Pond Conservation
For life in fresh waters

Developing a national pond surveillance strategy for localised species based on the results of PondNet 2012

Naomi Ewald, Penny Williams, Francesca Dunn and Jeremy Biggs

Draft 1
January 2013

For further information please contact:
Pond Conservation
Oxford Brookes University
Headington
Oxford OX3 0BP

This report should be cited as:
Ewald NC, Williams P, Dunn F, and Biggs J (2013). Developing a national pond surveillance strategy for localised species based on the results of PondNet 2012. Report to Natural England. Pond Conservation, Oxford.

Acknowledgements
We are enormously grateful to all the PondNet volunteers who collected field data on BAP species abundance using different methods in 2012. Without them, this report would not have been possible.

Contents

Summary

This report provides a summary of the results of one element of the PondNet trials[1]; used to design a statistically robust method for the survey of localised BAP pond plants as part of a volunteer surveillance network in England.

- Plants with a creeping habit including Pillwort, Coral Necklace and Marsh Clubmoss, should be monitored by recording the percentage cover of the species within the outer pond margin (up to the winter water line). However, small areas can be difficult to estimate in terms of percentage cover and therefore patch size should also be recorded for these species in order to improve the calculation.

- Species which grow as individual plants including Yellow Centaury and Tubular Water-dropwort, should be monitored by counting the number of individuals. However, large numbers of individuals are often recorded as an estimate and counts do not give an indication of cover in relation to habitat size, therefore the percentage cover of the species within the outer pond margin (up to the winter water line) should also be recorded.

- Based on the first year of the PondNet trials we recommend that the following number of ponds are selected randomly to monitor restricted species within known sites:

	Sites
○ Pillwort	50
○ Coral Necklace	50
○ Marsh Clubmoss	200*
○ Yellow Centaury	120*
○ Tubular Water-dropwort	30

*we expect the number of sites for Marsh Clubmoss and Tubular Water-dropwort to decrease - as the number of sites surveyed in year one of PondNet for these species was very low and may not be a true reflection of variability between sites.

- The presence/ absence of the target species in other ponds within the pond complex should also be recorded by PondNet volunteers, to give a measure of pond occupancy. As the number of pond complexes required to monitor pond occupancy is less than the number identified to monitor abundance, no new sites will need to be added to the network.

- The same sites will need to be monitoring annually (or other time period) in order to undertake a matched pairs analysis. Randomly sampling a different set of ponds each year increases the amount of variation and makes the size of the network required to reach the same level of power unfeasible.

[1] Pond Conservation (2012) PondNet - http://www.pondconservation.org.uk/Surveys/PondNet.

1 Introduction

1.1 Background and Aims

A network for highly localised pond plants (species known from less than 1000 1km grid squares) needs to be stratified to measuring species abundance at known ponds[2]. However, it is currently difficult to know what size this network of ponds needs to be in order to detect change in abundance, because there is little data on variability between populations, which is needed in order to undertake power analysis. This is further complicated by the fact that the choice of methodology can influence the number of samples required to achieve the same level of statistical power.

The 2012 PondNet trial in Hampshire selected ponds known to contain localised BAP pond plants (Tubular Water-dropwort *Oenanthe fistulosa*, Pillwort *Pilularia globulifera*, Coral Necklace *Illecebrum verticillatum*, Yellow Centaury *Cicendia filiformis* and March Clubmoss *Lycopodiella inundata*) to gather data on their abundance and to test different methodologies.

Power analysis was then undertaken to determine which methodology would be most appropriate for monitoring localised BAP pond plants.

1.2 Methods

1.2.1 Field methods

Using target plant species to assess different survey techniques

Plant abundance can be measured using a variety of different techniques. A volunteer surveyor (Francesca Dunn) recorded Pillwort (a creeping mat forming species) and Tubular Water-dropwort (growing as individual upright plants) at 8 and 5 ponds respectively. At each pond the abundance of each species was recorded as follows:

- percentage cover of the species within the whole pond - taken as the abundance of the species within the maximum winter water level of the pond.
- percentage cover of the species within the available niche - taking into account that some bodies of water will only ever support small populations of a species if the area of suitable habitat within the pond is small – e.g. the margin of a large permanent pond supporting Pillwort. The area of the whole pond may only constitute 10% of the total pond area; Pillwort could occupy 100% of the niche.
- 25cm^2, 50cm^2 and 75cm^2 quadrats - quadrats were randomly placed every 2m around the margin of the pond of the pond, so that the number of quadrats completed was proportional to the size of that pond[3]. In the quadrats:
    - Pillwort abundance was measured as percentage cover within the quadrat and an average abundance calculated.
    - Tubular Water plants were counted and the density/m^2 for each pond calculated as follows:

$$\textit{Density} = \frac{\textit{Total number of individuals of a species in all quadrats}}{\textit{Number of quadrats x area of each quadrat}}$$

[2] Williams P, Ewald NC, Cannon C, and Biggs J (2012). Developing a national pond surveillance strategy for widespread and localised species. Report to Natural England. Pond Conservation, Oxford.

[3] Croft MV and Chow-Fraser P (2009). Non-random sampling and its role in habitat conservation: a comparison of three wetland macrophyte sampling protocols, Biodiversity and Conservation. 18(9), pp.2283-2306.

- counts of individual plants (Tubular Water-dropwort only) - all plants within the maximum winter water line were counted (uncertainty when very large numbers of plants were present meant that counts over 50 individuals were considered to be an estimate).
- size of plant patches (Pillwort only) - the length and width of individual patches within the maximum winter water line were measured and then aggregated to give an area of cover for the pond (m^2).

The volunteer used the results of her efforts to complete an MSc Research Project[4].

Understanding differences in species abundance between sites to develop a statistically robust monitoring network

The abundance of Tubular Water-dropwort (5 ponds), Pillwort (17 ponds), Coral Necklace (12 ponds), Yellow Centaury (9 pond) and March Clubmoss (5 ponds) were recorded by different PondNet volunteers. Abundance was recorded as:

- percentage cover of the whole pond - area occupied by plants within the maximum winter water line.
- area covered (cm^2 or m^2) - creeping species only (Pillwort, Coral Necklace and March Clubmoss).
- species counts - species with individual upright flowering plants (Tubular Water-dropwort and Yellow Centaury).

Results were collated and analysed to determine the size of the network required to be confident we could detect change in species abundance if one occurred. Variability between sites as a result of surveyor bias is not considered here but has been investigation during QA of the sites[5].

Investigating the potential to monitor changes in pond occupancy

A surveillance network for localised species proposed by PondNet involves monitoring changes in abundance within known ponds. Inclusion of currently unoccupied or unknown ponds in the abundance analysis results in too many zero values, increasing the number of sites needed to detect change to unacceptable levels. However, by not monitoring these sites it is not possible to detect changes in pond occupancy which is required to monitor changes in range.

Although not the main focus of this investigation, the number of occupied and unoccupied ponds was recorded within 200m x 200m of the focal pond in 10 pond complexes. From this it was possible to look at variability between pond complexes in the percentage of occupied ponds and to determine how many of these complexes would need to be monitored in order to report on changes in pond occupancy.

1.2.2 Statistical analysis

Power analysis was used to determine the sample size needed to detect changes in abundance and pond occupancy. Type II errors (β) may occur if there is a failure to reject the null hypothesis, when in fact the alternative hypothesis is true. Power ($1-\beta$) is the probability of detecting an effect if one exists in the population, and is largely dependent on sample size N, effect size and levels of variance in sample groups σ^2.

---

[4] Dunn, F. (2012) Developing an appropriate methodology to monitor localised pond associated macrophytes in the New Forest, Hampshire. BMS11102 MSc Research Project. Supervisor: Dr. Robert Briers. Edinburgh Napier University.

[5] Williams P, Ewald N, Biggs J, Wilkinson J. 2013. Biodiversity of ponds: developing and testing new approaches to data collection in the voluntary sector. Year 1 interim report to Defra. Pond Conservation, Oxford.

t-tests were used to test the hypothesis that the difference in means between sampling years was zero (independent samples). Where the difference between the means $\overline{X}_1 - \overline{X}_2$ with a pooled standard deviation (1) and a standard error of the sample means (2) is compared against the t-statistic calculated by $T = \overline{d} / SE(d)$.

$$S_p = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}} \quad (1)$$

$$SE = S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \quad (2)$$

To calculate the sample size N, for power Z_β (where Z_α is the standard normal deviate at the α significance level) for detecting a true difference between the sample means:-

$$N = \left[\frac{Z_\alpha \sigma_0 + Z_\beta \sigma_1}{\mu_1 - \mu_0}\right]^2 \quad (3)$$

Paired t-tests were used for matched pairs analysis. Where the mean of differences between paired observations $\overline{d} = \overline{X}_b - \overline{X}_a$ with a standard error $\sum^{2} \square = \sigma_d^2 / N$ is compared against the t-statistic calculated by $T = \overline{d} / SE(d)$.

Therefore, to calculate the sample size N, for power Z_β (where Z_α is the standard normal deviate at the α significance level) for detecting a true difference:-

$$N = \frac{(Z_\alpha + Z_\beta)^2 \sigma_d^2}{\mu_1^2} \quad (4)$$

Analysis of power was undertaken in R[6] using the pwr package[7] and G*Power[8].

Firstly we investigated the pros and cons of different survey techniques. The mean difference in abundance between sampling years was specified as 10%, 20%, 30%, 40% and 50% of the original population size. The sample sizes required to achieve 60%, 65%, 70%, 75%, 80%, 85%, 90% and 95% power at each of these levels of change was calculated (0.05 significance level (level of α)). The sample sizes required by the different methodologies were then compared.

In the MSc project, the analysis of these data was based on a standard t-test (independent groups). We have taken this further, to compare the difference in sample size required to achieve different levels of power depending on whether different sites (independent groups (equation 3)) or the same sites (paired samples (equation 4)) were used. Next, we investigated the sample size needed to have statistical confidence in detecting change in species abundance for each of our target species assuming optimal sampling strategies. Finally, we investigated the sample size needed to have statistical confidence in detecting change in pond occupancy within sites.

---

[6] R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

[7] Champely, S (2009) R Package 'pwr': Basic functions for power analysis. V 1.1.1. Published 2012-10-29 08:59:31, URL http://cran.r-project.org/web/packages/pwr/pwr.pdf.

[8] Faul F (1992 – 2012) G*Power 3.1.5. http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/download-and-register

2. Results

2.1 Assessing different survey techniques

2.1.1 Pillwort

Abundance data for Pillwort were analysed, to determine the sample size required to detect different levels of change at different levels of power, using different collection methods (Table 1). The results were also analysed as independent and paired samples (i.e. in theory visiting different ponds each year or returning to the same pond each year) to understand how this affected sample size (Figure 1).

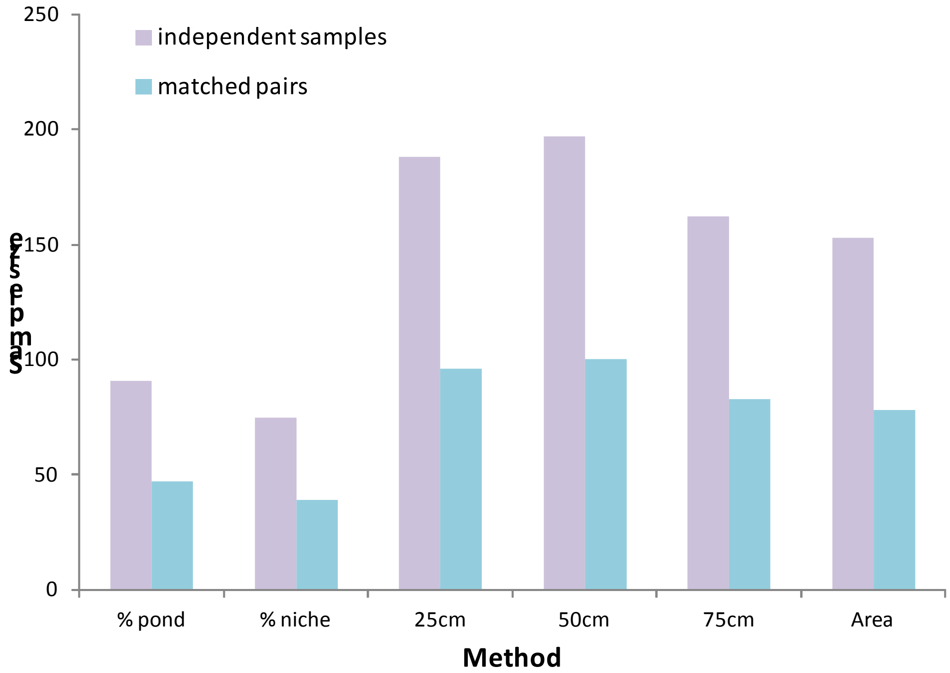

Figure 1. Comparison of the sample size required to detect a 30% change at 70% power using different methodologies to record the abundance of Pillwort.

Table 1. Pillwort (i) abundance measured as percentage cover of the whole pond

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	646	725	814	915	1035	1183	1385	1712	10	324	364	408	459	519	593	694	858
20	162	182	204	229	259	297	347	429	20	83	92	104	116	131	150	175	216
30	73	81	91	103	116	132	155	191	30	38	42	47	53	59	68	79	97
40	41	46	52	58	66	75	87	108	40	22	25	27	31	34	39	45	55
50	27	30	33	38	42	48	56	69	50	15	16	18	20	23	26	30	36

(ii) abundance measured as percentage cover of the available niche

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	528	593	665	747	845	967	1131	1399	10	265	298	334	375	424	485	567	701
20	133	149	167	188	212	242	284	350	20	68	76	85	95	107	123	143	177
30	60	67	75	84	95	108	127	156	30	31	35	39	43	49	56	65	80
40	34	38	42	48	54	61	72	88	40	18	20	23	25	28	32	37	46
50	22	25	28	31	35	40	46	57	50	13	14	15	17	19	21	25	30

(iii) 25cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1339	1503	1686	1896	2144	2453	2870	3550	10	671	753	845	950	1074	1228	1437	1776
20	335	376	422	475	537	614	718	888	20	169	190	213	239	270	308	361	445
30	150	168	188	212	239	273	320	395	30	76	85	96	107	121	138	161	199
40	85	95	106	119	135	154	180	223	40	44	49	55	61	69	79	92	113
50	54	61	68	77	87	99	116	143	50	29	32	36	40	45	51	59	73

(iv) 50cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1404	1576	1768	1988	2248	2572	3009	3722	10	703	789	886	996	1126	1287	1506	1862
20	352	395	443	498	563	644	753	931	20	177	199	223	250	283	323	378	467
30	157	176	197	222	251	287	335	414	30	80	89	100	112	127	145	169	209
40	89	99	111	125	141	162	189	234	40	46	51	57	64	72	82	96	118
50	57	64	72	80	91	104	121	150	50	30	33	37	42	47	53	62	76

(v) 75cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1153	1294	1452	1633	1846	2112	2472	3056	10	578	649	728	818	925	1057	1237	1530
20	289	324	364	409	462	529	619	765	20	146	164	183	206	233	266	311	384
30	129	145	162	182	206	236	275	340	30	66	74	83	93	104	119	139	172
40	73	82	92	103	116	133	155	192	40	38	42	47	53	60	68	79	97
50	47	53	59	66	75	85	100	123	50	25	28	31	35	39	44	51	63

(vi) area (m^2)

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1085	1218	1367	1537	1738	1988	2326	2876	10	544	610	685	770	870	995	1164	1440
20	272	305	342	385	435	498	582	720	20	137	154	173	194	219	250	293	361
30	121	136	153	172	194	222	259	320	30	62	70	78	87	98	112	131	162
40	69	77	86	97	110	125	146	181	40	36	40	45	50	56	64	75	92
50	44	50	56	62	70	80	94	116	50	24	26	29	33	37	42	48	59

The methodology which produced the highest level of power for any given sample size was estimating the percentage cover of Pillwort within its available niche. To achieve 70% power, with 30% change between years, 75 ponds would need to be surveyed. If, the same ponds were re-visited (matched pairs) the number of samples required was only 39 ponds.

When percentage cover was estimated as a proportion of the whole pond, the number of samples required for 30% change at 70% power increased slightly to 91 ponds (independent samples) and 47 ponds (matched pairs).

It was difficult to determine the percentage cover of Pillwort at very low abundance (Francesca Dunn *pers. comm.*) and it was sometimes easier to measure patch size and then calculate from this a percentage area of the whole pond.

Recording abundance using quadrats resulted in the need for the highest number of ponds to achieve the same level of power. Using the 50cm^2 quadrat, 197 ponds (independent samples) and 100 ponds (matched pairs) would need to be surveyed for 70% power at the 30% change level.

Sample size to achieve 70% power (30% change) using the 75cm^2 quadrat was similar to measuring patch size – for independent samples: 162 ponds for 75cm^2 quadrat and 153 ponds for area of patches – less for matched pairs: 83 ponds for 75cm^2 quadrat and 78 ponds for area of patches. But, this was still double the number of samples needed when compared with the percentage cover within niche technique.

2.2.2 Tubular Water-dropwort

Abundance data for Tubular Water-dropwort were analysed in the same way as Pillwort to assess different methods (Table 2) and independent and paired samples (Figure 2).

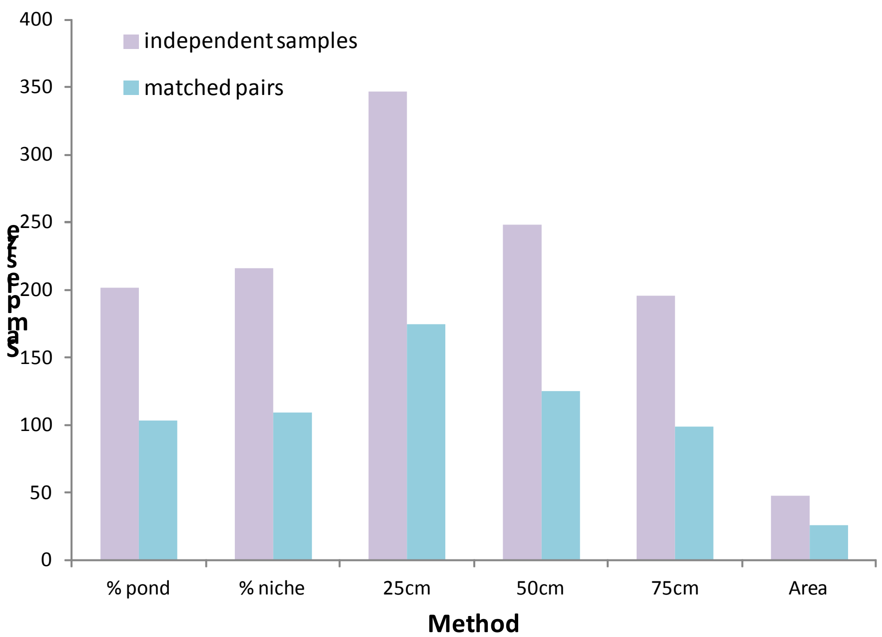

Figure 2. Comparison of the sample size required to detect a 30% change at 70% power using different methodologies to record the abundance of Tubular Water-dropwort.

Table 2. Tubular Water-dropwort (i) abundance measured as percentage cover of the whole pond

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1439	1616	1813	2039	2306	2637	3086	3817	10	721	809	908	1021	1154	1320	1545	1910
20	361	405	454	510	577	660	772	955	20	182	204	228	257	290	331	388	479
30	161	180	202	227	257	294	344	425	30	82	92	103	115	130	148	173	214
40	91	102	114	128	145	166	194	239	40	47	52	59	66	74	84	98	121
50	59	66	73	82	93	106	124	154	50	31	34	38	43	48	55	64	78

(ii) abundance measured as percentage cover of the available niche

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1534	1723	1933	2173	2458	2811	3290	4069	10	769	863	968	1088	1230	1407	1646	2036
20	384	431	484	544	615	704	823	1018	20	194	217	243	273	309	353	413	510
30	171	192	216	242	274	313	366	453	30	87	98	109	123	138	158	185	228
40	97	109	122	137	155	177	207	255	40	50	56	62	70	79	90	105	129
50	62	70	78	88	99	113	133	164	50	33	36	41	45	51	58	68	83

(iii) 25cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	2471	2775	3114	3501	3959	4529	5300	6554	10	1237	1389	1558	1752	1981	2266	2651	3279
20	619	694	779	876	991	1133	1326	1639	20	311	349	391	439	497	568	664	821
30	275	309	347	390	441	504	590	729	30	139	156	175	196	222	253	296	366
40	155	174	196	220	248	284	332	411	40	79	89	99	111	126	143	168	207
50	100	112	125	141	159	182	213	263	50	51	57	64	72	81	92	108	133

(iv) 50cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1765	1982	2224	2501	2828	3235	3786	4682	10	884	993	1113	1252	1415	1619	1894	2342
20	442	496	557	626	708	809	947	1171	20	222	250	280	314	355	406	475	587
30	197	221	248	279	315	360	421	521	30	100	112	125	141	159	182	212	262
40	111	125	140	157	178	203	238	294	40	57	64	71	80	90	103	120	148
50	72	80	90	101	114	130	152	188	50	37	42	46	52	58	67	78	96

(v) 75cm quadrats

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	1393	1564	1755	1973	2231	2552	2987	3694	10	698	783	879	988	1117	1278	1495	1848
20	349	392	439	494	559	639	747	924	20	176	197	221	248	281	321	375	464
30	156	175	196	220	249	284	333	411	30	79	89	99	112	126	144	168	207
40	88	99	111	124	140	160	188	232	40	45	51	57	64	72	82	95	117
50	57	63	71	80	90	103	120	149	50	30	33	37	41	47	53	62	76

(vi) area (m^2)

	Two independent means (t-test)									Two dependent means (paired t-test)							
	Power (%)									Power (%)							
Change (%)	60	65	70	75	80	85	90	95	Change (%)	60	65	70	75	80	85	90	95
10	339	381	427	480	542	620	726	897	10	171	191	215	241	272	311	364	450
20	85	96	107	121	136	156	182	225	20	44	49	55	62	70	79	92	114
30	38	43	48	54	61	70	81	100	30	21	23	26	29	32	36	42	52
40	22	25	28	31	35	40	46	57	40	13	14	15	17	19	21	25	30
50	15	16	18	20	23	26	30	37	50	9	10	11	12	13	14	17	20

Differences between methods to achieve the same level of power for Tubular Water-dropwort were less marked than for Pillwort. Abundance, measured as a the percentage in cover of the whole pond, the percentage cover of the available niche and measures of density within the 75cm^2 quadrat resulted in similar numbers of ponds to achieve the same level of power. To detect 30% change, this was around 100 ponds (matched pairs) and 200 ponds (independent samples).

Density measures in smaller quadrats would require an increase in sample size to achieve the same level of power, up to 125 ponds (50cm^2) independent samples and 175 ponds (25cm^2) matched pairs analysis.

In general the size of the network required to adequately account for the inherent variability in cover of this species between ponds was much bigger than for Pillwort, almost double the number of sites required.

Counting individual plants within the pond gave the highest level of power for any given sample size or species. At 30% change and 70% power only 26 ponds were required for matched pairs analysis. One drawback of this technique was that it was difficult to count individual plants at high abundance (Francesca Dunn *pers. comm.*).

2.2 Power analysis for monitoring changes in abundance

Abundance data for 5 BAP pond plant species were recorded from 40 ponds in the New Forest by PondNet volunteers. These data were analysed to determine the size of network required to detect different levels of change at specified levels of power (Table 3). As recommended, percentage cover of the whole pond was recorded for creeping species and species counts were made of individual plants. But, both methods are shown for all species for completeness (Figure 3).

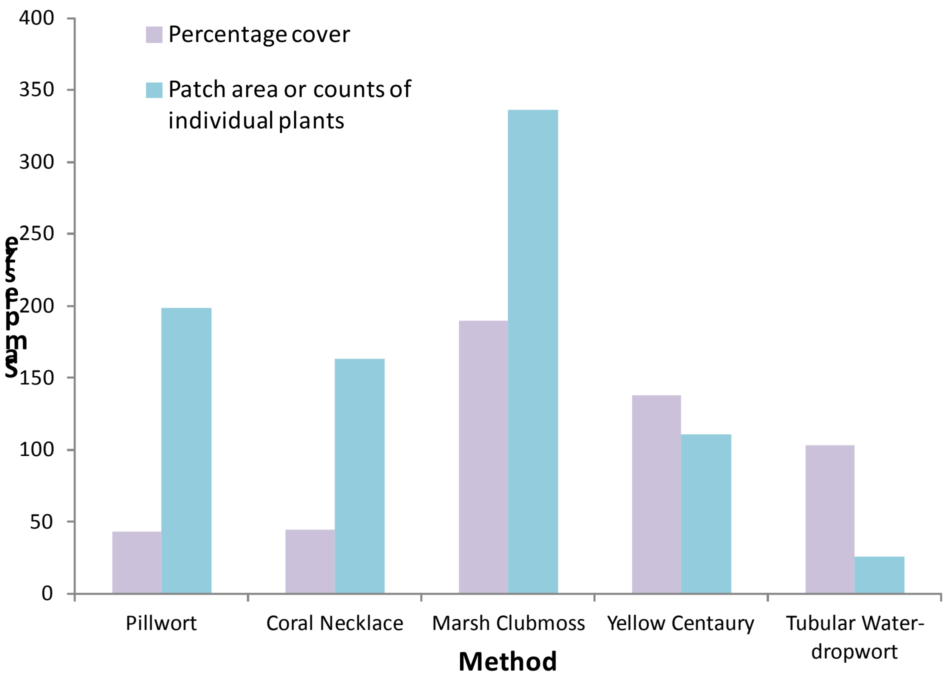

Figure 3. Comparison of the sample size required to detect a 30% change at 70% power for restricted BAP pond plant species recorded using (i) percentage cover of the whole pond and (ii) patch size area (m^2) (Pillwort, Coral Necklace and Marsh Clubmoss) or counts of individual plants (Yellow Centaury and Tubular Water-dropwort).

Table 3. Power analysis for restricted BAP pond plants (based on two dependent means (paired t-test)

(i) Pillwort

Percentage cover of whole pond

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	292	328	368	413	467	534	625	772
20	75	83	93	105	118	135	158	195
30	34	38	43	48	54	61	71	88
40	20	22	25	28	31	35	41	50
50	14	15	17	18	21	23	27	33

Patch area (m^2)

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	1408	1581	1774	1995	2256	2580	3019	3733
20	354	397	445	500	565	646	756	935
30	158	177	199	223	252	288	337	416
40	90	101	113	126	143	163	190	235
50	58	65	73	82	92	105	123	151

(ii) Coral Necklace

Percentage cover of whole pond

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	310	347	390	438	495	566	662	818
20	79	88	99	111	125	143	167	206
30	36	40	45	50	57	65	75	93
40	21	24	26	29	33	37	43	53
50	14	16	18	19	22	25	28	35

Patch area (m^2)

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	1154	1295	1453	1634	1847	2113	2472	3057
20	290	325	365	410	463	530	620	766
30	130	146	163	183	207	236	276	341
40	74	83	93	104	117	134	156	193
50	48	54	60	67	76	86	101	124

(iii) Marsh Clubmoss

Percentage cover of whole pond

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	1346	1511	1695	1906	2155	2465	2884	3567
20	338	379	425	478	540	618	723	893
30	151	170	190	213	241	276	322	398
40	86	96	108	121	137	156	182	225
50	56	62	70	78	88	100	117	145

Patch area (m^2)

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	2388	2681	3009	3383	3826	4376	5121	6333
20	599	672	754	847	958	1095	1282	1585
30	267	300	336	378	427	488	571	705
40	151	169	190	213	241	275	322	398
50	97	109	122	137	155	177	207	255

(iv) Yellow Centaury

Percentage cover of whole pond

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	971	1090	1223	1375	1555	1778	2080	2573
20	244	274	307	345	390	446	522	645
30	110	123	138	154	174	199	233	288
40	63	70	78	88	99	113	132	163
50	41	45	51	57	64	73	85	105

Count of individual plants

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	784	880	987	1109	1254	1435	1679	2076
20	197	221	248	279	315	360	421	520
30	89	99	111	125	141	161	188	232
40	51	57	64	71	80	91	107	132
50	33	37	41	46	52	59	69	85

(v) Tubular Water-dropwort

Percentage cover of whole pond

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	721	809	908	1021	1154	1320	1545	1910
20	182	204	228	257	290	331	388	479
30	82	92	103	115	130	148	173	214
40	47	52	59	66	74	84	98	121
50	31	34	38	43	48	55	64	78

Count of individual plants

Change (%)	Power (%)							
	60	65	70	75	80	85	90	95
10	171	191	215	241	272	311	364	450
20	44	49	55	62	70	79	92	114
30	21	23	26	29	32	36	42	52
40	13	14	15	17	19	21	25	30
50	9	10	11	12	13	14	17	20

2.2.1 Creeping species

Recording abundance for creeping pond plants by recording percentage cover as opposed to recording patch area produced the same results as in Section 1.3.1. The sample sizes needed to detect change with reasonable power were much smaller if percentage cover estimates were used. However, volunteers also noted that they found measuring patch size a useful technique to help in area estimation when the population size was small.

Pillwort

- Recorded from 17 ponds.
- 43 ponds would be required to detect 30% change at 70% power if measured using percentage cover of the whole pond. This would be 13% of ponds currently known for this species (340 ponds).
- If all 340 currently known ponds were monitored, we could achieve 95% power to detect a 20% change in abundance if one occurred, but only 65% power of detecting a 10% change.
- More subtle changes of less than 10% would not be detected through this form of monitoring, because the sample size required to give sufficient power would exceed the number of ponds known for this species.

Coral Necklace

- Recorded from 12 ponds.
- 45 ponds would be required to detect 30% change at 70% power if measured using percentage cover of the whole pond. Although data on the distribution of Coral Necklace in ponds is incomplete we estimate that this is around 25% of currently known ponds for this species.
- Similar results obtained for Coral Necklace and Pillwort suggest that using this methodology for pond plants with a creeping habitat may require a sample size of 50 ponds per species surveyed annually to provide a statistically robust network from which to monitor change.

Marsh Clubmoss

- Recorded from 5 ponds.
- 190 ponds would be required to detect 30% change at 70% power if measured using the percentage cover of the whole pond. This would be more than the number of currently known ponds (143 ponds) for this species.
- Four of the five ponds had a percentage cover of less than 1%; the last had a population size of 10% of the pond area. This variation between sites led to the very large sample size required for monitoring this species. Many ponds in the New Forest and nationally have larger populations than this, so the results presented here are unlikely to be a good basis for development of the monitoring strategy.
- The second year of PondNet 2013 will target additional ponds for Marsh Clubmoss to provide a better dataset for the analysis.

2.2.2 Upright species

Recording abundance for upright pond plants by counting individuals within the pond margin as opposed to recording percentage cover produced the same results as in Section 1.3.1. The sample sizes needed to detect change with reasonable power were much smaller if counts of individual plants were made. But, volunteers also noted that they were less confident they had accurately measured plant numbers in large populations, therefore measuring area covered should also be used as a measure of population size. This would also provide an assessment of the area of habitat occupied in relation to pond area.

Yellow Centaury

- Recorded from 9 ponds.
- 111 ponds would be required to detect 30% change at 70% power if measured using counts of individual plants. This would include all of the ponds currently known to support Yellow Centaury.
- Larger changes could be detected with greater power. A 50% change in abundance could be detected with 95% power using a sample size of 85 ponds.
- More subtle changes of less than 30% would not be detected through this form of monitoring, because the sample size required to give sufficient power would exceed the number of ponds known for this species.
- Relatively few sites were surveyed for Yellow Centaury in PondNet 2012 and the three ponds with population estimates of 200 plants were all located within the same pond complex. To have greater confidence in the results of the analysis more ponds from different pond complexes will be surveyed in 2013.

Tubular Water-dropwort

- Recorded from 5 ponds.
- 26 ponds would be required to detect 30% change at 70% power if measured using counts of individual plants. This would only be 3% of ponds currently known for this species (995 ponds).
- To detect 10% change at 95% power, 450 ponds would be required (45% of known ponds).
- This analysis was based on a limited number of sites. To have greater confidence in the results of the analysis more ponds for Tubular Water-dropwort will be surveyed nationally in 2013.

2.3 Monitoring changes in pond occupancy

BAP ponds plants were recorded as present or absent in ponds within a 200 x 200m area of the focal pond. Although, 40 focal ponds were surveyed to record species abundance, the presence/absence of species was only recorded from 10 pond complexes. Due to the small sample size the results were pooled regardless of species. On average, BAP plants occupied 57% of ponds within a complex. But, this varied from 17% of ponds occupied at one site by Pillwort to 100% of ponds occupied by Pillwort at another site.

Table 4. Power analysis to determine sample size required to detect change in pond occupancy of BAP species.

		Power (%)							
		60	**65**	**70**	**75**	**80**	**85**	**90**	**95**
Change (%)	**10**	112	125	140	157	178	203	237	293
	20	29	33	36	41	46	52	61	75
	30	14	16	17	19	22	24	28	34
	40	9	10	11	12	13	15	17	20
	50	6	7	8	8	9	10	11	14

- The results of power analyses suggest that 17 pond complexes per species would be required as part of a monitoring network to detect 30% change in pond occupancy within complex.
- The proposed network to monitor change in abundance (Section 1.3.2 and 1.4.2) suggests that 50 ponds per species would be sufficient. If ponds within the sample complex as the focal pond were also monitored – a network of 50 pond complexes to determine presence/ absence of the target species:
    - it would be possible to detect changes above 30% with 95% power.
    - a change of 20% could be detected with 80% power.
    - 10% change or less would not have sufficient power at this sample size.

3. Discussions and recommendations

3.1 Assessing different survey techniques

For creeping species, estimating percentage cover *within the available niche* resulted in the need for the smallest sample size to achieve the same level of power. In general, percentage cover estimates are often subject to surveyor bias but, with training, estimates can be standardised. However, estimating cover within the available niche of a species increases surveyor bias because of the difficulty in determining the area of the species niche before an estimate of cover is made.

- We recommend that percentage area of the whole pond is used to measure the abundance of creeping species, to limit surveyor bias even though it will slightly increase the number of ponds required as part of the monitoring network.

Estimating percentage cover for inexperienced volunteers becomes increasingly difficult at low densities or when species patches are scattered.

- We recommend that patch size is recorded by volunteers along with an estimate of pond area, to help volunteers calculate percentage cover.

For upright species, which tend to have a patchy distribution within ponds, measuring area resulted in the need for very large sample sizes to achieve sufficient power. However, volunteers may find it difficult to count numbers of individual plants at very high densities and counts will not provide information on the size of population in relation to the area of the pond.

- We recommend that counts of individual plants are made to assess the abundance of upright species. We also suggest that the proportion of the pond occupied by the species is recorded as percentage cover of the whole pond.

In order to minimise the number of samples required for a monitoring network, repeat visits to the same ponds (matched pairs analysis) will be required. If random visits are made to different ponds and the results between years analysed (independent groups), sample sizes would exceed the number of known ponds for very restricted species.

3.2 Power analysis for monitoring changes in abundance

Changes in the abundance (% cover) of creeping species such as Pillwort and Coral Necklace can be monitored using a network of 50 ponds per species (to achieve 70% power). Changes in the abundance of Marsh Clubmoss would require a substantially bigger network (200 ponds) because of the variability in population size between species.

Changes in abundance (species counts) for upright species such as Tubular Water-dropwort can be monitored using a network of 30 ponds per species. But, for other species such as Yellow Centaury the network would need to be up to 120 ponds to achieve the same level of power.

Results were based on analysis of data from the New Forest and for Marsh Clubmoss, Tubular Water-dropwort and Yellow Centaury a relatively small sample size.

- We recommend that PondNet 2013 concentrates on collation of data from a larger number of sites and where possible from the other PondNet regions. To confirm the findings of PondNet 2012.

3.3 Monitoring changes in pond occupancy

Provisional results suggest that it would be possible to detect 30% change (70% power) in pond occupancy within known sites with a network size of no more than 20 pond complexes per species. This is less than the recommended network size for measuring changes in abundance therefore no additional sites would need to be added to the network, but volunteers would need to visit as many ponds within a complex as possible in order to record occupancy as well as abundance.

There are still a number of outstanding questions:

- The results presented here were based on the collation of results from different species – individual species may have different patterns of pond occupancy within sites which have not been detected.
- The degree to which pond occupancy changes between years is not known. All the pond plant species investigated have a tendency to exist as meta-populations, appearing in ponds within the site when conditions are favourable. Variation between years and between sites may affect the sample size required to detect change at a country level.
- Pond occupancy within site will be affected by the total number of ponds. If the number of ponds within the site increases, the percentage of ponds occupied by a species will appear to decline if the number of occupied ponds remains the same.

In order to answer these questions, PondNet volunteers 2013 who are monitoring BAP pond plants will be encouraged to record:

- the number of ponds within the pond complex
- the number of ponds which they surveyed within the complex
- the number of ponds which were found to contain their target species

We will then analyse these results to refine the size of network recommended for each species to detect changes in pond occupancy.