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Over the moon: the effect of the lunar cycle on three species of newt in the UK

by

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Abstract

Lunar cycles provide several cues that can be recognised by animals, such as changes in light intensity, gravity and geomagnetism, all of which have been linked to changes in behaviour. Although amphibians are known to use celestial cues in orientation, the influence of the lunar cycle on amphibian behaviour has received comparatively little attention. The aim of this study was to determine whether the lunar cycle influences the capture rate and activity of three newt species: the great crested (*Triturus cristatus*), smooth (*Lissotriton vulgaris*) and palmate newts (*Lissotriton helveticus*). This study also aimed to discover how other external factors, including water temperature, light level and cloud cover influenced the capture rate and activity of each species. Capture data from a 9-year study was firstly used to identify how capture rates were influenced by the lunar cycle and external factors. This was then followed by a study focussing on two lunar cycles in 2019, which utilised results from trap captures and activity monitoring to determine the influence of the lunar cycle and external factors. During the 9-year study this study a combination of both lunar phase and temperature influenced the capture rates of newts, but with different combinations of factors important in different species. Likewise, over two complete lunar cycles in 2019, a combination of environmental factors influenced trapping rates, and this appeared to be species-specific. Direct observations on activity revealed that lunar phase, water temperature and light level all had an influence on the activity on each of the three newt species. In great crested newts there was increase in activity at higher temperatures and around both the full and new moon, whereas smooth and palmate newts were more likely to be active during periods of high illumination. Evidence for both capture rate and activity being influenced by multiple external factors means that surveys of newts may be biased unless they can account for each of these

factors. However, in high density populations of newts such as that studied here, interspecific interactions, limited space for newts within a trap and escapes from traps may mean capture rates are not reliable indicators of activity. I recommend that surveyors should take external factors, including influences of the lunar cycle, into account when interpreting and comparing results of newt surveys.

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Chapter 1: General introduction

Amphibian populations are in decline globally and since the first World Congress of Herpetology in 1989 (Kelhart, 2007) these declines are now widely recognised. In 2004 the Global Amphibian Assessment was completed, being the first comprehensive assessment of the global amphibian conservation status (Stuart *et al*., 2004). These assessments found that amphibian populations are in critical decline and declines are accelerating (Alford, *et al*., 2001; Houlahan *et al*., 2000; Stuart *et al*., 2004). Findings by the International Union for Conservation of Nature (IUCN Red List of Threatened Species, 2020), have identified that 41% of global amphibian species are currently threatened or have become extinct.

Habitat loss and fragmentation is well documented to be the biggest driver of amphibian decline and extinction (Cushman, 2006; D'Amen *et al*., 2010), but other threats have been reported, including global climate change (Blaustein *et al.*, 2001; Duan *et al.,* 2016), emerging diseases (Fisher *et al.,* 2009), environmental contaminants (Mann *et al.,* 2009), direct exploitation (Schlaepfer *et al.,* 2005) and invasive species (Kats & Ferrer, 2003; Kiesecker et al., 2001). Although extensive studies have been conducted on the causes of amphibian decline, these mechanisms are very complex and there is no one threat that is solely responsible (Beebee & Griffiths, 2005).

Human wildlife conflict is one of the biggest threats to amphibian species worldwide and in the UK these issues are usually associated with urban sprawl and development (Dickman, 2010). Increasing demands on housing and commercial development have created conflict and put pressure on several European Protected Species (EPS) and their habitat (Griffiths, 2004). Before development can occur on protected areas or where EPS are found, developers are legally required to conduct ecology surveys and undertake appropriate mitigation measures to reduce the impact on the species. This is in accordance with the Bern Convention (JNCC, 2019), the Conservation of Habitats and Species regulations (as amended) Regulations 2012 (JNCC, 2019) and the Wildlife and Countryside Act 1981 (Legislation.gov.uk, 2019).

This study will be focusing on the three newt species, including the great crested (*Triturus cristatus*), smooth (*Lissotriton vulgaris*) and palmate newts (*Lissotriton helveticus*), all of which are resident to the UK. All three species utilise freshwater bodies during breeding season (February to July), laying eggs on aquatic plants and overhanging vegetation. After hatching, larvae will spend several weeks growing and metamorphosing and once fully developed, leave the ponds (Beebee & Griffiths 2000). After leaving the ponds newts will spend late summer and winter on land and then hibernate under refugia during the winter months (Beebee & Griffiths 2000).

Great crested newts are the largest British newt species, reaching 15-18 cm in length and are widespread throughout England, but are rare or absent in many parts of Scotland (Jehle *et al.,* 2011). Although great crested newts are still widespread throughout the United Kingdom (UK), numbers are declining overall, due to loss of habitat and habitat degradation.

Smooth and palmate newts are the smaller newt species found in the UK, typically being 8-11 cm and 6-11 cm respectively (Beebee, 2013). Both species are widespread throughout the UK, being more abundant and colonising a wider range in habitat. Although many populations are found throughout the UK, human encroachment, habitat loss and habitat degradation threaten populations, although national trends are not available (Alford *et al.,* 2001; Beebee & Griffiths, 2005).

Surveys of British newts usually use one or more of six main survey methods: eDNA, trapping, spotlight searches, egg searches, dip netting and drift fences with pitfall traps. These methods differ in effectiveness which may depend on target species, habitat and region. Bottle traps and spotlight searches are the commonly used methods in newt surveys due to their effectiveness in determining population density and the reduced risk of disturbance to habitats compared to dip netting and drift fences (Beebee, 2013; Buxton *et al*., 2017; Griffiths, 1985a). Bottle trapping relies on active newts to enter traps and these trap captures may be an index of activity as well as population density. Amphibian behaviour determines how successful any survey method is, with both climate and seasonality being well-known to influence the biology of amphibians (Brooke *et al.,* 2000; Church, 1960b). Temperature, humidity, and rainfall have all been identified to influence activity, reproduction and feeding behaviour in amphibians (Brooke *et al*., 2000; Dolmen, 1983; Šamajová & Gvoždík, 2009). Although climate and seasonality have an influence, not all changes in behaviour can be explained by these factors alone (Grant *et al*., 2009).

Lunar cycles influence several geophysical changes on the earth that can be perceived by animals, which have been widely reported to cause biological and behavioural changes in the earth's flora and fauna (Brown, 1959). These lunar cycles can influence the behaviour of many terrestrial species, including foraging, breeding, habitat use, predation and migration (Campbell *et al.,* 2008; Dixon *et al*., 2006; James *et al*., 2000; Kronfeld *et al.,* 2013; Lillywhite & Brischoux, 2011; Naylor, 2018). Although these behaviours have been identified in many species, most of these studies focus on mammals, birds and some reptile species. Amphibians have received little attention regarding lunar mediated behaviour, although studies conducted between the 1960s-1980s identified that several amphibian species utilise celestial cues for orientation and migration (Ferguson *et al*., 1965; Jaeger & Hailman, 1973; Plasa, 1979).

For an understanding of the dynamics of newt metapopulations, researchers need to consider all influential factors on both observation and capture rate of each species. There is considerable surveying of great crested newts in the UK and Europe because of their protected status and the influence this has on building development. However, studies including the lunar cycle and its influence on newt behaviour is rare, although lunar phases have been identified as an influential factor in other amphibian species (Deeming, 2008; Grant *et al*., 2012). Due to the limited amount of studies including how the lunar cycle influences behaviour, results often lack consensus in terms of general affects.

With limited knowledge and controversial evidence regarding how the lunar cycle influences the behaviour of amphibian species, this project aims to address three fundamental questions: (1) How does the lunar cycle influence the capture rates observed within a nine-year population survey? (2) How does the lunar cycle and environmental factors affect captures at different phases of the lunar cycle? (3) How does the lunar cycle and environmental factors affect activity patterns at different phases of the lunar cycle?

Chapter 2: Patterns of newt captures in relation to the lunar cycle within a 9 - year population data set

2.0 Abstract

The influence of climate and seasonality on amphibians is widely recognised, with temperature, humidity and rain all affecting behaviour and morphology, whereas the moon and lunar cycles have been mostly overlooked. Lunar cycles provide several cues that can be recognised by animals, such as light intensity, geomagnetism and gravity. Using 9 years of capture data, which was collected at a research site in Kent, this study tested how environmental and lunar cycles influenced the capture rates of three newt species: great crested newts (*Triturus cristatus*), smooth newts (*Lissotriton vulgaris*) and palmate newts (*Lissotriton helveticus*). Both the lunar phase and temperature influenced the capture rate of each species, but complex interactions between lunar phase and temperature were identified, with these interactions being more important in some years than others. As these data were collected to explore long-term population dynamics rather than the impacts of the lunar cycle, this study was unable to conclusively determine which of the factors had the greatest influence on activity. Here these findings are discussed and the problems that occurred during the study and how to overcome these issues while collecting information on lunar related activity.

2.1 Introduction

The lunar cycle causes several geophysical changes on the earth that can be perceived by animals, such as the ocean tides, electromagnetic radiation, brightness of the lunar light and barometric pressure. Changes in these geophysical effects have been widely reported to cause biological and behavioural changes in the earth's flora and fauna. The behaviour and reproduction of marine and intertidal species are heavily influenced by the ocean tides, lunar cycles and light levels (Naylor, 1999; Park *et al*., 2006; Witt, 2013). Lunar cycles have been identified to influence behaviour in many terrestrial species, including breeding, predation, foraging, migration and habitat use (Campbell *et al.,* 2008; Dixon *et al*., 2006; James *et al*., 2000; Kronfeld *et al.,* 2013; Lillywhite & Brischoux, 2011; Naylor, 2018). Although these behaviours have been identified in many species, most of these studies focus on mammals, birds and reptile species, whereas amphibians have received little attention.

Amphibian biology and behaviour is well known to be influenced by climate and seasonality, with temperature, humidity and rain all having effects (Brooke *et al.,* 2000; Church, 1960b). Although climate and seasonality is well known to influence behaviour in amphibians, not all behaviour can be explained this way and the lunar phase has been linked to breeding, migration and orientation in several amphibian species (Able, 1980; Beebee, 2013; Diego-Rasilla & Luengo, 2002). Lunar phase and light level have been identified to influence the behaviour of several anuran species, including reproduction and mating in *Bufo melanostictus* and *Crinia geogiana* (Byrne, 2002; Church, 1960a), calling in *Cophixalus ornatus* (Brooke *et al*., 2000) and locomotor activity in *Bufo americanus* (Fitzgerald & Bider, 1974). Although the lunar phase has been recognised to influence amphibian behaviour (Fitzgerald & Bider, 1974; Grant *et al*., 2012), many of these results are controversial with evidence for both increased and decreased activity during each lunar phase (Ralph, 1957; Underhill 2018; Vignoli *et al.,* 2014).

A limited amount of studies has been conducted on the behavioural changes in newts (urodeles) in relation to the lunar cycle (Deeming, 2008; Grant *et al.,* 2009; Harper *et al*., 2018; Losson, 2010). Deeming (2008) reported that aquatic activity of *Lissotriton vulgaris* was highest just before the new moon, whereas in *Triturus cristatus*, captures were highest either side of the new moon, corresponding with the darkest nights. Grant *et al*. (2009) looked at the first sightings and peak arrivals of newts in breeding ponds, reporting that these were more frequent around the full and new moon, corresponding with the gravitational cycle. Other studies which focused on breeding and activity in newts suggested that the lunar phases and the geophysical changes the moon causes may have an underlying relationship with behaviour (Kröpfli et al. 2010; Paterson, 2018a, b). Consequently, there is no conclusive evidence on how the lunar cycle influences newts (Vignoli *et al*., 2014).

The aim of this paper is to determine the influence of the moon on three newt species (*Triturus cristatus, Lissotriton vulgaris* and *Lissotriton helveticus*), by interrogating a 9-year population data set at the University of Kent, Canterbury. Two hypotheses are proposed: (1) Does the lunar cycle influence the capture rate of each species? (2) How does temperature influence the capture rate of each species?

2.2 Methodology

2.2.1 Study Species and study site

Field work has been carried out in the north-western part of the University of Kent, Canterbury (TR 12977 59673), since 1999. The field site consists of 8 experimental ponds, with four ponds being were created in 1998, each being 2 m x 1 m, with a wedge-shaped profile, which is approximately 0.7 m at the deepest point and have a butyl liner for water retention (figure 2.1). These ponds were constructed parallel to each other with a 3-4 m strip of grassland separating them. Each pond has a butyl liner for water retention. In 2009 another row four ponds were constructed to the same dimensions parallel to the originals to study population levels and pond colonisation (Lewis, 2012).

The field site is in an open area, located close to urban areas, amenity grasslands and parkland, which are broken up by paths, roads, and hedgerows. Due to the location of the site, several sources of consistent artificial light can be seen from the ponds, this includes light from building and streetlamps, with the closest source being within 50 metres. The ponds are surrounded by unimproved grassland (bramble, *Rubus fruticosus* and nettle, *Urtica dioica*), which have been fenced off and secured to restrict public access. The ponds support a limited amount of vegetation, including filamentous algae, duckweed (*Lemna minuta*), overhanging grass and bramble. Low-level maintenance is carried out each year on the ponds, this is done to reduce any excessive build-up of vegetation. In December 2005 and 2014, and January 2019, all ponds were completely drained to simulate periodic desiccation. In 2005 and 2014 the butyl liners were also replaced. Each pond was then refilled with tap water and left to allow colonisation to occur naturally, with any overwintering newt larvae being replaced in the ponds they were originally situated.

Several ponds are located within one kilometre of the site, some of which have been identified to have breeding amphibian populations (Lewis, 2012; Sewell, 2006), which has allowed this site to be colonised naturally. A healthy population of all three native newt species have been recorded in the ponds since 2001, including: the great crested newt (*Triturus cristatus*), smooth newt (*Lissotriton vulgaris*) and palmate newt (*Lissotriton helveticus*). Non-native alpine newts (*Ichthyosaura alpestris*) are occasionally found at the field site, as the surrounding area supports low numbers (Lewis, 2012). In accordance with Schedule 9 of the Wildlife and Countryside Act, 1981 (as amended) (Legislation.gov.uk, 2019), these individuals are removed from the site. The field site supports a wide range of other species, including the common frog (*Rana temporaria*), grass snake (*Natrix natrix*) and a diverse range of bird, mammal and invertebrate species.

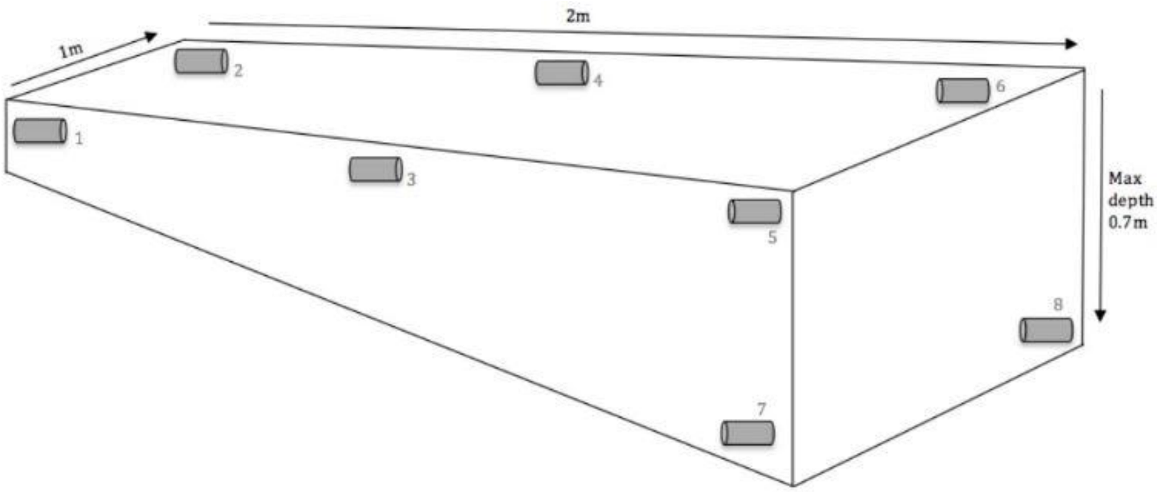

Figure 2.1 Cross section of a field site pond, showing its design and measurements. Placement of traps is included, with traps one to six being placed on the surface and traps seven and eight placed on the bottom of each pond.

2.2.2 Collection of trapping data

This project was undertaken and supervised by Richard Griffiths (1999 – 2019), funded by the University of Kent. This was conducted under a great crested newt survey and research licence, granted by Natural England, and held by Richard Griffiths. Guidance from Natural England was followed to ensure welfare of both target and non-target species (Natural England, 2014), with control measures regarding disease and non-native species being adhered to (ARG-UK, 2017).

Underwater bottle traps, described by Griffiths (1985a), were used for data collection. Each trap was constructed from a one litre plastic bottle, which was cut in two sections with the neck being inverted and clipped into the bottom half. Six surface traps were set in each pond, which were located around the edges and anchored to the sides with a cane to stop the trap from drifting (figure 2.2). At the start of the season when temperatures were lower (February to April) two fully submerged traps were also set on at the bottom of each pond, under traps five and six. These traps were no longer used after the third week of April as dissolved oxygen levels are reduced in warmer temperatures (Dejours, 1987), which affects the amount of time amphibians can stay submerged (Šamajová & Gvoždík, 2009).

Trapping throughout the study remained consistent, with trapping occurring weekly, starting from the last week of February until captures reduced to low levels, usually between June and July. The only weeks when trapping was not carried out was during extreme weather conditions, i.e. ponds covered with ice or during heatwaves. Bottle traps were set at 1930 hrs on Thursday nights and collected again at 0730 hrs on Friday mornings for each trapping session. All newts that were caught across the ponds were recorded and each individual was identified into both species and sex, then released back into the ponds they were trapped in.

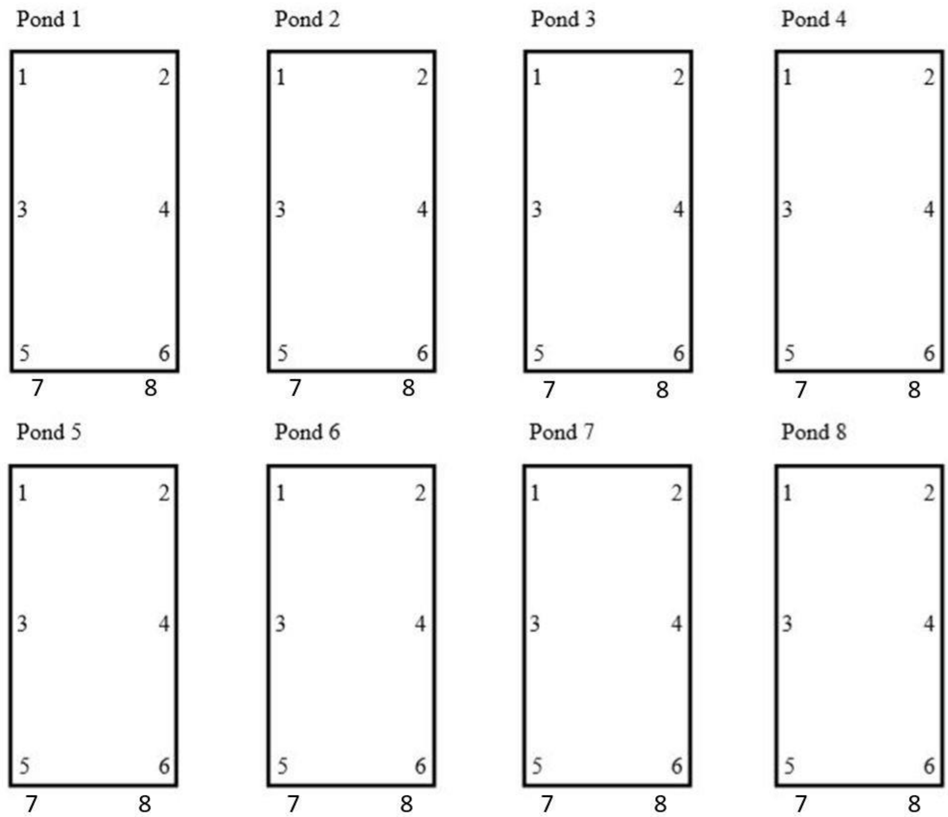

Figure 2.2. Layout at the Field Site Ponds in 2019, with ponds 1 to 4 being built in 1998 and ponds 5 to 8 in 2009. Each number indicates bottle-trap positions, with traps 1 and 2 located at the shallow end of each pond, 5 and 6 at the deep end. Bottle traps 7 and 8 are fully submerged and located under traps 5 and 6. These were only set at the start of the trapping season (January – April) while pond temperatures were low and dissolved oxygen levels were high.

2.2.3 Lunar and meteorological data

All lunar information was obtained from the United States Naval Observatory Astronomical Application Department (http://aa.usno.navy.mil/data/docs/MoonFraction.php) at midnight, universal time (GMT) with the coordinates: Canterbury, 51°29'N and 01°06'E. To express the changes in the lunar cycle, the percentage of the lunar disk that was illuminated for each trapping session was used (Brooke et al., 2000; Underhill, 2018). Information on sunrise, sunset, earth-moon distance, moonrise and moonset were also recorded during each trapping session.

Meteorological data including water temperature, humidity and rainfall were collected from the West Stourmouth weather station, station number: 03797, located 11 miles from the field site. Overnight water temperatures were taken from pond 3, both while setting and collecting the traps, using a min/max thermometer set 0.3 metres at the shallow end and a Tinytag plus 2 data logger set at 0.7 metres at the deepest point. During each trapping session any disturbances, predators or other species that may have influenced trapping behaviour were also recorded.

Water temperature was taken from the centre of the pond at a depth of 0.3 m, and these recorded temperatures ranged between 1°C and 20°C, with 90% of the trapping sessions occurring when the water temperature was between 5°C and 17°C (figure 2.4). Capture rates at both the higher and lower temperatures were low, so to reduce heterogeneity in modelling the capture rates, only captures made during the 'optimal' temperature range of 5°C and 17°C were used in the analyses.

2.2.4 Statistical analyses

To test for a relationship between the number of newts caught and external variables, IBM SPSS software (version 25) was used to conduct a generalized linear mixed model (GLMM), using a Poisson error distribution and log link function as the data were based on counts. As there are different numbers of newts in the ponds and newts could move freely between the ponds during the study, the ponds were not independent sampling units. Consequently, the total number of each species captured was treated as the response variable. Percentage of the lunar phase and average temperature during each study session are explanatory variables and were classed as fixed effects. This study utilised data from a nine-year period

(2010 – 2018), when all eight ponds were in place and after initial colonisation during the years following installation in 1999.

The analysis used the weekly trap captures of newts between February and July each year from 2010-2018. The weekly captures consisted of all individuals caught across the eight ponds and organised into species. Preliminary examination of the collected data identified extremely cold or hot weather, which usually occurred at the start or end of the survey period, resulted in very low captures that could bias the means (Fig. 2.4). To reduce the potential bias, only weeks when the water temperature was between 5°C and 17°C were included in the analysis. Likewise, as population sizes varied between years (Fig. 2.3), ‘year’ was included as a random factor when analysing the data (see below).

A generalised linear mixed model was identified as the most flexible approach to modelling this complex data set (McCulloch & Neuhaus, 2014). This analysis treated the lunar phase and temperature as fixed factors and the year as a random factor. By utilising this method, interactions between each of the three factors were calculated, identifying which had the greatest influence on capture each year.

2.3 Results

2.3.1 Captures

A total of 148 weekly bottle trap surveys were completed and 8220 newts were caught over the 9-year period (figure 2.3). Great crested newt captures slowly increased between 2010 and 2017 (figure 2.3a), with the average weekly captures between February and July being between 10 to 23. This included two to four days of high captures each year (30 to 50 newts). A sudden increase in captures occurred in 2018 (figure 2.3a), with the average weekly capture rate increasing to 43 newts (23 in 2017) with the highest capture rates being between 70 – 80 newts (35 to 47 in 2017).

Captures of smooth newts fluctuated throughout the study (figure 2.3b), with average captures ranging between 11 – 17 newts. Captures varied throughout the study, ranging from 18 to 30, usually occurring at the start of the season. An increase in average captures occurred in 2011, with average capture rate increasing to 23, with the highest captures being between 36 to 40 newts.

Captures of palmate newts varied between the years (figure 2.3c), with the average capture rate fluctuating each year. Average weekly capture rates ranged between 12 and 30 newts, with the highest capture rate being between 22 and 48.

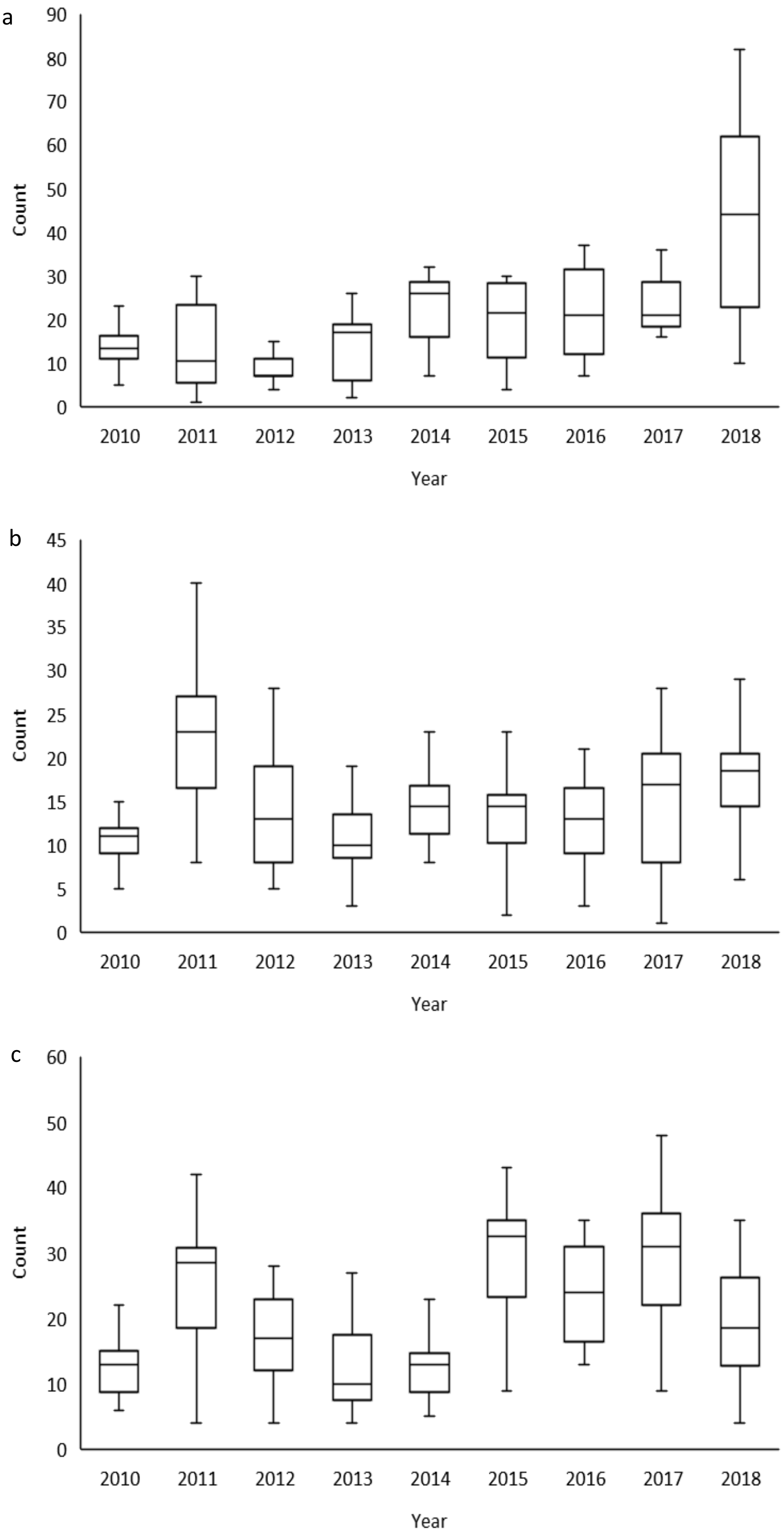

Figure 2.3. Box plots demonstrating the median, interquartile and total ranges of the population trends of three newt species for each year between 2010 and 2018, a) *Triturus cristatus*, b) *Lissotriton vulgaris*, c) *Lissotriton helveticus*. These box plots utilise all captures that occurred between February and July that were within the optimum temperature range (5°C and 17°C) and demonstrate the changes in population density throughout the study.

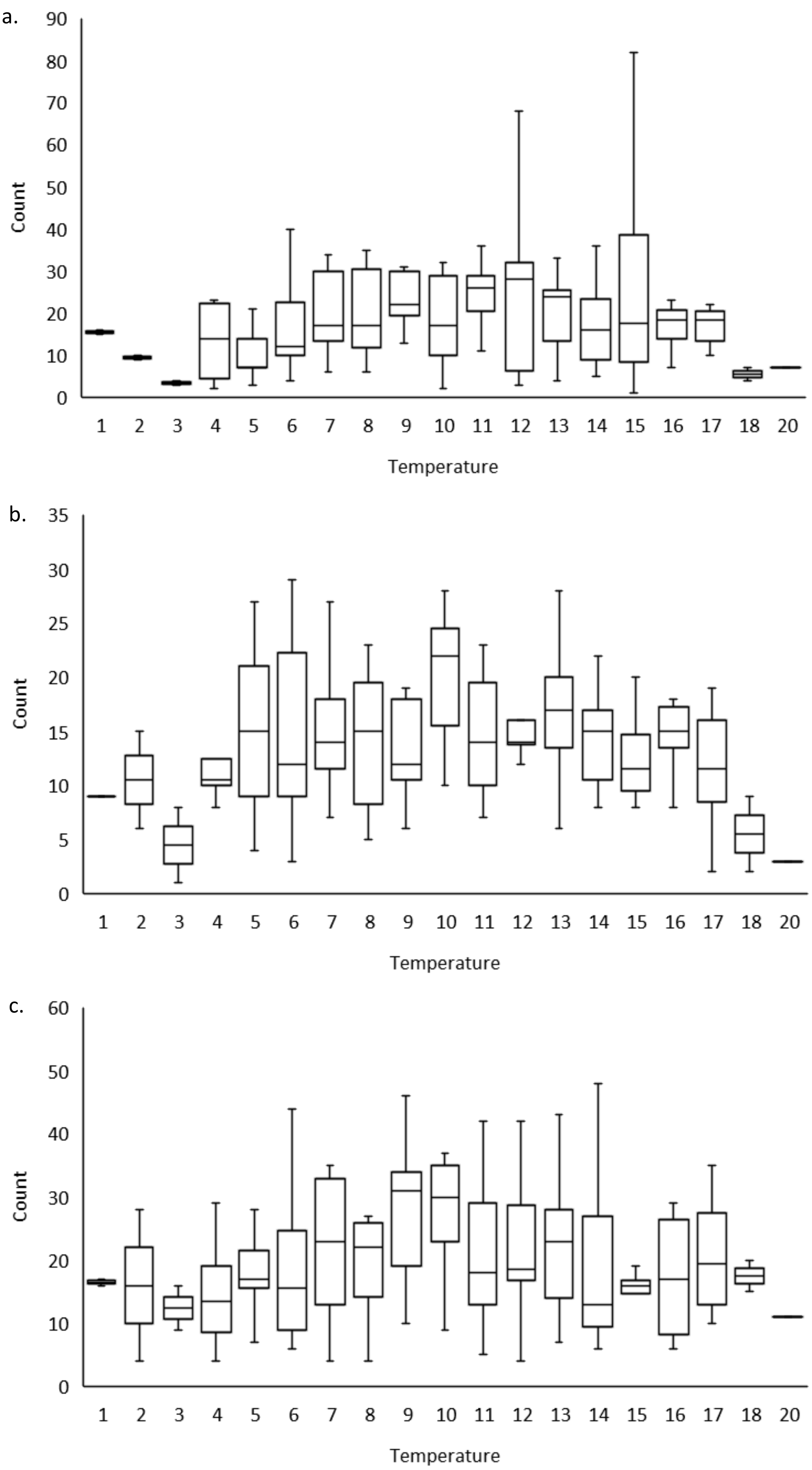

Figure 2.4. Box plots demonstrating the median, interquartile and total ranges in total trap captures for each of the three species in relation to temperature. Trap captures across the ponds were separated into species, including a) *Triturus cristatus*, b) *Lissotriton vulgaris*, c) *Lissotriton helveticus*. These box plots demonstrate the fluctuations in captures across the temperature range.

2.3.2 Comparison of captures in relation to lunar phase and temperature

Great crested newts

Both lunar phase and temperature influenced the capture rate of great crested newts, with complex interactions being identified and the importance of lunar and meteorological factors depending on the year. Lunar phase had the greatest impact on capture rate of great crested newts over the course of this study, but its impact varied between years, with six out of the nine years being influenced by the changes in lunar phase (table 2.1; appendix 2). By itself temperature had no influence on the capture rate of great crested newts for any of the nine years (appendix 2). However, the impact of temperature depended on the year, and there was also a three-way interaction that indicated that the combined impact of temperature and lunar phase depended on the year (table 2.1).

Table 2.1: Results from a generalised linear mixed model to determine how lunar phase and temperature influence the trapping patterns of the great crested newts between 2010 and 2018.

Source	F	df	P
Lunar phase percent	0.877	1	0.351
Temperature	3.541	1	0.062
Year * Lunar phase percent	4.743	8	<0.001
Year * Temperature	1.598	8	0.133
Lunar phase percent * Temperature	0.667	1	0.416
Year * Lunar phase percent * Temperature	5.412	8	<0.001

Smooth newts

The capture rate of smooth newts was influenced by both the lunar phase and temperature, with lunar phase influencing three out of nine years and temperature having an influence in one out of nine years (table 2.2; appendix 2). These results indicate that a combination of both the lunar phase percent and temperature has the greatest impact on the capture rate of smooth newts, with five out of the nine years being influenced by both (appendix 2). There was also a three-way interaction, demonstrating that the combined influence of temperature and lunar phase depended on the year (table 2.2).

Table 2.2: Results from a generalised linear mixed model to determine how lunar phase and temperature influence the trapping patterns of the smooth newts between 2010 and 2018.

Source	F	df1	df2	P
Lunar phase percent	2.068	1	116	0.153
Temperature	0.056	1	116	0.813
Year * Lunar phase percent	5.259	8	116	<0.001
Year * Temperature	4.300	8	116	<0.001
Lunar phase percent * Temperature	2.370	1	116	0.126
Year * Lunar phase percent * Temperature	6.558	8	116	<0.001

Palmate newts

Capture rate of palmate newts was influenced by interactions between lunar phase, temperature and years, with lunar phase influencing captures in two out of nine years and temperature also having an influence in two out of nine years (table 2.3; appendix 2). The three-way interaction therefore demonstrated that the combined influence of temperature and lunar phase depended on the year (appendix 2; table 2.3).

Table 2.3: Results from a generalised linear mixed model to determine how lunar phase and temperature influence the trapping patterns of the palmate newts between 2010 and 2018.

Source	F	df1	df2	P
Lunar phase percent	0.405	1	116	0.526
Temperature	0.699	1	116	0.405
Year * Lunar phase percent	3.815	8	116	<0.001
Year * Temperature	4.689	8	116	<0.001
Lunar phase percent * Temperature	0.287	1	116	0.593
Year * Lunar phase percent * Temperature	5.318	8	116	<0.001

2.4 Discussion

The data collected between 2010 and 2018 provide a clear indication that both lunar phase and temperature have an interactive influence on the capture rate of newts. Results from this study have identified that there are complex interactions between lunar phase and temperature, and these interactions are more important in some years than others (appendix 2). Yearly fluctuations in activity, temperature and population numbers need to be considered when trying to identify interactions with both lunar and meteorological factors. Whilst this study has identified that both lunar phase and temperature play an important role on the capture rate of each species, it is likely that other lunar or meteorological factors are influencing the capture rate, resulting in the differences in results each year.

This study found that the influence lunar phase had on the capture rate of newts varied between the years and in relation to temperature and had similar impacts on each of the three species. Neither lunar phase nor temperature had an influence on the capture rates of any of the species when analysing the data set as a whole (table 2.1, 2.2 & 2.3) , which is likely due to the differences in capture rates, population changes and meteorological factors across the nine year study. When the lunar phase was combined with year and temperature, results indicated that the lunar phase has an important influence on the capture rate of great crested newts, with lunar phase influencing the captures of six of the nine years (appendix 2). The lunar phase also influenced the capture rate of both the smooth and palmate newts, with lunar phase influencing the captures of smooth newts over 3 years and palmate newts being influenced for 2 years. These findings are not surprising as great crested newts are nocturnal in nature (Griffiths, 1983; Phillips, 2018), with the highest activity occurring during the darkest points in the night, compared to both smooth and palmate newts that have a more crepuscular behaviour (Griffiths, 1983; Phillips, 2018). Although lunar phase was expected to have an important influence on the capture rate of great crested newts, in 2011 and 2015, the lunar phase had no influence on the captures (appendix 2). It is likely that there were other external factors influencing the capture rate for these years.

As amphibians are ectothermic with body temperature regulated by external sources, it is evident that temperature has an important influence on all aspects of their behaviour, such as activity, feeding, reproduction and migration (Brooke *et al.,* 2000; Byrne 2002; FitzGerald & Bider, 1974; Griffiths, 1985b). Water temperatures below 5°C and above 19°C resulted in low trap captures for all three species that were excluded from analysis, but within this range captures for all three species remain temperature dependent. These results were, expected as multiple studies have indicated that temperature is an extremely important factor in amphibian activity and behaviour (Dolemen, 1983; Reading, 1998; Samajova, 2009).

Although the influence of environmental variables on all aspects of amphibian behaviour has been widely studied (Wells, 2007), the majority of these studies usually focus on only one or two different factors, such as temperature, light, cloud cover or rainfall. This study has been able to identify interactions between different factors, and determine what combinations have important impact on the capture rate of each species. When grouping and analysing lunar phase, temperature and yearly counts together, it is clear that all these factors combined have a greater influence on the capture rate of each species, compared to the factors separately (figure 2). Although these combined factors have a greater influence on the capture rates of each species, this combination of lunar phase and temperature does not account for all the variation in each year, and it is likely that other lunar or environmental factors are influencing these years.

Behaviour in amphibians is complex and studies have identified many different environmental and lunar factors that influence behaviour (Grant *et al.,* 2012; Höbel, 2017; Kusano *et al*., 2015). Light level has been identified to influence breeding, foraging and predator avoidance behaviour in amphibians (Baugh & Ryan, 2010; Grant *et al.,* 2012). Although the lunar phase influences the light level, other external factors may impact this, such as cloud cover, moon visibility in the night sky and artificial light (Dolmen, 1983; Grant *et al.,* 2012), all of which need to be taken into consideration as a whole, rather than separately. Dolmen (1983) suggested that high activity levels in smooth and great crested newts may be to some extent dependent on the temperature and lighting conditions earlier in the day, which can impact metabolic rate. This may influence the total capture rate of each species due to increased number of individuals escaping from the traps during the start of the evening (Nicholls, 2018). Other factors, including the presence of predators, competition, artificial light and habitat have all been linked to behavioural changes in amphibians (Dolmen, 1983; Grant *et al*., 2012; Harper *et al.,* 2018; Skei *et al*., 2006), but were not taken into consideration while the data was being collected.

2.4.1 Limitations

Several analytical problems were encountered during this project. The main problem identified was linked to the information available and how it was not designed to identify trap captures in relation to the lunar phase. Breeding periods of newts occur over a 10 to 14-week period, between February and May (Beebee, 2013; Inns, 2011) and as trapping sessions occurred on a weekly basis, there was (1) inconsistent coincidence between the trapping sessions and the lunar phases, and (2) seasonal variation in captures. Available data was then further reduced after identifying water temperatures below 5°C and above 19°C as having a major influence on the capture levels and were subsequently removed from further analysis. These weekly trapping sessions still did not account for changes in the lunar phase as they occurred on the same day each week, which resulted in unequal representation of different lunar phases within the data set for a given year, making extraction of lunar phase influences problematic. During the breeding season each species has a peak in population levels, usually lasting four to five weeks, between the end of March and the middle of May (Inns, 2011; Jehle *et al*., 2011). During this time there is usually an increase in trap captures, which again is likely to have had an influence on the results. Seasonal effects were not used in this model due to the limited amount of information available in this study and increasing the number of factors in the analysis would have resulted in overparameterization of the GLMM given the data available.

This study was only able to focus on two specific lunar and meteorological factors, which have been identified as influencing the behaviour of amphibian species (Grant *et al*., 2009; Vignoli & Luiselli, 2013). It is clear from the results that capture rates may be related to a combination of several different factors, including light level, barometric pressure, presence of predators and cloud cover, all of which were not recorded during the original study.

2.4.2 Conclusion

Despite using a data set that had the different lunar phases unevenly represented within it, all three species showed responses to the lunar phase that depended on temperature, but that also varied between years. Lunar phase and temperature may therefore have an important interactive influence on capture rate of each species, but that other factors, such as barometric pressure and cloud cover, may modify these effects.

This study only focused on how weekly trap captures were influenced by lunar and meteorological cues. However, lunar cues do not follow a weekly cycle and other important influences may have been missed. These limitations will be addressed in the next chapter, where external factors, including cloud cover, pond turbidity and vegetation will be identified and recorded.

Chapter 3: Analysis of newt captures over two lunar cycles

3.0 Abstract

Activity levels in amphibians vary throughout breeding season and are influenced by environmental and lunar cues. Although many studies have been conducted to identify which environmental cues trigger increased activity, the influence of the lunar cycle has been generally neglected. This study examined how the lunar cycle and environmental factors impact the capture rate of the great crested (*Triturus cristatus*), smooth (*Lissotriton vulgaris*) and palmate newt (*Lissotriton helveticus*). To measure the influence of lunar and meteorological factors on capture rate, lunar phase, water temperature and cloud cover were recorded throughout two lunar cycles during the breeding season. Variation in capture rate fluctuated throughout the study, with temperature important for great crested newts and cloud cover marginally important for smooth and palmate newts. Consequently, a combination of lunar and meteorological factors influences the capture rates of each species, with the importance of each factor likely to vary between species and according to season.

3.1 Introduction

Deciding when to breed is an extremely important decision in the life of any species, as reproduction is usually energetically costly (Höbel, 2017; Ryser, 1989). For any species to maximise fitness, reproduction timing should occur at the same time as favourable environmental conditions. By understanding the factors that determine reproduction intensity, we will have a better grasp of life history evolution, which will help predict how species may respond to climate change.

Activity patterns in amphibians have been well established (Beebee & Griffiths 2000; Dervo *et al*., 2016; Wells, 2007), with time of year and favourable environmental conditions triggering migration to breeding ponds, usually in the early spring (Harrison *et al.,* 1983). Since amphibians are ectothermic and semi-aquatic (Beebee & Griffiths 2000; Wells, 2007), many studies have regularly focused on environmental factors to explain how reproduction and activity is regulated. Temperature and rainfall are the most frequently identified factors influencing behaviour and population counts in amphibians (Brooke *et al.,* 2000; Byrne 2002; FitzGerald & Bider, 1974). However, other factors, including lunar cycle, light intensity, barometric pressure, humidity, moisture and wind, have all been identified as influencing behaviour (Deeming, 2008; Grant *et al*., 2012; Harrison *et al*., 1983).

The influence of the moon and its phases on amphibian activity and biology is unsurprising, as the moon has an impact on several climatic and environmental factors (Camuffo, 1999; Rich & Longcore, 2006). As most amphibians are either nocturnal or crepuscular in nature (Beebee & Griffiths 2000), light-dark cycles are likely to affect behaviour. Magnetic and gravitational field are linked to lunar cycles and moon distance (Naylor, 2001; Neumann, 1981). Although the lunar cycle was discovered to have an impact on the breeding and sexual cycle in *Bufo melanostictus* and *Rana cancrivora* in the 1960s (Church 1960a, b; 1961), this topic has been generally neglected.

Our aims in this paper are to provide field data on the influence of both lunar and meteorological factors on the capture rate of three newt species (*Triturus cristatus, Lissotriton vulgaris* and *Lissotriton helveticus*) over course of two lunar cycles. This study will build upon the work carried out in chapter two, looking at different meteorological factors with a focus on the lunar cycle. This may provide insights into survey results, which may influence future survey designs and conservation projects. The following key questions are proposed: (1) Are the capture levels of the study species influenced by the moon phase? (2) How does water temperature and cloud cover, influenced by the lunar phase, affect the capture rate of the study species?

3.2 Methodology

3.2.1 Study site and species

This project was undertaken and supervised by John Phillips and Richard Griffiths, funded by the University of Kent. This was conducted under a great crested newt survey and research licence, granted by Natural England, and held by John Phillips and Richard Griffiths. Guidance from Natural England was followed to ensure welfare of both target and non-target species (Natural England, 2014), with control measures regarding disease and non-native species being adhered to (ARG-UK, 2017).

The field site is located at the north-western part of the University of Kent campus, Canterbury, Kent (TR 12977 59673). The site is comprised of eight purpose-built experimental ponds which have been created for the study of colonization rates and population dynamics of amphibians (see chapter 2). Each pond was naturally colonised and support healthy populations of the three native newt species: the great crested newt (*Triturus cristatus*), smooth newt (*Lissotriton vulgaris*) and palmate newt (*Lissotriton helveticus*). Several other amphibian, reptile, bird mammal and invertebrate species are also found on the site, see chapter 2.

3.2.2 Collection of trapping data

This study took place over two lunar cycles during the aquatic stage of newt breeding season, from the 5th of March 2019 to the 27th of April 2019. A total of 16 trapping sessions occurred over the course of the study (eight per lunar cycle), with two trapping sessions occurring the evening before and the evening after four different lunar phases, including the new moon, 1st quarter, full moon and 3rd quarter.

The funnel traps followed the design described by Griffiths (1985a). Each trap was created from a 1 litre plastic bottle, with the top removed and inverted, then placed in the bottom section. Six funnel traps were set in each pond (figure 2.2) facing towards the centre of the pond and anchored to the edges using bamboo canes. Funnel traps were set at 19:30 hrs each evening and emptied the following morning at 07:30 hrs. Each trapping session recorded all newts caught across the eight ponds, where each individual was identified into species and sex, where they were then released back into the ponds they were originally caught.

3.2.3 Lunar, meteorological and environmental data

Moon data including phase, illumination, rise and set times, distance and other lunar cycles were obtained from the United States Naval Observatory Astronomical Application Department (http://aa.usno.navy.mil/data/docs/MoonFraction.php) at midnight, universal time (GMT) with the coordinates: Canterbury, 51°29'N and 01°06'E.

Meteorological data, comprising precipitation level (mm), using a standard rain gauge, air temperature (°C), using an external min/max thermometer and cloud cover percentage, all of which were recorded in the centre of the field site, next to pond three. All meteorological data was collected both while setting the traps at 1930 and once again when the traps were collected at 0730.

Overnight water temperatures were recorded in pond three while both setting and collecting the traps. This was done using a min/max thermometer set at the shallow end of the pond and a Tinytag plus 2 data logger set at 0.7 m at the deepest point: for the purpose of this study the min/max temperatures were used. Other environmental conditions, including pond turbidity using a Secchi disk with a scale from 1 to 3 (visible at 1 = bottom, 2 = middle, 3 = top) and vegetation density in the ponds (low – high) were recorded each trapping session. Disturbances that may have had an impact on the behaviour of the newts were noted, including the presence of predators and any increase in human presence.

3.2.4 Statistical analyses

A generalised linear model, utilising IBM SPSS software (version 25), was used to test the effects that three factors, that were identified using exploratory analysis (see appendix 1), had on the capture patterns of the great crested, smooth and palmate newts during each lunar cycle. These factors included the percent the lunar disk was visible, water temperature and cloud cover percentage. To determine which of these factors had the greatest influence on the capture rate of each species, the generalised linear model utilised combined captures for each of the three species across the eight ponds for each trapping session due to the differences in population levels in each pond, as well as their ability to move between ponds during the study. The median pond temperature and cloud cover was calculated from the recordings during the setting and collecting of the traps. As the trapping data comprised counts, the generalised linear model utilised a Poisson distribution model, with a log link function and all the factors were treated as fixed. Each cycle was modelled separately due to the changes in population levels, temperature range and cloud cover density, and the need to avoid overfitting models through the inclusion of too many predictors and interactions given the sample size. By conducting the generalised linear model in this way, the relative importance of each of the identified factors had on the capture rate of each species during each lunar cycle was determined.

3.3 Results

3.3.1 Capture rates over two lunar cycles

1545 newts were caught during this study, with 797 newts caught during the first lunar cycle (5^{th} - 30th March 2019) and 748 during the second (4^{th} - 28^{th} April 2019). Between the two lunar cycles the mean capture of great crested newts per trapping session increased from 51.1 during the first cycle to 63.4 during the second: an increase of 24%. The mean capture of smooth newts decreased between the first and second cycles, from 33.3 to 20.4 captures per trapping session: a decrease of 39%. The same trend was also seen in the mean captures of palmate newts, which went from 15.3 during the first cycle to 9.8 during the second; a decrease of 36%.

Water temperature throughout the study varied between 6°C and 14°C. Between 5th March and 13th April the water temperatures were between 6°C and 8.5°C, with a sudden increase at the end of the study between the 18th and 27th April, where the water temperatures increased to between 11°C and 14.5°C.

3.3.2 Comparison of capture rate to three external factors

Great Crested newts

During the first lunar cycle, none of the three potential predictors that were included in the model had an influence on captures (table 3.1; figure 3.1). During the second lunar cycle temperature was the only factor found to have an influence on the capture rate of great crested newts (table 3.1; figure 3.1). These results indicate that out of the factors compared, temperature was the only one to have an influence on great crested newt capture rates. As these results were only found in the April data set, it is likely that temperature combined with other unidentified factors are likely to be influencing the capture rate.

Table 3.1: Results from a generalised linear model to determine which of the three factors have an influence on the trapping patterns of the great crested newts, over the course of two lunar cycles.

First lunar cycle: 5-29 March 2019

Parameter	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
	B	Std. Error	Lower	Upper	Wald Chi-Square	P
Lunar Phase %	0.000	0.0035	-0.006	0.007	0.020	0.888
Temperature	0.111	0.1276	-0.139	0.361	0.757	0.384
Cloud Cover	-0.001	0.0017	-0.004	0.003	0.188	0.665

Second lunar cycle: 5-27 April

Parameter	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
	B	Std. Error	Lower	Upper	Wald Chi-Square	P
Lunar Phase %	0.000	0.0033	-0.006	0.007	0.001	0.974
Temperature	0.077	0.0294	0.019	0.134	6.806	0.009
Cloud Cover	0.001	0.0031	-0.005	0.008	0.208	0.649

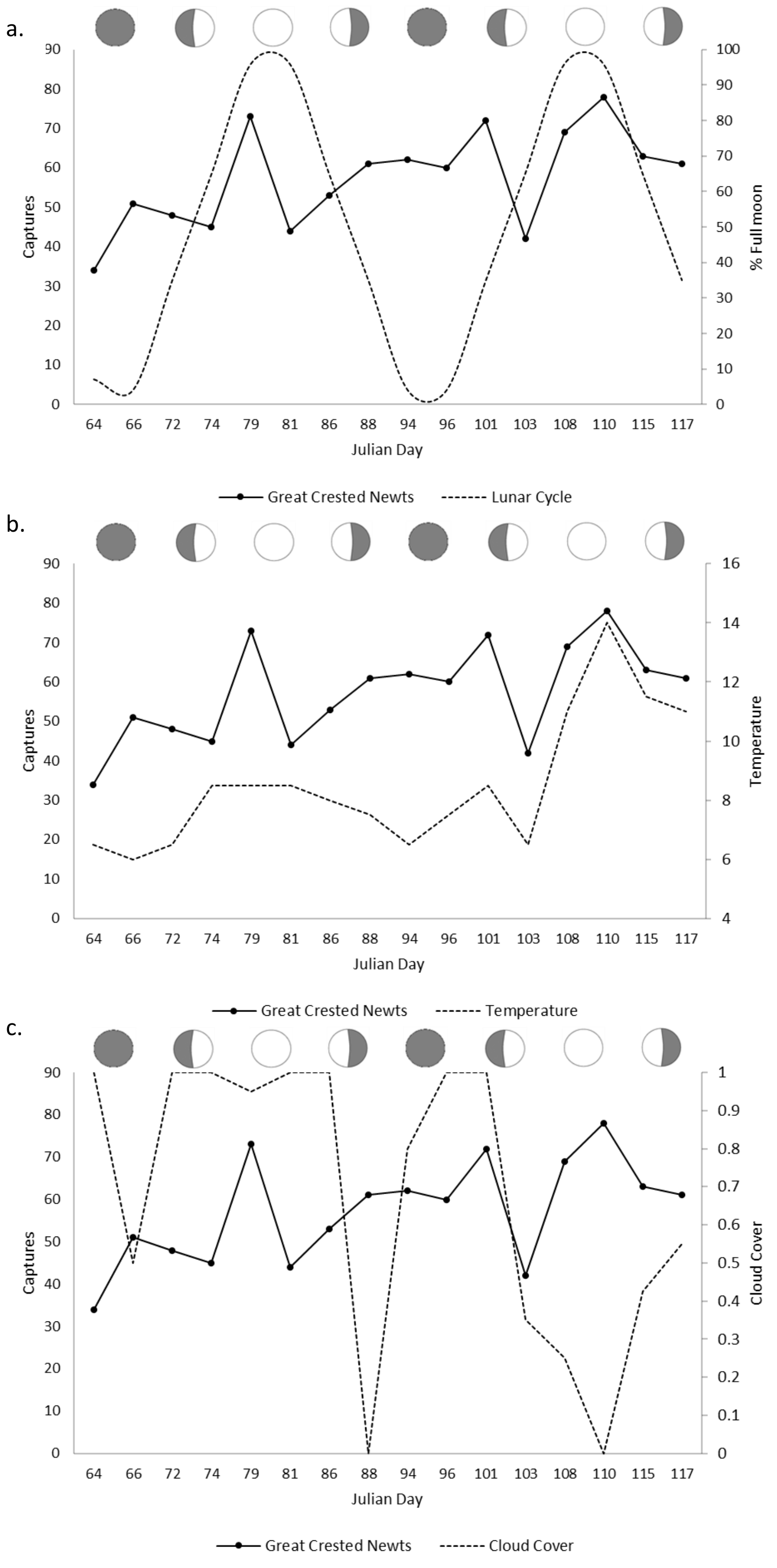

Figure 3.1: Combined activity trap counts of great crested newts, over the course of two lunar cycles (Julian days: first lunar cycle 65 – 87 and second lunar cycle 95 – 116), between March and April 2019, across eight study ponds. Combined trap counts are compared to the mean results of each of the three factors that have been identified, including: a) lunar phase, b) temperature, c) cloud cover.

Smooth newts

During the first lunar cycle cloud cover was the only factor found to influence the capture rate of the smooth newts, with high activity occurring when cloud cover percent was high (table 3.2; figure 3.2). During the second lunar cycle none of the three factors were found to influence capture rate. As cloud cover only had an influence on the captures during the first lunar cycle, it is likely that cloud cover combined with other unidentified factors are influencing the capture rate of smooth newts.

Table 3.2: Results from a generalised linear model to determine which of the three factors have an influence on the trapping patterns of the smooth newts, over the course of two lunar cycles.

First lunar cycle: 5-29 March 2019

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	0.000	0.0042	-0.009	0.008	0.011	0.916
Temperature	-0.005	0.1495	-0.298	0.289	0.001	0.976
Cloud Cover	0.005	0.0023	0.000	0.009	4.189	0.041

Second lunar cycle: 5-27 April

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	-0.002	0.0059	-0.014	0.009	0.136	0.712
Temperature	0.056	0.0488	-0.040	0.152	1.314	0.252
Cloud Cover	-0.004	0.0057	-0.015	0.007	0.532	0.466

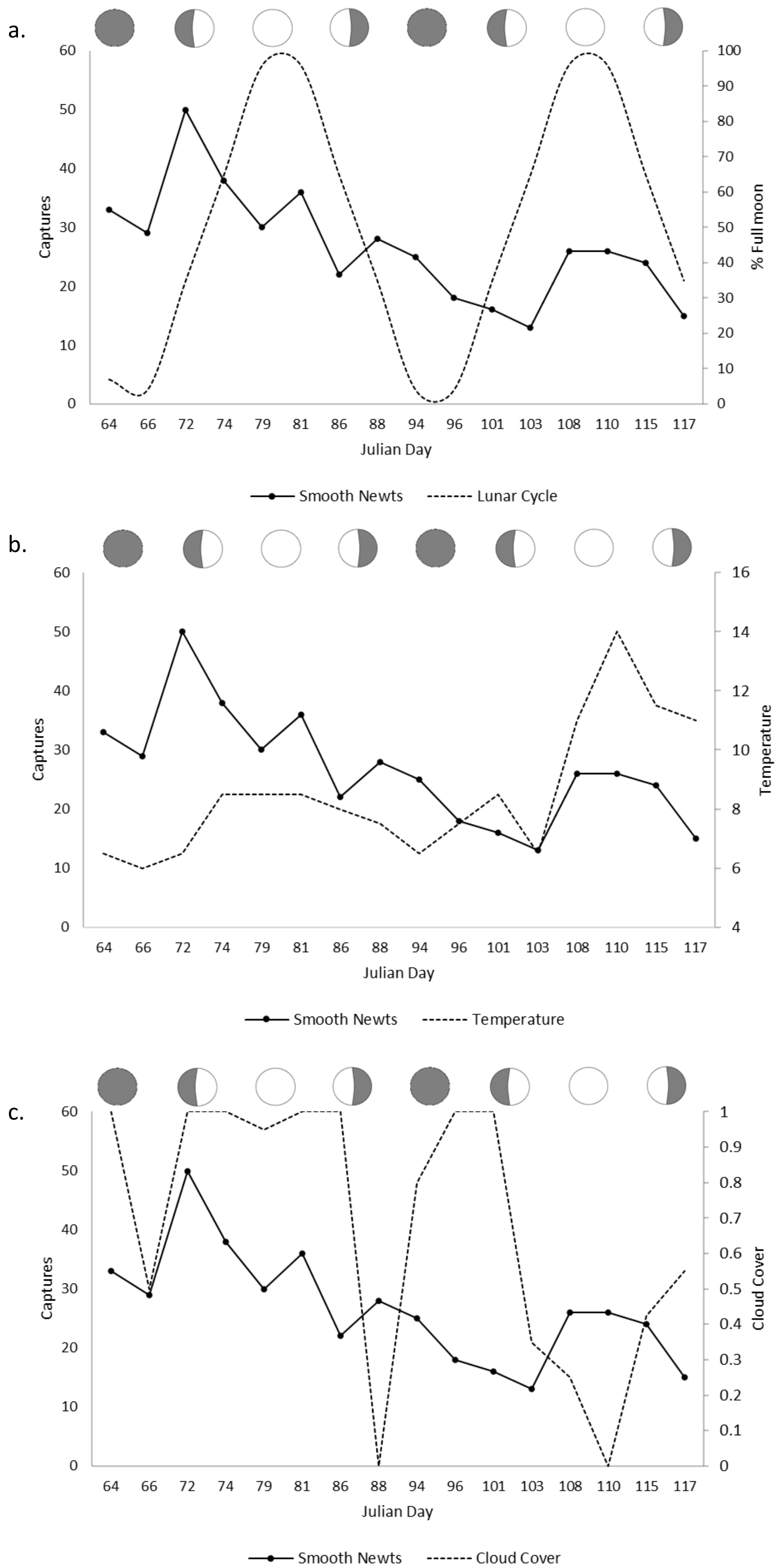

Figure 3.2: Combined activity trap counts of smooth newts, over the course of two lunar cycles (Julian days: first lunar cycle 65 – 87 and second lunar cycle 95 – 116), between March and April 2019, across eight study ponds. Combined trap counts are compared to the mean results of each of the three factors that have been identified, including: a) lunar phase, b) temperature, c) cloud cover.

Palmate newts

None of the three factors identified were found to have an influence on the capture rate of palmate newts during the first or second lunar cycle (table 3.3, figure 3.3). These results indicate no single factor that was identified influences the capture rate of palmate newts, although the effect of cloud cover was nearly significant in the first cycle. It is likely that a range of different factors are influencing the capture rates of palmate newts.

Table 3.3: Results from a generalised linear model to determine which of the six factors have an influence on the trapping patterns of the palmate newts, over the course of two lunar cycles.

First lunar cycle: 5-29 March 2019

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	B	Std. Error	Lower	Upper	Wald Chi-Square	P
Lunar Phase %	-0.005	0.0060	-0.017	0.007	0.733	0.392
Temperature	0.309	0.2200	-0.122	0.740	1.972	0.160
Cloud Cover	0.007	0.0036	0.000	0.014	3.520	0.061

Second lunar cycle: 4-27 April 2019

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	B	Std. Error	Lower	Upper	Wald Chi-Square	P
Lunar Phase %	-0.004	0.0083	-0.020	0.012	0.229	0.632
Temperature	0.077	0.0798	-0.080	0.233	0.923	0.337
Cloud Cover	0.005	0.0076	-0.010	0.019	0.378	0.539

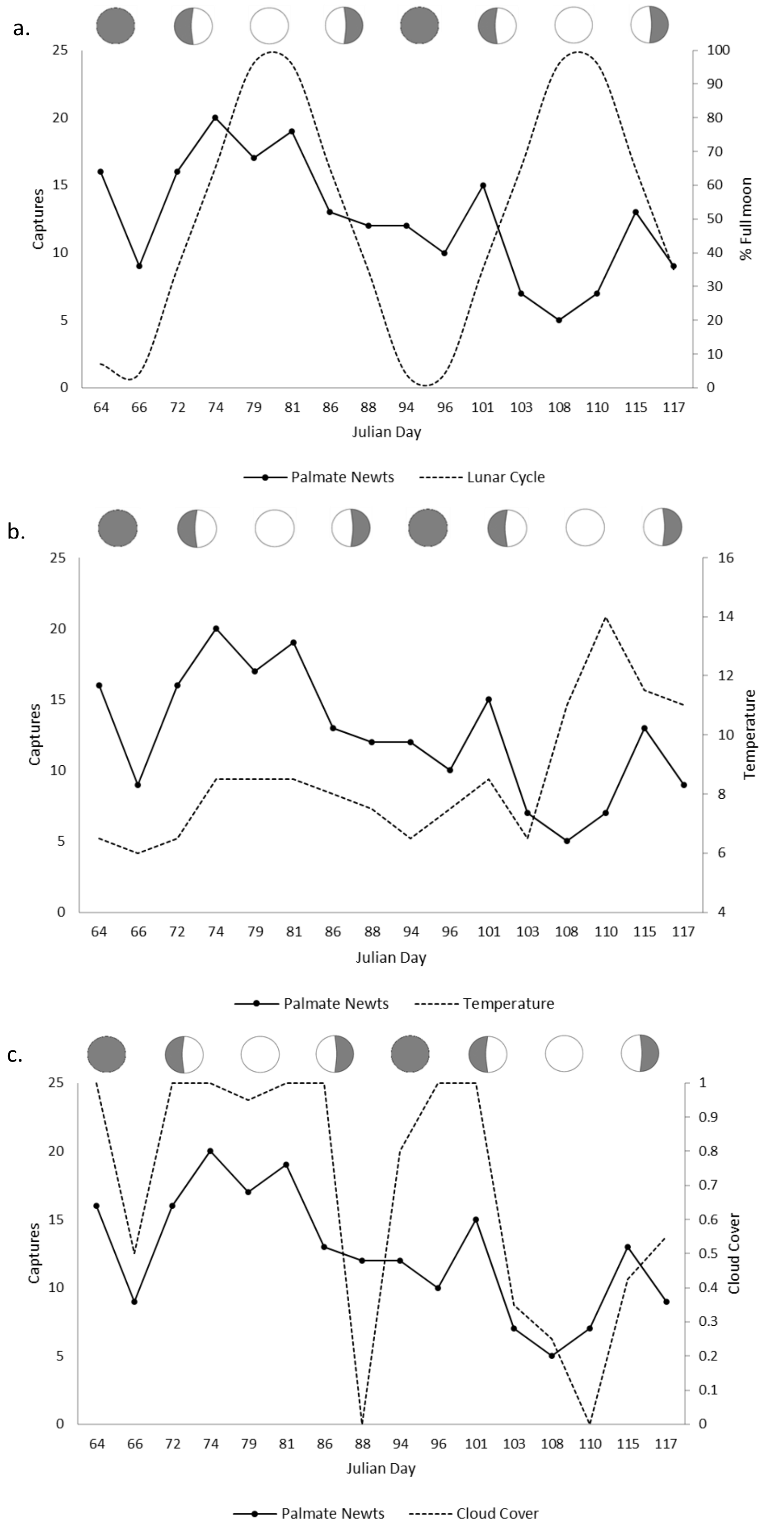

Figure 3.3: Combined activity trap counts of palmate newts, over the course of two lunar cycles (Julian days: first lunar cycle 65 – 87 and second lunar cycle 95 – 116), between March and April 2019, across eight study ponds. Combined trap counts are compared to the mean results of each of the three factors that have been identified, including: a) lunar phase, b) temperature, c) cloud cover.

3.4 Discussion

The data collected in this study indicate variation between the three species in how meteorological factors influenced capture rates of newts. Two out of the three factors analysed - water temperature (*T. cristatus*) and cloud cover (*L. vulgaris*) - had an influence on at least one of the three species. Lunar phase was the only factor over the course of two lunar cycles to have no influence on the capture rate of any species.

The impact of the lunar cycle and changes in lunar phase on the behaviour of amphibians has become more popular in recent years, which is seen by the increase in studies looking into its impacts (Deeming, 2008; Grant, 2012; Losson, 2010; Underhill, 2018; Vignoli, 2014). Although this subject and its impacts have become more popular, there is no consensus on how the lunar phase influences behaviour. This could be due to a number of different factors, including species, methods, statistics and region (Grant, 2012). Although there is no consensus on how the changes in lunar phase influences behaviour, it is generally believed that behaviour in nocturnal and crepuscular amphibian species are impacted by the changes in lunar cycle and the moon (Church, 1960a; Grant, 2012; Losson, 2010). With this in mind, the results from this study are surprising as the lunar phase had no influence on any of the species, for either lunar cycle. This conflicts with the results from chapter two, where lunar phase had an important influence on the capture rate of great crested newts and the interaction of both lunar phase and temperature had an important influence on the capture rate for both the smooth and palmate newts, over multiple years. Deeming (2009) also identified that funnel trap captures are influenced by the changes in the lunar phase, with an increase in captures of both great crested and smooth newts occurring around the new moon. These differences in findings may be due to the different methods used to collect the information, such as length of the study, number of traps used or time of year. Other underlying factors may also be influencing the results, such as temperature, population densities and migration times. This indicates that further study is required to get a better understanding of how lunar phase influences the capture rate of the three newt species.

As newts are ectothermic, slight changes in ambient temperature are known to have an important influence on behaviour and activity levels (Wright, 2009). The findings from this study are consistent with previous studies (Beebee, 2013; Griffiths, 1985b; Sweeny & Hastings, 1960), where water temperature was the only factor to have an influence on the capture rate of great crested newts. The influence of water temperature on the capture rate of great crested newts can be seen during the second lunar cycle, when the water temperature increased from 7°C to 13°C (figure 3.1b). This sudden increase in temperature resulted an increase in captures, and which is believed to be an increase in activity. Although this increase in temperature did not show in the statistical analysis of the smooth and palmate newts, both species had a slight increase in captures during this temperature increase (figure 3.2b & Figure 3.3b). Although previous studies have identified temperature to be an important influence of both the smooth (Griffiths, 1985; Patterson, 2018) and palmate newts (Patterson, 2018; Wright, 2009), the results from this study are consistent with the findings from chapter 2, where temperature interacts with other factors in terms of influencing capture rates.

Cloud cover is well known to influence multiple factors including light level, temperature and barometric pressure (Jaswal *et al.,* 2016; Rich & Longcore, 2006; Wetherald & Manabe, 1980), but the influence of cloud cover on amphibian behaviour is not well understood, with limited studies including or reporting their findings. This study has found that cloud cover is an important factor influencing the capture rate of smooth newts during the first lunar cycle (table 3.2), with high captures occurring during periods of high cloud cover (figure 3.2c). Although this result was found, it is likely that smooth newts may be reacting to the influence the cloud cover has on another factor, such as light level, humidity or barometric pressure, the inclusion of these in future studies may help identify these interactions (see appendix 1).

This study set out to determine the most influential factors over the course of two separate lunar cycles during March and April 2019, during the breeding season. This study found several, significant differences between these two lunar cycles, with there being a large shift in the captures of each species. Great crested newt captures increased by 35% between the first and second lunar cycle, which indicates that a migration of individuals occurred during this study. This increase corresponds to a decrease of nearly 40% in both smooth and palmate newts. Avoidance of traps containing great crested newts has been seen in both smooth and palmate newts and may be a result of direct predator avoidance or being driven away by great crested newt lekking behaviour (Nicholls, 2018; Wright, 2009). While comparing captures to lunar and meteorological factors, other factors such as behaviour modifications in relation to other species needs to be considered.

3.4.1 Limitations of the research

Although bottle trapping is widely used to determine capture patterns and population levels, it may not be the best method to determine activity and behavioural changes regarding lunar and meteorological factors. Nicholls (2018) showed that up to 30% of newts escape from traps during the course of an overnight trapping period. Great crested newts also tend to have a higher increase in trap retention towards the end of their activity periods (Nicholls, 2018), which may not represent activity levels throughout the trapping period.

This study focused on one site that has been deliberately constructed for research into colonisation and population dynamics of all three species of newt. Due to this site being created for this purpose, it is likely that these ponds have higher population densities compared to more natural areas. This may influence species behaviour due to increased competition for resources (Beebee & Griffiths, 2000; Jehle *et al.,* 2011). Due to pond location and size, there is a low number of predators utilizing the ponds regularly (*Natrix natrix* and various bird species), which may potentially modify lunar and meteorological related behaviour, due to increasing or decreasing activity during periods of high light and temperature.

During this study, three specific lunar and meteorological factors were identified as important influences in the capture rates of amphibians (Grant *et al*., 2009; Vignoli & Luiselli, 2013). It is evident from the results that there are other factors that may have an influence on capture rate that were not included in this study, such as light levels, barometric pressure, other astronomical cycles and the presence of humans and other predators.

3.4.2 Conclusion

This study showed that capture rates of the three species were influenced by different meteorological factors. This study suggests that several other lunar or meteorological factors may have an influence the capture rate of each species, including population density, activity timing, light level and barometric pressure. Moreover, the relationships between captures and the lunar cycle may vary according to the time of the year and newt breeding phenology.

This study used trap captures are as a surrogate for 'activity'. Although trapping is widely used to determine population levels and capture patterns, it may not the best method to determine activity and behavioural changes regarding the lunar cycle and other lunar and meteorological cues. The final chapter looks at direct observations of behaviour, which will be compared to the lunar cycle and other lunar and meteorological cues. This study will take time of activity, species activity and direct influence from lunar and meteorological cues into account.

Chapter 4: Analysis of newt activity over two lunar cycles

4.0 Abstract

Amphibian behaviour has been linked to different lunar and environmental cues. Results of these studies are often controversial, with responses to lunar and environmental cues often being highly species-specific. This study examines how the lunar cycle and environmental cues influence the aquatic activity of three coexisting newt species: *Triturus cristatus*, *Lissotriton vulgaris* and *Lissotriton helveticus*. Using data collected over the course of two lunar cycles during the breeding season, this study tested for lunar and meteorological effects on the activity and behaviour of the three study species. Lunar phase, temperature and light level were all identified to have an influence on the activity of each species, but with varying importance. Great crested newts had a greater response to both temperature and lunar phase, with an increase in activity occurring at higher temperatures and around both the full and new moon, whereas smooth and palmate newts were more likely to be active during periods of high illumination. These results suggest that a combination of multiple lunar and meteorological factors are likely to influence aquatic activity in newts and other amphibian species.

4.1 Introduction

The moon influences several geophysical changes on earth, including effects such as light levels, ocean tides and other more subtle variables such as barometric pressure, electromagnetic radiation and geomagnetic fields (Morgan, 1999). These impacts are widely recognised to cause behavioural and biological changes on the earth's flora and fauna (Naylor, 1999; Park *et al*., 2006; Witt, 2013).

Behaviour has been well-studied in newts (Griffiths, 1983, 1985b; Jehle *et al*., 2011), with migration to breeding ponds being triggered by rising temperatures, increase in the length of day, humidity and rainfall (Grant *et al*., 2012; Harrison *et al*., 1983). During the aquatic period, studies have identified that newts focus mainly on reproduction and feeding (Griffiths, 1985b), which requires both reproductive synchronization in each species to increase mating success while also reducing any risk of predation (Beebee, 2013). This behaviour is likely to be affected by several different factors including temperature, light levels and moon phase, all which have been identified to influence breeding behaviour (Grant, 2009; Taylor *et al.,* 2007). Although behaviour may change in relation to lunar and meteorological factors (Deeming, 2008; Grant *et al*., 2012), most studies focus on one specific influence, such as the lunar cycle or light level, rather than looking at a range of potential factors.

Although behaviour has been well studied, the majority of these studies either utilise trap captures as an indication of behavioural changes, or laboratory experiments. Both of these approaches have identified important factors that influence behaviour, but they are both likely to have an influence on natural behaviour due to their intrusive methods. Nicholls (2018) identified that a high number of newts are able to escape from bottle traps, with a higher number of escapes occurring at the start of the evening. It is therefore possible that interactions between captured newts may influence the escape rate and limit the number that it is possible to capture in a single trap.

Our aims in this paper are to identify how lunar and meteorological factors influence the activity of three newt species (*Triturus cristatus, Lissotriton vulgaris* and *Lissotriton helveticus*). This study will build upon the work carried out in both chapter 2 and 3, by looking at how aquatic activity compares to capture rates, with a focus on the lunar cycle. The following key questions are proposed: (1) Are the activity levels of newts influenced by the moon phase? (2) How does light level and temperature influence the activity of newts? (3) How does activity level change over the course of the night and is this influenced by lunar or meteorological factors?

4.2 Methodology

4.2.1 Study species and study site

This project was undertaken and supervised by John Phillips and Richard Griffiths, funded by the University of Kent. This was conducted under a great crested newt survey and research licence, granted by Natural England, and held by John Phillips and Richard Griffiths. Guidance from Natural England was followed to ensure welfare of both target and non-target species, with control measures regarding disease and non-native species being adhered to (ARG-UK, 2017).

The field site is located at the north-western part of the University of Kent campus, Canterbury, Kent (TR 12977 59673). The site is comprised of eight purpose-built experimental ponds which have been created for the study of colonization rates and population dynamics of amphibians (see chapter 2). Each pond was naturally colonised and support healthy populations of the three native newt species: the great crested newt (*Triturus cristatus*), smooth newt (*Lissotriton vulgaris*) and palmate newt (*Lissotriton helveticus*). Several other amphibian, reptile, bird mammal and invertebrate species are also found on the site, see chapter 2.

4.2.2 Collection of observational data

This study analyses the observational data from *Triturus cristatus*, *Lissotriton helveticus* and *Lissotriton vulgaris* over the course of two lunar cycles, between the 6th of March and the 27th of April 2019. A total of eight evening observations took place over the course of the two lunar cycles. Each study session occurred on the night of four different lunar phases, including the full, 1st quarter, new and 3rd quarter moons.

Observational data was collected from three of the eight ponds, with each pond being chosen for clarity of its water to facilitate observations before the study started (figure 4.1). Each pond had two, 2 cm wide, orange nylon strips placed across the width at the bottom of each pond, one located at the shallow end and one slightly deeper, approximately 30 cm apart. Each newt that passed completely over a nylon strip was counted. Newts were easily observed crossing the strips, due to the light colour of the strip against the pond bottom. As female smooth and palmate newts are difficult to distinguish in the ponds, they were recorded together. These observations were conducted on alternating evenings to the trapping sessions (chapter 3), so no bottle traps were set during the observations.

Ponds were illuminated by using three KC fire flashlights, casting approximately 1000 lumens (approximately 8 lux) of red light. Two of these torches were attached to bamboo canes and placed at either side of the pond, focusing on the nylon strips. The third torch was handheld and used for extra illumination to help identify newts and scan the pond for activity. Red light was utilised as it has been identified to have a reduced impact on scattering behaviour and flash blindness in amphibians, compared to white light (Buchanan, 1993; Harasti & Gladstone, 2013; Rich & Longcore 2006). Although red light is preferred, scattering is still likely to occur, so to reduce this impact torches were turned on at least 2-minutes before counts initiated, allowing individuals to return to normal behaviour and adjust to the increase in light.

Each pond had a 10-minute observation period, occurring within the first 30 minutes of each hour, between 2200-0300 hrs, resulting in 6 x 10 min observation periods per pond per night. Each observation period included a 5-minute transect count, where each individual that crossed over a strip was recorded, as well as the species and sex, with the remaining time used to identify behaviour, pond location and total number of individuals. The order in which the ponds were monitored was randomised every hour, this was done to reduce any biases that may occur in the time difference.

4.2.3 Lunar, meteorological and environmental data

Moon data including phase, rise and set times, distance and other lunar cycles were obtained from the United States Naval Observatory Astronomical Application Department (http://aa.usno.navy.mil/data/docs/MoonFraction.php) at midnight, universal time (GMT) with the coordinates: Canterbury, 51°29'N and 01°06'E.

Meteorological data were recorded hourly, after activity monitoring had occurred on each pond. Precipitation levels (mm) were recorded using a rain gauge located in a clear area next to the ponds. Light levels were recorded using a digital lux meter (TENMA, TEN01070), recording both the average and max lux levels, which were taken while standing next to pond three to maintain consistency. Pond temperatures were collected from pond three, using a min/max thermometer set at the shallow end of the pond and a Tinytag plus 2 data logger set at 0.7 metres at the deepest point in the pond, for the purpose of this study the min/max thermometer was used. Cloud cover was recorded by noting the percentage of the sky that was covered in clouds.

Before each study session pond turbidity and amount of vegetation in the pond was recorded. Pond turbidity was recorded using a Secchi disk and due to the depth of the pond a simple measure of 1 to 3 was used (visible at 1 = bottom, 2 = middle, 3 = top). Total vegetation in the pond was recorded by noting the percentage of the water that was covered and what species was most predominant.

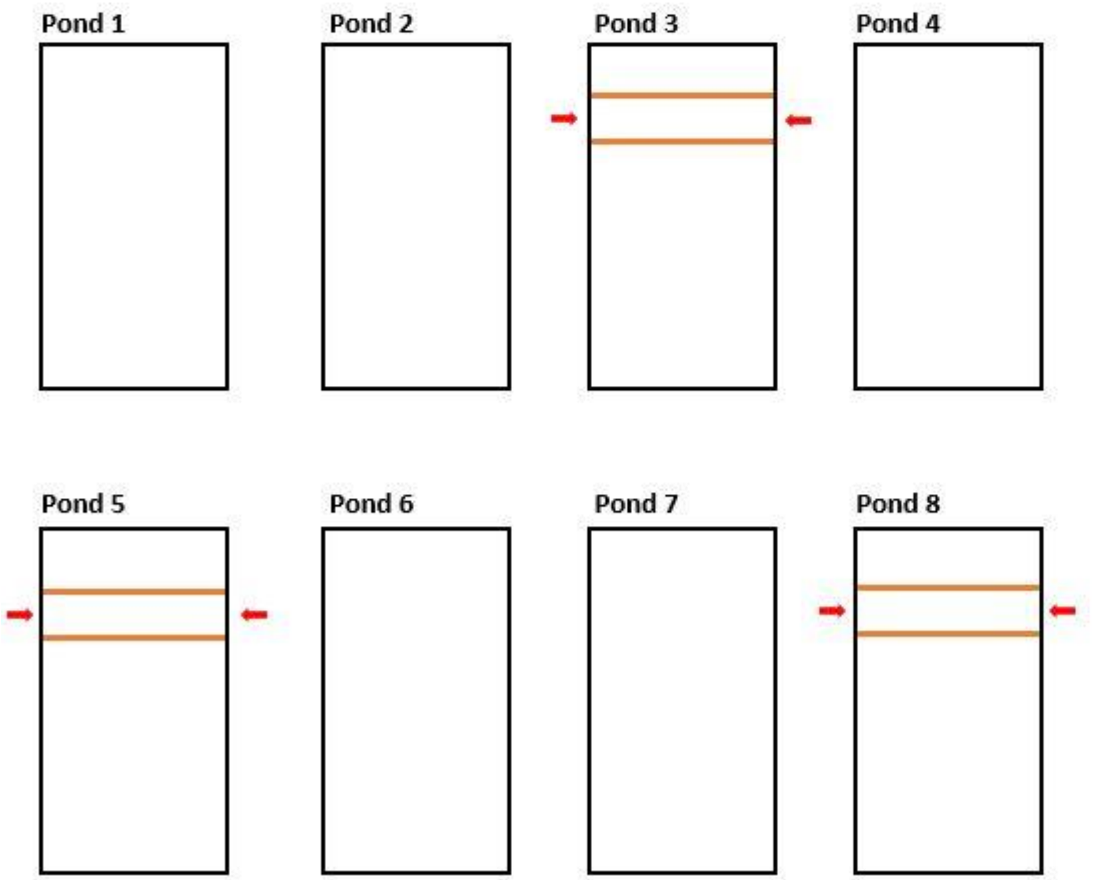

Figure 4.1: Plan of the layout at the field site in 2019. Ponds 3, 5 and 8 were the observation ponds chosen at random, with being the orange nylon strips placed at the bottom of each pond and being the red lights illuminating the ponds.

4.2.4 Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine the changes in activity between 2200 and 0300 hrs for the great crested newts and the combined results for the smooth and palmate newts for each lunar cycle. This was done to identify whether time after dusk had an influence on the activity rate of each species and if so, which time resulted in an increase or decrease in activity.

A generalised linear model, utilising IBM SPSS software (version 25), was used to test the effects that three factors had on the activity patterns of the great crested, smooth and palmate newts during each lunar cycle. These factors include lunar phase, water temperature and light level (lux). To determine which of these factors had the greatest influence on activity, total transect crossings across the three ponds were combined for each observational period (between 2200 and 0300 hrs), which were then separated into great crested newts and 'small' newts, including both smooth and palmate newts. These transect crossings were combined between the three ponds due to the population differences in each pond, as well as the ability for individuals to move ponds during the study. As transect crossings are counts, the generalised linear model utilised a Poisson distribution model, with a log link function and all the factors were treated as fixed. Each cycle was modelled separately due to the changes in population levels, temperature range and cloud cover density, and to avoid overfitting models with too many predictors and interactions in relation to sample size.

4.3 Results

4.3.1 General activity levels

Over the two lunar cycles a total of 144, 10-minute observations were carried out, which recorded over 2000 transect crossings. Great crested newts comprised 78% of the transect crossings, with a total of 1558 crossings being recorded. During the first lunar cycle 606 crossings were recorded, with an increase to 952 crossings during the second lunar cycle (figure 4.2): a 57% increase in activity. Smooth and palmate newts had a total of 443 transect crossings during the study, with 289 crossings recorded in the first lunar cycle, which decreased to 154 crossing during the second lunar cycle (figure 4.2): a 47% decrease in activity.

Throughout the study, water temperature ranged from 7°C to 14°C, which is in the optimal activity range of each species (see chapter 2), with only a slight drop of between 1°C and 2°C throughout each study session. Temperatures remained between 7°C and 10°C for the first six study sessions (6^{th} of March to the 12^{th} of April), with an increase to between 12°C and 14°C for the last two study sessions (19^{th} of April to the 26^{th} of April).

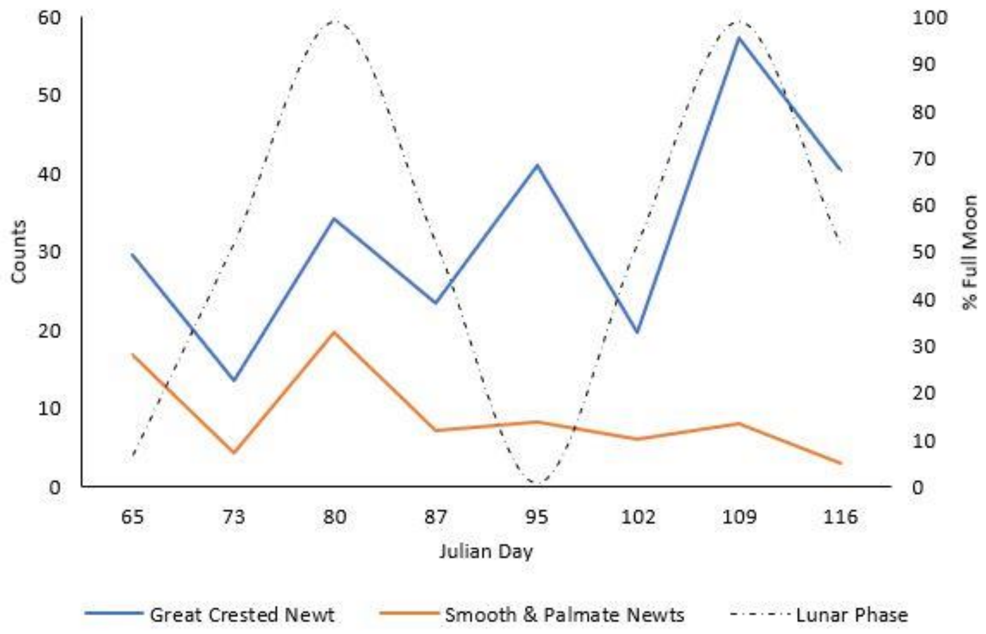

Figure 4.2. Combined activity counts of the great crested newts and combined smooth and palmate newts, over the course of two lunar cycles, between March and April 2019, across three study ponds. Activity is compared to the percentage of the moon that can be seen.

4.3.2 Comparison of activity in relation to time

4.3.2.1 Great crested newts

During both the first ($F = 0.285$, $p = 0.915$, df = 5,42 and second ($F = 0.677$, $p = 0.646$, df = 5,42) lunar cycles no differences were found in the mean activity between 2200-0300 hrs (figure 4.3). As a result, data collected at different times were pooled for further analyses.

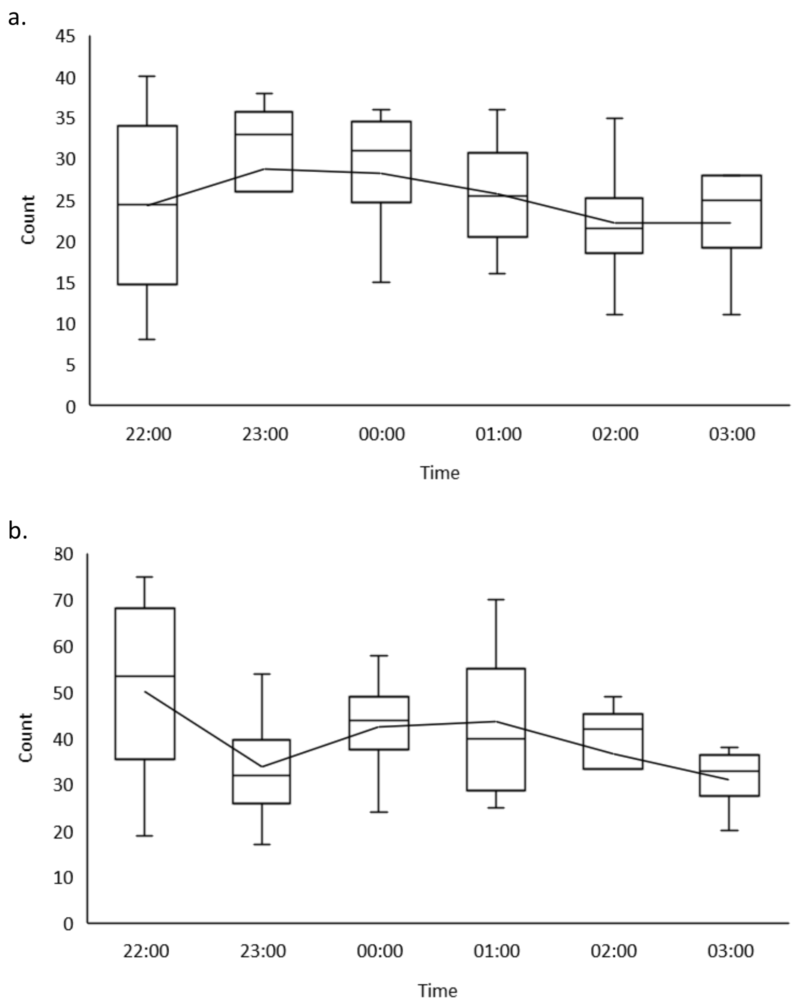

Figure 4.3: Box plot demonstrating the median, interquartile, and total ranges, for activity in the great crested newts. Activity counts across the three ponds were used for each lunar cycle, including: a) first lunar cycle (6th – 28th March 2019), b) second lunar cycle (05th – 26th April 2019), which were then separated into observation times between 1000 – 0300. These box plots demonstrate the changes throughout the observation periods.

4.3.2.2 Smooth and palmate newts

During both the first ($F = 0.221$, $p = 0.949$, $df = 5,42$) and second ($F = 0.457$, $p = 0.803$, $df = 5,42$) lunar cycle no differences were found in the activity between 2200-0300 hrs (figure 4.4). As a result, data collected at different times were pooled for further analyses.

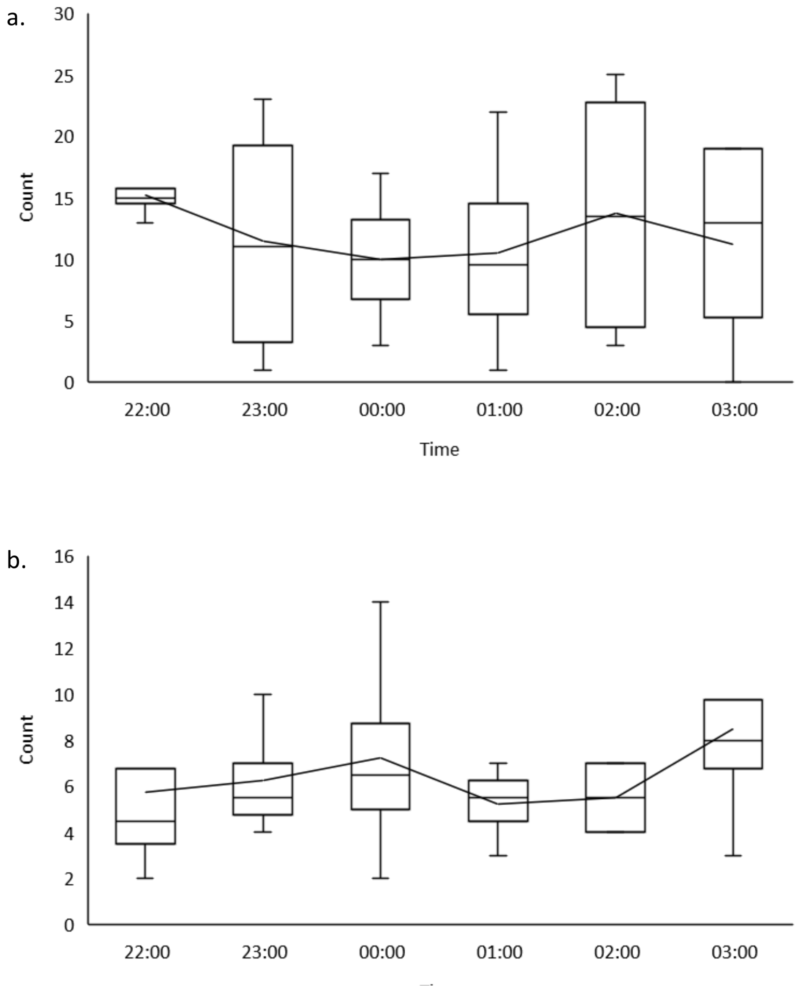

Figure 4.4: Box plot demonstrating the median, interquartile, and total ranges, for activity in the smooth and palmate newts. Activity counts across the three ponds were used for each lunar cycle, including: a) first lunar cycle (6th – 28th March 2019), b) second lunar cycle (05th – 26th April 2019), which were then separated into observation times between 1000 – 0300. These box plots demonstrate the changes throughout the observation periods.

4.3.3 Relationship of activity to lunar cycle and weather conditions

4.3.3.1 Great crested newts

During the first lunar cycle, all three factors had an influence on the activity, including lunar phase, temperature and light level (table 4.1; figure 4.5). During the second lunar cycle two of the three factors influenced the activity rate, including lunar phase and temperature (table 4.1; figure 4.3). This indicates that no one single factor is influencing the activity rate of great crested newts, but rather a combination of multiple different influences, including at least the lunar phase, temperature and light level.

Table 4.1: Results from a generalised linear model to determine which of the six factors have an influence on the activity patterns of the great crested newts (number of transect crossings per 5 min), over the course of two lunar cycles.

First lunar cycle: 6-29 March 2019

	Unstandardised Coefficients		**Interval (95%)**		**Hypothesis Test**	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	-0.004	0.0014	-0.007	-0.001	8.172	0.004
Temperature	0.272	0.0497	0.174	0.369	29.851	0.000
Light (Lux)	2.533	1.0825	0.411	4.655	5.476	0.019

Second lunar cycle: 5-27 April

	Unstandardised Coefficients		**Interval (95%)**		**Hypothesis Test**	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	-0.004	0.0016	-0.007	0.000	4.575	0.032
Temperature	0.147	0.0187	0.110	0.184	61.914	0.000
Light (Lux)	4.727	2.4728	-0.119	9.574	3.655	0.056

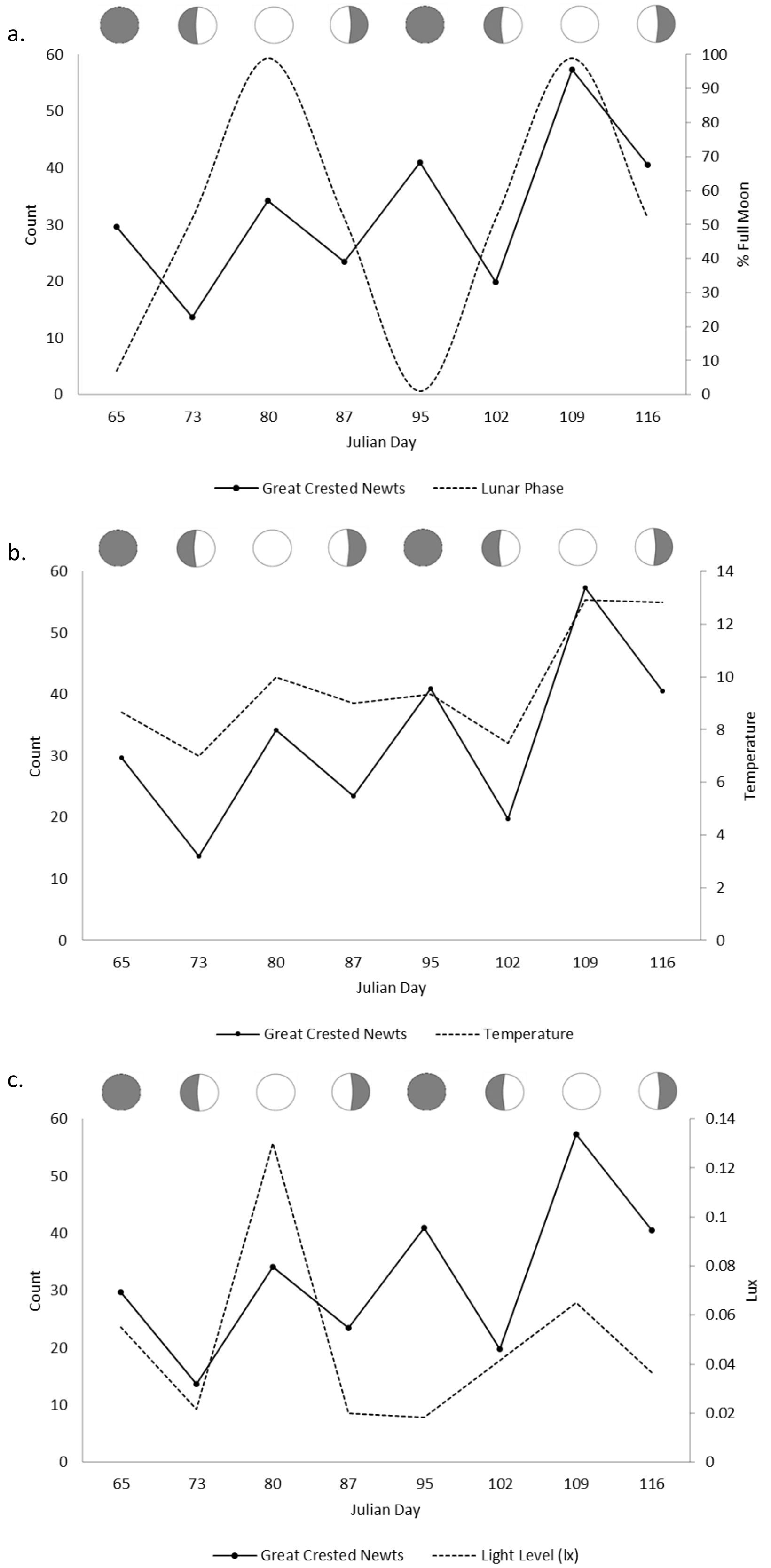

Figure 4.5: Combined activity counts of great crested newts, over the course of two lunar cycles (Julian days: first lunar cycle 65 – 87 and second lunar cycle 95 – 116), between March and April 2019, across three study ponds. Combined activity counts across the three ponds are compared to the mean results of each of the three factors that have been identified, including: a) lunar phase, b) temperature, c) light level.

4.3.3.2 Smooth and palmate newts

During the first lunar cycle, all three factors had an influence on the activity rate of the smooth and palmate newts, including lunar phase, temperature and light level (table 4.2; figure 4.6). During the second lunar cycle none of the modelled factors influenced activity rate. Due to the reduction of activity during the second lunar cycle it is likely that another factor or combination of factors not identified had a significant impact on the activity. These results indicate that no single factor determines the activity level of smooth and palmate newts, but more likely a combination of different influences.

Table 4.2: Results from a generalised linear model to determine which of the six factors has an influence on the activity patterns of the smooth and palmate newts, over the course of two lunar cycles.

First lunar cycle: 5-29 March 2019

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	-0.006	0.0020	-0.010	-0.002	10.189	0.001
Temperature	0.291	0.0773	0.140	0.442	14.185	0.000
Light (Lux)	5.994	1.5094	3.036	8.952	15.770	0.000

Second lunar cycle: 5-27 April

	Unstandardised Coefficients		Interval (95%)		Hypothesis Test	
Parameter	**B**	**Std. Error**	**Lower**	**Upper**	**Wald Chi-Square**	**P**
Lunar Phase %	-0.001	0.0039	-0.009	0.006	0.148	0.701
Temperature	-0.055	0.0406	-0.135	0.024	1.849	0.174
Light (Lux)	7.434	6.5321	-5.368	20.237	1.295	0.255

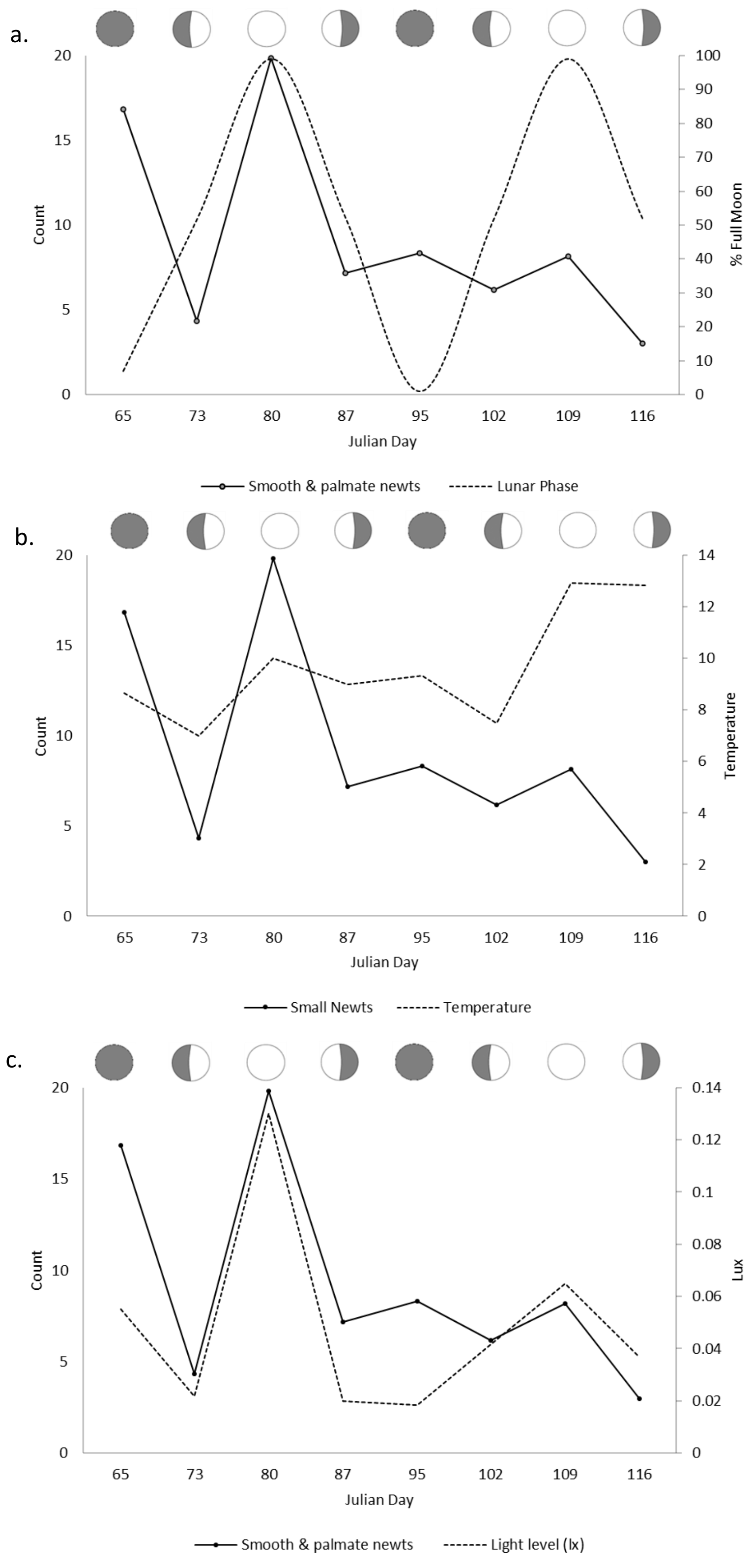

Figure 4.6: Combined activity counts of the smooth and palmate newts, over the course of two lunar cycles (Julian days: first lunar cycle 65 – 87 and second lunar cycle 95 – 116), between March and April 2019, across three study ponds. Combined activity counts across the three ponds are compared to the mean results of each of the three factors that have been identified, including: a) lunar phase, b) temperature, c) light level

4.4 Discussion

The data collected in this study provides a clear indication that both lunar and meteorological factors have an influence on the activity rate of the great crested, smooth and palmate newts. Although the influence of lunar cues and meteorological influences on amphibians is widely recognised, the influence is rather controversial. Evidence of behaviour modifications occurring during different lunar phases has been identified (Deeming, 2008; Grant et al., 2009), but contradictory evidence has been found, suggesting behaviour is influenced only by luminosity, with no evidence for endogenous rhythms being controlled by biological clocks (Vignoli et al., 2014). The difference in results is likely due to the difference in methods used, i.e. bottle traps (Deeming, 2008), migrations to breeding sites (Grant et al., 2009) and breeding phenology in relation to the lunar phase and light intensity (Vignoli et al., 2014). This study, by looking directly at aquatic activity, has been able to identify that lunar phase, temperature and light level, all have an important influence the activity of newts. This study was also able to identify that activity levels remain consistent for all three species between the hours of 2200 and 0300, with only minor fluctuations between these times.

This study found that lunar phase influenced the activity of all three species, with great crested newts having high activity occurring during both the new and full moon over both lunar cycles (figure 4.3a) and the smooth and palmate newts showing a similar pattern during the first lunar cycle (figure 4.4a). Grant *et al.* (2009) identified that first sightings and peak arrivals occurred more frequently around the new and full moon and less frequently during the 3rd quarter, which are comparable to the present results.

The level of moonlight may influence the behaviour of both nocturnal and crepuscular amphibians, yet true nocturnal light levels are rarely assessed (Underhill, 2018), and studies often identify that the new moon results in dark conditions and full moon with bright conditions, although light can be influenced by several different factors, including cloud cover and other light sources (Grant *et al*., 2012). This study demonstrated that lunar phase does not necessarily determine illumination levels during the night, with high levels of illumination (0.12 – 0.14 lux) occurring during both the new and full moons. The influence of illumination had a direct influence on the activity levels of both the smooth and palmate newts (figure 4.4c), with high levels of light resulting in increased levels of activity. This behaviour was also identified in great crested newts during the first lunar cycle (figure 4.3c) but it is likely that other external factors are having an influence on their behaviour. Periods of high lunar illumination have been identified to influence amphibian species in multiple ways, including feeding, predator avoidance, breeding, navigation and migration events. These influences cannot be generalised by taxonomic group or region but are highly species specific and relate directly to individual species ecology (Grant *et al*., 2012; Rand *et al*., 1997; Robertson, 1978). Both smooth and palmate newts rely on vision, smell and lateral line systems for the detection and avoidance of predators and in foraging (Buchanan, 1998; Deban & Wake, 2000; Grant *et al*., 2012). This means that any changes in light will likely influence how individuals orientate in their environment, to maximise foraging success and maximise information on potential predators or competition (Jaeger & Hailman, 1976). For nocturnal amphibians, illumination is known to influence breeding behaviour, with mate choice, movement, calling and visual displays either increasing or decreasing during periods of high ambient light (Baugh & Ryan, 2010). Both smooth and palmate newts are crepuscular in behaviour (Beebee, 2013; Phillips, 2018), suggesting that intermediate light intensities are optimal for foraging, breeding, orientation and migration (Jaeger & Hailman, 1973).

Temperature is widely recognised to have an influence on the behaviour of amphibians (Beebee, 2013; Wells, 2007), with temperature often being reported as one of the more important factors determining activity (Dolmen, 1983; Phillips, 2018; Šamajová & Gvoždík, 2009). This study supports the importance that temperature has on the activity of the great crested, smooth and palmate newts, with temperature influencing activity throughout the study. Temperature is an important factor in influencing the number of active newts during the migration period, which may have been the determining factor for the population increase during the second lunar cycle when temperature increased from an average of 8°c to 13°c.

Between the first and second lunar cycle there was a significant increase in great crested newt activity (57%), which relates to the increase in population density over the two lunar cycles (see chapter 3). In contrast, activity decreased in smooth and palmate newts between the first and second lunar cycle (47%). Wright (2009) identified the same negative impact great crested newts had on the behaviour of smooth and palmate newts. This negative relationship is likely to change the behaviour of both smooth and palmate newts, by either causing them to be more active between dawn and dusk or when great crested newt activity is reduced. Due to the likelihood of the great crested newt populations influencing the activity of smooth and palmate newts, it is difficult to determine which lunar and meteorological factors have the greatest influence on activity.

Periods of activity have been well studied in great crested, smooth and palmate newts, with great crested newts having nocturnal behaviour, with activity levels being highest between 2200 and 0300 hrs (Griffiths, 1985b; Phillips, 2018). Smooth and palmate newts are crepuscular, having high levels activity occurring during dawn and dusk, 0500 and 1900 hrs

(Beebee, 2013; Griffiths, 1985b; Phillips, 2018; Wells 2007). Although dawn and dusk were not included in this study, this behaviour is congruent with the results identified in this project, with both the great crested, smooth and palmate newts having similar activity levels between 2200 and 0300 hrs.

4.4.1 Limitations

Although hourly activity counts are a good method to identify changes in aquatic activity of newts, retrospectively there were some limitations to this study, including disruptive behaviour, count reliability and site influences.

Activity counts require the use of torches and spotlights to facilitate observations of individual aquatic activity levels. Jackson (1996) demonstrated that newts scatter in the presence of spotlights and increased light levels. Although red lights have been identified to reduce the scattering behaviour and flash blindness in amphibians (Buchanan, 1993; Harasti & Gladstone, 2013; Rich & Longcore 2006), when the torches were turned on some response was observed, with individuals appearing to move to deeper areas of the pond that were not illuminated. Although individuals returned to the illuminated areas within two to three minutes of the torches being turned on, it is likely that this had an influence on activity throughout the study period.

Although the observer has a high level of experience, count reliability and newt identification were heavily influenced by the pond turbidity, vegetation levels and weather. Although each study pond was generally clear and had low levels of vegetation, towards the end of the study, ponds became more turbid and vegetation levels increased. During periods of light rain or high wind levels, the water surface was disturbed, reducing the visibility of the nylon strips and the ability to count individual crossings. Although these disturbances are unavoidable, they may have impacted count reliability and individual identification during the study.

On two observations, high levels of activity were seen in one individual throughout the five-minute count, with consistent movement up and down the pond, which accounted for between 60% to 70% of transect crossings for each of those observations. Although these ponds often showed high levels of activity, the general activity counts were influenced. High activity in these individuals could have been due to cues other than lunar and meteorological factors, which may include human presence or the use of torches.

4.4.2 Conclusion

This study has identified that newt activity is related to a combination of lunar and meteorological cues, with each species being influenced by a combination of different factors. In addition, activity over the course of the night remained consistent throughout the study, with only slight variations occurring in each species. Lunar and meteorological cues have an important influence on the activity of newts, but other factors, including population density and activity start time, may have had a significant influence on the results.

This study focused on how activity was influenced by lunar and meteorological cues. In the general discussion the results from both the bottle trapping and activity counts are compared and key factors that influence newts are identified. These findings may help to influence the methods and effectiveness of population monitoring and conservation efforts in the future.

Chapter 5: General discussion

Although there has been a growing interest in how amphibians respond to lunar cycles, relatively few studies take all lunar influences into consideration, with studies focusing on single factors, such as the lunar phase or light level. This study took the main lunar influences and other known meteorological variables and compared them to both trap capture success and activity rates of three newt species during their aquatic stage. Throughout this study, several factors, i.e. lunar phase, water temperature, cloud cover, all influenced the activity level or trap capture success of each species in an interactive way that varied between years.

Our hypothesis that lunar phase influenced the capture rate and activity levels of each species is supported by the results. The lunar phase had a major influence on the great crested newts, with high activity occurring during both the full and new moons and a high capture rate occurring before and after the new moon. Although there are some differences in methods and results, these findings generally correspond with the results from Grant *et al*. (2009) and Deeming (2008), where activity increased around the new moon. Although the lunar cycle had an influence on the activity of the smooth and palmate newts, neither species was affected during trapping, which differs from the findings from Deeming (2008). Although these results indicate that the lunar phase may have an impact on the behaviour of both the smooth and palmate newts, it is likely that other external factors have more of an influence on trap captures.

Temperature is well-known to have an important influence on the behaviour of amphibians (Beebee, 2013; Griffiths, 1985b; Sweeny & Hastings, 1960), which is consistent with the findings during both trap capture and activity studies for all three species. This study showed that captures are lower when water temperatures are below 5°C and above 19°C. Even within this narrow range, trapping and activity levels of each species fluctuate with increasing and decreasing temperatures.

Cloud cover influences the activity rate of the great crested newts and the trap captures of the palmate newts, both of which increased during periods of high cloud cover and decreased during evenings with clear skies. Although cloud cover had an influence on captures, they were not consistent throughout the study. Cloud cover has an impact on several external factors, including light level, temperature and visibility of the moon, all of which have been identified to influence amphibian behaviour (Buchanan, 2006; Dai *et al*., 1997, 1999; Grant *et al.,* 2009). It is likely that individuals are not reacting directly to cloud cover, but rather a combination of cloud cover and other external factor but this requires further investigation.

Light level demonstrates a clear influence on the activity of smooth and palmate newts, where high levels of light results in increased activity. Although light level has been identified to influence the behaviour of amphibians (Grant *et al*., 2012; Rand *et al*., 1997; Robertson, 1978), most studies indicate that high levels of light result in reduced activity, often associated with predator avoidance (Baugh & Ryan, 2010; Buchanan, 1993; Rand *et al.,* 1997). As both smooth and palmate newts rely on vision, smell and lateral line systems for detecting predators, it is likely that light influences how individuals move in their environment (Buchanan, 1998; Deban & Wake, 2000; Grant *et al*., 2012). Due to smooth and palmate newts being photopositive in nature, (Beebee, 2013; Phillips, 2018), evenings with high levels of light are likely to result in extended periods of activity occurring throughout the evening (Jaeger & Hailman, 1973).

This study has identified that temperature, lunar phase and light level all have a clear influence on at least one of the study species, with cloud cover driving more variable responses. Although these factors have been identified, it has been made clear that meteorological and lunar-related activity is species-specific and needs to be taken into consideration in future studies.

This study utilised both trapping and activity counts, which are useful methods to indicate population counts and increases in activity, but it is likely that lunar-related factors have an influence on specific behaviours, such as foraging, breeding, competition or predator avoidance, which cannot be identified by these methods alone. Therefore, in order to better understand how individual behaviours are being influenced by the lunar and meteorological factors, I recommend three further avenues of research. Firstly, expand chapters 3 and 4 to include the full lunar cycle. This will provide a more rigorous analysis of the results, which may help identify the impact of each factor and enable the use of circular statistics (Fisher, 1995; Grant *et al*., 2009). Secondly, increase the number of study sites to include different habitats, population levels, predator abundance and which species can be found. By surveying several sites, it would be possible to determine if habitat and competitor and/or predator abundance has an impact on how newts respond to lunar and meteorological factors. Lastly, other aspects have been identified that may influence the behaviour of amphibians, that were not included in this study, including: barometric pressure, elevation and predator avoidance. Inclusion of these in future studies may prove to be an integral part in the capture and activity of amphibians (Buchanan, 2006; Grant *et al*., 2012; Hauselberger & Alford, 2005).

This study has identified that the trapping results identified in chapter three do not correspond to activity results in chapter four. This suggests that trapping is not a good measure of activity. This may be down to traps acting as more of a restraint rather than a trap, with a high turnover of newts occurring throughout the night (Nichols, 2018). When measuring activity levels traps may also impact the number of newts caught due to the upper limit a trap is able to catch. Wright (2009) also identified that higher number of great crested newts in traps reduces the number of small newts caught, suggesting interspecific interactions and/or limits on the number of newts a trap can capture. This study was carried out in 2019 when the newt populations reached their highest levels in 20 years and the ponds contained high densities. Trap captures may give a better indication of newt activity at lower population levels, which may explain the difference in results from previous studies (Deeming, 2008; Grant 2009). It may also explain differences in the apparent influence of environmental factors between the long-term analysis in Chapter 2 and the two lunar cycles analysed in 2019 in Chapter 3.

Generalised linear models are an effective tool to identify interactions between multiple fixed and random factors, but the number of factors these models are able to include depend on the number of data points, with between eight to ten data points recommended for each factor (Harrison et al., 2018). Due to this study focusing on the activity across two lunar cycles, the number of factors included were limited to a maximum of three. This study could have been expanded to include a total of three lunar cycles, but the increase in data points would have been offset by increased complexity, as populations counts, seasonal affects and multi-way interactions would have needed to be included, thereby overparameterizing the models.

Although the results from this study are limited, these findings can help influence the effectiveness of amphibian monitoring and conservation efforts. Many long-term amphibian studies do not take changes in the lunar cycle into account, often not even recording it as a variable. As this study has demonstrated, both the lunar cycle and other lunar changes have a considerable influence on both the capture rate and activity of three newt species. Long term monitoring that fails to take these into account may have a considerable impact on the study, giving misleading results. Including lunar variables in amphibian monitoring may provide more reliable results and create a better understanding of population trends. Due to the significant declines of many amphibian species around the world, population monitoring and understanding how species behaviour is influenced is critical in maximising conservation efforts and I encourage further study into lunar related behaviour.

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Appendices

Appendix 1
Exploratory analysis

Exploratory analysis conducted on the factors to be included in this model included, lunar phase, lunar phase percent, water temperature, cloud cover, light levels, moon visibility, moon distance and rain levels. Several other factors were also identified to influence the capture rate or activity of newts, including humidity, barometric pressure and other synodic cycles. These were not included in the exploratory analysis due to either the limited data collection methods (other synodic cycles), did not fit the model (humidity), or ability to reliably record the information (barometric pressure)

Correlation between lunar phase, moon visibility and moon distance were identified. Due to this both moon visibility and moon distance were removed from further analysis. Light level and cloud cover were also correlated. Chapter 3 included cloud cover in the analysis due to the ability to record the changes in cloud cover throughout the night and the potential ability to compare these results to chapter 2. Chapter 4 utilised light level in the analysis as any direct changes in light level had the potential to directly influence activity rate. These factors were chosen for each analysis as they either demonstrated the strongest signal or had the most reliable information.

Exploratory analysis determined that by utilising pond totals rather than the captures or activity monitoring from individual ponds resulted in the strongest fits to the data. Also, as ponds are not direct replicates, due to differences in vegetation, population levels, water clarity and the ability for individuals to move ponds, means that combining the ponds and treating it as one site was the best method for analysis and avoided problems of lack of independence between data points.

Separation of the two lunar cycles (March and April) was done as exploratory analyses showed stronger fits than when the months were combined. Issues arose when combining both lunar cycles due to changes in population levels between March and April, the sudden increase in temperature towards the end of the study, and resulting complex interactions between factors that overfitted the model in relation to the number of data points.

Appendix 2

Complete output of the generalised linear mixed model results in chapter 2 between 2010 and 2018. This analysis treated the lunar phase and temperature as fixed factors and the year as a random factor and calculated multiple levels of interactions for each the three species, including *Triturus cristatus, Lissotriton vulgaris* and *Lissotriton helveticus.* 2018 was used as a basis for comparison for all yearly analyses.

Triturus cristatus

Model Term	Coefficient	Std. Error	t	P	95% Confidence Interval		Exp (Coefficient)	95% Confidence Interval for Exp (Coefficient)	
					Lower	Upper		Lower	Upper
Intercept	2.729	0.2022	13.494	0.000	2.328	3.129	15.313	10.259	22.857
Lunar phase percent	0.013	0.0032	3.938	0.000	0.006	0.019	1.013	1.006	1.019
Temperature	0.042	0.0151	2.756	0.007	0.012	0.071	1.042	1.012	1.074
Lunar phase percent * [Year=2010]	-0.016	0.0067	-2.427	0.017	-0.030	-0.003	0.984	0.971	0.997
Lunar phase percent * [Year=2011]	-0.004	0.0064	-0.648	0.518	-0.017	0.009	0.996	0.983	1.009
Lunar phase percent * [Year=2012]	-0.019	0.0067	-2.783	0.006	-0.032	-0.005	0.982	0.969	0.995
Lunar phase percent * [Year=2013]	-0.039	0.0076	-5.215	0.000	-0.054	-0.024	0.961	0.947	0.976
Lunar phase percent * [Year=2014]	-0.016	0.0068	-2.402	0.018	-0.030	-0.003	0.984	0.971	0.997
Lunar phase percent * [Year=2015]	-0.007	0.0049	-1.484	0.141	-0.017	0.002	0.993	0.983	1.002
Lunar phase percent * [Year=2016]	-0.011	0.0046	-2.273	0.025	-0.020	-0.001	0.990	0.980	0.999
Lunar phase percent * [Year=2017]	-0.018	0.0049	-3.575	0.001	-0.027	-0.008	0.982	0.973	0.992
Lunar phase percent * [Year=2018]	0[b]								
Temperature * [Year=2010]	-0.042	0.0301	-1.412	0.161	-0.102	0.017	0.958	0.903	1.017
Temperature * [Year=2011]	0.066	0.0367	1.790	0.076	-0.007	0.138	1.068	0.993	1.148
Temperature * [Year=2012]	-0.032	0.0382	-0.828	0.409	-0.107	0.044	0.969	0.898	1.045
Temperature * [Year=2013]	-0.040	0.0368	-1.089	0.279	-0.113	0.033	0.961	0.893	1.033
Temperature * [Year=2014]	-0.040	0.0299	-1.324	0.188	-0.099	0.020	0.961	0.906	1.020
Temperature * [Year=2015]	-0.047	0.0260	-1.813	0.072	-0.099	0.004	0.954	0.906	1.004
Temperature * [Year=2016]	-0.034	0.0227	-1.476	0.143	-0.079	0.011	0.967	0.924	1.012

Temperature * [Year=2017]	-0.036	0.0297	-1.196	0.234	-0.094	0.023	0.965	0.910	1.024
Temperature * [Year=2018]	0[b]								
Lunar phase percent * Temperature	-0.001	0.0003	-3.725	0.000	-0.002	0.000	0.999	0.998	1.000
Lunar phase percent * Temperature * [Year=2010]	0.001	0.0006	2.225	0.028	0.000	0.002	1.001	1.000	1.002
Lunar phase percent * Temperature * [Year=2011]	0.000	0.0006	-0.437	0.663	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2012]	0.001	0.0007	1.175	0.242	-0.001	0.002	1.001	0.999	1.002
Lunar phase percent * Temperature * [Year=2013]	0.004	0.0007	5.660	0.000	0.003	0.005	1.004	1.003	1.005
Lunar phase percent * Temperature * [Year=2014]	0.002	0.0006	2.755	0.007	0.001	0.003	1.002	1.001	1.003
Lunar phase percent * Temperature * [Year=2015]	0.001	0.0004	1.492	0.139	0.000	0.001	1.001	1.000	1.001
Lunar phase percent * Temperature * [Year=2016]	0.001	0.0004	2.062	0.041	0.001	0.002	1.001	1.000	1.002
Lunar phase percent * Temperature * [Year=2017]	0.001	0.0005	2.934	0.004	0.000	0.002	1.001	1.000	1.002
Lunar phase percent * Temperature * [Year=2018]	0[b]								

Lissotriton vulgaris

Model Term	**Coefficient**	**Std. Error**	**t**	**P**	**95% Confidence Interval**		**Exp (Coefficient)**	**95% Confidence Interval for Exp (Coefficient)**	
					Lower	**Upper**		**Lower**	**Upper**
Intercept	2.623	0.1283	20.443	0.000	2.369	2.877	13.775	10.684	17.760
Lunar phase percent	0.000	0.0040	0.031	0.976	-0.008	0.008	1.000	0.992	1.008
Temperature	0.011	0.0169	0.639	0.524	-0.023	0.044	1.011	0.978	1.045
Lunar phase percent * [Year=2010]	-0.010	0.0065	-1.461	0.147	-0.022	0.003	0.991	0.978	1.003
Lunar phase percent * [Year=2011]	0.012	0.0051	2.406	0.018	0.002	0.022	1.012	1.002	1.023
Lunar phase percent * [Year=2012]	-0.004	0.0055	-0.659	0.511	-0.014	0.007	0.996	0.986	1.007
Lunar phase percent * [Year=2013]	-0.017	0.0067	-2.479	0.015	-0.030	-0.003	0.983	0.970	0.997
Lunar phase percent * [Year=2014]	-0.017	0.0077	-2.214	0.029	-0.032	-0.002	0.983	0.968	0.998
Lunar phase percent * [Year=2015]	0.009	0.0049	1.839	0.068	-0.001	0.019	1.009	0.999	1.019
Lunar phase percent * [Year=2016]	0.003	0.0051	0.542	0.589	-0.007	0.013	1.003	0.993	1.013
Lunar phase percent * [Year=2017]	-0.004	0.0051	-0.749	0.455	-0.014	0.006	0.996	0.986	1.006

Lunar phase percent * [Year=2018]	0[b]								
Temperature * [Year=2010]	-0.044	0.0255	-1.734	0.086	-0.095	0.006	0.957	0.910	1.006
Temperature * [Year=2011]	0.086	0.0245	3.518	0.001	0.038	0.135	1.090	1.038	1.144
Temperature * [Year=2012]	0.012	0.0265	0.445	0.657	-0.041	0.064	1.012	0.960	1.066
Temperature * [Year=2013]	-0.016	0.0271	-0.608	0.545	-0.070	0.037	0.984	0.932	1.038
Temperature * [Year=2014]	-0.015	0.0242	-0.623	0.534	-0.063	0.033	0.985	0.939	1.033
Temperature * [Year=2015]	-0.025	0.0234	-1.080	0.282	-0.072	0.021	0.975	0.931	1.021
Temperature * [Year=2016]	-0.017	0.0234	-0.713	0.477	-0.063	0.030	0.983	0.939	1.030
Temperature * [Year=2017]	-0.055	0.0288	-1.901	0.060	-0.112	0.002	0.947	0.894	1.002
Temperature * [Year=2018]	0[b]								
Lunar phase percent * Temperature	-0.001	0.0003	-0.174	0.862	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2010]	0.001	0.0006	1.996	0.048	0.001	0.002	1.001	1.000	1.002
Lunar phase percent * Temperature * [Year=2011]	-0.002	0.0005	-3.261	0.001	-0.002	-0.001	0.998	0.998	0.999
Lunar phase percent * Temperature * [Year=2012]	0.000	0.0006	-0.321	0.749	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2013]	0.001	0.0006	2.296	0.023	0.000	0.003	1.001	1.000	1.003
Lunar phase percent * Temperature * [Year=2014]	0.002	0.0008	2.452	0.016	0.000	0.003	1.002	1.000	1.003
Lunar phase percent * Temperature * [Year=2015]	-0.001	0.0004	-1.152	0.252	-0.001	0.000	0.999	0.999	1.000
Lunar phase percent * Temperature * [Year=2016]	0.000	0.0005	-0.458	0.648	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2017]	0.001	0.0005	2.196	0.030	0.000	0.002	1.001	1.000	1.002
Lunar phase percent * Temperature * [Year=2018]	0[b]								

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Model Term	**Coefficient**	**Std. Error**	**t**	**P**	**95% Confidence Interval**		**Exp (Coefficient)**	**95% Confidence Interval for Exp (Coefficient)**	
					Lower	**Upper**		**Lower**	**Upper**
Intercept	2.873	0.1793	16.028	0.000	2.518	3.228	17.694	12.406	25.237
Lunar phase percent	-0.001	0.0046	-0.324	0.747	-0.011	0.008	0.999	0.990	1.008

Temperature	-0.021	0.0208	-0.988	0.325	-0.062	0.021	0.980	0.940	1.021
Lunar phase percent * [Year=2010]	0.000	0.0073	-0.068	0.946	-0.015	0.014	1.000	0.985	1.014
Lunar phase percent * [Year=2011]	0.016	0.0062	2.517	0.013	0.003	0.028	1.016	1.003	1.028
Lunar phase percent * [Year=2012]	0.006	0.0059	1.040	0.300	-0.006	0.018	1.006	0.994	1.018
Lunar phase percent * [Year=2013]	-0.017	0.0076	-2.252	0.026	-0.032	-0.002	0.983	0.968	0.998
Lunar phase percent * [Year=2014]	-0.015	0.0091	-1.684	0.095	-0.033	0.003	0.985	0.967	1.003
Lunar phase percent * [Year=2015]	0.009	0.0055	1.672	0.097	-0.002	0.020	1.009	0.998	1.020
Lunar phase percent * [Year=2016]	0.004	0.0055	0.749	0.455	-0.007	0.015	1.004	0.993	1.015
Lunar phase percent * [Year=2017]	0.001	0.0056	0.160	0.873	-0.010	0.012	1.001	0.990	1.012
Lunar phase percent * [Year=2018]	0[b]								
Temperature * [Year=2010]	-0.017	0.0343	-0.488	0.627	-0.085	0.051	0.983	0.919	1.053
Temperature * [Year=2011]	0.158	0.0342	4.620	0.000	0.090	0.226	1.171	1.094	1.253
Temperature * [Year=2012]	0.040	0.0335	1.188	0.237	-0.027	0.106	1.041	0.974	1.112
Temperature * [Year=2013]	0.004	0.0373	0.111	0.912	-0.070	0.078	1.004	0.933	1.081
Temperature * [Year=2014]	-0.008	0.0337	-0.234	0.816	-0.075	0.059	0.992	0.928	1.061
Temperature * [Year=2015]	0.067	0.0265	2.526	0.013	0.014	0.120	1.069	1.015	1.127
Temperature * [Year=2016]	0.009	0.0263	0.361	0.719	-0.043	0.062	1.010	0.958	1.064
Temperature * [Year=2017]	0.003	0.0319	0.092	0.927	-0.060	0.066	1.003	0.942	1.068
Temperature * [Year=2018]	0[b]								
Lunar phase percent * Temperature	0.000	0.0004	0.318	0.751	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2010]	0.000	0.0006	0.676	0.500	-0.001	0.002	1.000	0.999	1.002
Lunar phase percent * Temperature * [Year=2011]	-0.002	0.0006	-3.128	0.002	-0.003	-0.001	0.998	0.997	0.999
Lunar phase percent * Temperature * [Year=2012]	-0.001	0.0006	-1.851	0.067	-0.002	-0.008	0.999	0.998	1.000
Lunar phase percent * Temperature * [Year=2013]	0.002	0.0007	2.354	0.020	0.000	0.003	1.002	1.000	1.003
Lunar phase percent * Temperature * [Year=2014]	0.001	0.0009	1.222	0.224	-0.001	0.003	1.001	0.999	1.003
Lunar phase percent * Temperature * [Year=2015]	-0.001	0.0005	-1.889	0.061	-0.002	0.001	0.999	0.998	1.000
Lunar phase percent * Temperature * [Year=2016]	0.000	0.0005	-0.401	0.689	-0.001	0.001	1.000	0.999	1.001
Lunar phase percent * Temperature * [Year=2017]	0.001	0.0005	0.960	0.339	-0.001	0.002	1.001	0.999	1.002
Lunar phase percent * Temperature * [Year=2018]	0[b]								