Analytical and methodological development for improved surveillance of the Great Crested Newt

WC1067

Appendix 4. Results of a pilot survey to test the use of eDNA outside of the recommended great crested newt survey window

Project contractors: Freshwater Habitats Trust, Spygen, Amphibian and Reptile Conservation and the Durrell Institute of Conservation and Ecology

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Results of a pilot survey to test the use of eDNA outside of the recommended great crested newt survey window

1. Summary

Three ponds in the New Forest, Hampshire, known to have supported great crested newt breeding populations in 2013, were visited on three separate occasions in October, November and December to make a preliminary assessment of the ability of eDNA to detect newts out of season. Traditional survey methods were used to assess the presence of great crested newts and compared with the eDNA technique applied in WC1067. The traditional survey methods were daytime visual and refugia searches, torching, egg searching and netting (i.e. excluded bottle trapping).

Newts were present in small numbers in two ponds (1-4 individuals detected by torch counting on 2 and 3 of the visits respectively), and completely absent from a third on all visits. eDNA scores were lower than in the breeding seasons, with the highest score being 1/12, compared to average eDNA scores for the three ponds of 9.3/12, 9.5/12 and 12.0/12 over four visits in the breeding season.

At the pond where no newts were seen, eDNA results were all negative. At the two sites where newts were seen, eDNA was positive at one of the sites and negative at the other.

Overall the results suggest that autumn season surveys are not suitable for identifying great crested newt breeding ponds. It would be valuable to explore whether samples collected in the late summer and early autumn were useful for identifying breeding ponds (e.g. in any future use of the eDNA approach).

2. Background

The main aquatic phase for adult great crested newts is from early spring – i.e. March through to September, although adults may be found in the water occasionally at almost any time of the year (Langton et al., 2001, Jehle et al., 2011). The great crested newt conservation handbook (Langton et al., 2001) notes that 'A proportion of great crested newts….may stay on until October and even, in some ponds, over winter amongst pond sediment and debris' (Figure 1). Larvae are also potentially present in ponds through to the end of the summer and early autumn.

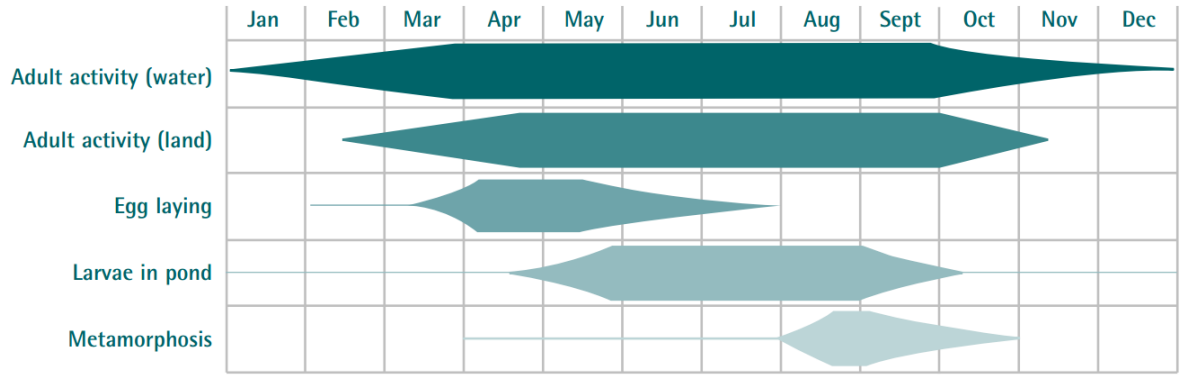

Figure 1. From great crested newt conservation handbook (from Langton *et al.* 2001).

Results from the main Defra eDNA project (Biggs et al. 2014) clearly show that newts can be detected very effectively with eDNA through until the end of June. On the 4th visit in the second half of June eDNA detected great crested newts in all 35 of the study ponds (New Forest and Wales), i.e. a detection rate of 100%.

The effectiveness of eDNA methods during the recommended survey season suggested that it would be worth assessing whether eDNA could continue to detect newts later into the autumn and winter.

There are two main potential limitations with late summer-autumn surveys:

(i) Most adults are expected to have left the pond by the end of August, or occasionally later (e.g. Griffiths and Mylotte 1987). With fewer adults present, eDNA detection rates may be significantly lower in the second half of the year.

(ii) Larvae are perhaps more likely to be detected by eDNA in the second half of the year than adults. If present, larval eDNA will show both that adults were present and that there was a successful breeding season. However, if breeding is unsuccessful and no larvae are present, a single late season eDNA sample could not be used to rule out the use of the pond by adult newts earlier in the year.

3. Objective of study

The aim to the work was to assess whether great crested newts could be detected using eDNA in the period October – December, detecting DNA shed by either larval, adult or immature stages; as a proof of concept study to indicate whether further testing of eDNA to detect newts outside of the breeding season was merited in future years. .

4. Methods

Three ponds in the New Forest, Hampshire, were included in the study. These had already been shown to support breeding great crested newt populations in 2013.

- Hatchet Triangle SU36840131
- Balmer Lawn hotel pond SU30580352
- Forest Road pond SU21650411

It was understood that 3 sites alone would not be statistically robust enough to look for a significant difference between eDNA and traditional techniques. However, the results would determine whether using eDNA outside of the traditional survey window warranted further investigation in future eDNA research work.

To assess the effectiveness of eDNA surveying, knowledge of the occurrence of newts by traditional methods was necessary.

Therefore, ponds were surveyed on **3 occasions** using **4 methods**: visual searching (which included a refugia search and looking for eggs), torching, netting and eDNA. The three visits were spaced as evenly as possible between 18/10/13 (when the eDNA kits arrived) and the 10/12/13 to allow time for eDNA to decay between visits.

To maintain independence between the techniques, a strict protocol was adhered to so that the following order of survey was maintained.

- *Refugia and visual search*: any newts seen on arrival at the pond, and any seen subsequently visually during egg searching, were recorded separately. A search of suitable refugia around the pond was also included. Newts seen at any stage of the torch survey were **not** included in this total.
- *Torching*: The standard method for torching, as per the great crested newt mitigation guidelines (English Nature 2001), were used. The amount of time spent torching and the conditions during the survey were also noted.
- *eDNA*: The eDNA sample was collected after the torch survey (to avoid disturbance to newts before torching), but before the pond was disturbed physically. The method for sample collection is detailed in Biggs et al., (2014).
- *Netting*: the following day, the standard method for netting, as per the great crested newt mitigation guidelines (English Nature 2001) was used. The amount of time spent netting was also noted.
- *Egg-searching*: This method included looking for eggs as other work was being done. The objective was simply to record whether eggs were present. If eggs were not found

during the other parts of the survey, a specific egg search was undertaken and the approximate length of time spent searching for eggs was recorded.

The results were recorded on a tailor made amphibian recording form (Appendix 1).

The information recorded included the following:

Survey conditions:

- Date of survey
- Start time (separately noting the start time of the torch survey)
- Length of survey time (separately noting the length of torch survey, netting survey and egg survey)
- % shoreline surveyed
- Survey conditions:
  - Air and water temperature
  - Whether rain was disturbing the water on scale 0, 1, 2, 3: 0 = No rain; 1 = rain yesterday; 2 = rained earlier on day of survey; 3 = raining during survey
  - Whether wind was disturbing water (yes or no)
  - Strong moonlight (yes or no)
  - Torch make, model and power - Clulite (1 million candle) and Fenix Tk11 (285 lumens)
  - Water clarity on scale 1-4: 1 = Clear; 2 = Moderately Clear; 3 = Moderately turbid; 4 = turbid
  - Vegetation cover on 1-4 scale: 1 = Little or no vegetation obscuring water; 2 = Water moderately obscured by vegetation; 4 = Water mainly obscured by vegetation; 4 = Water nearly or completely obscured by vegetation

Search results for each traditional technique:

- Number of great crested newts and other amphibians seen
- Sex and developmental stage (if possible adult, immature, larva, undetermined)
- Eggs found (Yes/No)

On the first visit an environmental recording form was completed which included the 10 metrics for calculation of a Habitat Suitability Index score (Appendix 2).

5. Results

Raw data from this extended great crested newt survey can be found in Appendix 3.

When recorded, great crested newts were only detected in the field using the torch survey technique (Table 1). It was also the most successful technique at finding other amphibian species, although 2 Palmate Newt were found on one visit whilst refugia searching and 8 Palmate Newts were found on one occasion using the netting technique.

At Hatchet Triangle, 2 great crested newt males were found whilst torching on visit 1 and visit 2, but none on visit 3. The eDNA results for this pond on were negative on all three visits.

At Balmer Lawn, no great crested newts were recorded, and only 1 Palmate Newt was recorded on the final visit. The eDNA results for this pond were all negative.

At Forest Road, great crested newts were recorded on every visit. The eDNA results on visit 1 and 2 were very weakly positive (eDNA scores of 1/12) and negative on visit 3.

Site	Visit	Date	Code		Great crested newts	Other newts	eDNA
Hatchet Triangle	1	29/10/2013	WM0	2373	2	7	0/12
	2	21/11/2013	WM0	2371	2	6	0/12
	3	12/12/2013	WM0	2378	0	8	0/12
Balmer Lawn	1	31/10/2013	WM0	2379	0	0	0/12
	2	19/11/2013	WM0	2375	0	0	0/12
	3	10/12/2013	WM0	2377	0	1	0/12
Forest Road	1	28/10/2013	WM0	2374	1	26	1/12
	2	18/11/2013	WM0	2372	4	34	1/12
	3	08/12/2013	WM0	2376	1	186	0/12

Table 1. Summary of the number of great crested newts and other newts counted during torch survey and the results of eDNA to detect the presence of great crested newts.

The results of Habitat Suitability Index scores (Appendix 4) for both Hatchet Triangle and Forest Road were excellent (>0.8) and good (0.79) for Balmer Lawn. Forest Road had the highest HSI score (0.96). The only difference between the ponds was the greater shade, fewer surrounding ponds and lower vegetation cover at Balmer Lawn, lower vegetation cover at Hatchet Triangle and almost perfect score for every variable at Forest Road.

6. Discussion

In the main survey eDNA results were positive for all sites on all four visits, and eDNA scores were higher than in the autumn survey. Mean eDNA scores over four visits for the three ponds in the breeding season were 9.3/12, 9.5/12 and 12.0/12 for Hatchet Triangle, Balmer Lawn and Forest Road, respectively.

This suggests that the eDNA signal was generally weaker in the autumn than in the breeding season.

Specifically for the three sites, eDNA and traditional surveys were broadly consistent at Forest Road (newts present on all three visits and detected by eDNA on two out of three visits) and Balmer Lawn (no newts detected by either method). At Hatchet Triangle traditional and eDNA survey did not agree, with newts present on two out of three visits but not detected at all by eDNA.

Forest Road supported a medium count great crested newt population in the breeding season (i.e. peak counts 10-99 individuals). The maximum number of newts recorded in the peak breeding season was only 14, but the small area and shallow depth of this pond may have increased the availability of eDNA to be sampled. Outside of the breeding season this pond appears to support overwintering newts, both great crested newts and a large population of Palmate/Smooth newts.

Balmer Lawn is an interesting pond, which has large numbers of great crested newts early in the breeding season (early May), being one of the first New Forest ponds to have breeding individuals (author pers. obs.). In the peak survey season the maximum number of great crested newts recorded was 72 (late May, a medium count population). However, the number of adults in this pond appears to decline rapidly and by the beginning of June only 2 newts could be detected. By the end of June no adult newts were detected and eDNA scores were lower, declining from 0.92, 1.00, 1.00 to 0.25 at the end of the season. Therefore, it is perhaps not surprising that no great crested newts were detected in this pond outside of the breeding season, giving credence to the negative eDNA result at this time.

Hatchet Triangle is a low count great crested newt population (i.e. peak counts less than 10 individual). In the peak survey season, the maximum number of newts found on any one occasion was 3 individuals. eDNA confirmed the presence of great crested newts in late May and early, middle and late June but the quantity of eDNA detected was less than at other

New Forest sites, being 0.83, 1.00, 0.50 and 0.75 respectively. Hatchet Triangle was also the largest pond in the winter survey, being twice the size of Forest Road pond and almost 4 times the size of Balmer Lawn pond. The discrepancy at this site could therefore be due to considerable dilution outside of the breeding season when less eDNA may be available to sample.

7. Conclusion

These results of the pilot autumn survey suggest that eDNA should not be relied upon as a technique to identify great crested newt ponds in the autumn. The question remains on its ability to detect newts in the summer months.

8. References

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

Jehle R, Thiesmeier B and Foster J (2011). The crested newt. Laurenti-Verlag, Bielefeld.

Langton TES, Beckett CL and Foster JP (2001). Great Crested Newt Conservation Handbook. Froglife, Halesworth.

Appendix 1: eDNA SURVEY RECORDING FORM

SITE INFORMATION: complete a new form for ***each pond*** on ***each visit***

Surveyor Name:		**Date of survey:**	
Organisation name: Address		**Great Crested Newt Licence Number:**	
Square: grid reference and name e.g. *SP1243 Square name* (see your map)		**Pond: grid ref** e.g. *SP 123 432*	
Pond name or number (e.g. *Pond 1, cf sketch map*)			

SURVEY CONDITIONS: note separately details of torch survey below

Start time (24hr clock)	:	Air temperature	°C	Water temperature	°C
Length of survey time (hh:mm)†		% Shoreline surveyed	%	Rain (score 0, 1, 2, 3)	
Wind disturbing water (yes/no)		Water clarity Score 1-4		Vegetation cover (1-4)	

Rainfall: 0=none, 1=yesterday, 2=earlier today, 3=during survey. *Water clarity:* 1=clear, 2=moderately clear, 3=moderately turbid, 4=turbid
Vegetation cover: 1=little or no vegetation obscuring water; 2=water moderately obscured by vegetation; 3=water mainly obscured by vegetation; 4=water nearly or completely obscured by vegetation
† Length of survey time = total time spent surveying the pond: i.e. pm - from the beginning of the visual search until all bottle traps set *plus* am – from collecting bottle traps until leaving the site.

SURVEY RESULTS: the focus of this survey is abundance monitoring of great crested newt – however if other species of amphibian are seen during the survey they can be recorded as present on this form

1. Daylight visual search Refugia search	**Tick or add numbers**				
	Male Adults	Female Adults	Immatures	Larva (e.g. tadpole, newt eft)	Unknown life stage/sex
Great Crested Newt					
Presence of other species (optional)					
Common Frog					
Common Toad					
Frog/Toad (not clear which)					
Palmate Newt					
Smooth Newt					
Smooth/Palmate (not clear which)					
Unknown newt					
Notes:					

2. Torching	**Tick or add numbers**				
	Male Adults	Female Adults	Immatures	Larva (e.g. tadpole, newt eft)	Unknown life stage/sex
Great Crested Newt					
Presence of other species (optional)					
Common Frog					
Common Toad					
Frog/Toad (not clear which)					
Palmate Newt					
Smooth Newt					
Smooth/Palmate (not clear which)					
Unknown newt					

Notes:					
Start time (24hr clock)	:	Length of survey time	mins	Torch power	
		Torch make/ model			

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eDNA SURVEY RECORDING FORM

3. eDNA water sample for great crested newt

Environmental DNA water samples should be taken using the kit and should conform to the **strict protocol** using sterile equipment and wearing the gloves provided with each kit.

	Confirm that a water sample collected for eDNA analysis?		**eDNA sample code** (the unique identifier code written on the labels)

Record any issues with the eDNA sample collection here:

4. Netting

	Tick or add numbers				
	Male Adults	Female Adults	Immatures	Larva (e.g. tadpole, newt eft)	Unknown life stage/sex
Great Crested Newt					
Presence of other species (optional)					
Palmate Newt					
Smooth Newt					

Notes:

Start time (24hr clock)	:	Length of survey time	mins	% margin netted	

5. Egg searching

GCN eggs recorded		If eggs are not found during the other parts of the survey, a specific egg search should be undertaken and **the approximate length of time spent searching for eggs should be recorded**.	(mins)

On the first visit we require you to fill in a separate HABITAT SUITABILITY INDEX form

Sketch map of site to identify pond location:

This work is supported by and in partnership with:

Defra, Natural England, JNCC, Natural Resources Wales, Welsh Government, Spygen, Amphibian and Reptile Conservation, Durrell Institute of Conservation and Ecology.

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Appendix 2: HSI RECORDING FORM

Your Name _____ **Date** _____

Square: grid reference and name e.g. *SP1243 Square name* (see your map) _____ **Pond: grid ref** e.g. *SP 123 432* _____

Pond name or number (e.g. *Focal pond , Pond 1, Pond 2*) _____

Please complete this Habitat Suitability Index (HSI) recording form for each pond where you have collected an eDNA sample. The sheet includes all 10 factors needed to calculate an HSI score for great crested newts.
Further guidance on how to record each factor can be found here: www.narrs.org.uk/documents/HSI%20guidance.pdf

1. Map Location

A = optimal
B = marginal
C = unsuitable

2. Number of ponds

Number of *other* ponds in a *1km radius circle* centred on your pond (omit ponds separated by barriers to amphibian dispersal e.g. large rivers, major roads, etc.) (1 : 25 000 maps)

3. Overhanging trees & shrubs

% pond *margin* overhung to at least 1m from the shore.

This is an estimate of how much of the pond margin is *directly* overhung by trees and shrubs which extend shade to at least 1m from the shore, i.e. that would be shaded if the sun was overhead.

4. Pond area

m^2

This can be a tricky measure – it is the *surface area of the pond when the water is at its highest level (usually in early spring)*. It will probably *not* be the current water level of the pond. The high water level line should be evident from wetland vegetation like rushes at the pond's outer edge. Measure area by pacing (single pace = 0.8-1m), or on-line maps e.g. Google Earth.

5. Pond dries?

1 = never dries
2 = rarely dries
3 = sometimes
4 = annually

1 = never dries , **2 = rarely dries**: no more than 2 years in 10 or only in drought,
3 = Sometimes dries: dries between three years in ten to most years,
4 = Dries annually. Deduce pond permanence from local knowledge (e.g. landowner) and personal judgement e.g. water level at the time of the survey. Ponds that dry out annually usually have a hard base.

6. Water quality for amphibians

1 = bad,
2 = poor
3 = moderate
4 = good

Bad = clearly polluted, only pollution-tolerant invertebrates, no submerged plants; **Poor** = low invertebrate diversity, few submerged plants; **Moderate** = moderate invertebrate diversity; **Good** = abundant and diverse invertebrate community.

7. Waterfowl impact

1 = major
2 = minor
3 = none

Major = severe impact of waterfowl e.g. few or no submerged plants, water turbid, pond banks have patches where vegetation removed, feed put down; **Minor** = waterfowl present, but little impact on pond vegetation, pond still supports submerged plants and banks are not denuded of vegetation; **None** = no evidence of waterfowl impact (moorhens may be present).

8. Fish presence

1 = major
2 = minor
3 = possible
4 = absent

Major = dense populations of fish known to be present; **Minor** = small numbers of Crucian Carp, goldfish or stickleback known to be present; **Possible** = no evidence of fish, but local conditions suggest that they may be present; **Absent** = no records of fish stocking and no fish revealed during survey.

9. Amphibian habitat

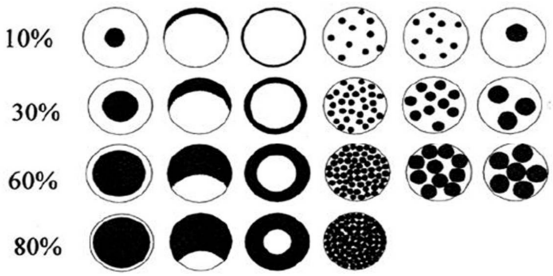
1 = none
2 = poor
3 = moderate
4 = good

None = clearly no suitable habitat within immediate pond locale; **Poor** = habitat with poor structure that offers limited opportunities for foraging and shelter (e.g. amenity grassland); **Moderate** = offers opportunities for foraging and shelter, but may not be extensive; **Good** = extensive habitat that offers good opportunities for foraging and shelter completely surrounds pond e.g. rough grassland, scrub or woodland.

10. Aquatic vegetation: includes emergent, floating and submerged plants

% cover

% of pond's water area occupied by vegetation reaching or breaking through the surface



Data protection and copyright agreement – If returning this form as a *paper sheet*, rather than on-line, please sign below: *I understand that the information that I provide on this form, including my name and contact details, will be entered onto a computer database. I agree to share any intellectual property rights that may pertain to the data submitted on this form.*

Signature:

Appendix 3: Results

3a. Survey conditions

Pond details		Survey conditions								Torching			Netting				Egg search
Grid ref	Pond name	Date	Wind disturb-ance?	Air temp	% shore surveyed	Water clarity	Water temp	Rain score	Vege-tation cover	Start time	Survey length (mins)	Bright moonlight ?	Date	Start time	Survey length (mins)	% margin netted	Time taken
SU36840131	Hatchet triangle	29/10/13	no	14.1	95	1	12.5	2	1	17:15	60	no	30/10/13	10:00	40	95	40
SU36840131	Hatchet triangle	21/11/13	no	13.1	95	1	9.5	1	1	17:30	70	no	22/11/13	10:20	45	95	45
SU36840131	Hatchet triangle	12/12/13	no	11.4	95	1	6.2	1	1	19:15	65	no	13/12/13	10:00	40	95	35
SU30580352	Balmer Lawn	31/10/13	no	14.4	98	1	13.0	2	1	17:20	78	no	01/11/13	11:00	40	98	35
SU30580352	Balmer Lawn	19/11/13	no	13.5	98	1	6.0	1	1	16:45	78	no	20/11/13	11:40	42	98	35
SU30580352	Balmer Lawn	10/12/13	no	11.3	98	1	5.9	1	1	19:45	65	no	11/12/13	11:00	35	98	30
SU21650411	Forest Road	28/10/13	no	17.6	99	1	11.8	1	1	17:18	80	no	29/10/13	11:00	40	99	30
SU21650411	Forest Road	18/11/13	no	15.1	99	1	9.8	1	1	16:30	83	no	19/11/13	11:00	45	99	30
SU21650411	Forest Road	08/12/13	no	10.4	99	1	5.0	0	1	18:00	60	no	09/12/13	10:00	45	99	30

Water clarity: 1=clear, 2=moderately clear, 3=moderately turbid, 4=turbid.
Rain score: 0=none, 1=yesterday, 2=earlier today, 3=during survey.
Vegetation cover: 1=little or no vegetation obscuring water; 2=water moderately obscured by vegetation; 3=water mainly obscured by vegetation; 4=water nearly or completely obscured by vegetation.

3b. Amphibian results

Pond name	Visit	Date	Technique	Great Crested Newt						Palmate Newt	Smooth Newt	Palmate/ Smooth Newt undet.	TOTAL OTHER NEWT	Common Frog	Common Toad
				Male	Female	Immature	Larvae	Undet.	TOTAL GCN						
Hatchet triangle	1	29/10/13	Visual						0	1	1		2		
Hatchet triangle	1	29/10/13	Torch	2					2	3	2	2	7		
Hatchet triangle	1	29/10/13	Egg						0				0		
Hatchet triangle	1	30/10/13	Netting						0				0		
Hatchet triangle	2	21/11/13	Visual						0				0		
Hatchet triangle	2	21/11/13	Torch	2					2	6			6		
Hatchet triangle	2	21/11/13	Egg						0				0		
Hatchet triangle	2	22/11/13	Netting						0				0		
Hatchet triangle	3	12/12/13	Visual						0				0		
Hatchet triangle	3	12/12/13	Torch						0	8			8		
Hatchet triangle	3	12/12/13	Egg						0				0		
Hatchet triangle	3	13/12/13	Netting						0				0		
Balmer Lawn	1	31/10/13	Visual						0				0		
Balmer Lawn	1	31/10/13	Torch						0				0		
Balmer Lawn	1	31/10/13	Egg						0				0		
Balmer Lawn	1	01/11/13	Netting						0				0		
Balmer Lawn	2	19/11/13	Visual						0				0		
Balmer Lawn	2	19/11/13	Torch						0				0		
Balmer Lawn	2	19/11/13	Egg						0				0		
Balmer Lawn	2	20/11/13	Netting						0				0		
Balmer Lawn	3	10/12/13	Visual						0				0		
Balmer Lawn	3	10/12/13	Torch						0	1			1		1
Balmer Lawn	3	10/12/13	Egg						0				0		
Balmer Lawn	3	11/12/13	Netting						0				0		

Pond name	Visit	Date	Technique	Great Crested Newt						Palmate Newt	Smooth Newt	Palmate/ Smooth Newt undet.	TOTAL OTHER NEWT	Common Frog	Common Toad
				Male	Female	Immature	Larvae	Undet.	TOTAL GCN						
Forest Road	1	28/10/13	Visual						0				0		
Forest Road	1	28/10/13	Torch	1					1	20		6	26		
Forest Road	1	28/10/13	Egg						0				0		
Forest Road	1	29/10/13	Netting						0				0		
Forest Road	2	18/11/13	Visual						0				0		
Forest Road	2	18/11/13	Torch					4	4	26	8		34		
Forest Road	2	18/11/13	Egg						0				0		
Forest Road	2	19/11/13	Netting						0				0		
Forest Road	3	08/12/13	Visual						0				0		
Forest Road	3	08/12/13	Torch					1	1			186	186		2
Forest Road	3	08/12/13	Egg						0				0		
Forest Road	3	09/12/13	Netting						0	8			8		

Appendix 4. Habitat Suitability Index Scores

Pond Name	Date		Map location	No. of ponds	Shade	Pond area	Pond perm-anence	Water quality	Water-fowl impact	Fish presence	Amphib habitat	Aquatic vege	HSI Score		
Hatchet triangle	29/10/13	Raw	A	4	15	3200	2	4	1	4	4	30			
		HSI	1.0	0.7	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.6	0.336	0.90	**EXCELLENT**
Balmer Lawn	30/10/13	Raw	A	1	80	900	2	4	1	4	4	15			
		HSI	1.0	0.4	0.6	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.099	0.79	**GOOD**
Forest Road	28/10/13	Raw	A	5	20	1600	2	4	1	4	4	70			
		HSI	1.0	0.8	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	0.638	0.96	**EXCELLENT**