Agile Frog Data Analysis Research Project 2015

Final Report - March 2015

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Summary

This report analyses population and environmental data collected during annual monitoring of the agile frog (*Rana dalmatina*) on Jersey from 1987-2014. In addition, other published and unpublished research is reviewed and used to produce recommendations for improving the monitoring protocols and further research.

The agile frog population at Ouaisné increased over the study period, and appeared to be responding to increased conservation management in recent years. There were insufficient data to detect a trend at Noirmont or at other reintroduction sites. Multiple regression analysis showed that population size – as assessed by annual spawn clump counts – was related to the number of head-started tadpoles released one and two years previously. The population at Ouaisné therefore seems to be responding well to head-starting.

Spawning of agile frogs occurs at relatively low temperatures, often just above freezing. Equally, the spawning peak usually occurs when temperatures are in the range 6-10 ^{o}C and no higher than 15 ^{o}C. First spawning tended to follow rainfall in the previous week, and peak spawning occurred after rainfall the previous day.

There were inconsistencies between years and between recorders in how spawn clumps were mapped and recorded, and in the site variables measured on each visit. We therefore recommend standardisation of the survey protocol. This can occur at one of three levels, depending on available expertise and resources (Table 2.1). At the most basic level, the number of spawn clumps should be recorded at each slack with general weather conditions noted and the water level categorised. At the intermediate level, we recommend that the location of each spawn clump's position is recorded and at selected slacks given an individual ID to allow monitoring. Furthermore, this level would include data on the lifestage and position of frogs, toads, newts and predators along with a larger set of environmental variables and more detailed measurement of the water level. The

most complete survey would involve all those measurements made at basic and intermediate levels, together with greater detail on the position of spawn in relation to the water body, and temperature from in situ data loggers which could be placed at well-studied slacks. In addition, at the advanced level the water bodies should be mapped on each visit, and notes on the health and behaviour of frogs and toads recorded in addition to recording sex ratio, snout-vent length (SVL) and body mass of individual animals. The latter measurements may provide an index of body condition; however a dedicated researcher would be required to carry this out. Furthermore there are associated impacts of disturbance to consider, and so we recommend this is preferably carried out on well-established populations.

A review of earlier studies showed that head-starting of tadpoles resulted in increased survival and larger metamorphs. The effects of spawn protection were mixed, and confounded by variation in predation between slacks. Nevertheless, it is likely that that this intervention also enhances survival, but to a lesser extent than head-starting. However, given the cost-effectiveness of spawn protection compared to head-starting, this technique needs further evaluation whilst continuing with its use where clumps are at risk of predation.

A provisional Habitat Suitability Index has been developed for the agile frog on Jersey, but may be limited in its application as it is based on very few extant sites. It is not possible to provide further recommendations on habitat suitability; however we make recommendations for further research which could improve our understanding of the ecology of Jersey's agile frog population.

Given the potential threat of emerging infectious diseases for amphibians, the existing biosecurity protocol for surveyors may need extending to include rangers and other individuals working at sites of interest. Further research that could usefully inform the action plan for the agile frog includes assessment of terrestrial habitat use and the potential use of garden ponds; further modelling of the effects of environmental factors on spawning and long-term population dynamics; and a re-evaluation of the benefits of spawn protection as a cost-effective intervention.

Based on spawning data and population viability analyses carried out by Racca (2004), we believe that Ouaisné is now at, or close to its carrying capacity. With this in mind, we recommend that this site now enters a monitoring stage with low level supplementation, and spawn protection still to be carried out on at-risk spawn. Spawn may still be removed from this site for head-starting and translocation to receptor sites. All other sites should continue to be supplemented through head-starting and / or spawn translocation.

Introduction

The agile frog (*Rana dalmatina*) can be found throughout much of southern and central Europe; however it is only present in a handful of locations in northern Europe including Jersey. Moreover, the Jersey population has both behaviourally and genetically diverged from its mainland counterparts, with a reduction in genetic variability (Racca, 2004).

In Jersey the agile frog population underwent a decline in the 1900's, with populations only present at seven ponds in the 1970's, and two ponds by the late 1980's; Ouaisné Common and Noirmont Pond (Gibson and Freeman, 1997; Racca, 2004). These declines have likely been the result of: i) a decrease in, and the pollution of ground water through intensive agricultural practices and water abstraction, ii) the modification, disturbance and loss of habitat through development, and iii) an increased predation pressure due to the introduction and proliferating populations of non-native predatory species and native waterfowl (Gibson and Freeman, 1997; AFG, 2001; Racca, 2004).

A pesticide spill in 1987 resulted in the loss of the Noirmont population, prompting the first intervention for the population (Gibson and Freeman, 1997). A partnership was formed between the States of Jersey Environment Department, and Durrell Wildlife Conservation Trust, with the Agile Frog Group (now Jersey Amphibian and Reptile Group) being formed in 1993. This partnership also involved several private individuals keen to conserve the frog population. This group devised an action plan in 2001 to provide achievable objectives for restoring the agile frog to Favourable Conservation Status by 2005 (AFG, 2001). To recover the population, a suite of management tools were used by the group including spawn protection, head-starting, habitat management and pond creation, and the translocation of spawn / tadpoles.

Between 1987 and 2014, these strategies have resulted in an estimated 48,700 froglets being head-started from spawn and released. Additionally, 67 spawn clumps have been protected in-situ since 2005 as a further management strategy for the population. Moreover, both Ouaisné

Common and Noirmont have received careful habitat management including slack deepening and clearance of encroaching vegetation as well as SSI (Ecological Site of Special Interest) status in 2007. The agile frog is listed on Appendix II of the Bern Convention and on Appendix IV of the EU Habitats Directive, as well as being protected in Jersey under the Conservation of Wildlife Law (2000).

The aims of this report were to:

1) Provide an analysis of the current agile frog spawn data for Ouaisné Transect, Ouaisné Slacks and Noirmont, with respect to (i) long-term trends; (ii) comparisons between sites; and (iii) factors affecting the timing of spawning. (See section 1).

2) Give recommendations on new/amended survey protocols, and identify the survey effort required for effectively monitoring agile frog spawning. (See section 2).

3) Carry out a more detailed analysis of spawn taken for head-starting and spawn clumps wrapped for protection with a view to identifying the best strategy (See section 3).

4) Identify what further research may be needed to determine a Habitat Suitability Index for agile frogs in Jersey and principles relating to an agile frog introduction strategy. (See section 4).

5) Provide any other recommendations. (See section 4.3).

Report

1. Site trends - Spawning, peak frog counts, and tadpole releases

1.1. Dataset

Data from 1987 to 2014 (Appendix 6) were included in this section for graphical purposes, while statistical analyses were carried out on data from 1997 through to 2014 unless otherwise specified. This was due to limited data availability and lack of spawning activity (or records thereof) previous to 1997. The variables included in analysis were: i) the number of clumps found each year; ii) the peak number of frogs counted each year; and iii) the number of head-started tadpoles / metamorphs released each year.

The sites included in the analysis were Ouaisné Transect (North Slack, South Slack, South/Lagoon, Lagoon exit to sump, and Sump), Ouaisné Slacks (Slacks 5, 5a, 6, 7, the main pond, Molinia bog, and the lined pond), and Noirmont Pond. Due to the small sample sizes for these groups of data, analysis was also undertaken for 'Ouaisné Total' (results from Ouaisné Transect plus Ouaisné Slacks), and for the 'Total' (all sites together). In recent years data have become available for Les Creux and Woodbine Corner as a result of reintroduction efforts, but these are not included here.

Climatic variables were also considered in our analysis as potential predictors of spawning. These data were provided by the Meteorological Section of the States of Jersey Department of the Environment, and consisted of daily temperature and rainfall.

1.2. Analysis

All analyses and graphical outputs were created using R 3.0.2. (R Core Team, 2013). The data were initially tested to see if they met parametric assumptions (Appendix 5).

1.3. Results

1.3.1. Spawn clump trends over time

Trends in the number of spawn clumps over time show a positive increase (Figure 1.1). Relationships between the number of clumps and the year (1987-2014) were assessed using linear regression with year as the independent variable. Missing cases were excluded from the analysis. All sites but Noirmont have a clear significant positive relationship between the year and the number of clumps found (Table 1.1).

A higher R^2 value means a stronger ability to predict Y from X. Ouaisné Transect has the highest R^2 value, meaning the relationship between year and clump is stronger than at other sites, and that the year provides a strong explanation for the variance in the number of clumps ($R^2 = 0.79$).

The regression equations are given in Appendix 7, and can be used to calculate estimates of future spawn numbers in a given year.

Table 1.1 Relationships between the number of spawn clumps to year (1987-2014)

Site	R^2	*F*	DF	*p*
Ouaisné Transect	0.79	100.2	26	<0.001
Ouaisné Slacks	0.45	21.43	26	<0.001
Ouaisné Total	0.59	37.27	26	<0.001
Noirmont	0.29	4.093	10	>0.05
Total	0.55	31.87	26	<0.001

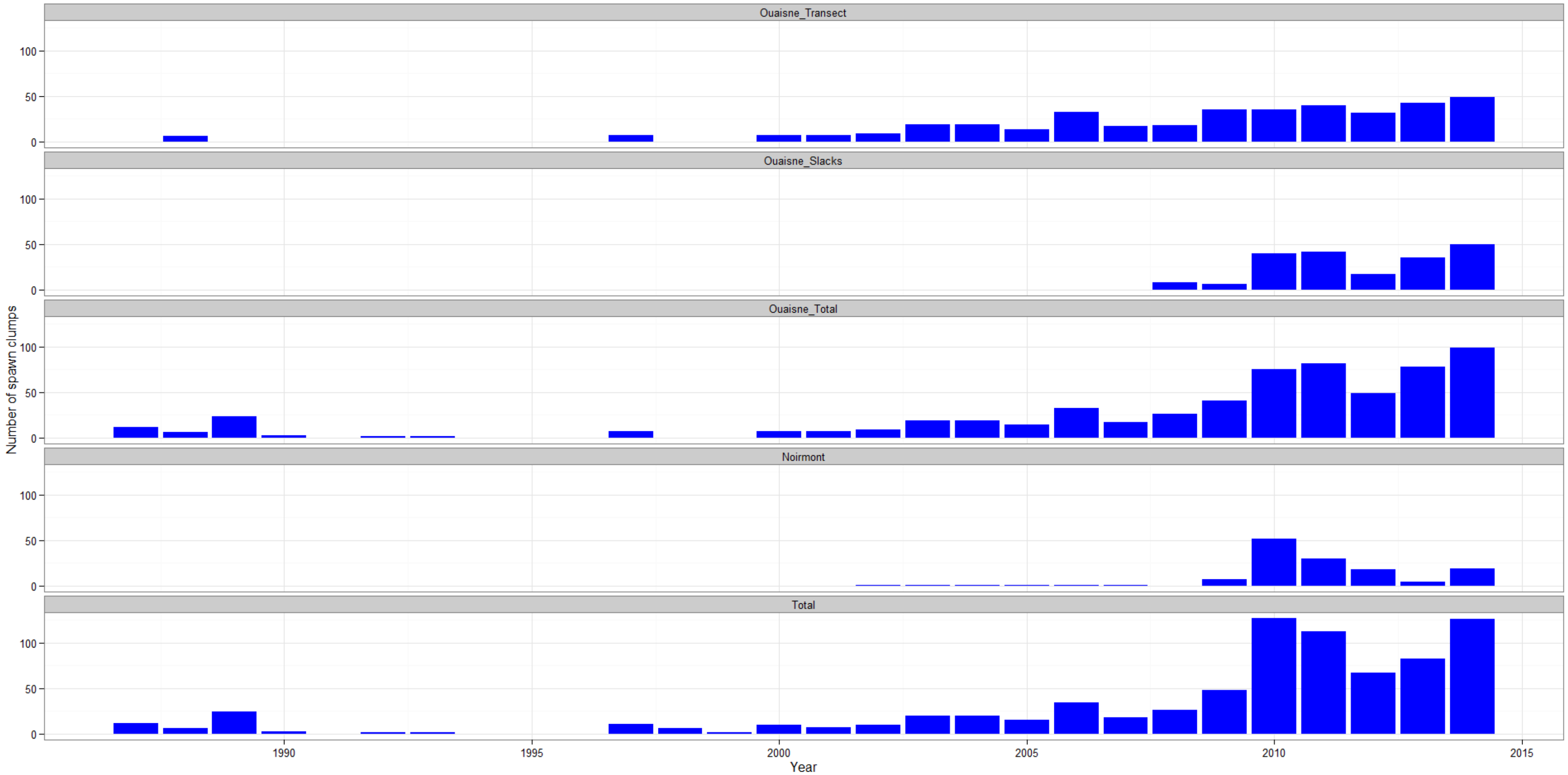


Figure 1.1 Number of spawn clumps per year at Ouaisné Transect, Ouaisné Slacks, Ouaisné Total, Noirmont Pond, and Total.

1.3.2. Peak frog counts over time

Trends in the peak number of frogs (the most counted on one occasion in one season within a site; Figure 1.2) show that there is some indication of an increasing number of frogs each year. Greater detail on sex and age structure would allow for further investigation of recruitment in the population.

Figure 1.2 Peak frog count per year at Ouaisné Transect, Ouaisné Slacks, Ouaisné Total, Noirmont Pond, and Total (2001-2014).

1.3.3. Number of head-started tadpoles released each year

Trends in the number of head-started tadpoles released each year (Figure 1.3) show that slack-level data on releases is limited, particularly as releases were largely constrained to more recent years. As part of the reintroduction strategy, other sites have also received releases in attempts to create a metapopulation; however they are not displayed here. The figure is still included to show the trends over time, and the relatively recent increase in the number released.

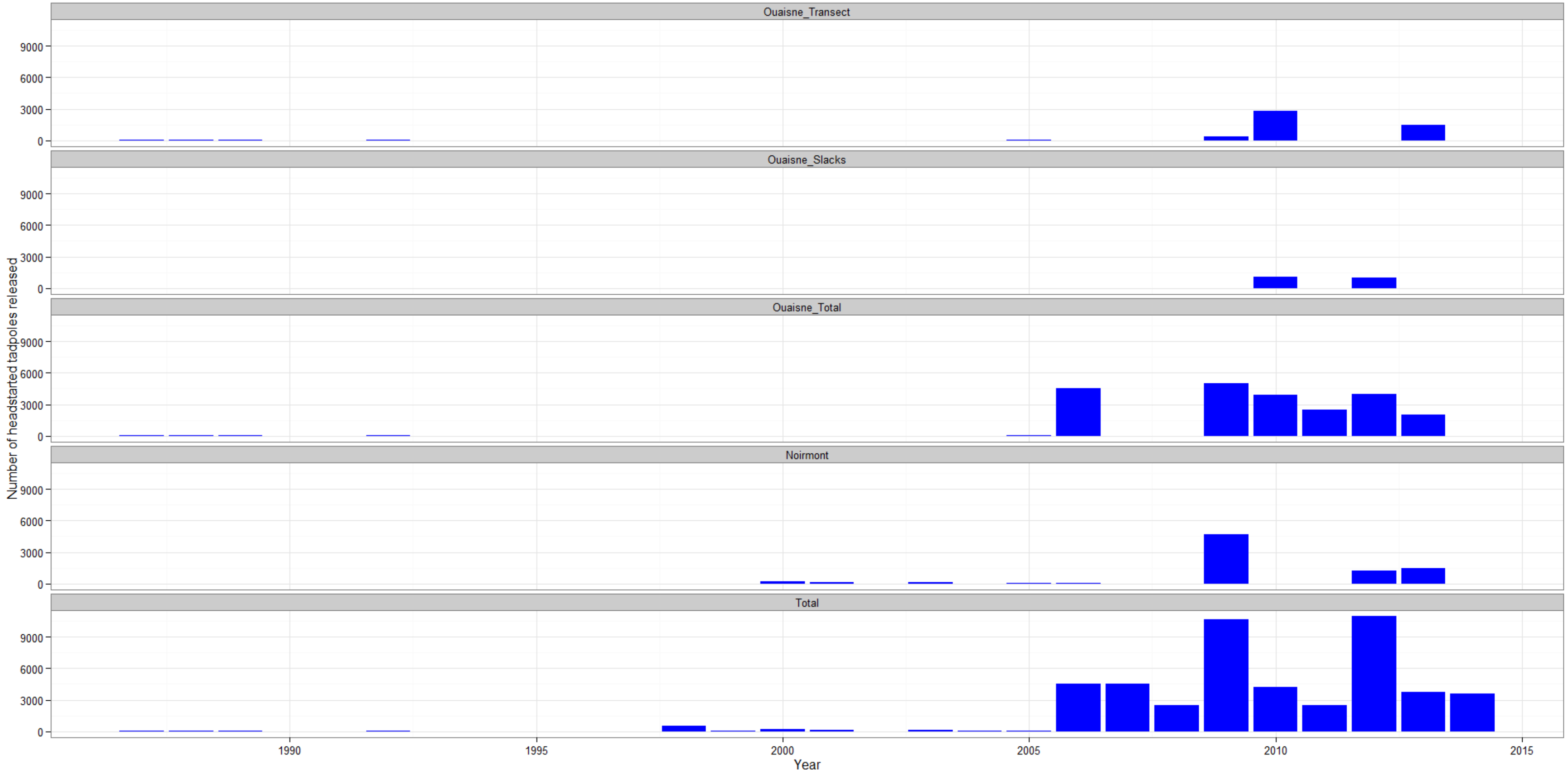


Figure 1.3 Number of head-started tapoles released at each site from 1987-2014.

1.3.4. *Timing of spawning*

The timing of spawning (first, last, and peak; the greatest increase in spawn numbers) from 1997-2014 (Table 1.2, Figure 1.4) on average starts in mid-February, but it can vary by about a month from early February to early March. Likewise, the last spawning occurs in the second half of March on average, but can vary from mid-February through to early April. The peak spawning typically occurs around the end of February and early March, but can also be quite variable from mid-February through to mid-April.

Table 1.2 Summary of spawn timing (days from 1st January each year) for each site (1997-2014).

Site	Timing	Min	Max	Mean	SD
Ouaisné Transect	First	36	64	48.4	8.6
	Peak	44	81	58.5	10.6
	Last	53	91	74.8	9.5
Ouaisné Slacks	First	41	53	49.1	4.4
	Peak	58	74	67.1	6.5
	Last	72	91	80.3	5.9
Ouaisné Total	First	36	64	47.8	8.7
	Peak	47	81	63.8	10.5
	Last	53	91	75.5	9.6
Noirmont	First	33	106	60.8	20.1
	Peak	42	106	66.1	16.5
	Last	42	106	72.5	15.7
Total	First	33	64	46.2	9.3
	Peak	47	81	63.8	10.5
	Last	53	106	77.1	11.9

1.3.5. Duration of spawning

Spawning duration from 1997-2014 (Table 1.3, Figure 1.4) tended to last around 31 days from start to finish, but can extend to twice that length. The shortest spawning periods tend to be at Noirmont. However, this may be due to a limited dataset due to the relatively recent reintroductions there, and the comparatively small population contributing to spawning when compared to the areas at Ouaisné.

Table 1.3 Summary of spawning duration for each site (1997-2014).

Site	Min days	Max days	Mean	SD
Ouaisné Transect	5	46	26.35	12.24
Ouaisné Slacks	21	42	31.14	7.95
Ouaisné Total	5	46	27.71	13.36
Noirmont	<1	43	11.75	14.85
Total	5	66	30.82	16.45

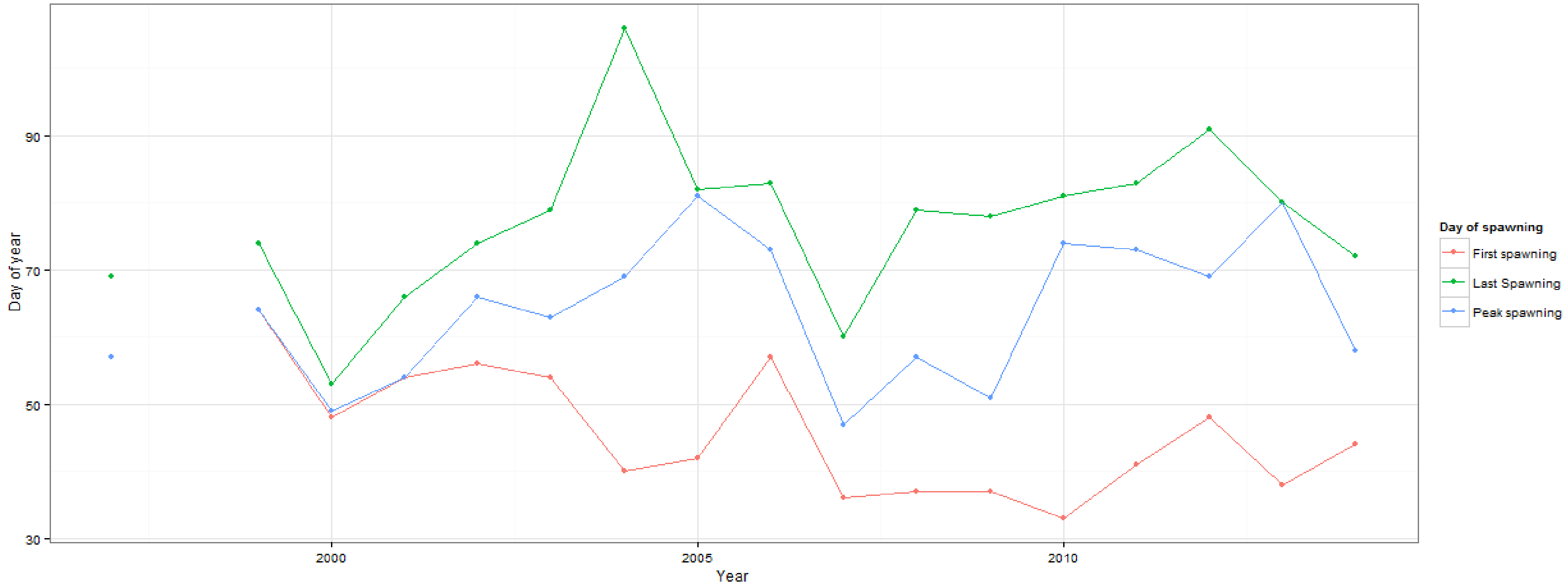


Figure 1.4 Summary of total spawning timing from 1997-2014, displaying the first day of spawning, last day of spawning, and the peak spawning day (the day the greatest increase in spawn occurred). Days are displayed as number of days since the beginning of the year (Julian date). A graph showing the timings at each site can be found in Appendix 8.

1.3.6. *Environmental factors affecting spawning*

We investigated the influences of temperature and rainfall one day and one week before first and peak spawning from 1997 to 2014 (Table 1.4, Table 1.5, Figure 1.5, Figure 1.6). There is a great deal of variability in environmental conditions around spawning, but in general peak spawning is associated with increasing temperatures. In summary, spawning can occur when temperatures fall to nearly zero, and peak spawning occurs when temperatures average 6-10 ^{o}C, with a peak temperature of just over 15 ^{o}C (Table 1.4, Figure 1.5). Rainfall tended to be higher one day before peak spawning and less so for first spawning. Equally, one week before first spawning typically had higher rainfall than peak spawning (Table 1.5, Figure 1.6).

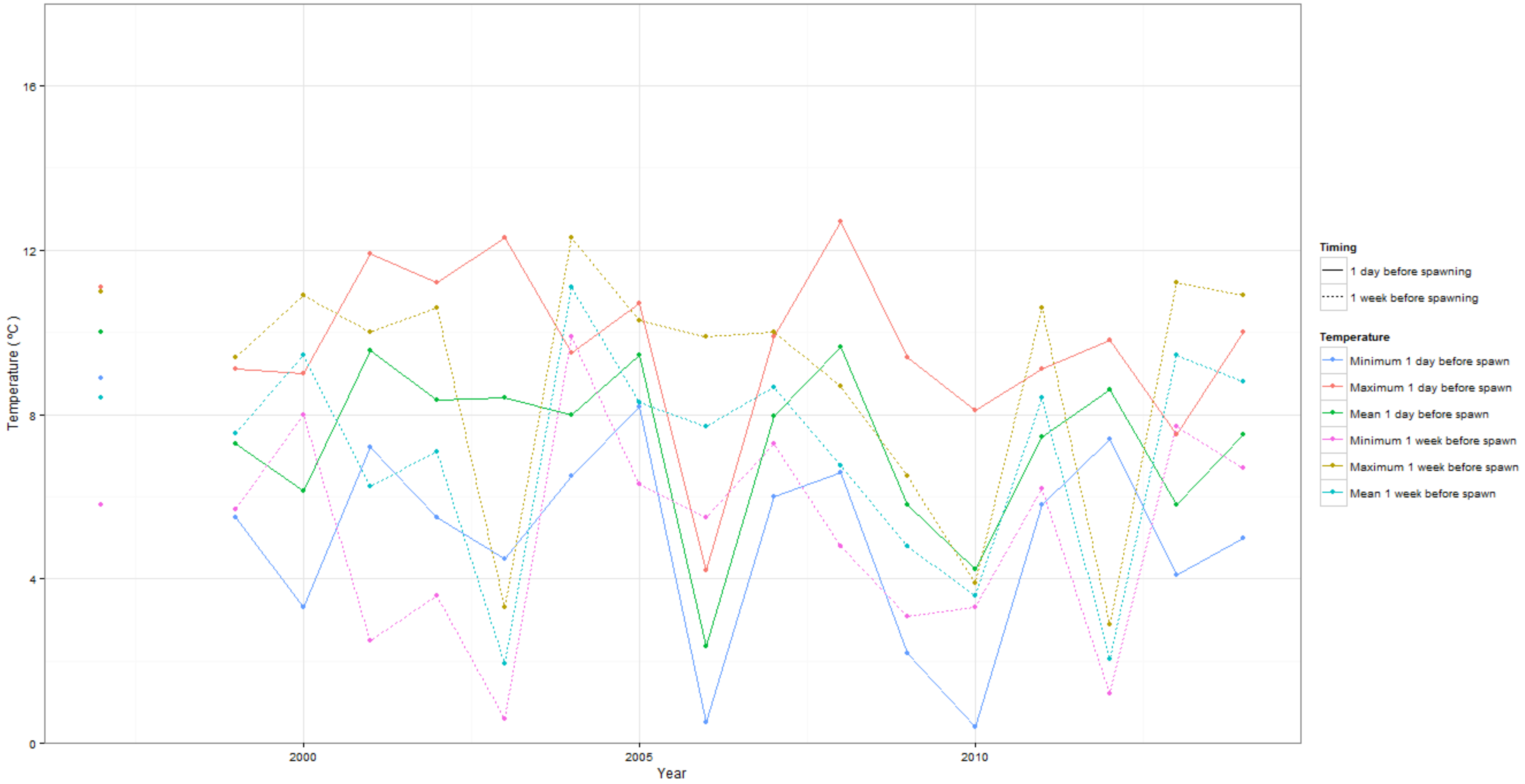
Table 1.4 Summary of temperature influences upon spawning (1997-2014).

Site	Temperature (^{o}C)	Timing of spawn and measurement			
		1 day before	1 day before	1 week before	1 week before
		First	Peak	First	Peak
Ouaisné Transect	Min	0.2	0.6	0.6	0.9
	Max	12.7	15.2	12.3	13.6
	Mean	6.9	7.9	7.4	7.0
Ouaisné Slacks	Min	2.2	2.8	0.9	0.9
	Max	12.1	15.6	10.6	12.7
	Mean	7.3	9.1	6.2	6.5
Ouaisné Total	Min	0.2	0.6	0.6	0.8
	Max	12.7	15.2	12.3	13.6
	Mean	6.9	8.1	7.1	6.9
Noirmont	Min	0.4	2.5	1.2	1.2
	Max	14.9	14.9	12.9	12.9
	Mean	8.0	8.3	7.3	8.4
Total	Min	0.4	0.6	0.6	0.8
	Max	12.7	15.2	12.3	13.6
	Mean	7.4	8.1	7.1	6.9

Table 1.5 Summary of rainfall influences upon spawning

Site	Rainfall (mm)	Timing of spawn and measurement			
		1 day before	1 day before	1 week before	1 week before
		First	Peak	First	Peak
Ouaisné Transect	Min	0.3	0.0	0.0	0.0
	Max	6.8	9.9	12.5	12.5
	Mean	2.4	3.9	3.8	4.1
Ouaisné Slacks	Min	0.0	0.0	0.0	0.0
	Max	9.7	9.2	12.7	6.2
	Mean	3.0	2.8	2.9	1.1
Noirmont	Min	0.0	0.0	0.0	0.0
	Max	10.7	10.7	12.5	7.0
	Mean	3.2	3.0	3.3	2.4
Ouaisné Total	Min	0.2	0.0	0.0	0.0
	Max	6.8	9.9	12.5	8.8
	Mean	2.1	2.6	3.4	3.5
Total	Min	0.0	0.0	0.0	0.0
	Max	9.4	9.9	12.5	8.8
	Mean	2.8	2.6	3.8	3.5

a)



Temperature (°C)
0
4
8
12
16
2000
2005
2010
Year
Timing
1 day before spawning
1 week before spawning
Temperature
Minimum 1 day before spawn
Maximum 1 day before spawn
Mean 1 day before spawn
Minimum 1 week before spawn
Maximum 1 week before spawn
Mean 1 week before spawn

b)

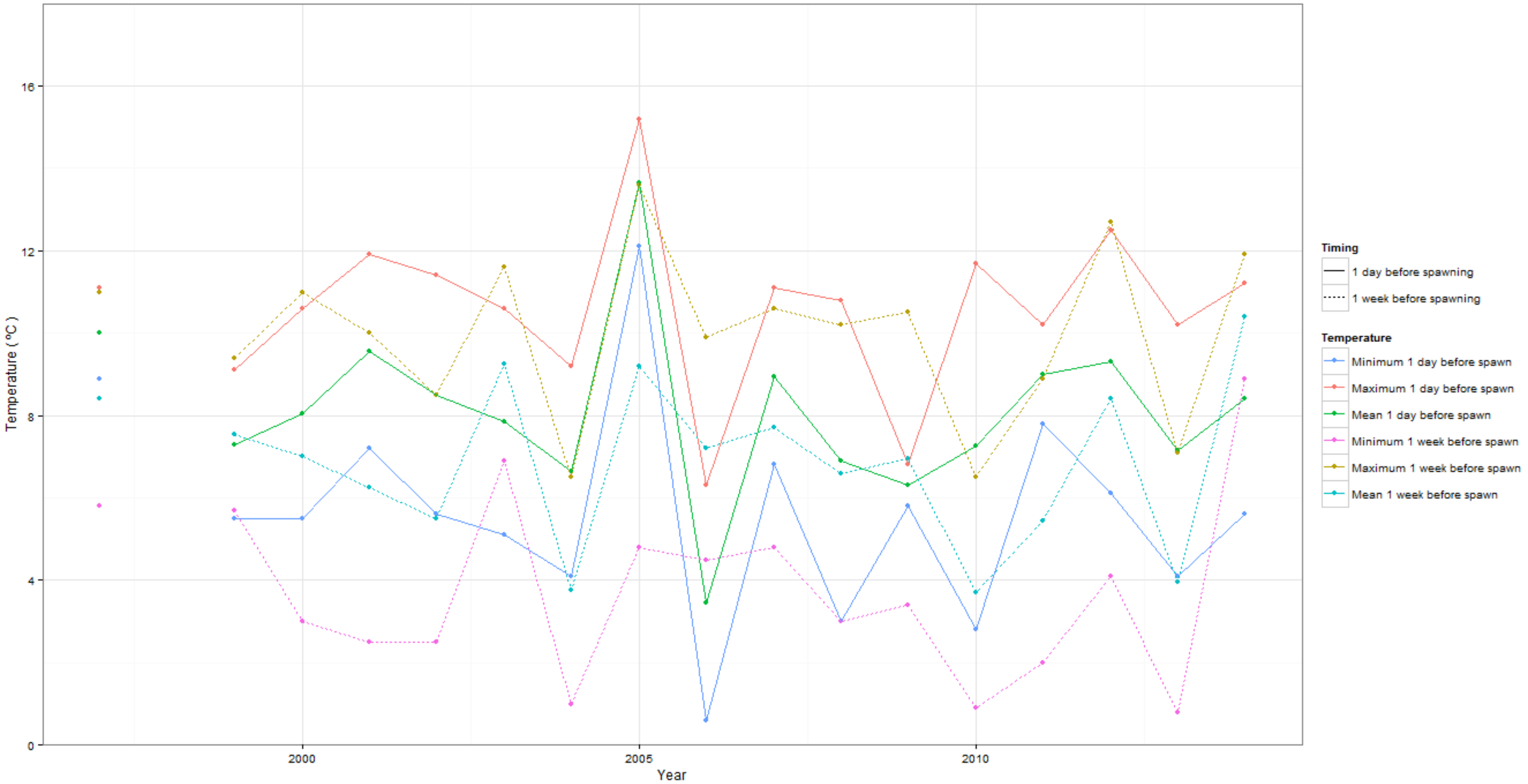


Figure 1.5 Total first (a) and peak (b) spawning thresholds and relationships to temperature (ºC). The spawning thresholds for each site can be found in Appendix 8.2.

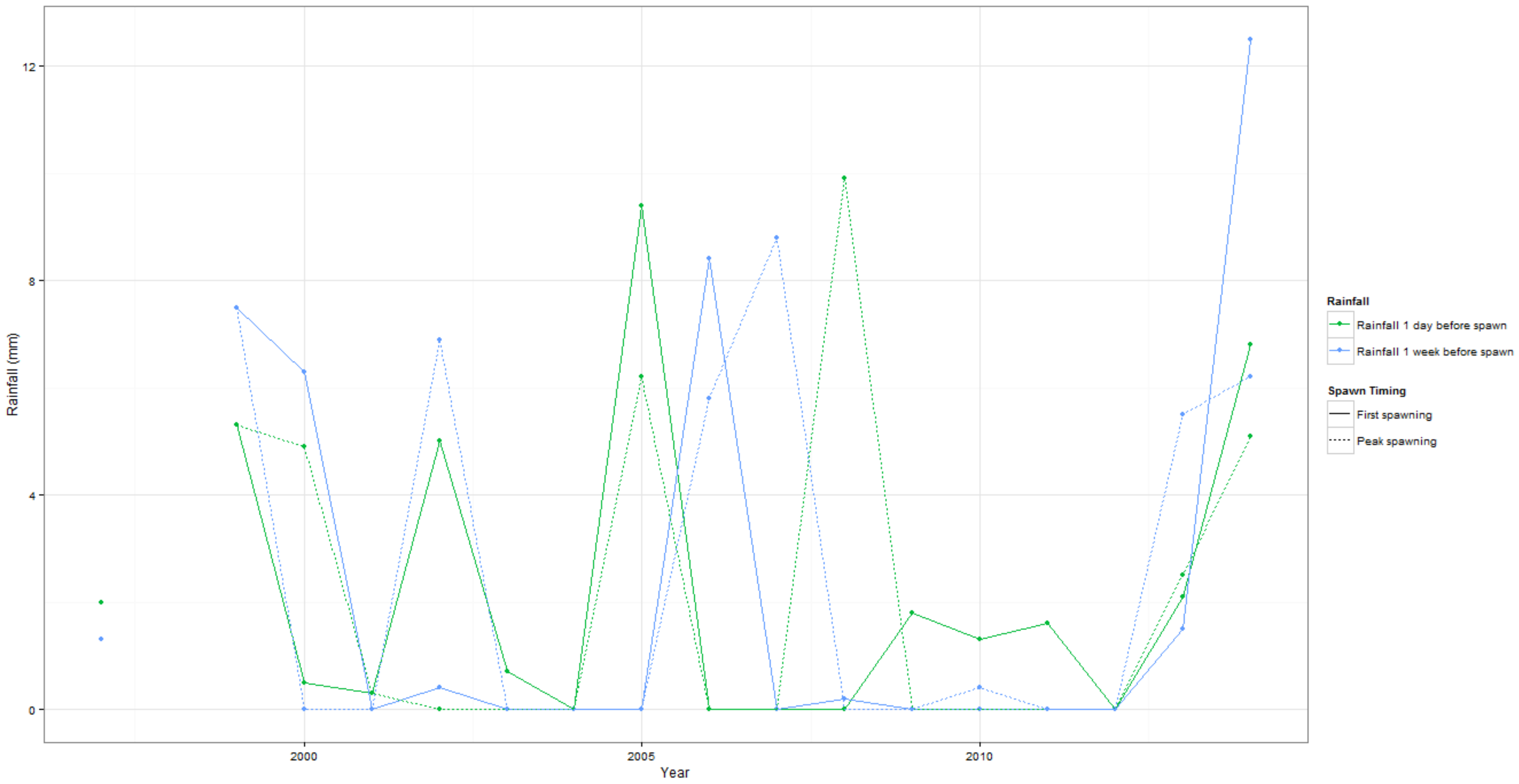


Figure 1.6 Relationship between total spawning periods and rainfall (mm) from 1997-2014. A graph displaying all sites can be found in Appendix 8.3

1.4. Discussion

1.4.1. Spawn clump trends over time

The relationship between the number of spawn and the year is particularly strong at Ouaisné Transect. While remaining significant, the relationship is weaker for Ouaisné Slacks, Ouaisné Total, and Total spawn clumps. These relationships suggest that spawn numbers will continue to increase year on year with the exception of Noirmont where there was no significant relationship. This is likely due to the limited number of years spawn has occurred at Noirmont since the recovery programme was implemented.

Although the trend over time was one of a general increase, there are other factors which influence the number of spawn produced in a given year. It is also worth noting that the R^2 value is affected by the number of data points, and so those sites or site levels with fewer data points will end up with lower R^2 values. Therefore sites and site levels which have had spawn clumps for more years (i.e. Ouaisné Transect, Ouaisné Total and Total) will benefit from a higher R^2 as overall the linear regression line will be closer to a larger proportion of the data points. With this in mind, although the R^2 value is lower at Ouaisné Slacks ($R^2 = 0.45$), this is likely to just be a reflection of the lower number of years that spawn has been recorded there. Moreover, Ouaisné Total ($R^2 = 0.59$) and the Total dataset ($R^2 = 0.55$) have lower R^2 values than Ouaisné Transect ($R^2 = 0.79$) due to the spawning that occurred during the late 1980's; most of which we were unable to allocate to specific slacks from the available data. This spawning will have influenced the fit of the linear regression line, resulting in a poorer fit overall and therefore a lower R^2 value.

From a management perspective, the results suggest that the recovery efforts have been effective in producing a steady increase in clump numbers over time at all sites except Noirmont (but see above). Moreover, the regression equations can be used to make predictions about future numbers of spawn which may allow for greater preparation prior to each season in terms of the resources that may be required. It must be noted however that the sites with lower R^2 values will result in less reliable predictions as the relationship between year and clump number is weaker.

1.4.2. Peak frog counts over time

The use of peak frog data as an indicator of minimum population size has been used in several amphibian studies (e.g., Cooke and Oldham, 1995; Buckley and Foster, 2005). However, it may not be particularly accurate due to the issues associated with spotting and counting frogs. Moreover, without knowing the population demographics it does not give an idea of the effective population size (N_e). Nonetheless it is used here in the absence of demography data.

Overall the peak frog count has increased over the study period albeit with a fair amount of fluctuation. This may be explained by observer bias, shifts in population size over time, or staggered arrival of frogs resulting in a misrepresentation of the number of frogs utilising the slacks. At Ouaisné Transect the peak frog count has been fairly stable since the mid 2000's, whereas Ouaisné Slacks shows a fairly recent increase which could be due to recruitment following release efforts in previous years. Combined, this has resulted in Ouaisné as a whole displaying an increasing trend in the peak frog count, as well as for the Total as the data from Ouaisné has a strong influence on the overall dataset. Noirmont, however, has seen a great deal of fluctuation in the peak number of frogs recorded, which may again be associated with the recruitment taking place from releases in previous years. The recent decrease in peak frog count at this site indicates that releases here should be ongoing in order to supplement and stabilise the population.

1.4.3. Number of head-started tadpoles released

The number of released individuals each year has been fairly irregular, with large peaks in 2009 and 2012. This reflects the larger number of spawn clumps that were collected in these years (27 and 37 respectively). Moreover, these numbers represent a large proportion of the spawn recorded in those years (56.3% and 55.2% respectively). This indicates that conditions in the slacks in those years were poor leading to a greater need for intervention. In particular, it is noted that there was a water shortage in these years (T. Liddiard, pers. comm. 2015). When taking this into account, the number of individuals released per year since 2006 has otherwise been fairly stable at around 2500 - 4500 individuals; the equivalent of 10 – 24 clumps per year.

A further factor affecting the number of spawn clumps removed for head-starting and the resulting releases is that of the capacity of the head-starting facilities at Durrell Wildlife Conservation Trust (DWCT) as well as the resources they have to manage the captive population.

1.4.4. Timing of spawning

The results suggest that monitoring of the slacks should begin in early February, although other parameters such as temperature and rainfall should be taken in to account. Monitoring of spawn deposition should then continue until the end of March, after which it is unlikely further spawning will occur. Beyond this time, monitoring should focus on the success of the spawn taking note of the time taken until hatching occurs as we have little data on this. However, Racca (2004) reported hatching to begin two to four weeks after spawn was laid, with hatching lasting up to 22 days for any individual spawn. These data allow for estimates to be made of hatching in the field. The peak spawning period was shown to occur between mid-February and mid-March, and so counts could be focused around this period when resources for surveying the whole season are limited.

There was some variation between the sites (Ouaisné and Noirmont) and site levels (Transect and Slacks), with Noirmont in particular displaying the most variation in timing over the years. However, this was due to a single clump laid at Noirmont in 2004 which was later than any other spawning event recorded (day 106). Otherwise spawning tends to occur at a similar time across the sites, although spawning at Ouaisné Slacks tended to be later than other sites. The reasons for this are unknown; however they may be associated with water level (i.e. the Main Pond holding water all year round).

1.4.5. Duration of spawning

As the population has increased over the years so has the duration of the spawning. In general, the spawning period rarely exceeds six to seven weeks in length, but in the earlier periods of monitoring the spawning period could occur in less than one week. There was little variation in the spawning duration between the sites, with a typical duration of around four weeks at all sites except Noirmont which hosts a smaller population that can contribute to spawning events. As the population increases at Noirmont we would also expect the spawning period to be of similar duration to that observed at Ouaisné.

1.4.6. Environmental factors affecting spawning

Environmental conditions were variable in relation to spawning, although peak spawning tended to occur at higher temperatures than first spawning. Spawning does still occur on days when temperatures fall to just above freezing, although it appears that mean daily temperatures above 6 ^{o}C are preferable, with maximum temperatures for spawning at around 15 ^{o}C. Levels of rainfall were fairly similar across sites one day before first and peak spawning, with marginally higher rainfall one day before peak spawning overall. Rainfall one week before first spawning was consistently greater or equal to that experienced one week before peak spawning, indicating that rainfall may be an important trigger for agile frogs to head to their spawning grounds.

There was some variation in temperature between the sites, with Ouaisné Slacks tending to have warmer minimum temperatures one day before spawning than at other sites. Nevertheless, maximum and mean daily temperatures one day before first and peak spawning were fairly similar across all sites. These similarities were also reflected for minimum, maximum and mean temperatures when investigating daily temperatures one week before first and peak spawning.

Environmental data collected locally at the slacks would allow for a more detailed insight in to the conditions that trigger and influence spawning events.

2. Survey methods

2.1. Current survey protocols

Current survey methods consist of three surveys being conducted per week; one at night, and two during the day. Evening visits are used to assess the numbers of spawn clumps present with incidental recording of adult frogs as well as newts, toads, and predators such as ducks. Daytime visits are used to carry out any spawn management (protection or removal) that may be required, as well as to act on any threats such as water pollution incidents and desiccation of water bodies.

The environmental variables collected during surveys are largely based on those used by Racca (2004), but at a coarser scale of monitoring than could be achieved by a dedicated researcher due to resource limitations.

As surveys are not necessarily conducted every night during the spawning period, it has been difficult to draw relationships between the number of spawn clumps and a specific date and its associated conditions, as the spawn may have been present for several days prior to survey.

Details on the number of spawn clumps left in-situ that were either protected, or not protected, were not stored in a manner by which comparisons can be easily made between methodologies and their success. Thus they were not analysed in this report (but see section 3). Issues also occur with the delimitation of each slack, as these are dependent on water levels and surveyor bias, the current classifications may not provide enough consistency to conduct reliable assessment of each slack over time.

2.2. Recommendations for improvement

The existing survey methods could be improved in a number of ways, but it is important to consider the resources required for surveying. With this in mind, we suggest three different levels of survey effort (Table 2.1) which can be applied depending on the resources available. Existing methods for surveying in terms of movement of spawn and biosecurity should be maintained from existing protocols.

Table 2.1 Suggested levels of survey and recording effort depending on resources available. Categorical variables are shown in ***bold italics***.

Survey effort		
Basic [a]	***Intermediate*** [b]	***Advanced*** [c] *
SURVEY FREQUENCY		
One night-time visit per week.	Two night-time and two day-time visits per week.	Nightly surveys and two day-time visits per week.
COUNTS / RECORDING		
(i) Spawn clumps, number of frogs, and other species** to be counted	**(i) +** **(ii)** Previously recorded spawn presence or absence checked **(iii a)** Clumps given unique ID at **selected** slacks*** **(iv)** Position of frogs and other species (***water; land; other***) **(v)** Details on lifestage (***spawn; adult; juvenile; metamorph; tadpole; calls; unknown***)	**(i) + (ii) +** **(iii b)** Clumps given unique ID at **all** slacks **(iv) + (v) +** **(vi)** sex (***male; female; unknown***), behaviour (***amplexus; migration; calling; NA***) and health (***live; sick/injured; dead***) recorded for all species of interest
CLUMP LOCATIONS MAPPED		
(i a) Marked on site map (Appendix 9.3). Overwrite each site map each time. Improved slack delimitation	**(i b)** Marked on site map including management (Appendix 9.3). Save new map for each visit. Improved slack delimitation	**(i b)** Marked on site map including management (Appendix 9.3). Save new map for each visit. Improved slack delimitation
SPAWN CONDITION AND MANAGEMENT		
—	**(i)** spawn condition assessed by embryo colour (***dark-brown*** - *live*; ***white/grey*** - *dead*; ***cloudy jelly / cotton-wool appearance*** - *unfertilised/fungal infection*) **(ii)** Management strategy recorded (***left in-situ; protected - branch placement; protected - mesh wrapped; protected - moved to cage; translocated - within site; translocated - to other site; removed for head-starting***)	**(i) + (ii) +** **(iii)** Predation (***yes; no; unknown***) **(iv)** Proportion hatched at end of season (***none; 1-25% hatched; 26-50% hatched; 51-75% hatched; >75% hatched; unknown***) **(v)** Spawn diameter (cm) **(vi)** Date of hatching
ASSESSMENT OF WATER BODIES AT THE SITE		
Water level (***low*** - *water level less than a third of maximum*; ***medium*** - *water level one to two thirds of maximum*; ***high*** - *water level above two thirds of maximum*)	**(i)** Water levels recorded using water gauge pre-placed at deepest point in slack.	**(i) +** **(ii)** Pond shape/extent drawn on to map.
*ENVIRONMENTAL DATA FROM MET OFFICE***** (*min, max, and mean daily temperature (ºC); min, max, and mean precipitation (mm); moon phase*)		
✓	✓	✓
SPAWN CLUMP POSITION IN RELATION TO WATER BODY		
—	—	**(i)** Depth of water (cm) at point of spawn clump **(ii)** Depth of clump (cm) from water's surface to top of clump **(iii)** Shortest distance from clump edge to water's edge (cm)

Continued on next page

Survey effort		
Basic [a]	***Intermediate*** [b]	***Advanced*** [c] *****
	LOCAL ENVIRONMENTAL DATA (*General weather conditions; % cloud cover; wind; min, max, mean local air temperature (ºC); Min, max, mean water temperature (ºC)*)	
(i) General weather conditions (***0 - clear***; ***1 - overcast; 2 - hazy***; ***3 - fog***; ***4 - drizzle***; ***5 - rain***; ***6 - hail***; ***7 - snow***) **(ii)** % cloud cover - estimated to nearest 10%	**(i) + (ii) +** **(iii)** Wind (***0 - smoke rises vertically; 1 - wind direction shown by smoke drift; 2 - wind felt on face; leaves rustle; 3 - leaves or small twigs in constant motion; 4 - raises dust and loose paper; small branches move; 5 - large branches in motion***) **(iv a)** Min, max, mean local air temperature (ºC) over survey period with max/min thermometer	**(i) + (ii) + (iii) +** **(iv b)** Min, max, mean local air temperature (ºC) daily over the season with datalogger*** **(v)** Min, max, mean water temperature (ºC) daily over the season with datalogger***
	HABITAT MANAGEMENT RECORDED	
—	Details of habitat management recorded which may influence the suitability of each slack	Details of habitat management recorded which may influence the suitability of each slack

[a] data recorded on to site map (Appendix 9.3)

[b] data recorded on to site map and recording form '***4. Anuran survey form Intermediate***' (Appendix 9)

[c] data recorded on to site map and recording form '***5. Anuran survey form advanced***' (Appendix 9.2)

* The advanced level of survey is likely to require a dedicated researcher

** To include toads, newts, and predatory species such as ducks

*** We recommend north and south slack, and Noirmont as the focal areas for detailed spawn monitoring. As clump numbers increase or available resources change, the number of areas monitored in this way can be adjusted.

**** Data can be downloaded at the end of the season and the associated spreadsheet filled in.

2.3. Survey timing

Surveys to detect clumps should begin in early February; however the start of spawning will fluctuate depending on temperature and rainfall, with temperatures needing to be above 0°C, and in some cases can occur as late as early March. Moreover, spawning usually follows or coincides with rainfall which triggers activity in the amphibians. Monitoring of spawn deposition should then continue until the end of March, after which it is unlikely further spawning will occur. The final spawning normally occurs in the second half of March, but can vary from February through to early April. Therefore if wishing to just detect the final number of spawn, survey effort can be focused between late February and April. Peak spawning typically occurs between late February and late March, and so visits in this period should allow for detection of maximum spawning activity.

It has also been noted that inconsistencies have occurred between partner organisations involved in the project regarding spawn data counts, and so we recommend in future that greater efforts are made to standardise the recording procedure. The recommendations made below in section 3 regarding giving spawn clumps unique ID numbers should remove the potential for this issue to occur in future.

Spawn survival (hatch rates and predation) should be recorded at the time of hatching to give an indication of the success of each monitored spawn clump in conjunction with details on the way in which they were managed. This will then allow future analysis of the effects of different management strategies as well as differences between slacks in survival.

2.4. Data recording / storage

A limiting factor for the analysis carried out for this report was the inconsistency in collection and reporting of the data. In order for future analyses to be conducted effectively, and with comparative ease, it is of utmost importance that the data are collected in a standardised way.

To address this issue template recording forms are provided in electronic format (*Agile frog monitoring.xlsx*, *Ouaisné Spawn Map Template 2015.doc*, and *Noirmont Spawn Map Template 2015.doc*) as well as being displayed in Appendix 9. Furthermore data should be stored so that it can easily be retrieved for analysis. A new spreadsheet is therefore provided (*Agile frog monitoring.xlsx*) which will allow for data to be extracted easily for future analysis. This spreadsheet has been designed for ease of use and data extraction. Improvements to slack delimitation have already been addressed through placement of stakes with reflective tape to allow field recorders to identify a given position in the slack with ease (as marked on the spawn mapping sheets in section 9.3).

3. Spawn management strategies

3.1. Current spawn management options

Three strategies are currently in place to improve egg and larval survival:

i) Spawn protection in the form of spawn wrapping or the use of cages

ii) Spawn removal for head-starting at Durrell Wildlife Conservation Trust

iii) Branch placement to provide some protection from ducks

Each method has associated costs and benefits in terms of resources, and the impact upon tadpole survival and subsequent effective population size (Table 3.1). The effect of branch placement upon spawn survival is not included here, but it warrants further research.

Table 3.1 Summary of associated costs and benefits of different spawn management strategies (based on data from Racca, 2004 and Jameson, 2009)

<table>
<tr><th></th><th colspan="3">Management Strategy</th></tr>
<tr><th rowspan="2"></th><th rowspan="2">Spawn protection</th><th colspan="2">Head-starting</th></tr>
<tr><th>Early release</th><th>Late release</th></tr>
<tr><td>Benefits</td><td></td><td></td><td></td></tr>
<tr><td>Protection from predators</td><td>✓</td><td colspan="2">✓✓</td></tr>
<tr><td>Increased survival</td><td>✓</td><td>✓</td><td>✓✓</td></tr>
<tr><td>Increased mass</td><td>O</td><td>✓</td><td>✓✓</td></tr>
<tr><td>Increased snout-vent length</td><td>O</td><td>✓</td><td>✓✓</td></tr>
<tr><td>Increased body condition</td><td>O</td><td>✓✓</td><td>✓</td></tr>
<tr><td>Costs</td><td></td><td></td><td></td></tr>
<tr><td>Financial</td><td>✓</td><td colspan="2">✓✓</td></tr>
<tr><td>Time</td><td>✓</td><td colspan="2">✓✓</td></tr>
<tr><td>Effort</td><td>✓</td><td colspan="2">✓✓</td></tr>
</table>

'O' - no effect, ✓ - some effect, ✓✓ - large effect

3.2. Analysis

Analysis of spawn management strategies based on the "*Agile Frog Data 1997-2014 For Analysis.xlsx*" file was not possible due to the inconsistencies in recording and the way in which the data are presented. It is therefore not possible to follow each clump from start to finish in terms of its management, and its resulting success or failure. Instead we investigated the effects of releasing head-started individuals in to the population (3.3.1), undertook analysis on the number of clumps protected from the spawn location maps recorded each year (3.3.2), and focus on the results from work carried out by Jameson (2009).

Future monitoring should include marking of each spawn clump so that it can be uniquely identified. Any spawn management should then be recorded and the resulting hatching success and survival categorised (see Table 2.1). Such monitoring is time consuming, and so we recommend only carrying it out on selected slacks where regular monitoring occurs (e.g. Ouaisné Transect). Furthermore, as the number of clumps continues to increase over time, the number of slacks being monitored in this way may need to be reduced due to the available resources for doing so.

3.3. Results

3.3.1. Number of spawn clumps related to releases of head-started tadpoles / metamorphs

Relationships between the number of clumps laid and releases in previous years were assessed using linear regression. Missing cases were excluded from the analysis (Table 3.2). Releases over the three years prior to the survey were positively related to the number of spawn clumps (Figure 3.1). Consequently, the number of spawn clumps per year appears to be related to the number of head-started tadpoles released in earlier years. These effects may not be obvious at the individual pond / slack level due to the limited data available for site-specific releases. The regression equations are available in Appendix 7.

Table 3.2 Relationships between the number of tadpoles/metamorphs released in previous years and the number of spawn clumps

Site	Release year	R^2	*F*	DF	*p*
Ouaisné Transect	-1	0.44	7.072	9	<0.05
	-2	0.26	2.759	8	>0.05
	-3	0.39	5.031	8	>0.05
Ouaisné Slacks	-1	0.996	974.4	3	<0.001
	-2	0.66	5.696	3	>0.05
	-3	1	1.829e+31	2	<0.001
Ouaisné Total	-1	0.47	10.7	12	<0.01
	-2	0.60	16.72	11	<0.01
	-3	0.47	8.966	10	<0.05
Noirmont	-1	0.95	124.9	6	<0.001
	-2	0.88	38.21	5	<0.01
	-3	0.86	25.58	4	<0.01
Total	-1	0.59	36.2	25	<0.001
	-2	0.67	48.02	24	<0.001
	-3	0.41	16.27	23	<0.001

These relationships were further investigated using multiple regression to determine which years of release were the best predictors of spawn clump numbers in subsequent years. Due to limited data at individual sites, analysis was only possible for the combined data for all sites (Table 3.3). When the number of clumps was predicted it was found that individuals released in the previous year (β = 0.44, p < 0.001) and individuals released two years before (β= 0.54, p < 0.001) were significant predictors. Individuals released three years before was not a significant predictor (β= 0.15, n.s.). The overall model fit was R^2 = 0.88, demonstrating that the number of individuals released in the three previous years accounts for 88% of variation in the number of spawn clumps. The number of head-started individuals released two years prior to the spawn counts appears to have the biggest influence on the model, but releases one year previous to spawn also make a significant contribution.

Table 3.3 Results from multiple regression of tadpole / metamorph releases one, two, and three years before upon clump numbers

	ΔR^2	*B*	*SE B*	β	*p*
Constant	0.88	4.66	3.64		>0.05
Release in previous year		0.006	0.001	0.44	<0.001
Release two years previous		0.007	0.001	0.54	<0.001
Release three years previous		0.002	0.002	0.15	>0.05

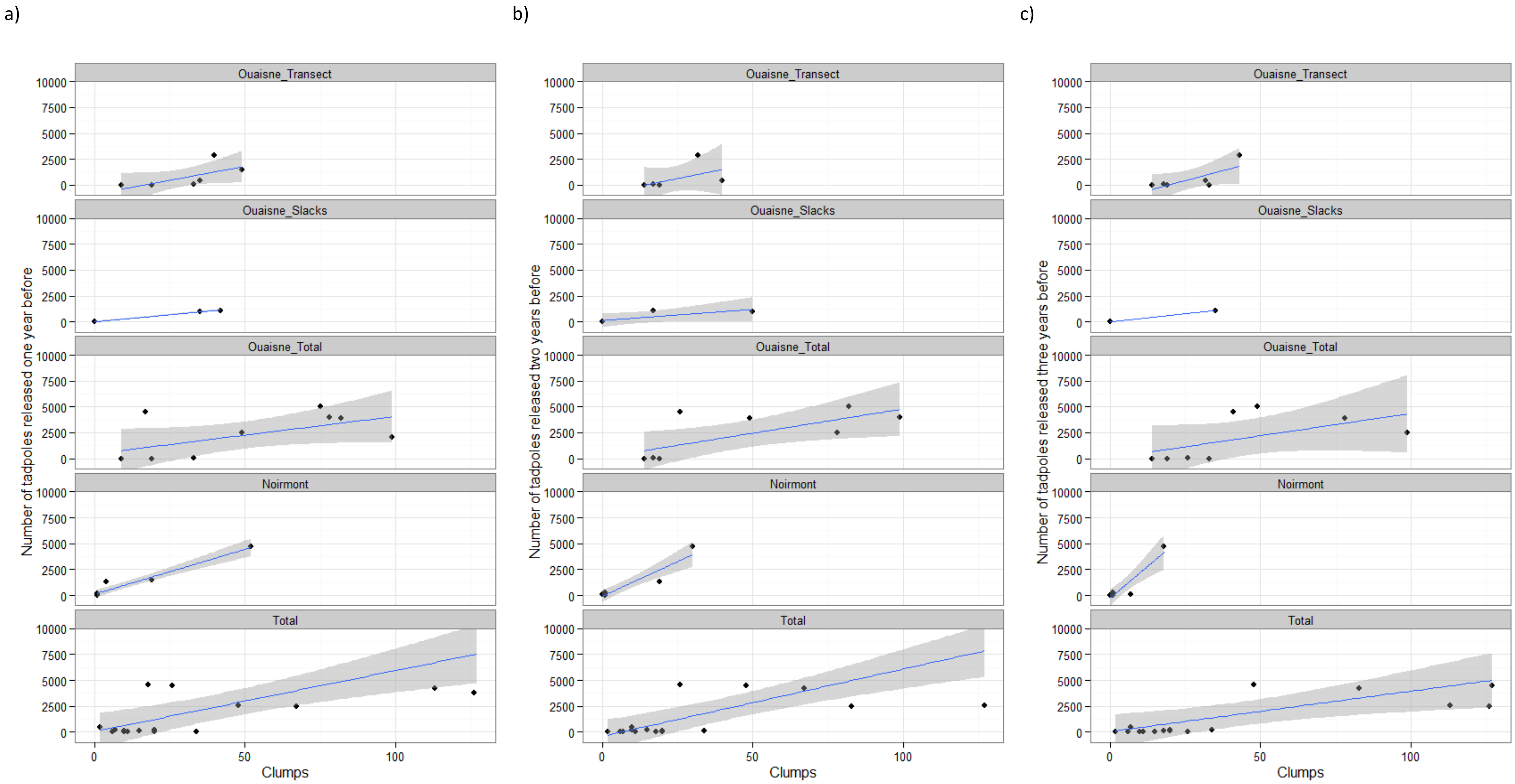


Figure 3.1 Relationship between the number of clumps and head-started tadpole releases in a) one, b) two, and c) three years previous. Graphs display a linear regression line with 95% confidence interval. The graphs show that at all sites, there has been a positive relationship between the number of spawn clumps found in a given year, and the number of head started individuals released in previous years.

3.3.2. Number of spawn clumps related to number of spawn protected in previous years

Relationships between the numbers of spawn clumps and spawn protection in previous years was assessed using linear regression. Data were extracted from spawn location maps to give numbers of spawn protected each year. These data were only available from 2005 to 2014, with no spawn shown as protected in 2007 or 2010. Missing cases were excluded from the analysis. Significant relationships were not present between the number of clumps protected and the number of spawn clumps found in subsequent years, apart from at Ouaisné Slacks (Table 3.4, Appendix 7.3).

Table 3.4 Relationships between the number of spawn clumps protected in previous years and the number of spawn clumps.

Site	Protection year	R^2	*F*	DF	*p*
Ouaisné Transect	-1	0.531	4.53	4	>0.05
	-2	0.171	0.619	3	>0.05
	-3	0.590	2.882	2	>0.05
Ouaisné Slacks	-1	0.200	0.501	2	>0.05
	-2	0.997	363	1	<0.05
	-3	0.787	3.704	1	>0.05
Ouaisné Total	-1	0.060	0.386	6	>0.05
	-2	0.103	0.573	5	>0.05
	-3	0.018	0.071	4	>0.05
Noirmont	-1	-	-	-	>0.05
	-2	-	-	-	>0.05
	-3	-	-	-	>0.05
Total	-1	3.412e-05	0	6	>0.05
	-2	0.146	0.851	5	>0.05
	-3	0.047	0.195	4	>0.05

3.3.3. Previous studies

The study carried out by Jameson (2009) found some variation in survivorship in cohorts originating in different slacks (North and South) (X^2=28.9, df=1, p<0.001). Overall however, head-started tadpoles were found to have greater survivorship than those left in-situ (X^2=124.4, df=1, p<0.001). Furthermore, unprotected clumps in-situ produced a greater number of metamorphs than those that were protected (X^2=179.4, df=1, p<0.001), but this may have been confounded by differences between the slacks in predators.

The timing of release (early or late) of head-started tadpoles had little effect upon survivorship (X^2=3.0, df=1, p>0.05), although the cohort from South Slack had greater survivorship in the late-release (X^2=8.5, df=1, p=0.01).

Late-release tadpoles generated larger metamorphs than the early-release cohorts, which in turn had greater mass than those left in-situ (Friedman Test; North Slack (protected) X^2=26.0, df=2, n=19, p<0.001, South Slack (unprotected) X^2=25.2, df=2, n=20, p<0.001). Furthermore, snout-vent length followed the same pattern (North slack, X^2=24.0, df=2, n=19, p<0.001 and South slack, X^2=27.1, df=2, n=20, p<0.001) as just described for mass.

The North slack early-release cohort had the highest body condition index (BCI = mass/length^3 x 1000) (X^2=13.1, df=2, n=19, p<0.001), with late-release head-started tadpoles having the second best BCI. In the South Slack however no statistical difference was found (X^2=4.9, df=2, n=20, p=0.086) (Jameson, 2009).

3.4. Discussion

3.4.1. Number of spawn clumps related to releases of head-started tadpoles / metamorphs

Releases of head-started tadpoles / metamorphs in the three years previous to a spawn count were good predictors of subsequent spawning. In particular, the number of individuals released two years previously had the greatest effect on the resulting number of spawn. At local site levels however, these trends were not always clear which is likely due to the low number of years that releases have occurred (Table 3.2, Figure 3.1).

The use of multiple regression produced similar results, providing evidence that the number of individuals released in previous years has a significant impact on future spawning trends. Moreover, releases two years prior to spawning have the greatest effect upon the subsequent number of spawn clumps. This is consistent with males maturing at 20-21 months (SVL of 42-63 mm, mass 8-30 g) (Riis, 1997). In comparison, females mature at 55-73 mm (SVL), and have been shown to weigh between 19-54 g when laden with eggs, and 12-35 g following oviposition (Riis, 1997). This suggests that frogs take two years to reach sexual maturity. These ages of maturity are also reported in previous studies (Racca, 2004; Sarasola-Puente *et al.,* 2011). However, Sarasola-Puente *et al.* (2011) commented that occasionally sexual maturity was attained in males at only one year of age. Although females generally reached sexual maturity at two years of age some females were not mature until three years of age (Sarasola-Puente *et al.,* 2011). Racca (2004) also noted that some non-breeding one year old males return to breeding ponds so counts of individuals during breeding the season should ideally include classification of age structure (Table 2.1).

3.4.2. Number of spawn clumps related to number of spawn protected in previous years

The analysis carried out to investigate the influence of spawn protection on future number of clumps resulted in insignificant results. This may be due to the low number of spawn clumps protected (67 over the nine year period) and low statistical power. As suggested in section 2, a comparison of the success of different management strategies would be of greater use for informing future management decisions.

3.4.3. Previous studies

These results show that head-starting is the most effective strategy for tadpole survival, with late-release cohorts being marginally more effective in terms of survival. Little evidence was produced to indicate that in-situ protection is a beneficial spawn management strategy aside from protected spawn resulting in larger metamorphs than unprotected spawn (Jameson, 2009). It is worth noting however that this study did not assess predator density or abundance which could have led to differences between slacks. Similarly, previous assessment of spawn protection carried out in 1998-1999 found it to be an ineffective strategy, with the protective enclosures proving harmful to the eggs (Racca, 2004). The study carried out by Racca (2004) however suggested that spawn protection resulted in an increase in recruitment, with 2.4% recruitment in 2001, increasing to 17.1% and 7.5% recruitment in 2002 and 2003 respectively when spawn was protected. Furthermore, Population Viability Analysis (PVA) carried out by Racca (2004) highlighted the positive impact spawn protection had upon the population (extinction risk = 0.02; median time to extinction > 50 years), as without it models predicted that the viability of the population was greatly diminished (extinction risk = 1.0; median time to extinction = 10 years). Moreover, Racca (2004) states that enhanced productivity can be achieved through spawn protection, and because it is inexpensive, the strategy should be continued.

In consideration of these mixed results, we recommend further investigation into the effects of spawn protection, particularly as there may be underlying effects contributing to the results presented by Jameson (2009) such as predation. Possible considerations when studying the effects of spawn protection are that the spawn in protective cages or bags may be exposed to density-dependent factors affecting survival. Spawn protected in bags will also receive a greater amount of disturbance through handling which may influence egg and tadpole survival (Jameson, 2009).

Despite the need for further investigation of spawn management strategies, we recommend continuation of both spawn protection and head starting where resources allow (see section 3.5).

3.5. General discussion and management recommendations

There are a number of associated costs involved in protecting or headstarting spawn, and so it is even more pertinent that clear population targets are identified so that resources can be applied elsewhere, and a monitoring phase can be entered. As a site reaches carrying capacity, the need for intervention may be greatly reduced for the purpose of securing that particular population. However, management of spawn may still be beneficial in providing low level supplementation and protection to a population, as well as providing a source population for releases and translocations into other areas as well as opportunities for research.

Racca (2004) undertook Population Viability Analysis (PVA) during her PhD work on the agile frog population, and from this was able to make suggestions that between 20 and 120 individuals would be required to create a viable population in the medium term (extinction risk < 0.05, over 50 years) at any given site. Moreover, this would be best achieved using captive-bred metamorphs, or to a lesser extent, individuals that have received protection (Jameson, 2009) which exhibit greater fitness than spawn which is not managed. Better still is the use of one-year old frogs when available as they have a higher rate of survival than metamorphs (Racca, 2004), with all stages being released over a three-year period when translocating to new areas (Racca, 2004). Racca (2004) also stated that up to 60 metamorphs per three-year period could be used directly from Ouaisné for translocations which could in the long-term negate the need for head-starting. However, this should only be done if spawn protection is still in place in order to maximise success of clumps.

Of all the sites used by Jersey's agile frog population, Ouaisné is currently the most important in terms of its population, which has grown considerably since interventions began. The assumed carrying capacity for this site is around 200 individuals (Racca, 2004). In 2014 99 spawn clumps were recorded at Ouaisné as a whole, and this record is set to be broken in 2015. If we consider that each reproductive female produces one spawn clump, then we can safely assume that there are at least as many mature female frogs on a site as there are spawn clumps in a given year. Although Racca (2004) had to assume a sex ratio of 1:1 in her PVA work due to limited field data, other research carried out in Europe has found adult sex ratios during breeding aggregations to be male biased with a mean of 2.12 ± 0.41, with the operational sex ratio also being male-biased (Lodé *et al.,* 2005). These data would suggest that given the number of clumps recorded at Ouaisné now each year, the site is at - or at least very close to - its carrying capacity.

Further consideration must be given to the survival rate of individuals within the population, as this has an influence on the need for supplementation. Using the Robson and Chapman method (Krebs, 1999), Racca (2004) calculated an annual adult survival rate of 0.23 (based on males due to lack of females caught). The use of skeletochronology produced a slightly higher estimate of adult survival (0.32), and so the survival rate used in Racca's (2004) models was 0.275 ± 0.064. These rates are very low compared to survival studies on other frog species which have survival probabilities of 0.38 - 0.68 (Gibbons and McCarthy, 1984; Biek *et al*., 2002). These low survival rates for Jersey's frogs led to Racca (2004) commenting that it would be necessary to have constant metamorph recruitment in order to produce a viable population.

Based on the points above, we recommend that Ouaisné enters a monitoring phase with low-level supplementation to compensate for the poor survival rate. Moreover, at-risk spawn on the site should still be protected, and spawn may still be removed for head-starting or translocation to other potential receptor sites. Furthermore we make recommendations for further PVA to be carried out based on up to date records in order to inform the management of agile frog sites in Jersey, and to try and determine their carrying capacity with greater detail.

4. Rana dalmatina Habitat Suitability Index (HSI)

4.1. Previous research

We base this account of the development of a Habitat Suitability Index (HSI) on Radiguet (2012), and make recommendations for future work. However, a limitation in developing a robust HSI for Jersey's agile frogs is the low number of occupied sites. Moreover, as Jersey's agile frogs appear to inhabit different habitat to their mainland counterparts it is not possible to apply findings from other studies to a HSI for Jersey.

Radiguet (2012) identified suitable habitat, water acidity, water quality, macrophyte cover, pond depth, shoreline shade, risk of disturbance, waterfowl presence, fish occurrence, and date of desiccation to be the key components. These variables could be used to assess habitat suitability in Jersey, but due to the limited number of ponds from which to gain HSI data it is impossible to provide a robust set of HSI components.

4.2. Further research

Despite the aforementioned issues with producing a HSI for Jersey's *Rana dalmatina* population, the following research may improve the validity of existing and further HSI's:

- Investigate the role that garden ponds in Jersey play in the agile frog population and their suitability. This research would be of great interest as not only would it provide further ponds to include in developing the HSI, it could reveal information regarding suitability of management strategies, particularly as there are many gardens bordering known agile frog habitat.
- Study of the terrestrial life-stage to ensure that the terrestrial aspects and limits of 800 m dispersal are relevant to Jersey's agile frog population.

4.3. Further recommendations

We recommend the following research activities are carried out in order to improve our understanding of the agile frog population in Jersey, and to inform future management:

- Carry out a cost benefit analysis of each management strategy (spawn protection and head starting) currently being used for the agile frog
- Long-term analysis of spawn management strategies and resulting survivorship
- Investigate the terrestrial ecology of Jersey's agile frogs; distance of migration, terrestrial habitat use, and connectivity to breeding areas. This would require a dedicated researcher and the use of mark-recapture or radio-telemetry on the now well-established population at Ouaisné.
- Investigate the use of garden ponds by Jersey's agile frogs
- Conduct further population viability analysis to identify target population size at each site

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Appendix

5. Data analysis

The following steps were undertaken to ensure the dataset met parametric assumptions prior to analysis:

i. Initial exploratory analysis tested the data to determine whether variables were normally distributed.
ii. Levene's Test was used to test for homogeneity of variance to ensure correlations could be reliably calculated. Due to the relatively low sample size for each variable, the combined dataset of all sites was tested together as the sample sizes for each subset were to be too small for analysis.

For the number of spawn clumps, the variances were similar across survey years (1997-2014), $F(17,67)=1.76$. Over the same time period, variances were similar for the number of head-started tadpoles released, $F(17,31)=1.30$, as well as for releases the year before, $F(17,28)=1.22$, two years before, $F(17,25)=1.09$, and three years before, $F(17,61)=1.90$. The peak frog count also had similar variance across years, $F(13,44)=0.71$.

Regression of variables was undertaken using linear and multiple regression where needed to model the relationships between variables. The residuals of our regression models were checked for normality using q-q plots to ensure they met the appropriate assumptions.

6. Summary of agile frog data

Data from Ouaisné Transect, Ouaisné Slacks, the combined numbers for the whole of Ouaisné, for Noirmont, and the combined numbers for all of these sites. Totals also include data from Woodbine and Les Creux.

Year	Location	Clumps	Peak Frog Count	Clumps removed for head-starting	Head-started tadpoles released
1987	Ouaisné Transect	0		0	75
1987	Ouaisné Slacks	0		0	
1987	Ouaisné Total	12		0	75
1987	Noirmont				
1987	**Total**	**12**		**0**	**75**
1988	Ouaisné Transect	6		0	75
1988	Ouaisné Slacks	0		0	
1988	Ouaisné Total	6		0	75
1988	Noirmont				
1988	**Total**	**6**		**0**	**75**
1989	Ouaisné Transect	0		0	75
1989	Ouaisné Slacks	0		0	
1989	Ouaisné Total	24		0	75
1989	Noirmont				
1989	**Total**	**24**		**0**	**75**
1990	Ouaisné Transect	0		0	
1990	Ouaisné Slacks	0		0	
1990	Ouaisné Total	3		0	
1990	Noirmont				
1990	**Total**	**3**		**0**	**0**
1991	Ouaisné Transect	0		0	
1991	Ouaisné Slacks	0		0	
1991	Ouaisné Total	0		0	
1991	Noirmont				
1991	**Total**	**0**		**0**	**0**
1992	Ouaisné Transect	0		0	75
1992	Ouaisné Slacks	0		0	
1992	Ouaisné Total	2		0	75
1992	Noirmont				
1992	**Total**	**2**		**0**	**75**
1993	Ouaisné Transect	0		0	

1993	Ouaisné Slacks	0	0	
1993	Ouaisné Total	2	0	
1993	Noirmont			
1993	**Total**	**2**	**0**	**0**
1994	Ouaisné Transect	0	0	
1994	Ouaisné Slacks	0	0	
1994	Ouaisné Total	0	0	
1994	Noirmont			
1994	**Total**	**0**	**0**	**0**
1995	Ouaisné Transect	0	0	
1995	Ouaisné Slacks	0	0	
1995	Ouaisné Total	0	0	
1995	Noirmont			
1995	**Total**	**0**	**0**	**0**
1996	Ouaisné Transect	0	0	
1996	Ouaisné Slacks	0	0	
1996	Ouaisné Total	0	0	
1996	Noirmont			
1996	**Total**	**0**	**0**	**0**
1997	Ouaisné Transect	7	0	
1997	Ouaisné Slacks	0	0	
1997	Ouaisné Total	7	2	
1997	Noirmont			
1997	**Total**	**11**	**2**	**0**
1998	Ouaisné Transect	0	0	
1998	Ouaisné Slacks	0	0	
1998	Ouaisné Total	0	0	
1998	Noirmont			
1998	**Total**	**6**	**0**	**505**
1999	Ouaisné Transect	0	0	
1999	Ouaisné Slacks	0	0	
1999	Ouaisné Total	0	1	
1999	Noirmont			
1999	**Total**	**2**	**1**	**55**
2000	Ouaisné Transect	7	1	
2000	Ouaisné Slacks	0	0	
2000	Ouaisné Total	7	1	
2000	Noirmont			244

2000	**Total**	**10**		**1**	**244**
2001	Ouaisné Transect	7		0	0
2001	Ouaisné Slacks	0		0	0
2001	Ouaisné Total	7		0	0
2001	Noirmont			0	130
2001	**Total**	**7**	**10**	**0**	**130**
2002	Ouaisné Transect	9		0	0
2002	Ouaisné Slacks	0		0	0
2002	Ouaisné Total	9		0	0
2002	Noirmont	1			0
2002	**Total**	**10**	**18**	**0**	**0**
2003	Ouaisné Transect	19		0	0
2003	Ouaisné Slacks	0		0	0
2003	Ouaisné Total	19		0	0
2003	Noirmont	1		0	170
2003	**Total**	**20**	**8**	**0**	**170**
2004	Ouaisné Transect	19	17	1	
2004	Ouaisné Slacks	0	0	0	
2004	Ouaisné Total	19	17	1	
2004	Noirmont	1	0		
2004	**Total**	**20**	**17**	**1**	**80**
2005	Ouaisné Transect	14	9	4	46
2005	Ouaisné Slacks	0	0	0	
2005	Ouaisné Total	14	9	4	46
2005	Noirmont	1	5		32
2005	**Total**	**15**	**14**	**4**	**78**
2006	Ouaisné Transect	33	4	24	
2006	Ouaisné Slacks	0	0	0	
2006	Ouaisné Total	33	4	24	4500
2006	Noirmont	1	14		52
2006	**Total**	**34**	**18**	**24**	**4552**
2007	Ouaisné Transect	17	11	10	
2007	Ouaisné Slacks	0	3	0	
2007	Ouaisné Total	17	14	10	
2007	Noirmont	1	0	1	
2007	**Total**	**18**	**14**	**11**	**4500**
2008	Ouaisné Transect	18	29	7	
2008	Ouaisné Slacks	8	3	0	

2008	Ouaisné Total	26	32	11	
2008	Noirmont	0	1		
2008	**Total**	**26**	**33**	**11**	**2530**
2009	Ouaisné Transect	35	16	24	410
2009	Ouaisné Slacks	6	3	0	
2009	Ouaisné Total	41	19	24	5026
2009	Noirmont	7	20	3	4675
2009	**Total**	**48**	**39**	**27**	**10637**
2010	Ouaisné Transect	35	18	6	2835
2010	Ouaisné Slacks	40	2	0	1100
2010	Ouaisné Total	75	20	7	3935
2010	Noirmont	52	43	3	
2010	**Total**	**127**	**63**	**10**	**4192**
2011	Ouaisné Transect	40	22	8	
2011	Ouaisné Slacks	42	19	0	
2011	Ouaisné Total	82	41	10	2463
2011	Noirmont	30	20		
2011	**Total**	**113**	**61**	**10**	**2463**
2012	Ouaisné Transect	32	16	15	
2012	Ouaisné Slacks	17	7	0	1000
2012	Ouaisné Total	49	23	24	3976
2012	Noirmont	18	9	7	1270
2012	**Total**	**67**	**32**	**37**	**10941**
2013	Ouaisné Transect	43	16	8	1500
2013	Ouaisné Slacks	35	19	0	
2013	Ouaisné Total	78	35	8	2048
2013	Noirmont	4	2	2	1487
2013	**Total**	**83**	**37**	**11**	**3752**
2014	Ouaisné Transect	49	21	9	
2014	Ouaisné Slacks	50	21	0	
2014	Ouaisné Total	99	42	10	
2014	Noirmont	19	10		
2014	**Total**	**126**	**52**	**13**	**3571**

7. Regression equations

7.1. Number of spawn clumps related to year

Ouaisné Transect: *no. clumps = -3418.785 + 1.720*year*, R^2 = 0.79, F(1, 26) = 100.2, p <0.001.

Ouaisné Slacks: *no. clumps = -2445.649+ 1.226*year*, R^2 = 0.45, F(1, 26) = 21.43, p <0.001.

Ouaisné Total: *no. clumps = -5313.773+ 2.667*year*, R^2 = 0.59, F(1, 26) = 37.27, p <0.001.

Noirmont: *no. clumps = -4247.392 + 2.121*year*, R^2 = 0.29, F(1, 10) = 4.093, p = 0.0706.

Total: *no. clumps = -6954.302 + 3.490*year*, R^2 = 0.55, F(1, 26) = 31.87, p <0.001.

7.2. Number of spawn clumps related to head-started tadpoles / metamorphs

Ouaisné Transect:

*no. clumps = 13.050+ 0.013*individuals released in previous year*, R^2 = 0.44, F(1,9) = 7.072, p = 0.03.

*no. clumps =11.137 + 0.008*individuals released two years previous*, R^2 = 0.256, F(1,8) = 2.759, p = 0.14.

*no. clumps = 11.825 + 0.011*individuals released three years previous*, R^2 = 0.386, $F(1,8)$ = 5.031, p = 0.30.

Ouaisné Slacks:

*no. clumps = -0.053 + 0.037*individuals released in previous year, R^2 = 0.997, $F(1,3)$ = 974.4, p <0.001.*

*no. clumps = 0.572 + 0.031*individuals released two years previous, R^2 = 0.655, $F(1,3)$ = 5.696, p = 0.10.*

*no. clumps = 1.776e-15 + 3.182e-02*individuals released three years previous, R^2 = 1.0, $F(1,2)$ = 3.64e+31, p < 0.001.*

Ouaisné Total:

*no. clumps = 18.458 + 0.012*individuals released in previous year, R^2 = 0.471, $F(1,12)$ = 10.7, p <0.01.*

*no. clumps = 13.647 + 0.012*individuals released two years previous, R^2 = 0.603, $F(1,11)$ = 16.72, p <0.01.*

*no. clumps = 15.591 + 0.011*individuals released three years previous, R^2 = 0.473, $F(1,10)$ = 8.966, p <0.05.*

Noirmont:

*no. clumps = -0.727 + 0.011*individuals released in previous year, R^2 = 0.954, $F(1,6)$ = 124.9, p <0.001.*

*no. clumps = 1.299 + 0.007*individuals released two years previous, R^2 = 0.887, $F(1,6)$ = 47.29, p <0.001.*

*no. clumps = 1.480 + 0.004*individuals released three years previous, R^2 = 0.863, F(1,5) = 31.6, p <0.01.*

Total:

*no. clumps = 12.364 + 0.010*individuals released in previous year, R^2 = 0.592, F(1,25) = 36.2, p <0.001.*

*no. clumps = 13.022 + 0.011*individuals released two years previous, R^2 = 0.667, F(1,24) = 48.02, p <0.001.*

*no. clumps = 17.166 + 0.011*individuals released three years previous, R^2 = 0.414, F(1,23) = 16.27, p <0.001.*

7.3. Number of spawn clumps related to number of spawn protected

Ouaisné Transect:

*no. clumps = 49.084+ -1.576*no. spawn protected in previous year, R^2 = 0.531, F(1,4) = 4.53, p =0.10.*

*no. clumps = 50.880 + -1.335*no. spawn protected two years previous, R^2 = 0.171, F(1,3) = 0.619, p = 0.50.*

*no. clumps = -45.0 + 7.0*no. spawn protected three years previous, R^2 = 0.590, F(1,2) = 2.882, p = 0.23.*

Ouaisné Slacks:

*no. clumps = 8.421 + 7.211*no. spawn protected in previous year, R^2 =0.200, F(1,2) = 0.501, p = 0.55.*

*no. clumps = 32.714 + 2.357*no. spawn protected two years previous,* $R^2 = 0.997$, $F(1,1) = 363$, $p < 0.05$.

*no. clumps = 63.0 + -10.0*no. spawn protected three years previous,* $R^2 = 0.787$, $F(1,1) = 3.704$, $p = 0.31$.

Ouaisné Total:

*no. clumps = 66.928 + -1.069*no. spawn protected in previous year,* $R^2 = 0.060$, $F(1,6) = 0.386$, $p = 0.56$.

*no. clumps = 54.166 + 1.181*no. spawn protected two years previous,* $R^2 = 0.103$, $F(1,5) = 0.573$, $p = 0.48$.

*no. clumps = 74.2712+ -0.386*no. spawn protected three years previous,* $R^2 = 0.018$, $F(1,4) = 0.071$, $p = 0.80$.

Noirmont:

It was not possible to analyse data from Noirmont as only one season (2009) had clumps protected.

Total:

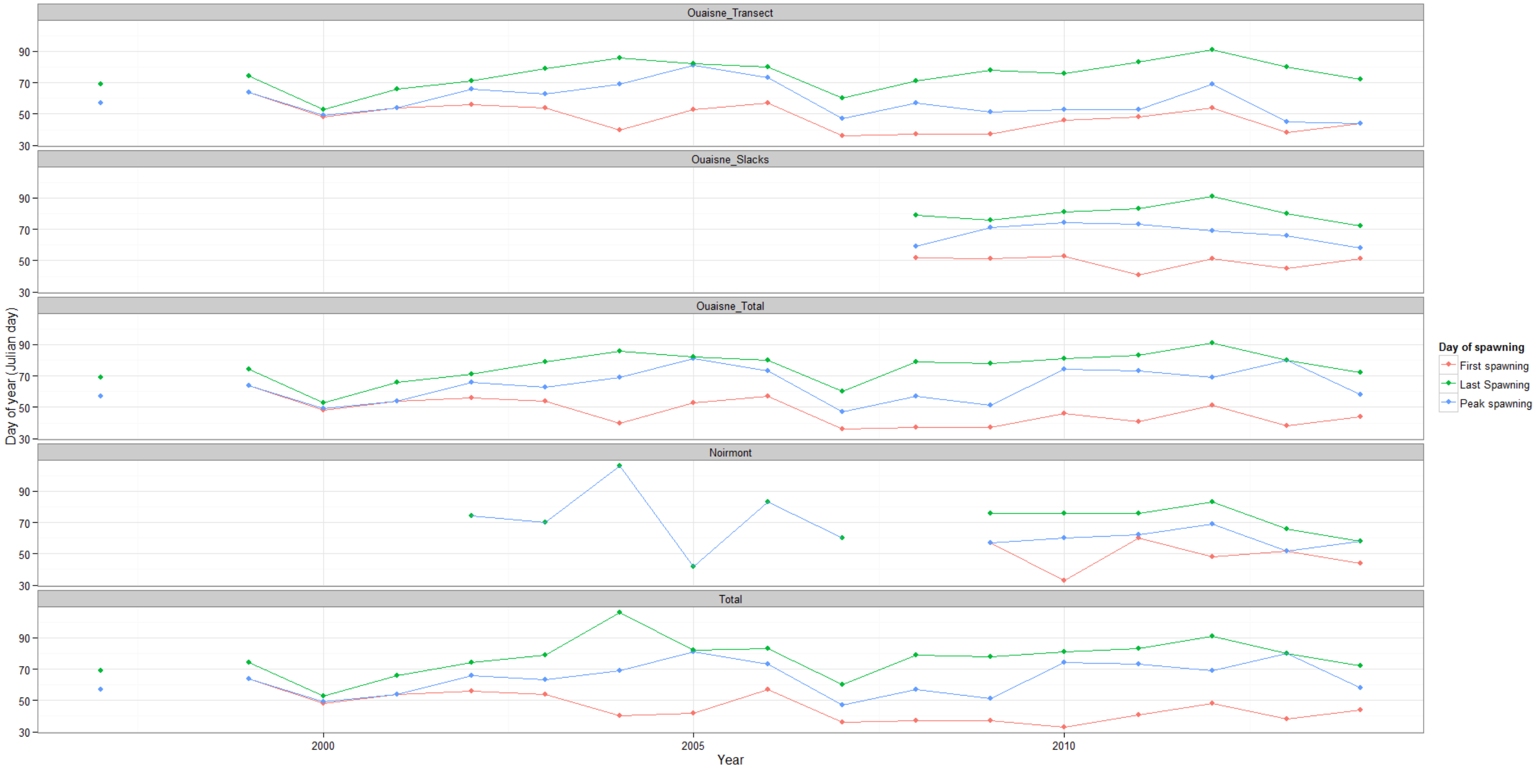
*no. clumps = 75.709 + 0.035*no. spawn protected in previous year,* $R^2 = 3.412e\text{-}05$, $F(1,6) = 0$, $p = 0.99$.

*no. clumps = 66.541 + 1.972*no. spawn protected two years previous,* $R^2 = 0.146$, $F(1,5) = 0.851$, $p = 0.40$.

*no. clumps = 102.711 + -0.886*no. spawn protected three years previous,* $R^2 = 0.047$, $F(1,4) = 0.195$, $p = 0.68$.

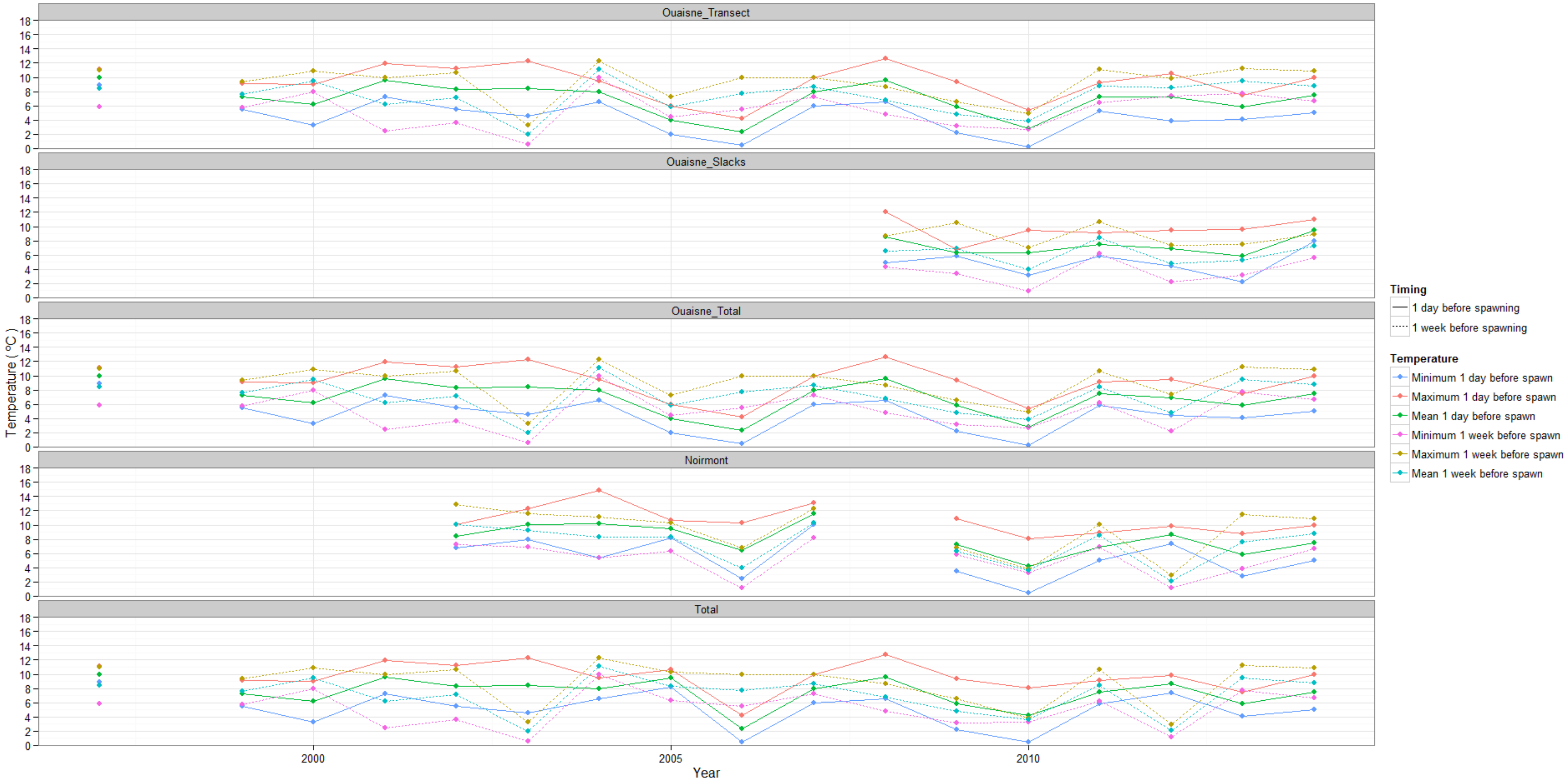
8. Graphs

8.1. Spawn timing from 1997-2014. Displaying the first day of spawning, last day of spawning, and the peak spawning day (the day the greatest increase in spawn occurred). Days are displayed as number of days since the beginning of the year (Julian date).

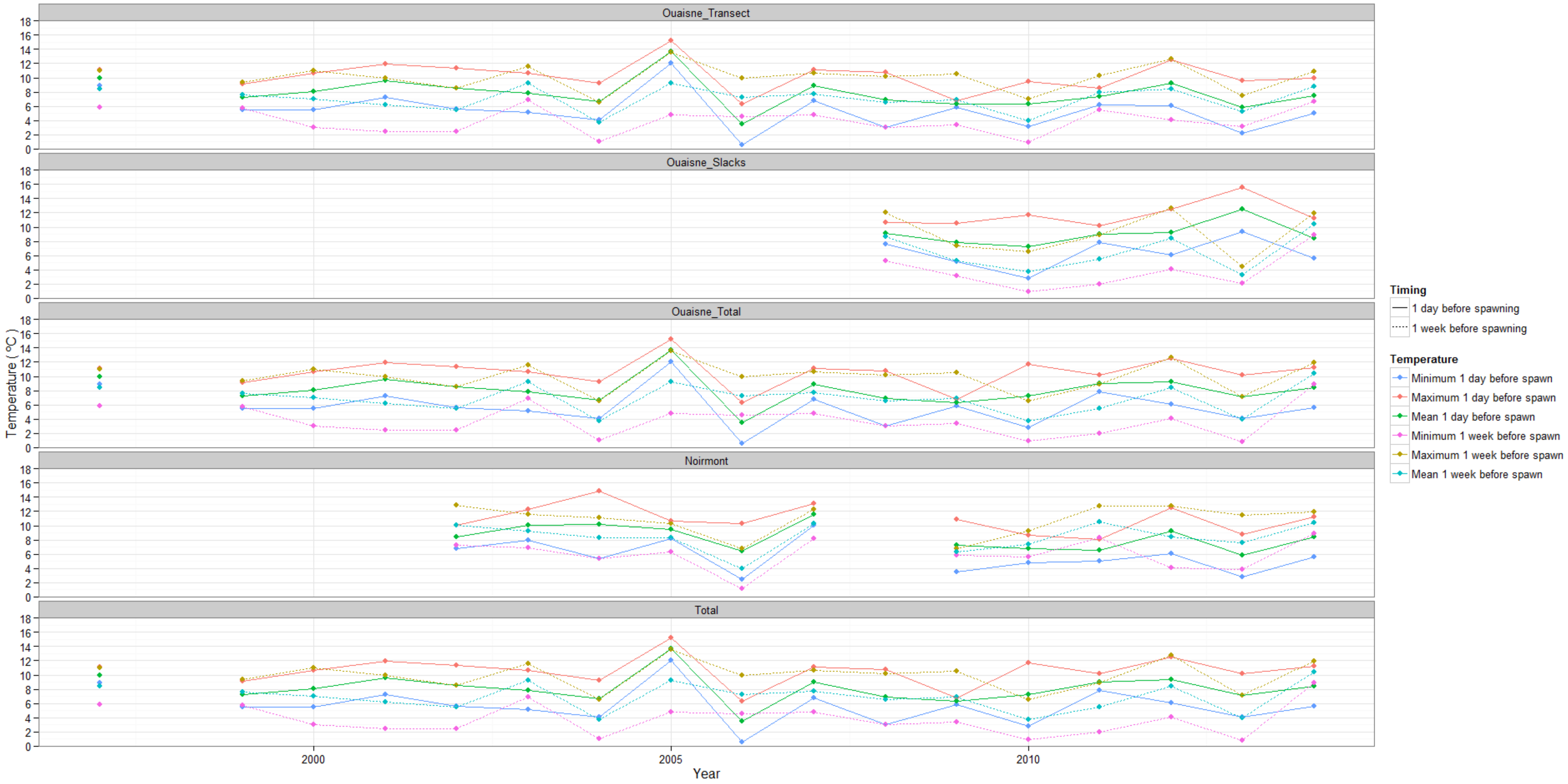


8.2. First (a) and peak (b) spawning thresholds and relationships to temperature (ºC)

a)

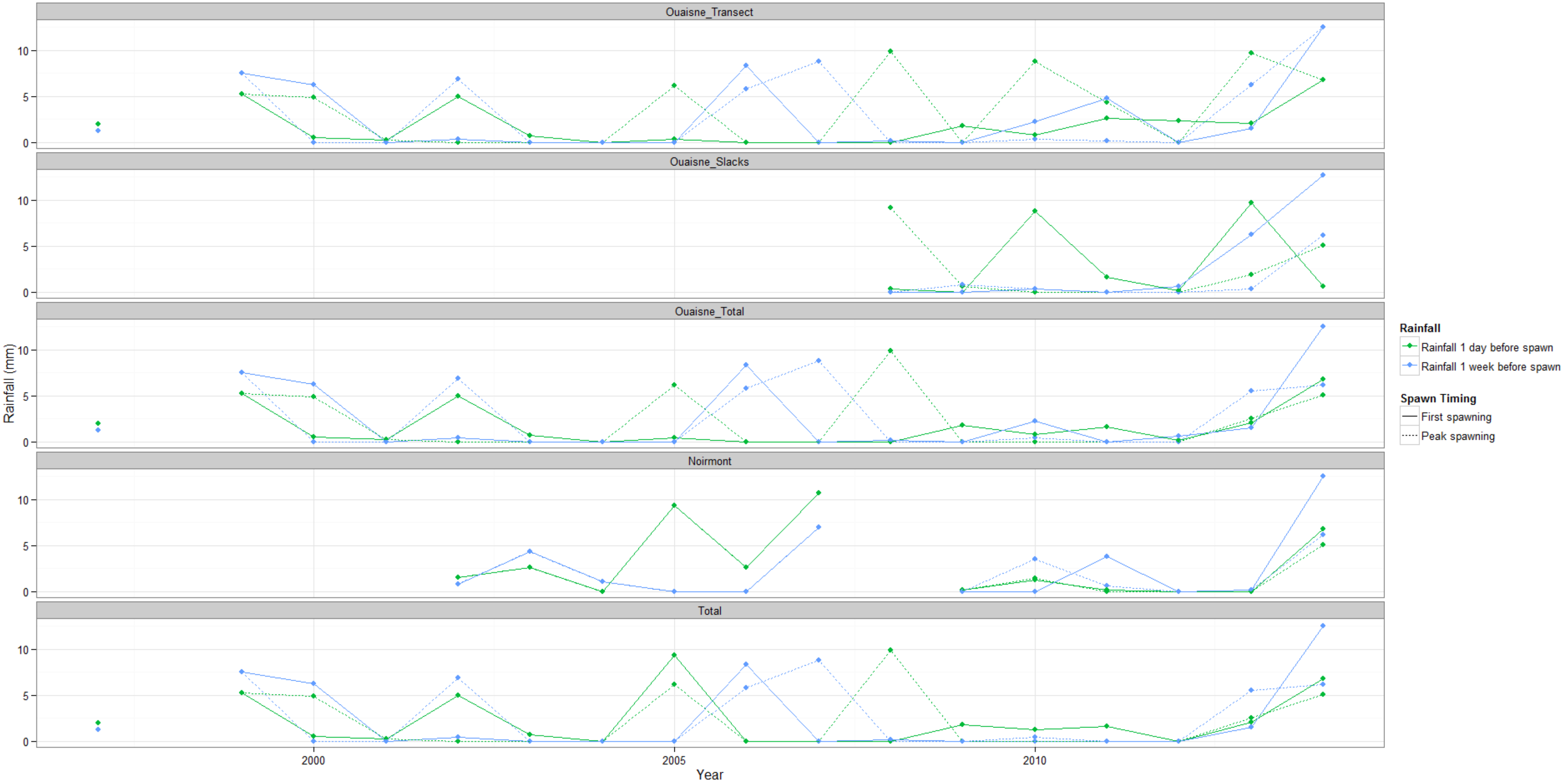


b)



Ouaisne_Transect
Ouaisne_Slacks
Ouaisne_Total
Noirmont
Total
Temperature (°C)
Year
2000
2005
2010
Timing
1 day before spawning
1 week before spawning
Temperature
Minimum 1 day before spawn
Maximum 1 day before spawn
Mean 1 day before spawn
Minimum 1 week before spawn
Maximum 1 week before spawn
Mean 1 week before spawn

8.3. Relationship between spawning periods and rainfall (mm) from 1997-2014



9. Template recording forms

9.1. Intermediate level survey form (Agile frog monitoring.xslx tab 4. Anuran Survey Form Intermed.)

Name		Date		Start time		End time	
Weather code:	Cloud cover %:	Wind code:	Local Air T°C	Min.	Max	Mean	Codes and categories on reverse
No. Calls	Frog						
	Toad						
	Site/Area						

Site	Area	Water level	Species	Qty	Spawn ID	Lifestage	Position	Spawn condition	Spawn mgmt.	Comments

Codes and entry options for relevant columns

Codes	Weather code	Wind code	Lifestage	Position	Spawn condition	Spawn management
0	Clear	Smoke rises vertically	Spawn	Water	Poor	Left in-situ
1	Overcast	Wind direction shown by smoke drift	Adult	Land	Moderate	Protected - branch placement
2	Hazy	Wind felt on face; leaves rustle	Juvenile	Other	Good	Protected - mesh wrapped
3	Fog	Leaves or small twigs in constant motion	Metamorph		Unknown	Protected - moved to cage
4	Drizzle	Raises dust and loose paper; small branches move	Tadpole			Translocated - within site
5	Rain	Large branches in motion	Calls			Translocated - to other site (specify location in comments)
6	Hail		Unknown			Removed for head-starting
7	Snow					

9.2. Advanced level survey form (Agile frog monitoring.xslx tab 5. Anuran Survey Form Advanced)

Name		Date		Start time		End time	
Weather code:	Cloud cover %:	Wind code:		Local Air T°C	Min:	Max:	Mean:

Codes and categories on reverse

No. Calls	
	Frog
	Toad
	Site/Area

Site	Area	Water level	Species	Qty	Spawn ID	Lifestage	Position	Health	Sex	Behaviour	Comments

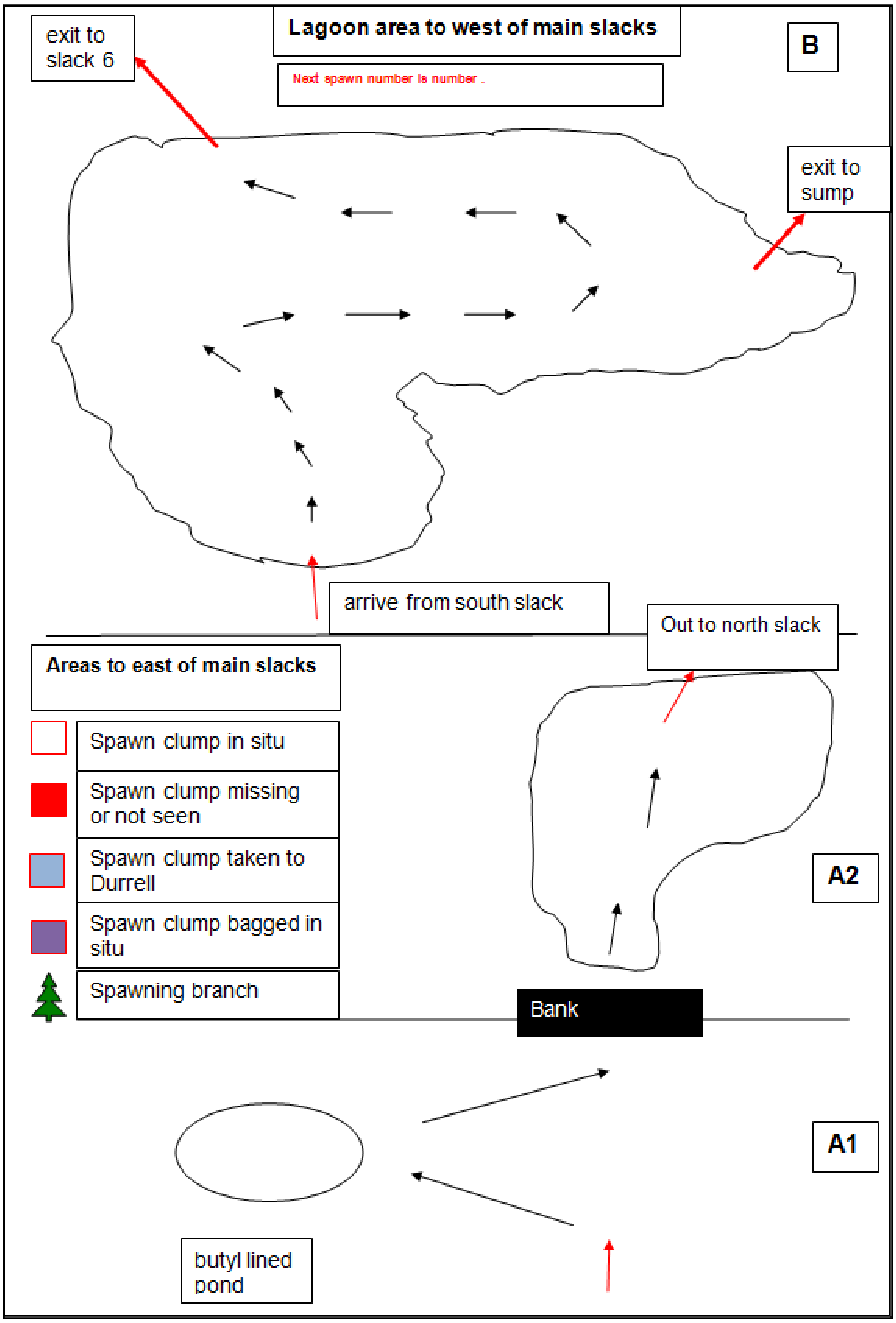
Date	Spawn ID	Condition	Depth (cm)		Distance (cm)	Diameter (cm)	Management	Predation	Hatched (amount)	Hatch date	Comments
			Water at spawn	Top of spawn							

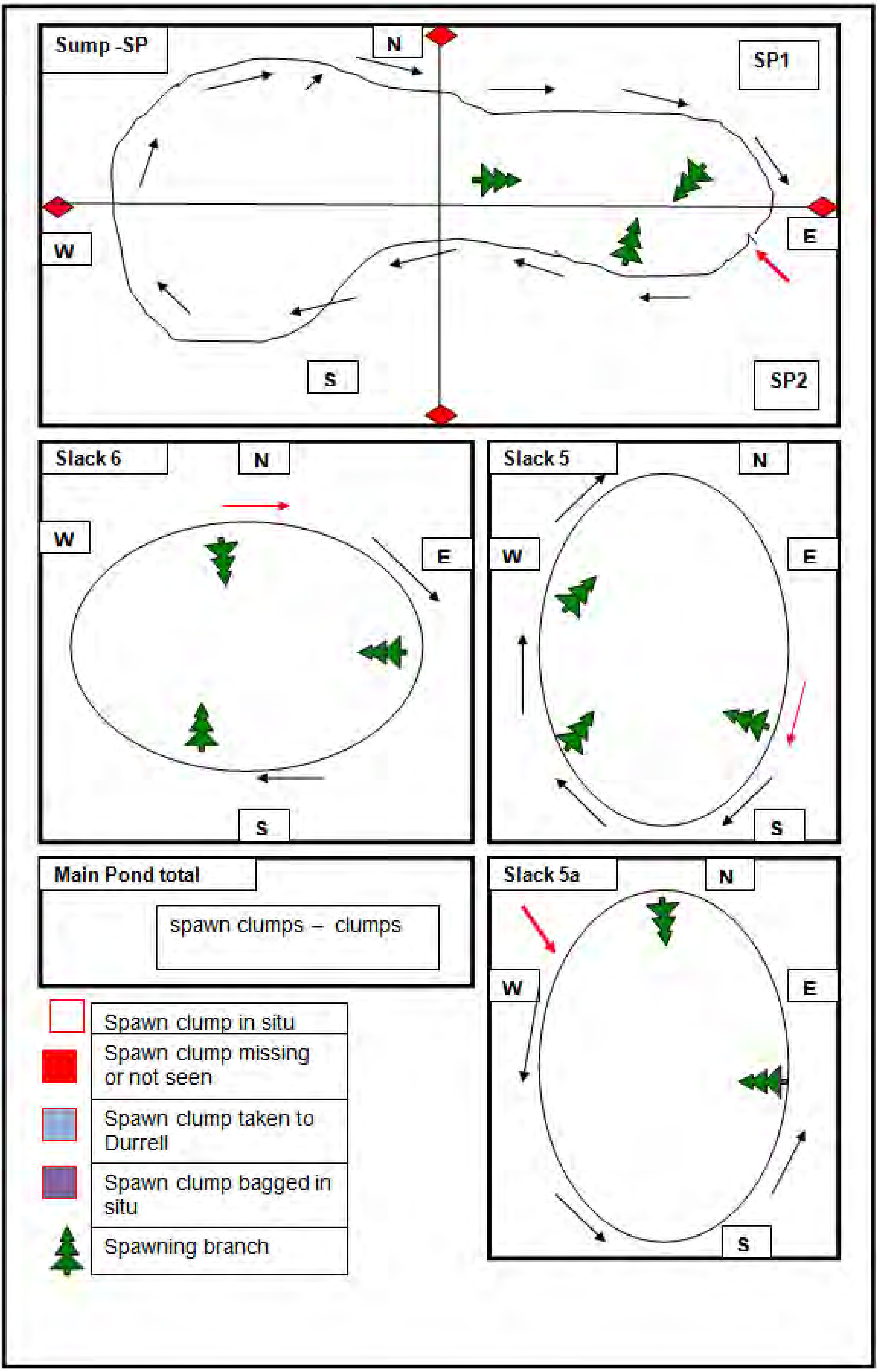
Codes and entry options for relevant columns

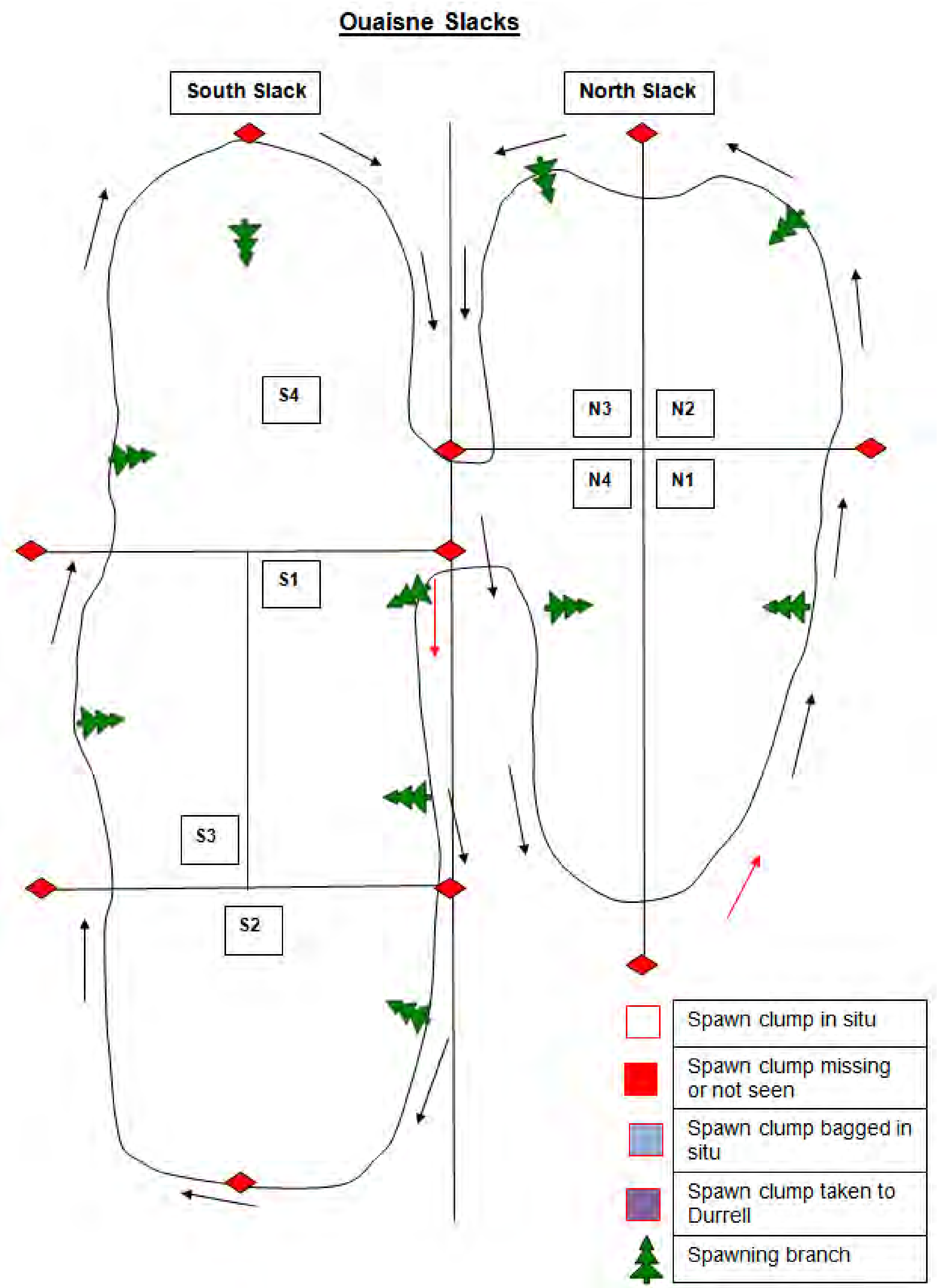
Codes	Weather code	Wind code	Lifestage	Position	Spawn condition	Spawn management	Hatched (amount)
0	Clear	Smoke rises vertically	Spawn	Water	Poor	Left in-situ	None
1	Overcast	Wind direction shown by smoke drift	Adult	Land	Moderate	Protected - branch placement	1-25% hatched
2	Hazy	Wind felt on face; leaves rustle	Juvenile	Other	Good	Protected - mesh wrapped	26-50% hatched
3	Fog	Leaves or small twigs in constant motion	Metamorph		Unknown	Protected - moved to cage	51-75% hatched
4	Drizzle	Raises dust and loose paper; small branches move	Tadpole	**Health**	**Behaviour**	Translocated - within site	>75% hatched
5	Rain	Large branches in motion	Calls	Live	Amplexus	Translocated - to other site (specify location in comments)	Unknown
6	Hail		Unknown	Sick/injured	Migration	Removed for head-starting	
7	Snow			Dead	Calling		

9.3. Spawn mapping sheets

9.3.1. Ouaisné (Ouaisné spawn map template 2015.doc)




Sump -SP
N
SP1
W
E
S
SP2
Slack 6
N
W
E
S
Slack 5
N
W
E
S
Main Pond total
spawn clumps – clumps
Slack 5a
N
W
E
S
Spawn clump in situ
Spawn clump missing or not seen
Spawn clump taken to Durrell
Spawn clump bagged in situ
Spawning branch


Ouaisne Slacks
South Slack
North Slack
S4
S1
S3
S2
N3
N2
N4
N1
Spawn clump in situ
Spawn clump missing or not seen
Spawn clump bagged in situ
Spawn clump taken to Durrell
Spawning branch

9.3.2. Noirmont (Noirmont spawn map template 2015.doc)