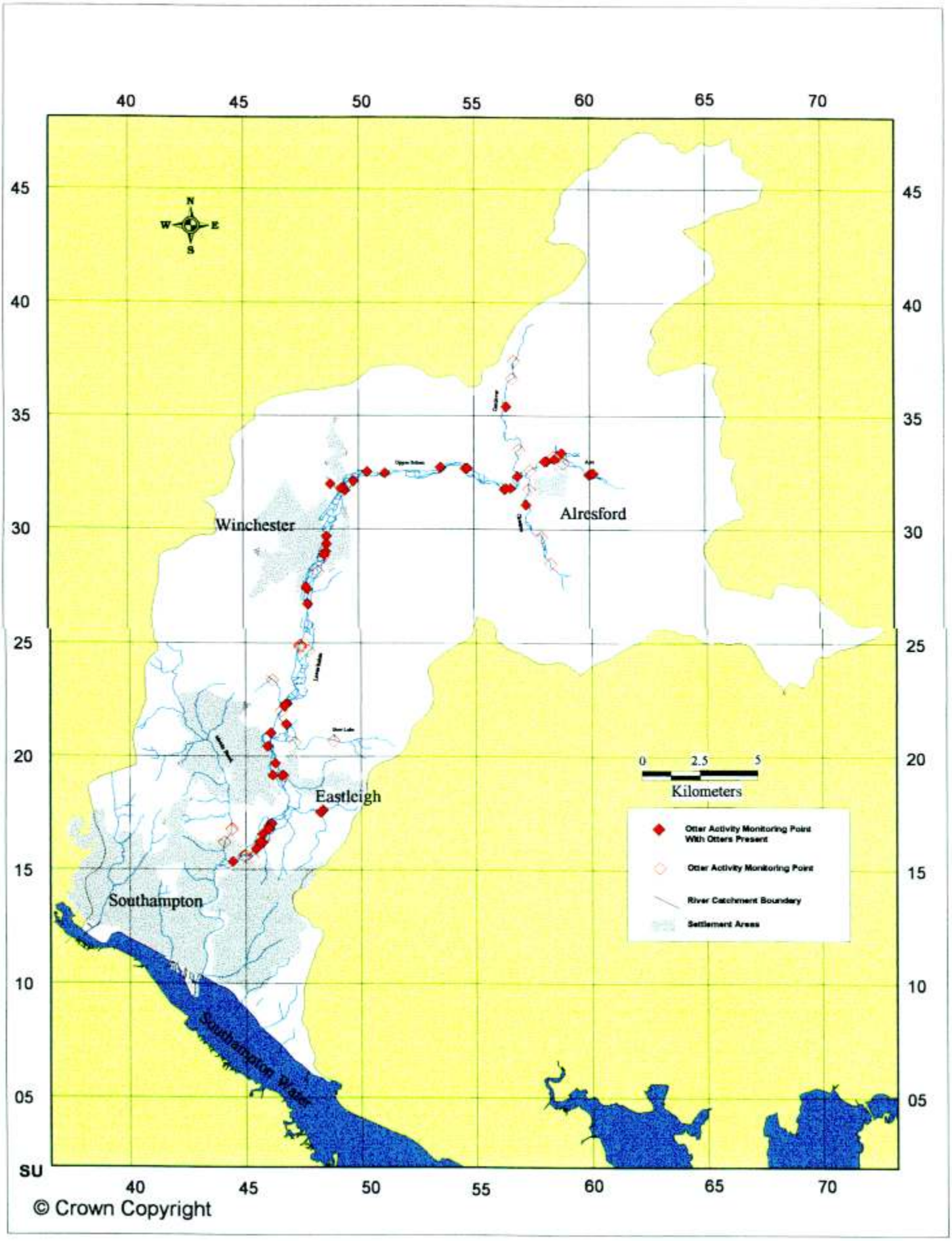
Three Bridges Stream flows north from Luxhay Reservoir, under the M5 to the Tone at its confluence with Hillfarrance Brook. Two otters have been found on this watercourse, S13F and S09M. S09M has been identified twice from spraint collected at Stoford, in December 1997 and May 1998. In February 1998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

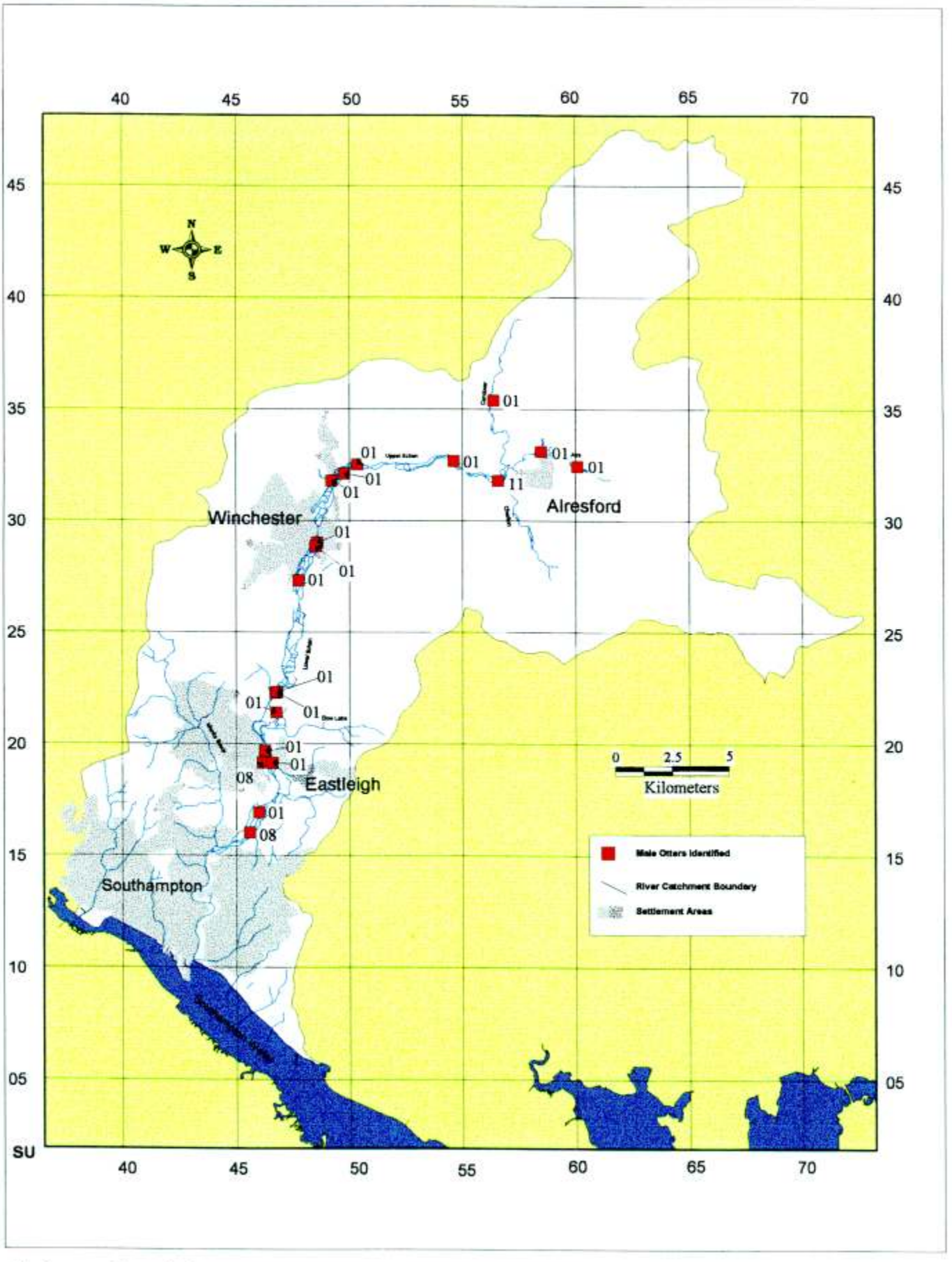
No individual DNA profiles/otters have been recorded on more than one tributary.

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.

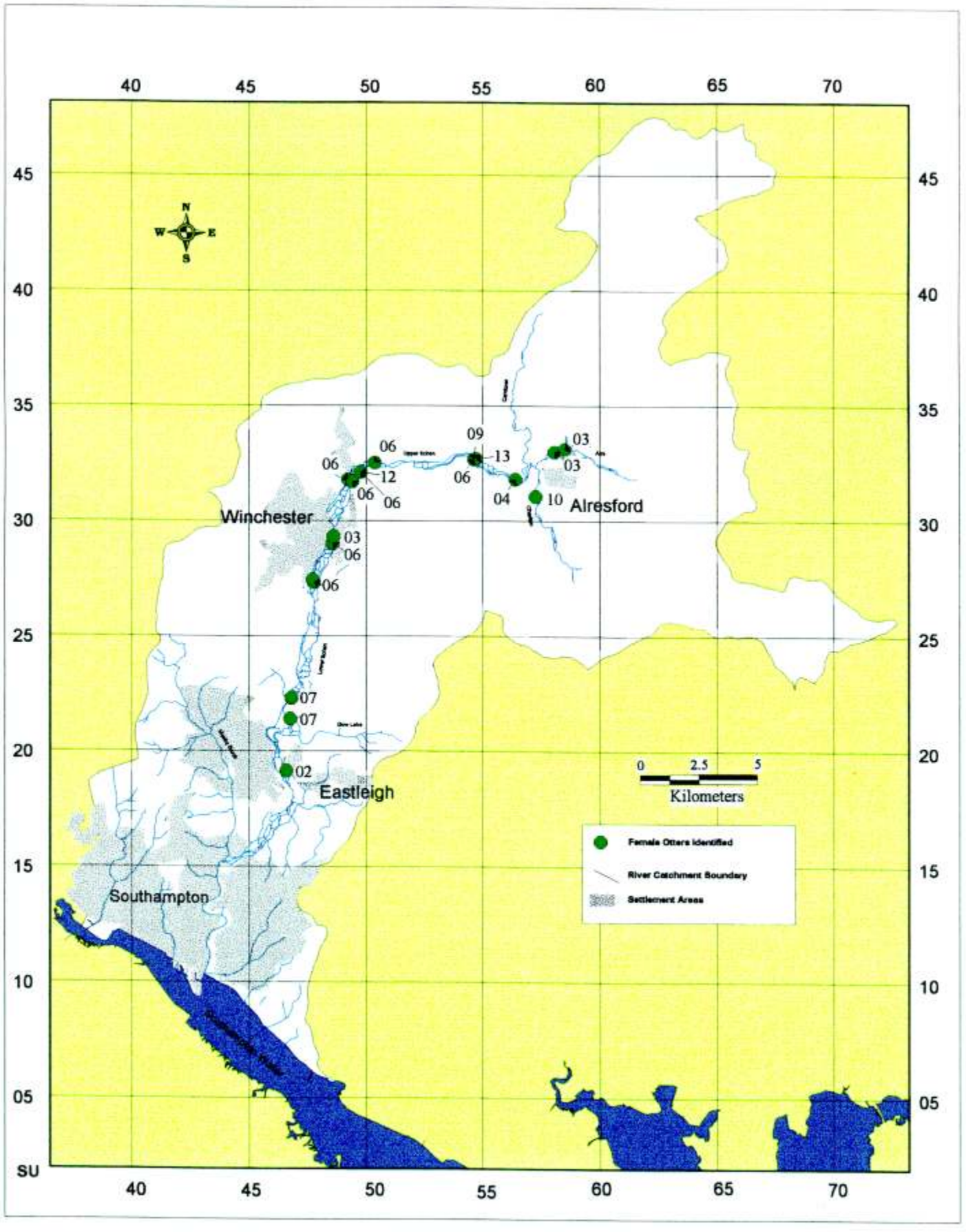
Map D1 - River Itchen Catchment: Monitoring Sites



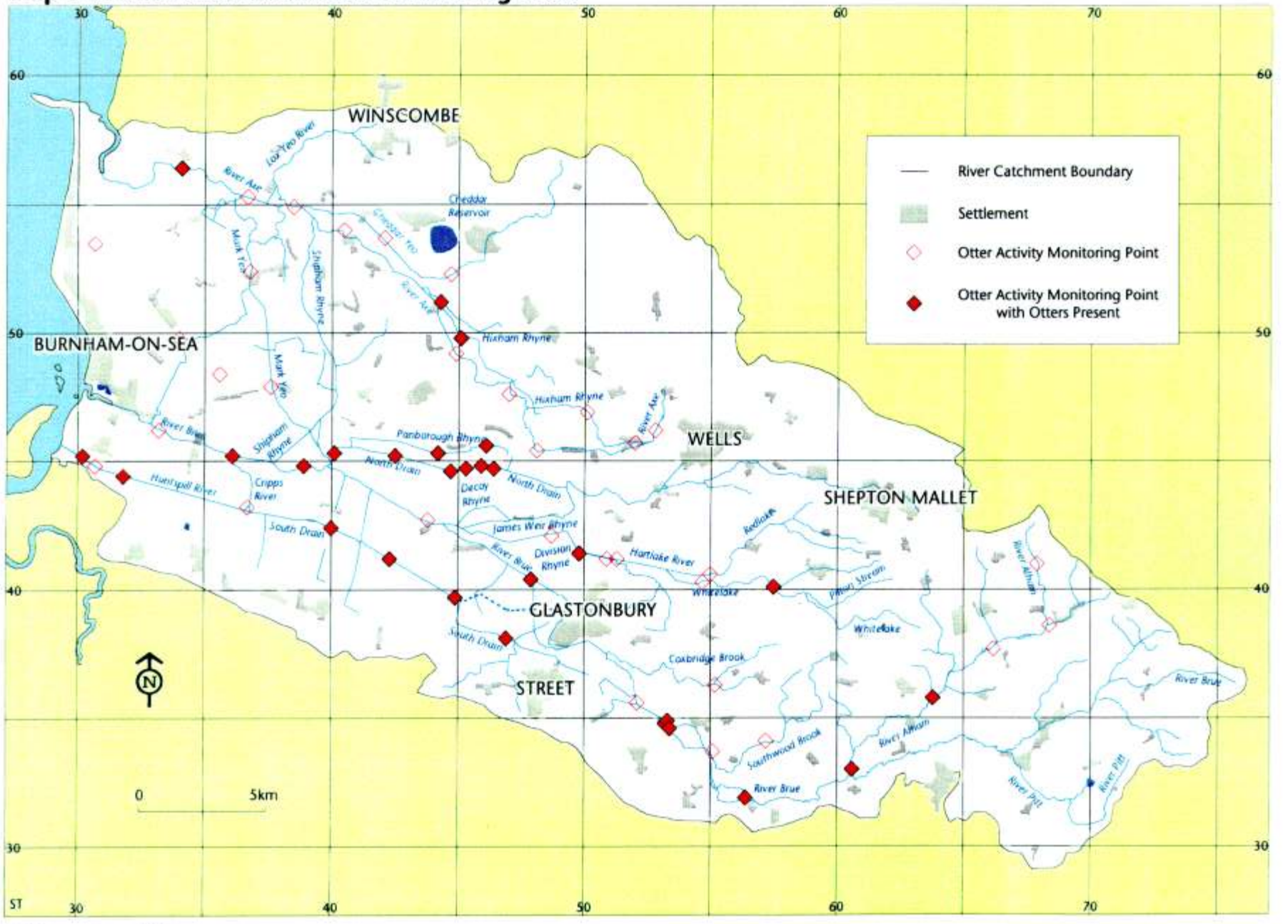
Map D2 - River Itchen Catchment: Male Otters Identified



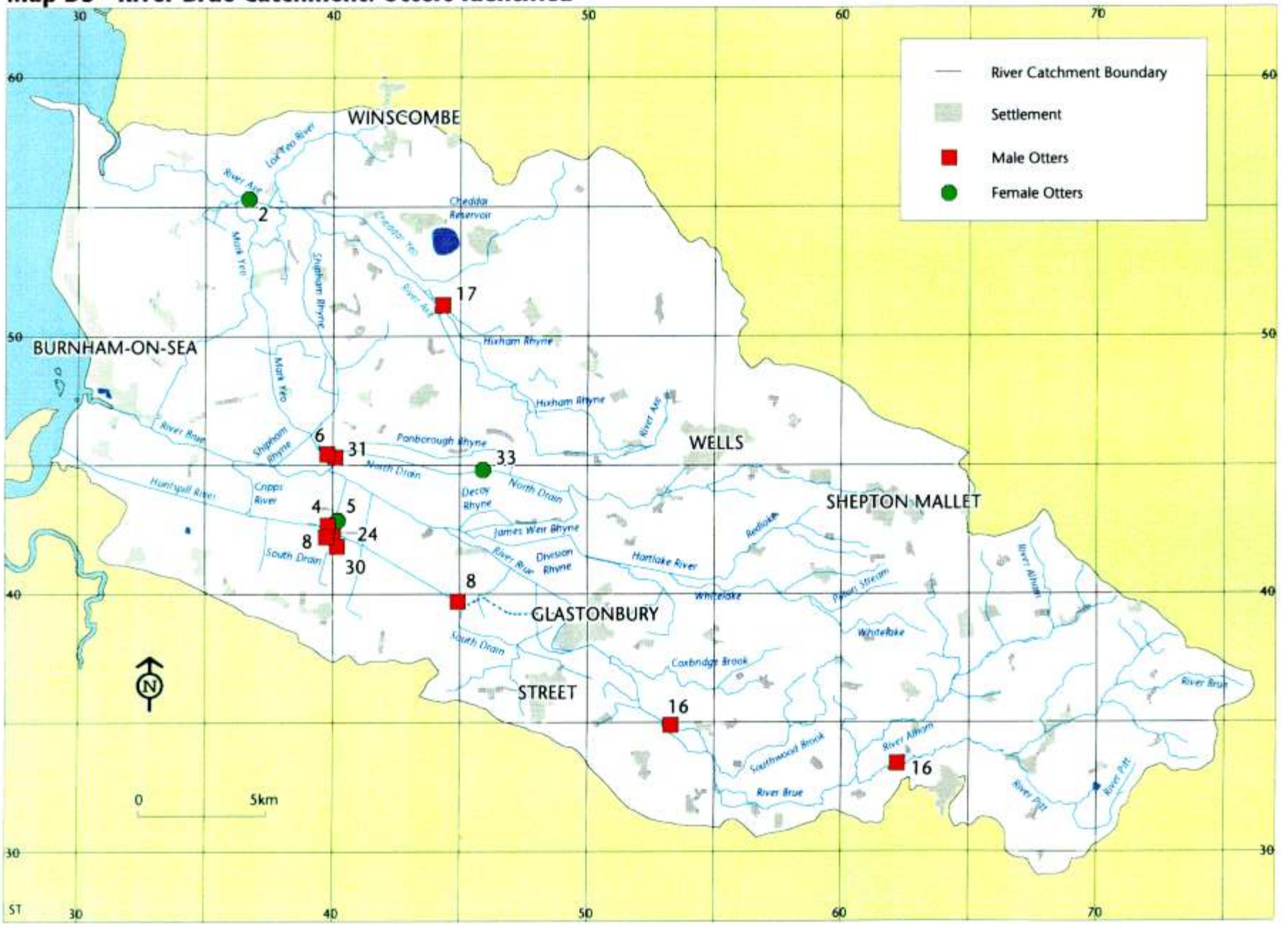
Map D3 - River Itchen Catchment: Female Otters Identified



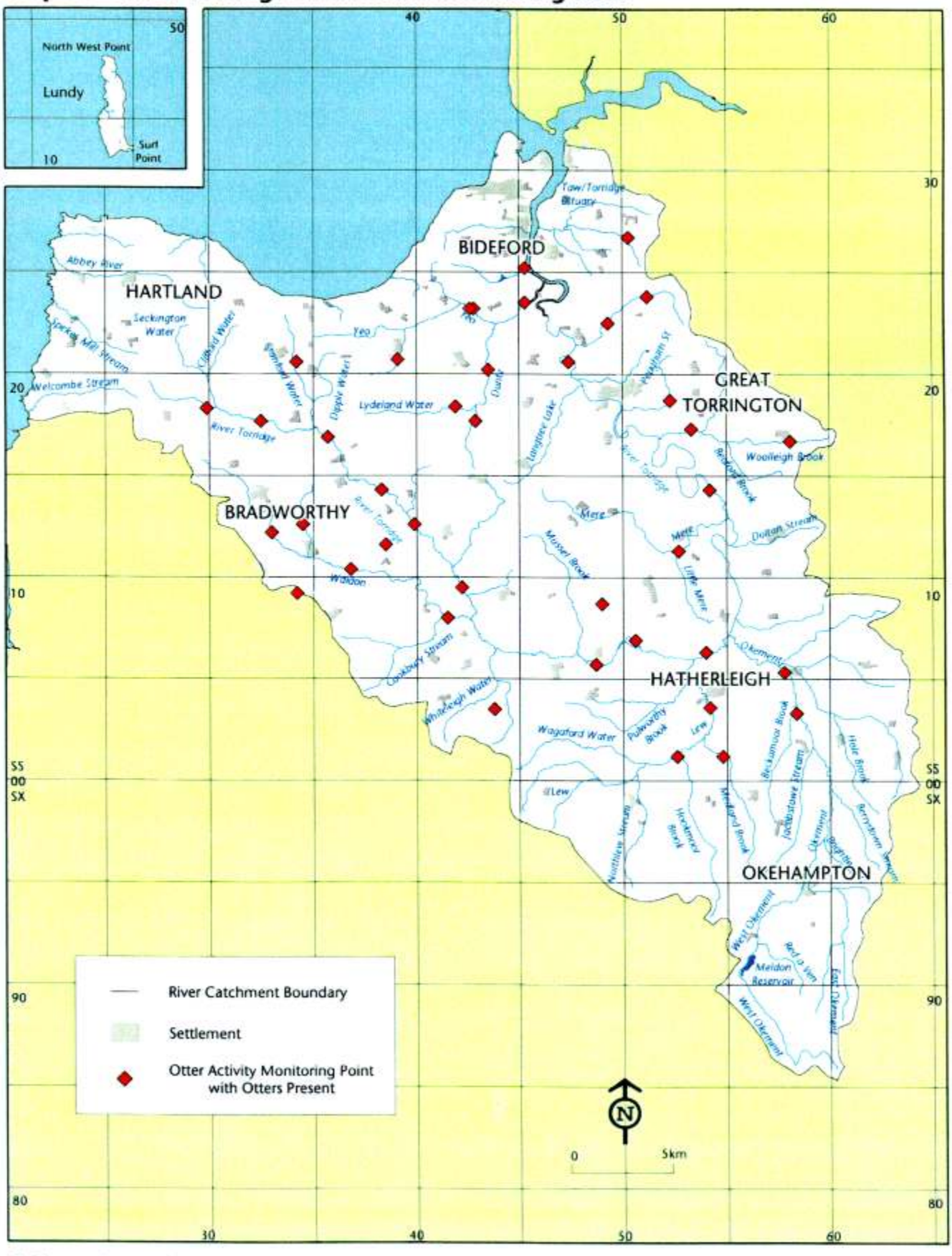
Map D4 - River Brue Catchment: Monitoring Sites



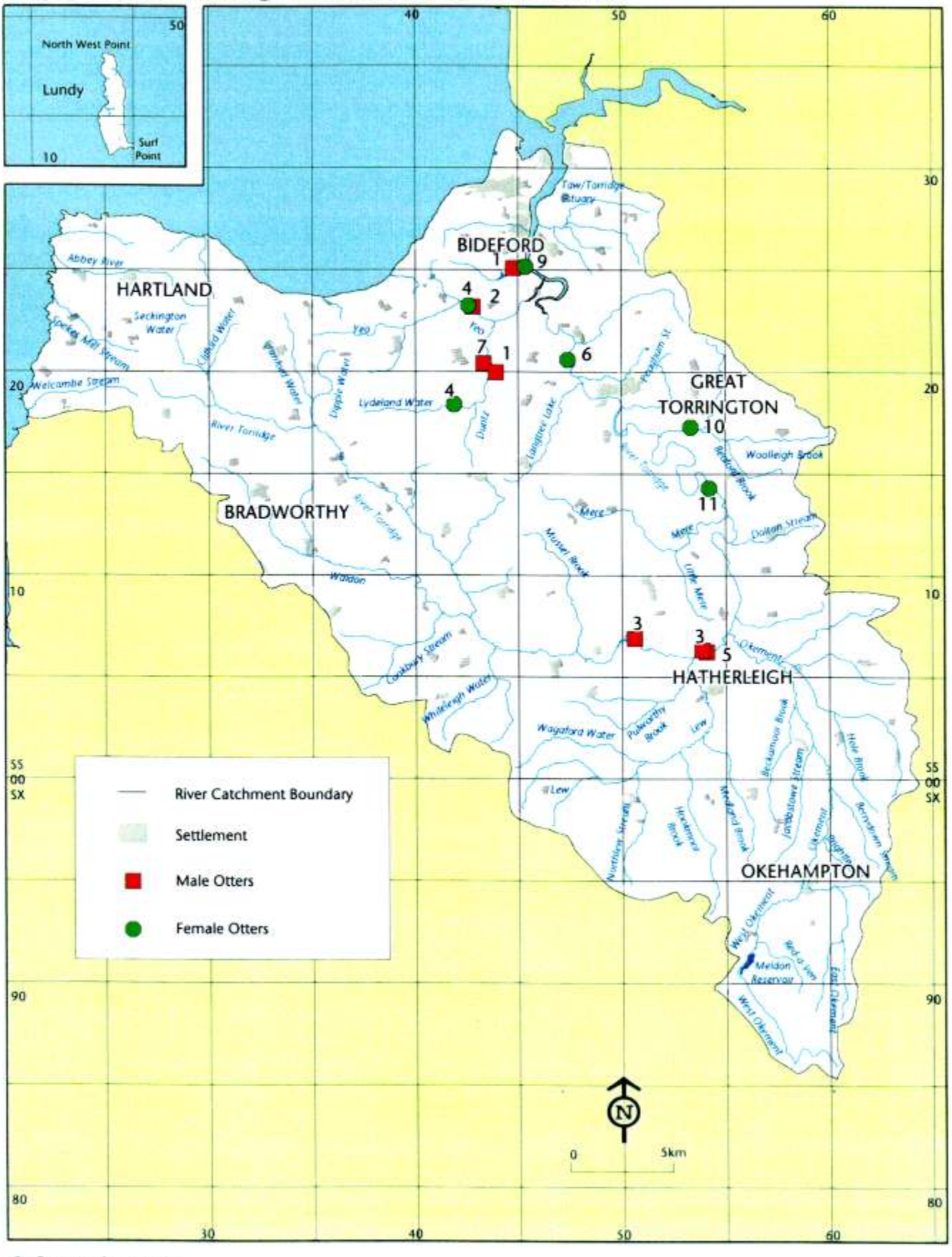
Map D5 - River Brue Catchment: Otters Identified



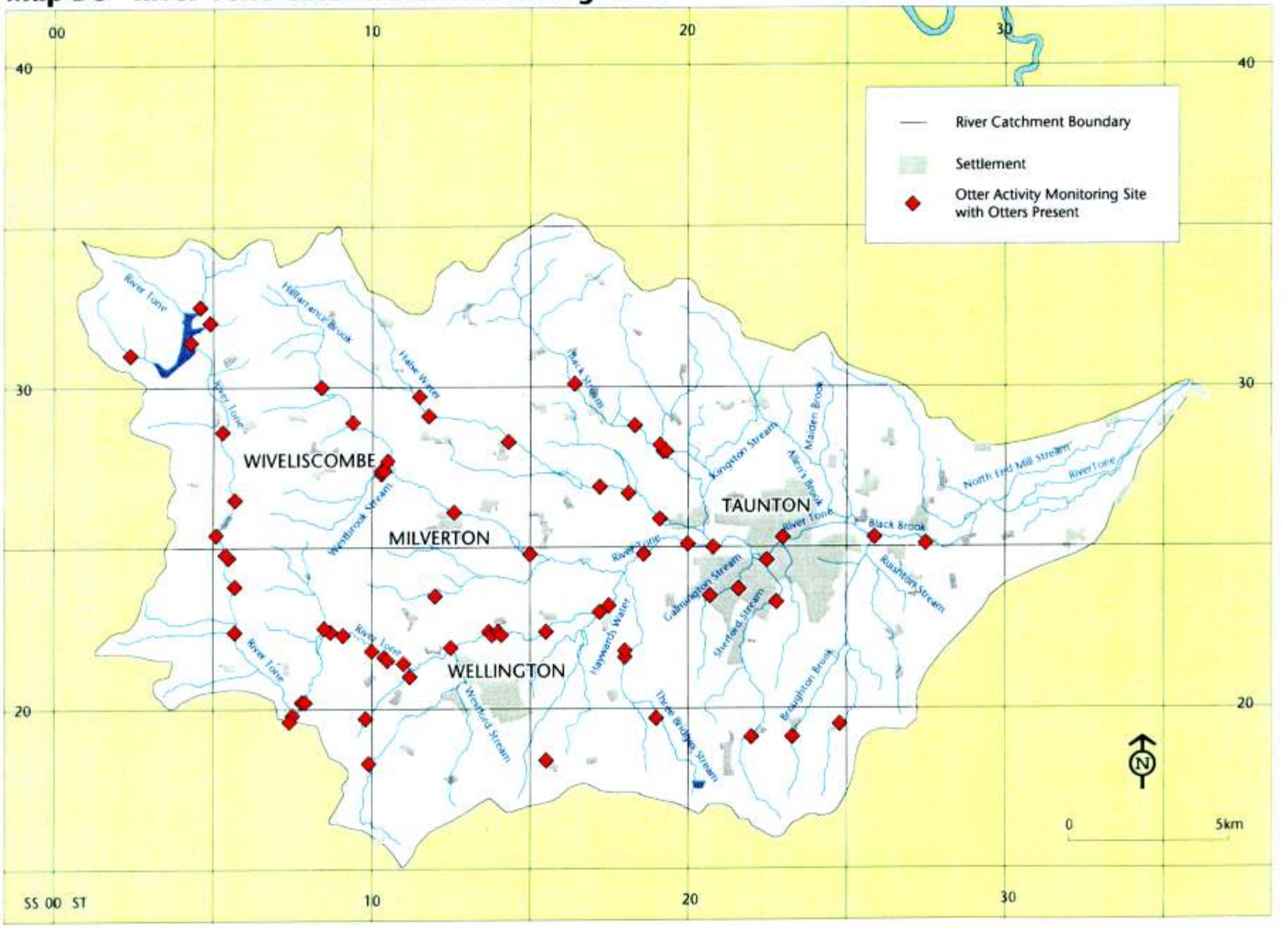
Map D6 - River Torridge Catchment: Monitoring Sites



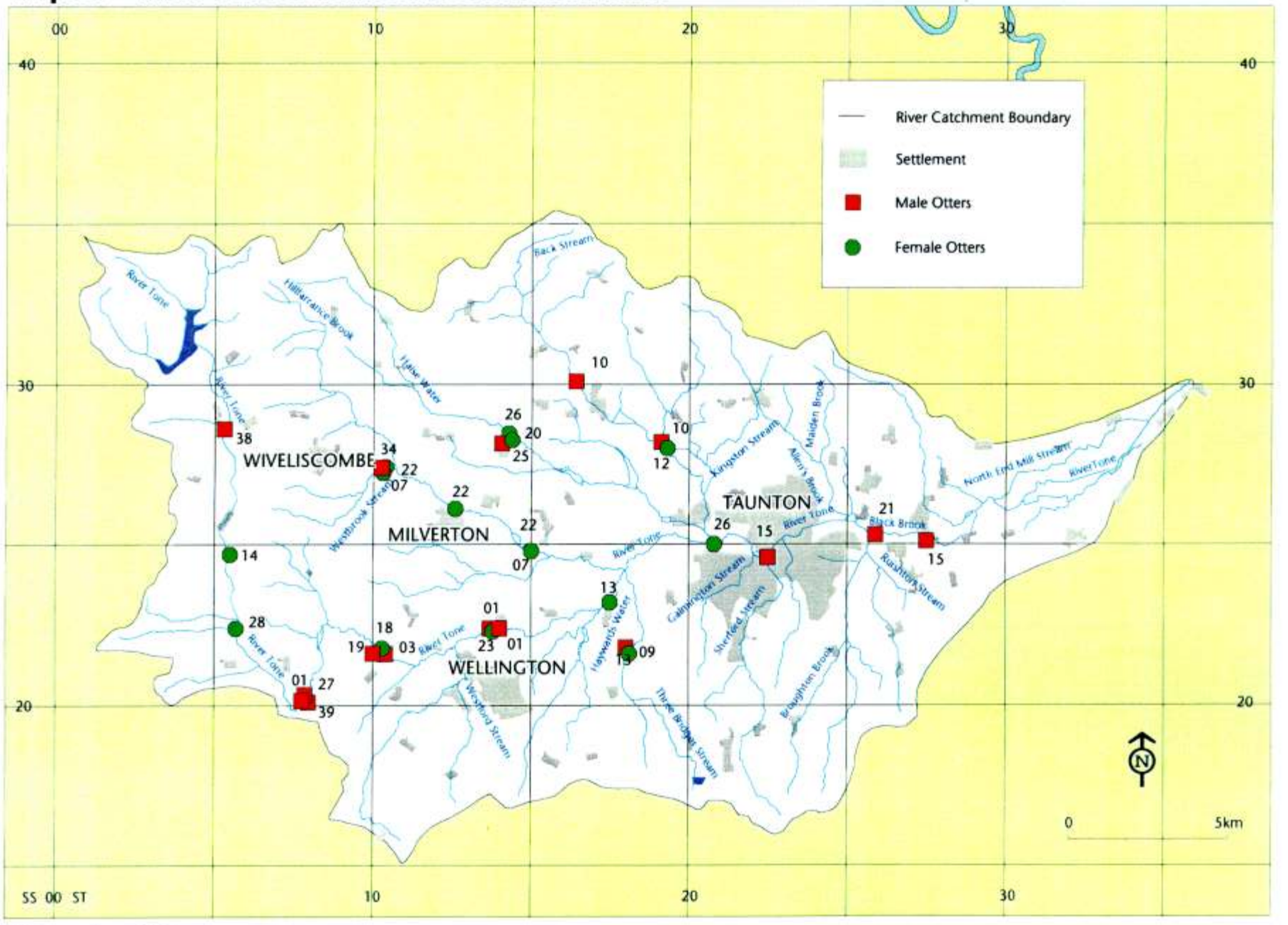
Map D7 - River Torridge Catchment: Otters Identified



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

RIVER TONE AND TRIBUTARIES

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**

RIVER ITCHEN

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

RIVER ITCHEN

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

RIVER ITCHEN

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

Otter DNA Fingerprint
Results 1997 to 1998

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:

SAMPLE TYPE COLLECTED *Spraint / Anal Jelly / None*		SITE NAME	WATERCOURSE
TUBE NO	DATE TIME	GRID REF	TRACKS SIZE (mm)
SUBSTRATE UNDER SAMPLE *Rock / Gravel / Sand /Mud/ Other (describe):*		DEGREE OF EXPOSURE OF SAMPLE *e.g. Under Bridge / Trees / Open* ALSO *was it in Sun / Shade*	
WEATHER CONDITIONS ON DAY OF SURVEY		WEATHER CONDITIONS DURING PREVIOUS WEEK	
COMMENTS - please add any information that may be relevant to the condition of the sample collected or other signs of otter activity			

SAMPLE TYPE COLLECTED *Spraint / Anal Jelly / None*		SITE NAME	WATERCOURSE
TUBE NO	DATE TIME	GRID REF	TRACKS SIZE (mm)
SUBSTRATE UNDER SAMPLE *Rock / Gravel / Sand /Mud/ Other (describe):*		DEGREE OF EXPOSURE OF SAMPLE *e.g. Under Bridge / Trees / Open* ALSO *was it in Sun / Shade*	
WEATHER CONDITIONS ON DAY OF SURVEY		WEATHER CONDITIONS DURING PREVIOUS WEEK	
COMMENTS - please add any information that may be relevant to the condition of the sample collected or other signs of otter activity			

SAMPLE TYPE COLLECTED *Spraint / Anal Jelly / None*		SITE NAME	WATERCOURSE
TUBE NO	DATE TIME	GRID REF	TRACKS SIZE (mm)
SUBSTRATE UNDER SAMPLE *Rock / Gravel / Sand /Mud/ Other (describe):*		DEGREE OF EXPOSURE OF SAMPLE *e.g. Under Bridge / Trees / Open* ALSO *was it in Sun / Shade*	
WEATHER CONDITIONS ON DAY OF SURVEY		WEATHER CONDITIONS DURING PREVIOUS WEEK	
COMMENTS - please add any information that may be relevant to the condition of the sample collected or other signs of otter activity			

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.

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Environment Agency, Hampshire & Isle of Wight Area
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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 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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