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- **Title:** Long-term population ecology of the great crested newt in Kent
- **Abstract:** Climate change has been recognized as one of the causes of global amphibian population declines. Amphibians may be particularly susceptible to climatic changes, as a result of their ectothermic life style and dependence on moisture. Climatic factors may affect amphibian population dynamics deterministically or stochastically, and can act at both local and regional levels. Using capture-mark- recapture (CMR) methods, population dynamics of great crested newts over two decades were compared between two separate populations in Canterbury, Kent in order to explore local and regional drivers of population change. Accurate individual identification is a basic assumption of capture-mark-recapture methods. A comparison of manual and computer-assisted photo identification programs verified that the spot patterns of individual newts did not change significantly through time, and were sufficiently varied to reliably identify individual newts. At a metapopulation located within an agricultural landscape, capture-mark-recapture modelling revealed variations in survival, detectability, and population size between years. Low annual survival of adult newts was related to mild, wet winters which impacted the metapopulation at the regional level. Therefore, survival varied between years but not between subpopulations. Regardless of this regional effect, the four subpopulations were generally asynchronous in their dynamics, but the persistence of the metapopulation depended on a single source pond that was the smallest water body within the system. At a further population two miles away, survival since 2001 was constant and high every year despite mild, wet winters. Management practised through draining and refilling the ponds did have an apparent effect on the number of newts captured over the subsequent years. Population increase could be due to the decrease in predatory invertebrates following pond desiccation and a subsequent increase in recruitment levels. Body condition may be linked to the survival of amphibians. However, there was no influence of climatic conditions on the body condition at either of the populations studied. Nevertheless, body condition was related to survival at one of the populations, and body condition was lower in ponds with high densities of newts. Consequently, the persistence of the two populations relies on a combination of (1) local, population-specific factors - such as population density and pond desiccation, and (2) regional factors, such as climate that affect recruitment and survival from each pond. However, conservation actions at the local scale may offset reduced larval recruitment and adult survival at the regional scale.

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Long-Term Population Ecology of the Great Crested Newt in Kent

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ABSTRACT

Climate change has been recognized as one of the causes of global amphibian population declines. Amphibians may be particularly susceptible to climatic changes, as a result of their ectothermic life style and dependence on moisture. Climatic factors may affect amphibian population dynamics deterministically or stochastically, and can act at both local and regional levels. Using capture-mark-recapture (CMR) methods, population dynamics of great crested newts over two decades were compared between two separate populations in Canterbury, Kent in order to explore local and regional drivers of population change. Accurate individual identification is a basic assumption of capture-mark-recapture methods. A comparison of manual and computer-assisted photo identification programs verified that the spot patterns of individual newts did not change significantly through time, and were sufficiently varied to reliably identify individual newts. At a metapopulation located within an agricultural landscape, capture-mark-recapture modelling revealed variations in survival, detectability, and population size between years. Low annual survival of adult newts was related to mild, wet winters which impacted the metapopulation at the regional level. Therefore, survival varied between years but not between subpopulations. Regardless of this regional effect, the four subpopulations were generally asynchronous in their dynamics, but the persistence of the metapopulation depended on a single source pond that was the smallest water body within the system. At a further population two miles away, survival since 2001 was constant and high every year despite mild, wet winters. Management practised through draining and refilling the ponds did have an apparent effect on the number of newts captured over the subsequent years. Population increase could be due to the decrease in predatory invertebrates following pond desiccation and a subsequent increase in recruitment levels. Body condition may be linked to the survival of amphibians. However, there was no influence of climatic conditions on the body condition at either of the populations studied. Nevertheless, body condition was related to survival at one of the populations, and body condition was lower in ponds with high densities of newts. Consequently, the persistence of the two populations relies on a combination of (1) local, population-specific factors - such as population density and pond desiccation, and (2) regional factors, such as climate that affect recruitment and survival from each pond. However, conservation actions at the local scale may offset reduced larval recruitment and adult survival at the regional scale.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 THREATENED AMPHIBIANS

Amphibians comprise a threatened species group and are declining throughout their distribution range (Fischer et al. 2009). The reasons for diminishing amphibian populations have been investigated and disputed widely and appear to vary among species in different parts of the world (D´Amen et al. 2011). Possible reasons for these declines include changing climate, habitat modification, over-exploitation, introduction of alien species, diseases and emissions of environmental hazards (Kiesecker et al. 2001; Teixeira & Arntzen 2002; Collins & Storfer 2003; Lips et al. 2005; Cushman 2006; Hamer & McDonnell 2008). In Europe, 59% of amphibians have decreasing populations, and nearly 25% of amphibian species are considered threatened (Temple & Cox 2009). Here, the most evident causes for decreasing numbers and loss of populations are habitat destruction and landscape fragmentation, followed by pollution with climate change, invasive alien species and pathogens (Griffiths et al. 1996; Oldham & Swan 1997; Wood et al. 2003; Cushman 2006; Edgar & Bird, 2006; Temple & Cox 2009).

Previous studies have demonstrated that many populations and species have vanished from even seemingly pristine natural habitats within recent decades (Ron et al. 2003; Burrowes et al. 2004; Gallant et al. 2007). Although direct human activities such as habitat destruction or fragmentation may drive amphibians to extinction (Chanson et al. 2008), it was the strong decline or even extinction of amphibian populations without any noticeable human influence that first raised concern among amphibian ecologists and conservationists (Blaustein & Wake 1990). Nevertheless, it may be challenging to differentiate population declines from short-term ‘troughs’ because amphibian populations may display strong year-to-year natural fluctuations (Pechmann et al. 1991). Long time-series of amphibian populations are therefore much needed. The analysis of such long time-series would give a better indication actual population trends. Equally, analysing long-term data may reveal some of the factors that regulate the dynamics of amphibian populations, and ultimately control population distribution and abundance. This study is one of the longest population studies on amphibians in Europe.

1.2 GLOBAL AMPHIBIAN DECLINES

Global declines of amphibians refer to the occurrence of population declines and even extinctions of amphibian species across the world (Whittaker et al. 2013). Alford et al. (2001) suggested that the declines had been occurring at a global scale since 1990 and this situation goes beyond the overall biodiversity crisis (Pechmann & Wilbur 1994). Indeed, inspection of 935 amphibian populations across the world, Houlahan et al. (2000) highlighted that amphibian declines were on a global scale. Concern over amphibian population declines was raised at the First World Congress of Herpetology, held in Canterbury, UK in 1989 (Wake 1991). This was followed in 1990 by a meeting in California, USA, in which herpetologists reported on apparent declines in amphibians from many parts of the world (Wake 1991). The congress caused a renewed interest in amphibian conservation as within a few years declines were reported for more than 500 amphibian species out of an estimated total of just over 4000 at the time (Beebee & Griffiths 2005).

According to the IUCN, almost a third of the world's amphibians are believed to be at risk of extinction and are now more threatened and declining more rapidly than either birds or mammals (Stuart et al. 2004). Of 6000+ species, 165 are believed to be extinct with a further 1,896 species threatened (Stuart et al. 2008). Common amphibian species are also demonstrating population declines such as common toad (*Bufo bufo*) in Europe (Johnson et al. 2011) and the northern leopard frog (*Lithobates pipiens*) in several states of the USA (Bonardi et al. 2011). Among amphibians, newts and salamanders (Caudata) are the most endangered, with 47 % of threatened species occurring within this group (Stuart et al. 2008). Most species are classified as Least Concern under the International Union for the Conservation of Nature (IUCN) Red List (Stuart et al. 2008) but they have also shown declines over parts of their range. This is particularly the case for the great crested newt (*Triturus cristatus*), a flagship species at the European Union level, and one of the amphibians to be specially protected under the Habitats Directive (Edgar & Bird 2005). The great crested newt declined remarkably during the latter part of the twentieth century, primarily as a result of agricultural intensification (Langton et al. 2001). They are rather more demanding in their habitat requirements than other widespread amphibian species in Europe, and as a result they have declined more markedly.

1.3 CLIMATE CHANGE AND AMPHIBIANS

Global mean temperature has risen by 0.6°C throughout the past 100 years, which is the hottest period of the preceding millennium (Jones et al. 2001). Over the last three decades, precipitation and temperature abnormalities have been recorded to negatively influence the population dynamics of various organisms and thus associated to a decline in biodiversity (Cayuela et al. 2017). As an ectothermic animal which is able to maintain body temperature by attaining outside heat itself, all aspects of amphibian life history are strongly subjected to the external environment, including climate (Morris 1992). Amphibians are appropriate biological models for examining this issue as species inhabit various positions along the fast–slow continuum (Cayuela et al. 2017). Amphibian maximum longevity ranges from a few years to a century, and their yearly fecundity varies from a few eggs to clutches of more than 25,000 (Wells 2010). Global climate change as a cause of amphibian declines has been studied in relation to increasing temperatures. However, research suggests that changing climatic conditions will impact synergistically with other factors including UV-B radiation due to thinning of ozone layer and diseases that impact on populations (D'Amen & Bombi 2009). For example, the western toad (*Bufo boreas*) in North America seems to experience egg survival of only up to 50% due to the synergistic effects of UV-B, pathogenic fungi and lower water levels due to changes in climate (Kiesecker & Blaustein 1995). Pounds et al. (2006) predict that amphibian populations, specifically in tropical regions, will decline in warm years by changing disease dynamics. In Europe, Bosch et al. (2007) examined 28 years of data on 10 amphibian species within central Spain and concluded that an increase in temperature and moisture during the breeding season stimulate the growth of *Batrachochytrium dendrobatidis*, the fungal agent of the disease chytridiomycosis, resulting an increase in infected species.

Pounds et al. (1999) link the extinction of an entire species, the golden toad (*Bufo periglenes*) in Costa Rica, to global warming effects on montane dry-season mist frequencies. Animals that occupy tropical montane cloud forests may be particularly susceptible to rapid climate change that may change pattern of cloud formation and thereby the availability of water (Still et al. 1999). Within the UK, climate change is predicted to bring hotter, drier summers and mild, wet winters (DEFRA 2009). Increasing mean temperature, for example, could affect amphibians by increasing mortality and lower fecundity in female survivors. In a population study, Reading (2007) discovered a correlation between a decline in the body condition of female common toads (*Bufo bufo*) and their annual survival. There was a relationship between mild winters and body size, resulting in the production of fewer eggs. In a study in Kent, male great crested newts (*Triturus cristatus*) exhibited a lower estimated annual survival after increased air temperatures in winter (Griffiths et al. 2010).

The outcome of climate change is diverse, and effects can be beneficial as well as negative to amphibian breeding phenology. Changes in temperature and precipitation on pond hydrology and hydroperiod (the duration of a temporary/ephemeral pond retain water) may produce severe effects on amphibians (Corn 2005). As a consequence of early drying of temporary or ephemeral ponds, amphibian larvae may experience a shorter time to complete their metamorphosis (Rowe & Dunson 1995). Changes in pond hydrology and breeding phenology may influence growth rates of amphibian larvae (Boone et al. 2002). The relationships between amphibians and their predators may change because predation on amphibian larvae is related to size. Earlier breeding could increase risk of exposure to extreme temperatures from more variable early spring weather (Corn & Muths 2002). On the contrary, an alteration in breeding activity to earlier in the season may extend time for development and growth of amphibian larvae. Large sized individuals may have higher fitness/body condition and survive over winter better than small sized individuals (Reading & Clarke 1999). In adult, skip breeding can reduce annual variation in adult survival by decreasing the cost of reproduction on survival when extreme environmental conditions occur (Cayuela et al. 2016).

Generally since 1960s, spring activities have occurred progressively (Walther et al. 2002). Global meta-analyses over 1,700 species predict spring events will be earlier by an average of 2.3 days per decade (Parmesan & Yohe 2003). Several studies have indicated a tendency towards breeding earlier by some amphibian species. One of the longest data series for amphibians - 140 years of surveillance by volunteers in Finland was documented by Terhivuo (1988), who conclude that common frogs (*Rana temporaria*) bred 2 to 13 days earlier in the 1980s compare to 1840s, taking account of variation due to latitude. The most noticeable changes in the breeding time were recorded in England, where the three species of newt (*Triturus cristatus, T. vulgaris*. *T. helveticus*) started breeding 5–7 weeks earlier in 1990–1994 compared to the first five years, 1978–1982, and two anuran species (*Bufo calamita* and *Rana* kl. *esculenta*) produced eggs in the ponds 2–3 weeks earlier (Beebee 1995). These trends correlate with warmer spring temperature increasing since the 1970s associated with the North Atlantic Oscillation (Forchhammer et al. 1998).

1.4 GREAT CRESTED NEWT

1.4.1 Classification and Description

The great crested newt, *Triturus cristatus* (Laurenti 1768) is classified in the order Caudata, the tailed amphibians, which contain 10 families containing about 550 species (AmphibiaWeb 2017). They are part of the family Salamandridae that is distributed across Europe, Asia Minor and the Middle East (Griffiths 1996). The crested newt, also called the northern crested newt or warty newt, is the largest newt found in Britain. Females are larger than males and can measure up to 16 cm while males measure 14 to 15 cm. The great crested newt's back and flank is dark black-brown in colour, with a warty skin surface dotted with tiny white spots. The belly is either orange or yellow-coloured with large, black blotches, which have a distinctive pattern in each individual. The great crested newt is sexually dimorphic, especially during the breeding season. The males develop a silver-grey stripe on the middle of the tail on each side. A black jagged crest running along their back, then a separate smoother-edged crest runs to the tip of tail. These breeding embellishments develop in the water (Griffiths & Mylotte 1988). These sexual characteristics are reduced during the terrestrial phase (Baker 1992). Females lack a crest, tail flash and swollen cloaca, but have a yellow-orange stripe along the lower edge of the tail.

1.4.2 Breeding Migration

The seasonal activity and migration of crested newts was recorded more than 100 years ago (Durigen 1897). The newts migrate to breed in water (Kupfer & Kenitz 2000), and the migration usually takes place between February and April (Langton et al. 2001). The first migrations usually occur after dark when night air temperature is above 4-5 ^{0}C, with migratory activity peaking during and immediately after consecutive humid nights (Jehle et al. 2011). Temperature threshold for crested newts' migration is higher than the smaller species (Griffiths & Raper 1994).

Langton et al. (2001) documented that the distance covered in one night can reach 120 m, or in exceptional cases up to 1000 m. The migration of all newts is not synchronous and animals can be recorded migrating several months after first reaching the pond (Langton et al. 2001). On average, male newts arrive at a pond a few days earlier before females presumably enhancing their chances for mating success (Jehle et al. 2011). Arntzen (2002) documented data from 27 years of field work at more than 500 ponds in western France, and report that males also have a tendency to leave the ponds before females, regardless of a slightly longer overall aquatic phase. In a pond subject to large water level fluctuations and high newt densities, the mean aquatic phase lasted only 60 days (Jahn 1995). On the other hand, Griffiths and Mylotte (1987) report an average aquatic phase of seven months (March-September) in an upland area which is longer than normally observed. By August, most crested newts have emigrated back onto land and adults usually stay within 250 m surrounding of the breeding pond during the winter terrestrial phase (Langton et al. 2001). Metamorphosed larvae leave the ponds from early August (English Nature 2001).

Apart from reproduction, crested newts also exploit their breeding ponds for feeding and this plays an important role for resource acquisition (Jehle et al. 2011). Cooke (1974) recorded that adult crested newts consume tadpoles that are maximally 45 – 50 mm long and weigh up to 1 g. Hagstrom (1971) documented two smooth newts in the stomach of a female crested newt of 127 mm in length. Griffiths and Myotte (1987) by using stomach flushing documented crested newts feeding on small prey items such as waterfleas and seed shrimps and larger prey size such as leeches, snails and common frog tadpoles. Jehle et al. (2011) suggest that energy budgets during the aquatic phase are largely influenced by the high activity levels required for reproduction. Sinsch et al. (2003) observed that females gained weight during the aquatic phase, whereas males lost weight. Mullner (1991) and Stoefer (1997) recorded a weight gain for both sexes, but with large underlying individual-specific variation involving gains as well as losses.

Amphibians seem to orientate to the breeding ponds by a combination of methods such as the sun, stars, moon, polarized light and landmarks, conspecifics vocalizations, earth magnetic fields, or home pond odour (Griffiths 1996; Hayward et al. 2000). Newts have been suggested to move in a non-random way towards the ponds and can locate a breeding pond based on pond odour (Joly & Miaud 1993) and travelling in straight lines away from the pond and orientate towards preferred habitat in the area (Malmgren 2002). Their preferred habitats include deciduous woodland, hedgerows, shrubs and trees (Jehle & Arntzen 2000). The movement direction also remains similar over the years (Malmgren 2002). This information later can be used to determine optimum habitat and used in conservation purposes (Dodd & Cade 1998).

1.4.3 Distribution

The great crested newt has a broad range, covering the majority of central and northern Europe including southern Scandinavia. Its range extends to the west into central France, the Benelux countries and Great Britain (Beebee & Griffiths 2000). In Great Britain, the great crested newt is widely distributed across much of lowland England **(**Figure 1.1**)**. The species is absent from the far southwest of England and parts of Wales and Scotland (Jehle et al. 2011). Wilkinson et al. (2011) estimated the number of breeding ponds in the Great Britain to be 61,000 across the country. Large, well- established ponds that are that have plenty of weed cover but are devoid of fish seem to be favoured. The crested newt will sometimes utilise garden/ornamental ponds, but to a lesser extent than the smooth newt or common frog.

Although the distribution of crested newts is well understood, a relatively small proportion of populations have been formally recorded (Jehle et al. 2011). Survey effort has varied substantially over time and across the Great Britain, corresponding to recording projects and surveyor numbers (Gleed-Owen et al. 2005). The UK's largest crested newt populations tend to form in abandoned mineral extraction sites such as chalk, clay and rock quarries. These sites usually contain large, unshaded, fish-free ponds that offer ideal breeding conditions for newts which immigrate from smaller ponds in nearby areas (Jehle et al. 2011). Some crested newt populations in the Great Britain are very large. One at Hampton Nature Reserve, Peterborough is said to be the biggest in the world with over 30,000 adults according to one survey (Beebee & Griffiths 2000). Nevertheless, there is no doubt that the species has declined dramatically over the past 50 years and may still be decreasing faster than any other species of British amphibian or reptile (Beebee & Griffiths 2000).

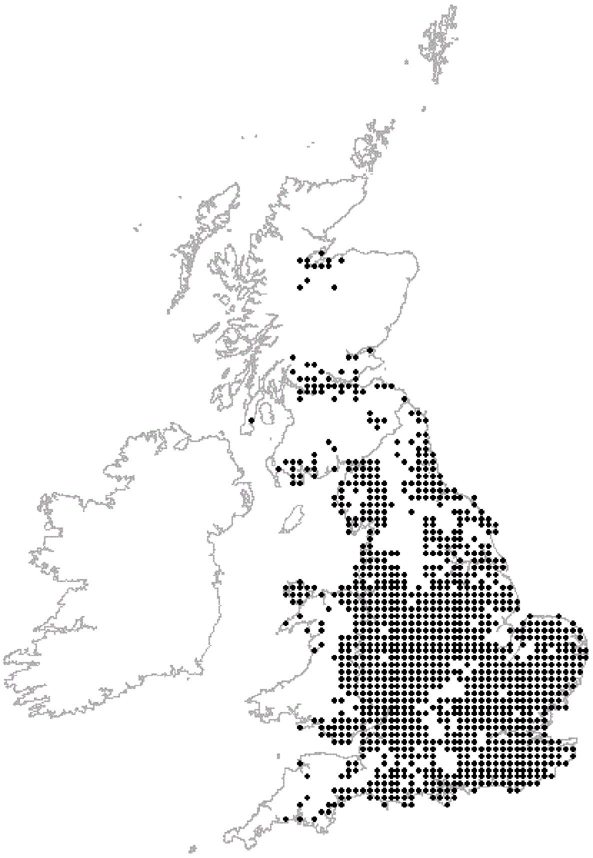


Figure 1.1 Distribution of the great crested newt in Britain from JNCC (2017), based on various data sources

1.4.4 Conservation Status

In view of the declining conservation status across Europe, great crested newts are listed on Annexes II and IV of the EU Natural Habitats Directive and Appendix II of the Bern Convention. In the UK the species is protected under Schedule 5 of the Wildlife and Countryside Act 1981 and Schedule 2 of the Conservation of Habitats and Species (Amendment) Regulations, 2012, (Regulation 40). These laws set out activities that constitute offences. The newts are protected against killing, injuring, taking, taking or damaging eggs, certain forms of disturbance (for instance at a breeding pond or during hibernation), and possession and sale are prohibited. Due to their legal protection, great crested newts often come into conflict with human development. In such cases there is a legal obligation to carry out some form of mitigation, involving population assessments, possibly translocations and the construction of new habitats. The great crested newt is one of only four amphibians that are protected by the UK Biodiversity Action Plan and, guidance on development in relation to crested newts can be found in 'Great Crested Newt Mitigation Guidelines' handbook (English Nature 2001) which should be followed by developers.

1.5 METAPOPULATIONS

Modern metapopulation theory originates from a framework laid out by Levins (1969). The underlying concepts were that area could be split into patches of habitat enclosed by an incompatible environment, the matrix, which individuals can live but not reproduce. Therefore, dispersing or migrating animals link subpopulations in various habitat patches into a network, a metapopulation. To support viable populations, many ponds depend on the immigration of individuals from neighbouring ponds. Populations that produce a large number of offspring can supply neighbouring populations with some of their young, overall increasing the number of individuals inhabiting a given pond network. Such points are considered in the metapopulation concept, which looks simultaneously at sets of populations (or subpopulations) that are to some extent, connected with each other. However, it is still debatable whether typical amphibian breeding ponds represent true metapopulations or not. They are often either completely isolated from each other, or linked to an extent that population size fluctuations become coupled (Jehle et al. 2005). Metapopulation concepts play an important role in explaining the local occurrence of crested newts. Great crested newts have generally been considered as existing in metapopulations (e.g. Miaud et al. 1993; Griffiths & Williams 2000; Malmgren 2002). For example, Griffiths (2004) used a Population Viability Analysis (PVA) to show that the crested newt persisted better in several connected small ponds compared to a single and isolated large pond.

In reality, spatial variation in patch size and habitat quality results in variation in demographic rates and local extinction probability among populations (Coulson et al. 1999). Additional models have been developed which relax Levins' assumption that all populations are correspondingly susceptible to local extinction. Source-sink models are based on the 'rescue-effect' which refers to the process by which dispersal from large and/or more productive populations prevents the extinction of smaller and/or less productive populations (Brown & Kodric-Brown 1977). Source-sink metapopulation structure takes into account differences in the quality of habitats (Gaggioti 1996). 'Source' populations occupy high quality habitats; as a consequence they are self-sustaining and may provide surplus individuals that disperse to less productive 'sink' populations. Sink populations occupy low quality habitats, where within-population reproduction is inadequate to balance mortality. The persistence of sink populations is governed by continued immigration from source populations (Thomas & Kunin 1999). The number of sink populations is reliant on the species dispersal rate and the excess productivity of source populations. In species with high rates of dispersal, sink populations may be predominant (Donovan et al. 1995).

In species where the probability of local extinction is high, existence as multiple populations is expected to increase regional persistence, as the chance of simultaneous extinction in all populations is low compared to the risk of extinction in single population (Hanski 1997). If the dynamics of the subpopulations are synchronized, then they will have a tendency to function as a single large population, and there will be a possibility that all populations will decline - and even go extinct - at the same time (Griffiths & Williams 2001). Environmental stochasticity frequently performs on a regional scale and may cause populations to fluctuate in synchrony (Harrison & Taylor 1997). The effects of this regional correlation in environmental conditions can be reduced if stochastic variation in local habitat quality results in local asynchrony (Sutcliffe et al. 1996). Synchrony among populations may also result when dispersal among populations occurs at a high rate (Sutcliffe et al. 1996). If dispersal among populations is so high that individuals are equally likely to breed in a neighbouring patch as in the original patch, then the dynamics of the populations will not be independent and the population forms a patchily distributed, continuous population (Ruxton et al. 1997). Therefore, asynchrony between subpopulations can ensure that sources of colonizers will always compensate for extinctions elsewhere within the metapopulation system (Griffiths & Williams 2001). A metapopulation consists of a group of populations whose dynamics are predominantly independent, where an individual has a much lower probability of mating with an individual from a neighbouring population than with one from its original population (Stacey et al. 1997).

For an understanding of the dynamics of crested newt metapopulations, researchers need to quantify the amount of migration between ponds. However, studies that allow firm quantitative conclusions are very rare, because it is logically difficult and very time consuming to follow the fate of hundreds to thousands of individuals (adults and juveniles) at several breeding ponds over several years. Griffiths et al. (2010) summarised a long-term study on four ponds separated from each other by between 200 m and 800 m. Adult population size fluctuations were largely asynchronous between populations, and only a small proportion of adults (< 1% of captures) migrated between ponds. However, recruitment of offspring to the whole metapopulation relied on a single source, and the three other ponds were characterised by frequent reproductive failures. The study reveals that inter-pond connectivity mediated through recruitment is a vital parameter in determining the survival of population sat suboptimal sites.

An indirect approach to reconstructing migration rates between ponds is offered by genetic inferences. Jehle et al. (2005) investigated 15 ponds that were separated from each other by between 400 m and 6000 m, using DNA fingerprints to identify those eggs that were the result of mating between a resident and an immigrant newt. Recent dispersal occurred only between five population pairs, and mostly took place from large to small populations without any migrant moving in the opposite direction, supporting the idea of source-sink processes within the metapopulation system. Pair-wise genetic distances between ponds were also correlated to some extent with between-pond geographical distances. This shows that at a larger temporal scale, closely co-located populations are more connected to each other than more distant ponds. Baker and Halliday (1999) report that newly created ponds did not become colonised by crested newts when they were more than 400 m away from existing populations, suggesting that in their case regular movements were only performed within shorter distances. In a large-scale mitigation project in Hampshire, UK, 898 newt individuals were intercepted between 250 m and 500 m from the breeding pond (Redgrave 2009).

Metapopulation considerations are important when assessing the ability of populations to persist. By collecting ecological data in and around a large number of ponds, Rannap et al. (2009) revealed that the distance to the nearest occupied pond was the second-most important after the diversity of invertebrate fauna out of 27 variables that determine whether a given pond harboured crested newts or not. Halley et al. (1996) used a mathematical model to show that crested newt populations only have a high probability of surviving more than 20 generations when they are large (consisting of at least 40 females), or when they lie at a distance of less than 500 m from an adjacent site. Their model predicted that no small population (< 10 individuals) would survive further away than 750 m from a source pond, whereas large populations (> 100 individuals) only need to be within 1,500 m of a source pond. Such estimations are in line with Griffiths & Williams (2000) and Griffiths (2004), who used Population Viability Analysis (PVA) to predict that when isolated, even large populations have a high probability of becoming extinct over a 50-year period. Several smaller populations result in a slightly higher probability for local crested newt persistence than a single large population, and persistence vastly increases when smaller populations are not isolated from each other. Griffiths et al. (2010) used twelve years of empirical demographic data to project the fate of four connected subpopulations into the next ten years. Extinction risks varied considerably between populations, depending most strongly on juvenile dispersal and adult survival.

Taken together, these studies demonstrate that the occasional exchange of individuals between populations is essential to enable the local long-term survival of crested newts. However, merely protecting existing networks of ponds might not be a sufficient management strategy to ensure population persistence. Karlson et al. (2007) used a simulation model that also incorporated ecological pond parameters to predict the fate of 18 crested newt populations. According to their results, active management such as pond restoration and the construction of new ponds is required for the survival of crested newts beyond the next 50 years, as existing ponds become gradually more unsuitable through shading and natural succession.

1.6 POPULATION REGULATION IN AMPHIBIANS

In order to conserve amphibians in the future, researchers need to be able to distinguish between natural population declines, which are a normal part of the life history of these animals, and declines which are instead due to anthropogenic causes (Bancila et al. 2010). To achieve this objective, one of the most important steps is to fully understand the population dynamics of amphibians. Populations can be regulated through density-dependent (intrinsic) and density-independent (extrinsic) factors (Turchin 1999, Sibly et al. 2005, Knape & de Valpine 2011). Density-dependent factors include predation, competition, waste accumulation, parasitism and disease; their effect increases with population density. Density-independent factors include climatic and environmental changes, the effect of which does not depend on population density.

Bjørnstad and Grenfell (2001) Begon and Mortimer (1981) concluded that the distinction between density-dependent (biotic) and density-independent (abiotic) factors is appropriate, since these two factors can act together to influence population density. For example, if climatic variation causes a restriction in the habitat of a certain species, this will also cause an increase in density, and thus in competition. The final effect on population abundance will be a consequence of both the climatic variation and the increased competition (Turchin 1999). Amphibian populations to certain extent may be prone to environmental stressors as long as their mortality is compensatory rather than additive (Bancila et al. 2016). Low survival induced by an environmental stressor may lead to reduce mortality in another life cycle stage that there is low or no net effect of the stressor (Lebreton 2005). There may be no effect on the population viability although an environmental stressor may affect individuals (Vonesh & De la Cruz 2002). Many studies reported the importance of density dependence at various life stages of amphibians. In the larval stage, food competition is typical and leads to reduced size at metamorphosis or to decreased larval survival, which may influence juvenile and adult performance (Schmidt et al. 2012). Indeed, similar actions may also initiate density dependence in terrestrial phases since adult body size can vary with abundance in adult amphibians (Green & Middleton 2013).

According to Odum (1971), density-independent factors, typical of low- diversity habitats, tend to bring out variations in population size which are often drastic, and cause a shifting of carrying-capacity levels. Density-independent factors also tend to occur irregularly and on the contrary, density-dependent factors tend to be regular and cyclic. Density-dependent effects are more important in high-diversity habitats and tend to maintain population sizes in an equilibrium state. For example, Gill (1979) studied the density-dependent regulation of natural breeding populations of the red-spotted newt, and suggests that the original populations were at carrying capacity, and that density dependence in annual survival was a mechanism by which breeding population sizes of newts adjusted to fluctuations in density.

In stage-structured life cycles, density-independence survival is characterized by a linear relationship between survival to the next life stage and density. However, most studies have examined mechanistic stressor effects on specific stages with less consideration for how stage-specific density dependent bottlenecks might confer population-level resilience to perturbation. With density-independent survival, the effect of stressors on one stage is directly translated to the next, and potentially to emergent population dynamics.

Other factors also contribute to the regulation of animal populations (Begon & Mortimer 1981). Genetic effects can be responsible for differences in reproductive rate between generations in the same population, which can have regulatory effect. Territoriality is another way in which animal populations partition resources, and thus minimise intraspecific competition. Spatial dynamics, which involves immigration or emigration in response to density variations, has the same function. The complexity of the interactions between all the above-mentioned factors makes it very hard to understand which mechanisms regulate the fluctuations of a particular population. In amphibians, the situation is complicated by the fact that these animals exhibit a complex life cycle (Wilbur 1980). Thus includes several life stages (egg, larva, eft, immature and, adult) that inhabit very different ecological niches. Therefore, regulation may occur at one or more stages, and its mechanisms will probably be different at each stage. In the UK, compared to that other species of amphibian, the ecology of crested newts has been relatively well studied in recent years and researchers are starting to piece together the various factors that regulate populations (Beebee & Griffiths 2000).

1.7 RESEARCH OBJECTIVES

Although various aspects of the great crested newt ecology have been studied, there are still many areas that are poorly understood. Effective conservation of declining crested newt populations requires a detailed understanding of their population dynamics. This includes knowledge of metapopulation dynamics and the impacts of climate change on the populations. The general aim of this study is to increase knowledge and understanding of the impact of climatic changes on ecology of the great crested newts within a natural and semi-natural area in the south-east Kent. The following specific objectives will be investigated to meet the aim of this study:

(a) To evaluate the performance of manual identification and automatic recognition software in the context of a long-series mark-recapture study of the great crested newt, and understand the limitations and key issues associated with the techniques;

(b) To explore the population structure and metapopulation dynamics of the great crested newts within an agricultural area;

(c) To investigate the colonisation and population dynamics of great crested newt in newly created ponds; and

(d) To examine the effect of climatic conditions preceding the annual breeding season on body condition of the great crested newt and assess impacts on apparent annual survival.

1.8 THESIS STRUCTURE

Chapter 2: Study Sites and General Methods

The study area consisting of two main sites are explained along with general methods for capturing and identifying adult great crested newts. This chapter also described the Cormack-Jolly-Seber capture-mark-recapture (CMR) models using the software MARK (White & Burnham 1999) and defined the candidate models.

Chapter 3: Accuracy of Photo Identification of Newts by Manual and Computer Identification Methods

The long-term capture mark-recapture (CMR) studies at one of the study sites (Field Site) allow this chapter to evaluate the use of belly pattern photos in the identification of the great crested newts. This chapter explains the limitations of the performance of expert and non-expert respondents while identifying the newts using manual photo identification. Also, the performance of five computer identification packages using different quality images, number of available reference images, and the belly pattern stability over time were assessed and compared.

Chapter 4: Metapopulation Dynamics of the Great Crested Newt in an Agricultural Landscape

Although the great crested newt often exists in sub-divided populations, there have been few long-term population studies within such sub-divided systems. By using a 19-year dataset, this chapter investigates the impact of climate change on the inter-year survival of four sub-populations and determines whether factors impacting metapopulation persistence of crested newts operate at regional or local levels.

Chapter 5: Colonisation and Population Dynamics of the Great Crested Newt in Newly Created Ponds

This study combined capture-mark-recapture data collected over a 16-year period at an artificial pond system. This chapter examined the persistence of the great crested newt populations within a single and a very small area and discusses whether the population dynamics are linked to the pond characteristics or climatic factors.

Chapter 6: Interactions between Body Condition Index and the Survivorship of the Great Crested Newt in Two Populations

As body condition is one of the parameters related to survival and reproductive success of great crested newts, the ‘scaled mass index’ (Peig & Green 2009, 2010) was used to estimate the body condition in two populations. The inter-annual variation in post hibernation body condition were examined and compared between these two study sites. The relationships between body condition, inter-year survival, and climatic conditions experienced previously and how these factors may impact population dynamics were explored in this chapter.

Chapter 7: General Discussions

This chapter will discuss the factors that that govern the dynamics of the great crested newt populations at both study sites. The analysis of such long time-series would allow this chapter to conclude whether the dynamic of the population was driven alone by intrinsic density dependence, extrinsic factors, or a combination of factors. Implications for understanding of the ecology of this species and its conservation will be discussed in light of results obtained from each area of study.

CHAPTER 2

STUDY SITE AND METHODS

2.1 STUDY SITES

The main study area constituted a 5 km x 5 km grid, originating at TR 1100590 in the southwest corner and extending to TR 1600640 in the northeast and, situated to the north of Canterbury in southeast England. This area was located within the Blean plateau, an area characterised by cold, wet soils derived from the underlying London clay and at elevations of 50 – 75 metres above mean sea level (Sewell 2006). Within the area were mixed landscape of woodland fragments, and agricultural lands that were used for grazing, fruit orchards and arable. Two pond systems were the subjects of investigation, and these are described separately within this thesis in Chapters 4 and 5 (Figure 2.1).

Chapter 4 is based on information collected at four farmland ponds occupied by great crested newts near Canterbury (TR135619), where a metapopulation of great crested newts has been studied over 19 breeding seasons (from 1995 until 2013). It is situated on the Blean plateau at Well Court farm, approximately 3.5 km north of Canterbury at an elevation of approximately 70 m. The surrounding landscape comprised mixed arable farmland and orchards that are intersected by hedgerows, formal garden, and semi-natural woodland to the north, offering a mixed range of terrestrial habitats for the newts. Land use to the south of the site is the residential village of Tyler Hill. For most of the study period, the farm was used for market gardening and orchards, with some land used for arable crops, principally wheat

(Sewell 2006).

Chapters 3 and 5 are based on information collected over 18 years at another site (the Field Site) where weekly trapping was also conducted during the breeding season from 1999 till 2016. This study site is located at the north-western part of the University of Kent campus (TR 130597). The immediate surrounding habitat to this site consists of rough grassland and surrounding this area was the University grounds, mown on a regular basis, residential with houses and gardens, sports fields and a paddock. The population of crested newts is small and in most years has not been greater than sixty individuals. Following pond creation in 1999, male crested newts were first recorded in 1999, and females from 2000. The origin of these colonisers is unknown, but may have been from an occupied pond 220 m to the north, or a formerly occupied pond 300 m to the southeast. The additional years of data collected from these two study sites since these earlier studies have enabled more detailed analysis of population trends. The lists of the previous publications and theses which originated from Well Court farm and Field Site are summarized in Table 2.1.

Table 2.1 Lists of publications from two study sites

Year	**Author**	**Publications/Theses**	**Study site**
2017	Buxton et al.	Is the detection of aquatic environmental DNA influenced by substrate type? *PLOS ONE* 12: p. e0183371	Field Site
2017	Buxton et al.	Seasonal variation in environmental DNA in relation to population size and environmental factors. *Scientific Reports* 7: p. e46294	Field Site
2012	Lewis	An evaluation of mitigation actions for great crested newts at development sites. PhD thesis, University of Kent	Field Site

2010	Griffiths et al.	Dynamics of a declining amphibian metapopulation: Survival, dispersal and the impact of climate. *Biological Conservation* 143: 485-491	Well Court
2009	Wright	Assessing the status of newts within ponds using bottle traps: colonisation, behavioural interactions and microhabitat preference. MSc thesis, University of Kent	Field Site
2006	Sewell	Great crested newts (*Triturus cristatus*) as indicators of pond biodiversity. PhD thesis, University of Kent	Well Court
2002	Walsh	Population dynamics of the Great Crested Newt (*Triturus cristatus*) in south-east England: An eight year study. MSc thesis, University of Glasgow	Well Court
2002	Young	Population ecology and behavioural interactions of smooth newts, *Triturus vulgaris*, and common frogs, *Rana temporaria*, in garden ponds. PhD thesis, University of Kent	Field Site
2000	Griffiths & Williams	Modelling population dynamics of great crested newts (*Triturus cristatus*): A population viability analysis. *Herpetological Journal* 10: 157-163	Well Court
1996	Bonetti	Geographical variation in growth, size and body condition between amphibian populations. MSc thesis, University of Kent	Well Court
1999	Williams	Metapopulation dynamics of the crested newt, *Triturus cristatus*. PhD thesis, University of Kent	Well Court

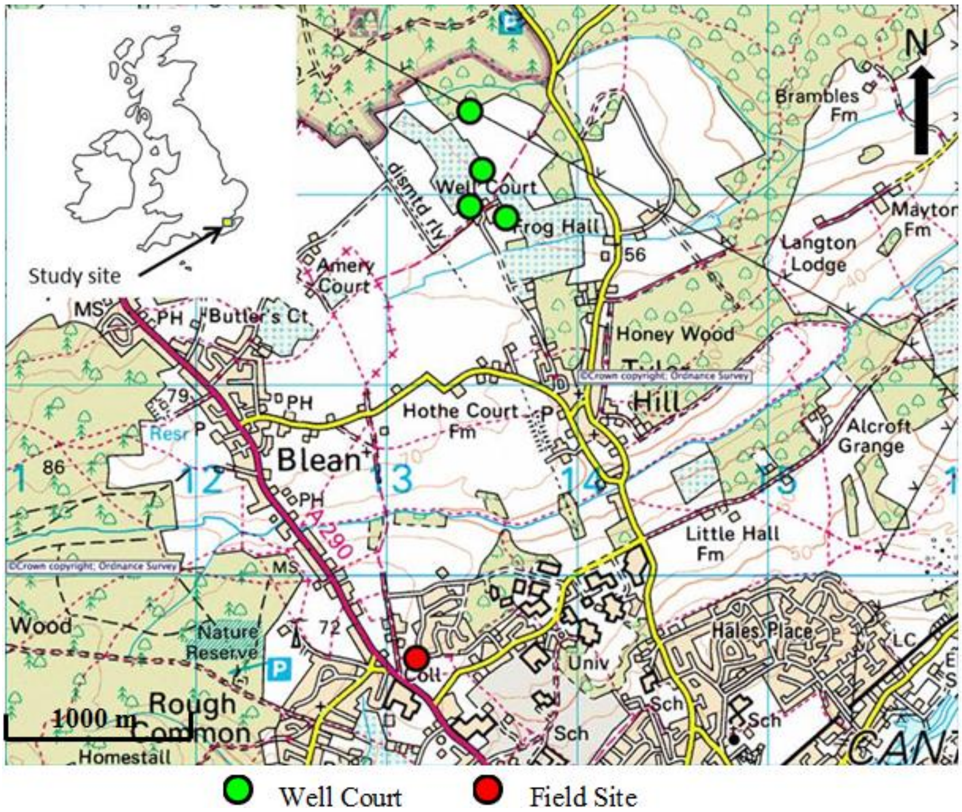


Figure 2.1 Ponds in the two main study areas, with Well Court pond sites indicated by green dots and Field Site by red dot. Map was adapted from Ordnance Survey

2.1.1 Well Court Farm, Blean

The study site comprises four pond systems on the Blean plateau called Well Court, approximately 3.5 km north of Canterbury, Kent (Grid reference TR135619) at an elevation of approximately 70 m. The study site is surrounded on a landscape consisting of mixed arable land and orchards intersected by hedgerows, and with an area of semi-natural woodland to the north. The general surrounding area comprised arable farmland, formal garden, orchards and woodland, offering a mixed range of terrestrial habitats for the newts. Land use to the south of the site is the residential village of Tyler Hill. Distances between the ponds range from 200 m to 800 m. The four pond system was probably isolated as the next nearest newt pond was further than the normal dispersal distance (i.e. > 1 km from any of the studied ponds) of great crested newts (Griffiths et al. 2010).

Well Court is a privately owned farm and as a result, the ponds are protected from any outside urban effect. However, pesticides and fertilizers were applied to adjacent fields. The metapopulation was studied over four ponds or groups of ponds; the Garden ponds, Pylon pond, Snake pond and Swimming pool (Figure 2.2). The ponds studied differed in size and characteristics and were created for different reasons (Figure 2.3). The linear distances from Garden ponds to the Snake pond and Snake pond to the Pylon pond are 200 m and 375 m, respectively.

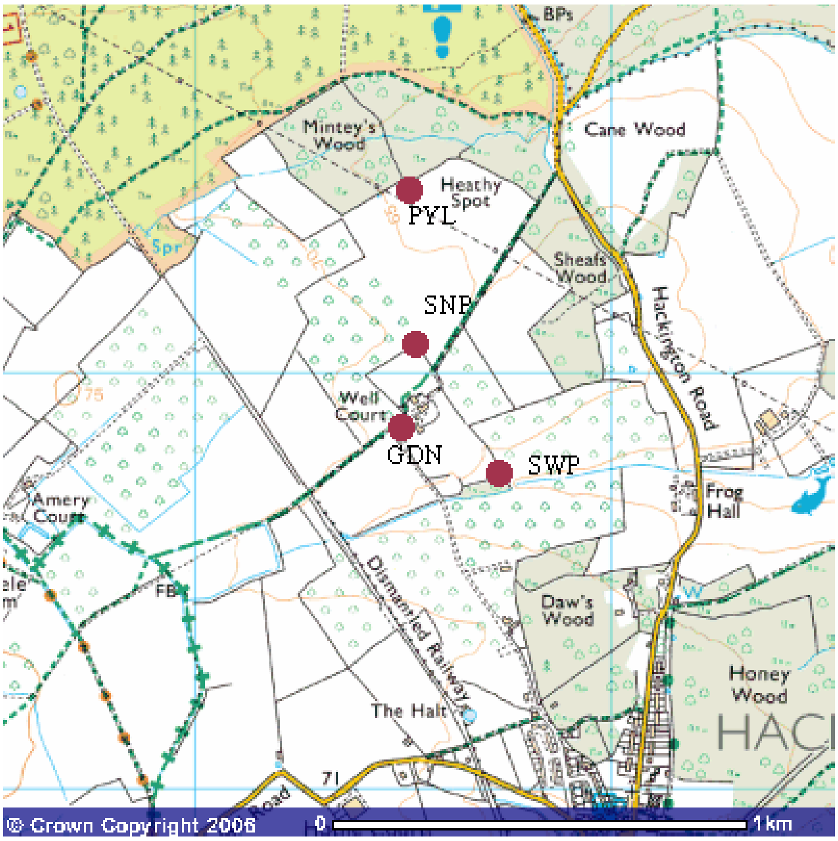


Figure 2.2 Ordnance Survey map showing the survey area of Well Court farm. The position of each of the survey ponds is shown with a red dot and initials of the pond

Figure 2.3 Four pond systems in Well Court Farm, Blean with (a) Garden ponds, (b) Snake pond, (c) Swimming pool, and (d) Pylon pond

The Garden ponds

The Garden ponds at Well Court Farm were created in 1970 (Williams 1999). The Garden ponds system comprised three artificial butyl-lined ponds situated in the farmhouse garden and has a surface area between 4.5 and 32 m with a maximum depth of 1 m. Garden pond 1, the biggest one, has a surface area of about 25 m^2, while Garden pond 2 is the smallest one (about 7 m^2). Garden pond 1 is situated within 2 m of two other garden ponds and divided by a narrow path. Garden ponds 2 and 3 have low numbers of newts and are also prone to drying out in mid-season. Great crested newts move without restriction between them, based on the evidence of weekly capture records. Hence, all newts captured at these ponds were grouped together as describing a population covering a single site. There are flower-beds and ornamental plants around these ponds and the grass is cut regularly. The aquatic vegetation consisted of *Nymphaea alba* (white water lily), *Ranunculus lingua* (greater spearwort) and *Crassula helmsii* (pigmyweed). Introductions of North American fathead minnow (*Pimpaphales promelas*) were reported in 1997 (Williams 1999) and goldfish (*Carrasius auratus*) have also been recorded in various years, but no fish occurred from 2007 onwards. The Garden ponds are located approximately mid-way between the Snake pond and the Swimming pool, approximately 230 m.

The Snake pond

The pond is situated among fields and orchards and its shoreline is surrounded by very thick hedges. This is a large semi-natural pond with a variable shape and surface area of roughly between 160 m^2 and 270 m^2, depending on water levels. However, this pond fluctuates in depth between weeks and the maximum water depth is roughly 2 m. Water levels decreased during most summers and in September 2005 the pond was nearly dry (Sewell 2007). The aquatic vegetation includes *Sparganium erectum* (branched bur-reed), *Nymphaea* spp. (water lily), *Berula erecta* (water parsnip), *Eleocharis* spp. (hairgrass), *Elodea* spp. and *Lemna minor* (tiny- leafed duckweed). The nearest pond to the Snake Pond was the Garden pond system; approximately 230 m away. Very little aquatic vegetation was noted in this pond over the study period.

Swimming pool

This is an old, abandoned concrete Swimming pool, surrounded by a damp woodland area. Its shape is rectangular and its size area is about 50 m^2. The water surface is covered by a thick layer of tiny-leafed duckweed (*Lemna minor*) and algae. Although the pool is more than 2 m deep, the water level dropped considerably during the season, leaving a gap of c. 50 cm between the water surface and the soil level. Newts that try to leave the pond in such a situation may well be able to climb up the vertical walls by clinging to the rough surface, or they may use the stalks of grass and plants around the pond edges.

The Pylon pond

This is a rectangular semi-natural pond with a surface area of about 185 m^2 and has a maximum depth of about 1.5 m. It is bordered by a narrow belt of hedgerow which separates it from arable fields on three sides. The fourth side is bordered by deciduous woodland. Through the peak season, most of the water body was covered by submergent or emergent vegetation, but the water level fell during the summer months. There is an array of macrophyte species present in this pond with *Ranunculus aquatilis* (water crowfoot) as an abundant plant and also including the invasive species, *Crassula helmsii* (pigmyweed), *Rorippa nasturtium-aquaticum* (watercress) and *Solanum dulcamara* (woody nightshade). The nearest pond to the Pylon pond was the Snake pond, located 350 m to the south.

2.1.2 Field Site, University Of Kent

The study site is situated on the north-western part of the of the University of Kent campus at Canterbury (Grid reference TR129596) (Figure 2.4). The site consisted of eight artificial ponds, each of 2 m x 1 m x 0.7 m deep size located within a fenced enclosure (Figure 2.5). Four ponds were constructed in February 1998 using PVC liner, filled with tap water and left to colonise naturally. In 2008, four new ponds were created adjacent to the four old ponds. Each of the ponds had a wedge-shaped profile, with the bottom sloping from a shoreline to a deeper end (about 0.7 m)

(Figure 2.6). The ponds were laid out parallel to each other in a row and separated by 4 m strips of grassland.

The water bodies were colonised by duckweed (*Lemna*) and filamentous algae, and overhanging grass provided egg-laying sites. There were no other emergent or submerged aquatic plants. The ponds were surrounded by bramble (*Rubus fruticosus agg.*), common nettle (*Urtica dioica*), chamomile (*Chamaemelum nobile*), thistle (*Cirsium vulgare*) and rough grassland. The surrounding area was mostly residential with houses and gardens, sports fields and paddocks. There were several other ponds within one kilometre, the nearest being 450 m away. All three native species of newt (*Lissotriton vulgaris, L. helveticus* and *Triturus cristatus*) and one non-native species, *Ichthyosaura alpestris* naturally colonised the ponds and used them as breeding sites. In the early years, there was the occasional frog but no other species of amphibian were found in these ponds in recent years. Aquatic invertebrates comprised pond skaters (*Aquarius remigis*), backswimmers (*Notonecta glauca*), beetles (*Dytiscus marginalis*), dragonflies (*Aeshna cyanea*) and damselflies (*Coenagrion pulchellum*).

Male crested newts were first recorded in 1999, and females from 2000, since then the population has been monitored and all individuals can be individually recognised (Lewis 2012). The origin of these colonisers is unknown, but may have been from an occupied pond 450 m to the north, or a formerly occupied pond 300 m to the southeast (Sewell 2006).

Figure 2.4 The red pin showing study site consisted with eight artificial ponds within a fenced enclosure, map was adapted from Earthstar Geographics SIO

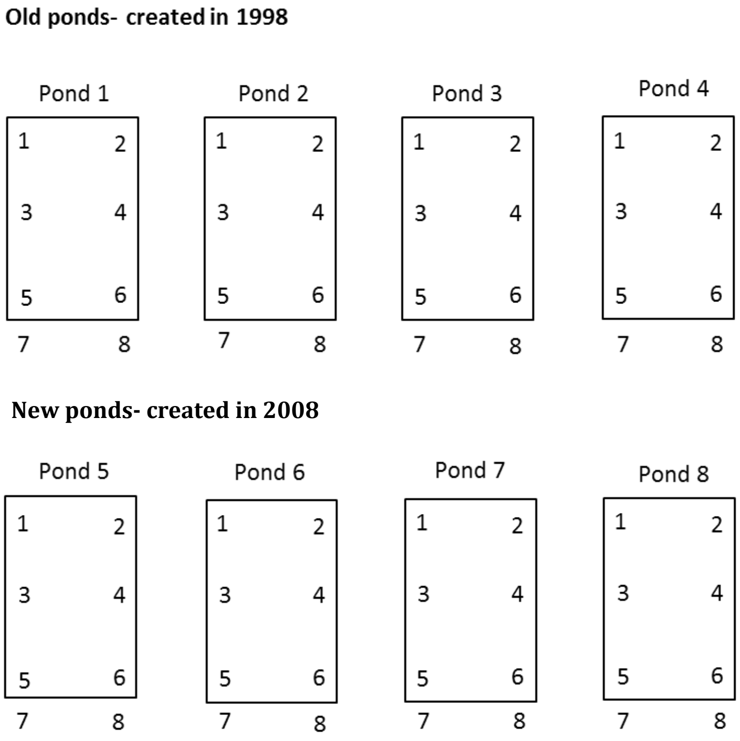


Figure 2.5 Plan of the layout at the Field Site Ponds, adapted from Lewis (2012). The numbers within the ponds indicate bottle-trap positions. Traps 1 and 2 are at the shallow end of each pond, 5 and 6 at the deep end. Traps 7 and 8, when used are laid below traps 5 and 6

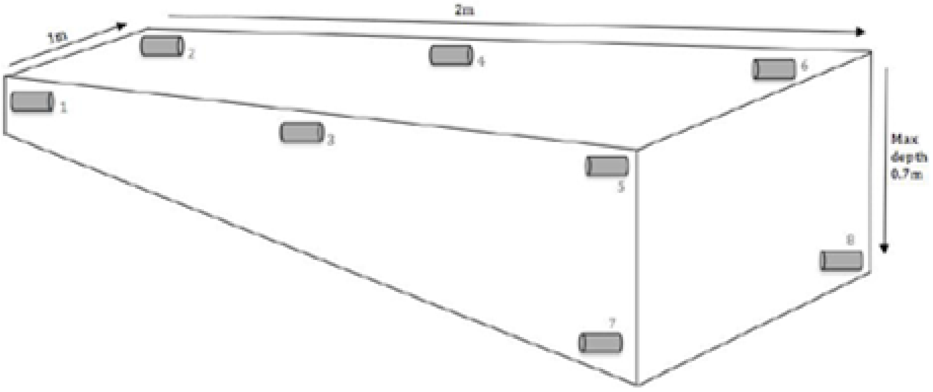


Figure 2.6 A cross section of a Field Site pond showing its tapered design and measurements, from Wright (2009). Also, trap placement within each pond

2.2 DATA COLLECTION

The chosen method for this study was trapping during the aquatic breeding season. Field surveys at Well Court were carried out annually from 1995 until 2013, and at the Field Site from 1999 until 2016. My own data collection at Well Court was conducted in 2013 only (permission for access was withdrawn after 2013) and, at the Field Site between 2014 and 2016. However, analyses at both of the study sites include the full data set. Trapping were commenced on the Thursday night/Friday morning most closely matching the night of 28th February/1st March in each year. Trapping then continued at weekly intervals until the end of the aquatic period (i.e. when no further newts were trapped), this usually occurred at the end of July although there was variation from year to year.

Newts at the Well Court ponds were captured by using funnel traps as described by Griffiths (1985), spaced at two metre intervals along the shoreline of the ponds. Traps were numbered and the positions of each trap marked on a plan to allow for continuity of data between years. The size of trap used was based on a 1 litre plastic bottle, with the funnel inverted into the body and held in place by a cane inserted through the trap and into the bottom of the pond. Where this was not possible for artificial ponds where the pond had a plastic or similar liner, traps were secured to nearby vegetation or stones by means of a piece of string.

Trapping was carried out in a similar manner at the Field Site ponds, except that the interval between traps was one metre. The trap arrangement is described in detail in Chapter 4 (Well Court) and Chapter 5 (Field Site). Traps were set between 2000 and 2200 hrs and were then checked and emptied between 0730 and 0900 hrs the next morning depending on the season.

2.3 NEWT IDENTIFICATION

Newts trapped were recorded according to pond, trap number, species and sex, and released back into the pond where they were caught. Smooth and palmate newts were counted but not marked or measured in any way. The great crested newts were placed in a rectangular transparent plastic tray and restrained by a foam rubber sponge (Figure 2.7). They were then measured; this included the snout-vent length (from the tip of the snout to the hind margin of the cloaca) and the tail length (from the hind margin of the cloaca to the tip of the tail). This was achieved using a 30 cm ruler, with measurements being taken to the nearest 1 mm. The great crested newts were then dried of excess water using a paper towel and measured using a digital balance to the nearest 0.1 g.

Each crested newt exhibits a belly pattern that is unique to that individual. Thus, identification of each crested newt captured was enabled by photographing the belly pattern (Hagstrom 1973). Each trapped individual therefore has a picture of its belly taken using a digital camera and the photos obtained are compared with records from previous weeks and years, enabling detailed capture histories for all individuals to be constructed. For simplicity and quicker individual identification, pictures of males and females in the ponds were compiled on separate sheets and given names.

Figure 2.7 Example of photo utilised for individual photo identification

2.4 WEATHER RECORDS

Climatic data were taken from the UK Meteorological Office website for the nearest weather station which is Manston, approximately 15 miles from the study site. Previous exploratory correlations between annual survival and possible predictor variables at Well Court by Griffiths et al. (2010) revealed four potentially important climatic factors. These were: winter air frosts (AF), winter mean minimum temperature (WT), spring rainfall (SR) and non-aquatic season rainfall (NAR).

Winter air frost was defined as the total number of days of air frost in the months of December, January and February combined. Winter mean minimum temperature was defined as the mean daily minimum temperature (^{0}C) over the period December-February each winter. Spring rainfall was defined as the combined rainfall (mm) in the months of March, April and May each year, whilst non-aquatic period rainfall was defined as total rainfall (mm) over the remainder of the calendar year (January-February plus June-December), the period in which most newts were expected to be absent from ponds. Dividing the rainfall data into spring rainfall (SR) and non-aquatic season rainfall (NAR) was regarded as applicable given the different impacts of rainfall on aquatic and terrestrial stages of newts.

2.5 MARK RECAPTURE ANALYSIS

To estimate between-year survival and detection rates, individual capture histories were used after annualising all the weekly capture data for each year (i.e. an individual was scored as ‘1’ if captured in any week in a particular year and ‘0’ if not captured at all in the year). As well as examining survival and detection probabilities, the study also sought to test whether these probabilities varied between sexes. Capture histories for all males and females were constructed over a nineteen- year period for the Well Court and sixteen-year period for the Field Site. Juveniles were excluded from this analysis because the belly pattern may change as the animal grows, making identification unreliable. As survival rate calculation depends on survival between years, nineteen years of data effectively give eighteen (i.e. n – 1) estimates of annual survival.

The probabilities of survival and detectability were estimated using Program MARK (White & Burnham 1999). MARK was used to estimate differences in the rate of survival and detectability within the population, both over time and between the sexes, using the Cormack-Jolly-Seber (CJS) model. The CJS model has four basic assumptions, as follows:

1. Every animal present in the population at time *(i)* has the same probability of recapture *(p_i)*;
2. Every marked animal in the population immediately after time *(i)* has the same probability of surviving to time *(i + 1)*;
3. Marks are not lost or missed; and
4. All samples are instantaneous relative to the interval between occasions *(i)* and *(i + 1)* and each release is made immediately after the sample.

Pre-defined and customised models were used in MARK, allowing analyses that were both time and group dependent. Model assumptions followed Williams et al. (2002), whilst model notation followed Lebreton et al. (1992), as follows:

Phi_i	survival probability from time *i* to *i* +1;
p_i	probability of detection (i.e. capture or recapture) at time *i*;
(g)	group (sex and/or pond) dependent survival or detection;
(t)	time dependent survival or detection;
*(g*t)*	both group dependent and time dependent survival or detection; and
(.)	constant survival or capture rate.

Up to sixteen candidate models could be run from each data set as follows:

Phi(.) p(.)	Survival and detection rates constant

Phi(.) p(g)	Survival rate constant, detection varies by group
Phi(.) p(t)	Survival rate constant, detection varies over time
*Phi(.) p(g*t)*	Survival rate constant, detection varies by both group and time
Phi(g) p(.)	Survival rate varies by group, detection rate constant
Phi(g) p(g)	Survival and detection rates vary by group
Phi(g) p(t)	Survival rate varies by group, detection varies over time
*Phi(g) p(g*t)*	Survival rate varies by group, detection by both group and time
Phi(t) p(.)	Survival rate varies by time, detection rate constant
Phi(t) p(g)	Survival rate varies by time, detection varies by group
Phi(t) p(t)	Survival and detection rates vary over time
*Phi(t) p(g*t)*	Survival rate varies over time, detection by both group and time
*Phi(g*t)p(.)*	Survival rate varies by group and time, detection rate constant
*Phi(g*t)p(g)*	Survival rate varies by group and time, detection varies by group
*Phi(g*t)p(t)*	Survival rate varies by group and time, detection varies by time
*Phi(g*t)p(g*t)*	Survival and detection rates both vary by group and time

The number of groups used also varied as follows:

Single group	No variation between sex or pond
Two groups	Variation between sexes
Four groups	Variation between the four ponds tested
Eight groups	Variation between both pond and sex

By definition no model including *g* or *g*t* could be operate from a single group analysis, meaning that a total of 52 different ways of running the data were performed within MARK (i.e. 3 x 16, plus 1 x 4).

In addition to these, models were also run with climatic data as covariates. Goodness-of-fit (GOF) was tested by means of 1000 bootstrap iterations of the best-fitting model. Probabilities were calculated based upon deviance ranks and mean deviance and variation inflation factor (*c-hat*) from GOF simulations and were used to calculate new *c-hat* values for the model. In order to keep estimates conservative the highest *c-hat* value was chosen and the data adjusted accordingly. *C-hat* is a measure of the over-dispersion or under-dispersion of the data with a value of 1 considered a perfect fit, > 1 indicating over-dispersion and < 1 indicating under-dispersion (Cooch & White 2001).

A number of candidate models were tested and the best fits chosen using Akaike's Information Criterion (AIC) or quasi-likelihood (QAIC) rank and weighting (Burnham & Anderson 1992). The QAIC is an AIC parameter resulting from *c-hat* adjusted data (Anderson et al. 1994). The best-fitting model was taken as that which best explains the variation in the data whilst having the fewest parameters. To determine the best model, this study took a ΔQAICc of < 2 as evidence of substantial fit, values between 3-7 as indicating less support and a ΔQAICc > 10 as having little or no support (Burham & Anderson 1992). Models with a ΔQAICc of 10 or over were discounted and are not reported in this study. The estimated population for each year was calculated by multiplying the number of individuals caught in each year by 1/detection probability for that year (Griffiths et al. 2010).

CHAPTER 3

ACCURACY OF PHOTO IDENTIFICATION OF NEWTS BY MANUAL AND COMPUTER IDENTIFICATION METHODS

ABSTRACT

Accurate individual identification is a basic assumption of capture-mark-recapture methods. When overlooked, errors can bias demographic parameter estimates. The study was conducted using 15 years of images captured at the Field Site population. Expert newt researchers more consistently identified the same newt than non-experts when the same images were repeated, and were more cautious identifying two different individuals with similar patterns. However, both experts and non-experts had difficulty identifying newts from poor quality images. Computer-assisted methods using five software packages proved that the spot patterns of individual newts did not change significantly through time, and were sufficiently varied to allow their individual identification. The accuracy of each software package gradually decreased using low quality images and increased with the increase number of reference images. With a database comprising 136 individuals, manual identification was more accurate and quicker than software packages. However, if the number of images increases, manual identification will become unfeasible and software packages can be successfully employed for the identification of individuals in large populations of animals.

3.1 INTRODUCTION

Individual identification of animals is necessary in many types of ecological studies (Nietfeld et al. 1994). One of the most basic mechanisms of animal population biology is the capability to identify and track individual animals over time and space (Bolger et al. 2012). Estimates of population size, survival and reproduction rates, and migration rates typically involve identifying previously marked or sighted individuals (Nichols 1992). Conventionally, animal identification involves capturing and placing clear and distinctive marks on them (Williams et al. 2002). The most common method for identifying individual amphibians is by applying artificial marks such as PIT-tags, visible implanted elastomers, bands, or tattoos (Mettouris et al. 2016). However, ethical and methodological objections to such methods have been raised and there is broad interest in developing alternative methods. Some of these invasive techniques may lead to stress and influence the survival or behaviour of the studied animal (Antwis et al. 2014). In addition, there have been concerns about ethical and animal welfare issues that arise from their broad use (Perry et al. 2011).

One alternative is to use photo-identification methods (PIMs) to identify individuals based on their natural markings. Photo-identification methods (PIMs), which use photographs of unique natural markings to identify individuals (Bradfield 2004), avoid most of the ethical issues associated with artificial marking methods, and it can be quick, easy, and inexpensive to generate images in the field. Nowadays, photography remains one of the quickest and affordable ways of "labelling" substantial numbers of individuals in a population (Harting et al. 2004). In recent years, camera trapping is widely used, especially to study medium-to-large terrestrial mammals and birds, arboreal mammals, small mammals and herpetofauna. Camera trapping is the use of remotely triggered cameras that automatically take images and/or videos of animals or other subjects passing in front of them (Rovero et al. 2013).

Images captured are usually stored in a library to be visually examined later for matches and to develop individual capture history files (Speed et al. 2007). Although it is possible to manage small numbers of photographs manually, the task becomes increasingly inefficient and error-prone when library sizes are large (Arntzen et al. 2004). Therefore, many studies involving photographic identification now apply one or more of the following techniques to minimize the amount of manual matching: 1) classification of unique characteristics into a searchable database (e.g. Petersen 1972); 2) semi-automated matching with pattern recognition algorithms (e.g. Gamble et al. 2008); or 3) computer-assisted extraction of morphometric information (e.g. Araabi et al. 2000).

Researchers entering this rapidly expanding field should consider several major factors before deciding if, and what type of visual recognition method is appropriate for their study context. A basic understanding of the history, limitations, and key issues associated with photographic identification will serve to improve experimental design and analysis, facilitate comparisons between studies, and efficiently advance the methodology and technology associated with this field.

Volunteers – or ‘citizen scientists’ - have contributed for centuries to ecological surveys (Cohn 2008), and have been increasingly engaged by scientists over the past few decades to assist and support scientific research (Silvertown 2009). A central aspect of volunteer involvement is the collection of data, which often contribute significantly to research projects, especially when guided by experienced scientists

(Foster-Smith & Evans 2003). Clearly, the accuracy and consistency of data collected by volunteers is a critical aspect of these projects, as data are often used to support scientific publications and management planning decisions. Volunteers are associated with the image of the well-meaning 'amateur', however volunteers can often be dedicated individuals already working or studying in a relevant field area and may either have or achieve high standards of expertise (Foster-Smith & Evans 2003). If sufficiently trained, volunteers can gather high quality data (Newman et al. 2003), but the reliability of data collected by novice volunteers has sometimes been disputed (Cohn 2008).

In photo-identification studies, the probability of recognizing, and thus recapturing, a marked individual is dependent on three major factors: 1) individual distinctiveness; 2) stability of the pattern; and 3) quality of the photograph (Hammond et al. 1990; Friday et al. 2000). The first of these factors, distinctiveness, is based on another primary assumption of CMR studies - that each individual in a population has a distinct mark by which it can be identified. "Distinctiveness" has also been defined as "recognisability" (Hammond et al. 1990), "unique information content" that each individual contributes to distinguishing itself from others (Burghardt 2008) and "the degree of visibility of permanent marks" allied to the "ease of individual identification" (Forcada & Aguilar 2000). Across species, distinctiveness is commonly age or sex dependent. Most amphibians, pinnipeds, and birds, for instance, fail to develop a stable pattern until they reach sexual maturity because juveniles often exhibit different pigmentation patterns to adults (e.g. Forcada & Aguilar 2000). Similarly, with morphological marks, it is typically older manatees, and female otters that have the highest degree of scarring available to photograph (Langtimm et al. 2004; Finerty et al. 2007). Within a species there is natural variation in morphology and/or pigmentation among individuals (Friday et al. 2000). The more divergent the pattern or shape between two photos, the easier it is for an observer or a computer to identify, code, or rank the individuals as different (Agler 1992). Lastly, if not accounted for, gradations in distinctiveness can potentially cause both human and computer processing errors or bias in matching (Harting et al. 2004).

Another source of processing error in identifying individuals relates to the quality of the photographic image. In general, quality has been broadly defined with respect to several secondary factors: 1) clarity, sharpness, or focus (Friday et al. 2000); 2) contrast or the degree of difference between blacks and whites (Friday et al. 2000); 3) noise or the amount of unnecessary background information in the frame with the individual of interest (Gamble et al. 2008); 4) resolution or the amount of available pixel information (Markowitzet et al. 2003); 5) glare or specularity (Gamble et al. 2008); and 6) relative size of the animal of interest in the picture frame (Sears et al. 1990). Weather, water depth or turbidity, patterns in the background environment, and ambient light are just a few natural environmental variables that affect photographic quality (Markowitzet et al. 2003; Langtimm et al. 2004). Photographing individuals in their natural environments typically precludes the controlled lighting, uniform background, and limited movement that would otherwise improve photographic quality. In fact, it is this unpredictability of photographic quality and its complex relationship with distinctiveness that continues to prevent widespread use of fully automated matching systems (Shirley et al. 2010).

On the other hand, automatic recognition software approaches based on pattern recognition hold the promise to become useful for monitoring efforts and mark-recapture studies for a wide range of animals that differ individually in body pattern traits (Drechsler et al. 2015). For large populations, computer-assisted photo-recognition programs may be needed as the time required for manual matching may be excessive (Knox et al. 2013). Pattern recognition software may increase the speed of correct identifications (relative to manual matching) regardless of the user's experience (Gamble et al. 2008). Pattern recognition programmes are useful for monitoring efforts and mark-recapture studies for a wide range of animals that differ individually in observable patterns (Drechsler et al. 2015). While numerous computer-assisted programmes have been introduced, there have been few studies to thoroughly test their performance across large datasets. Therefore, performance tests in large datasets are crucial if automatic approaches aim to be competitive and challenge the predominant manual approaches still used in many mark-recapture studies (Drechsler et al. 2015). Nevertheless, computer-based methods vary in their degree of accuracy and all require some additional visual confirmation that the correct match has been identified by the program (Knox et al. 2013). This study tested the performance of recognition software based on standardized images in the context of a large mark-recapture study in the great crested newt.

3.1.1 Aims and Objectives

The general purpose of PART 1 was to assess how experience in using manual photo-identification may affect the correct identifications of individual newts. The specific objectives were:

1) to compare expert and non-expert ability to detect distinctiveness of the belly pattern of the newts;
2) to test expert and non-expert accuracy in identifying good and low quality

images of newt belly pattern; and

3) to evaluate the performance consistency of experts and non-experts in identifying the great crested newt using the belly pattern images.

The general aim of PART 2 was to evaluate the performance of the computer-assisted photo-identification packages that are available for the public to access. The primary objectives were:

1) to determine whether pattern recognition software can be used to distinguish the same individual newts over several years;
2) to test whether good and low quality images of the ventral patterns of individual newts can be used to reliably distinguish between individuals accurately;
3) to assess how the number of reference images can improve the accuracy of pattern recognition software; and
4) to describe the advantages and limitations of this approach.

3.2 MATERIALS AND METHODS

3.2.1 Study Site and Photograph Collection

The identities of newts captured were individually recorded using a simple photographic recording system. This record is based on 3542 images from 156 adult great crested newts captured and photographed in ponds at a Field Site on the University of Kent campus at Canterbury (Grid reference TR132595) throughout 2000 and 2016 during the aquatic breeding season (March-July). Each individual was placed into a small, transparent plastic dish and restrained by a cover of foam rubber to prevent them from turning over. Great crested newts were held between the forefingers and a photograph taken of the ventral surface.

Images of individual belly patterns were taken subsequently and catalogued using digital camera 12.1 megapixels Canon Power Shot A1100 IS equipped with 6.2-24.8 mm lenses with ‘Macro’ setting. Images were taken by experts and trained volunteers, and the photographic process lasted approximately 30 seconds for each newt. Afterwards newts were released into the ponds to which they were captured. The images are of varying resolution, in 24-bit colour. Newts were individually identified by distinct belly pattern spot and several photographs of the same newt were taken in each session.

Previous study by Gowans and Whitehead (2001) highlighted the importance of obtaining high quality images, so animals were ‘clean’ (i.e., free of debris that could obscure natural marks) and excess water was removed using paper towels. Apart from that, animals were secured in a position that did not conceal ventral markings. A minimum of 4-5 images per specimen were taken to ensure correct focus and framing. However, poor quality images were also captured and have been used in reliability tests of good and low quality images. The belly pattern of newts was compared with the previous year’s record and the new individuals were sexed and coded individually. A minimum of three high quality images were selected from each capture event. Selected images were saved in a new directory for individual identification.

3.2.2 Manual Photo-Identification Evaluation

This survey is designed to investigate the accuracy of photo-identification techniques in identifying individual great crested newts. A total of 214 respondents took part in the study: 83 experts and 131 non-experts. An expert was defined as a person who has experience capturing and handling newts in the wild; non-experts had no such experience. Participants ranged in age from 18 to 65; however the great majority were from the 25-34 age group range. Data from this study have been divided into three main sections to test the reliability score between the good and poor quality images, the pattern distinctiveness of the belly pattern, and the consistency of experts and non-experts in answering sequential test involving the same images of the same individual that were presented twice.

The time taken to identify each newt was recorded in seconds. The low quality images (Figure 3.1) were defined as images having either a flash reflection, out of focus and out of frame, newts in a position that distorted ventral marking or newts were unclean (contain debris that could obscure natural marks). In the pattern distinctiveness section, each respondent was shown two different newts images that had some pattern similarity and could potentially be confused (e.g. central continuous line; scattered small spots - Figure 3.2). Every respondent was shown 20 pairs of images from each test section through an online questionnaire and asked to decide whether they think the images came from the same individual or not. There was no maximum or minimum time involved in answering this test but the duration that each respondent spent to complete the test was recorded.

Additionally, a total of 30 newts captured in the 2015 sampling season were used to determine how long it will take to identify individual using manual identification.

From this sample, 20 newts were recorded as recaptures and 10 newts were new. 136 belly pattern images from the previous sampling season (2008-2014) were used for reference in the identification process. Figure 3.3 shows some of the belly pattern images from the previous newts that were recorded at the Field Site.

(a) (b)

Figure 3.1 Example of two good quality images (1a left and 1b left) and two poor quality images (1a right and 1b right). Poor quality image of 1(a) right had flash reflection, 1(b) right was out of focus

(a) (b)

Figure 3.2 (a) and (b) are examples of images from 4 different individuals that have some similarity but unclear distinctiveness of belly pattern

Figure 3.3 Example some of the belly pattern images that were used for reference in newt identification

3.2.3 Computer-assisted Pattern Recognition Program Evaluation

Recently, different automatic pattern recognition algorithms have been developed (e.g.: Speed et al. 2007; Van Tienhoven et al. 2007; Sacchi et al. 2010), but few approaches have been tested in large datasets. In this study, five available pattern-recognition packages that are suitable for the great crested newts were tested. The list and descriptions of the programmes are as follows:

1) Amphident

This study used the trial version of this software that is available at http://www.amphident.de/en/pages/download.html. The test version of Amphident supports all features of the full version. The only limitation is that the test version can only work with databases that contain at most 200 patterns. Basically, Amphident is developed based on algorithm in improved technique of cross-correlation comparisons to find similar sign (e.g., a specific spot pattern) in a large arrangement of related signals again. A thorough explanation and how the program work is illustrated by Matthé et al. (2008). The program also came with the option to crop certain angle of the belly pattern from the images, if some parts of the picture are of low quality. Based on a similarity values for a particular pattern (i.e., for an individual), the program suggests user maximum the best 30 ranked matching patterns in a descending order from the database. The user then requires to compare by eye and decided whether the pattern is already contained in the database, that is, whether an individual has been recaptured or not.

2) APHIS

APHIS **(**Automatic Photo-Identification Suite) was specially designed by Moya et al. (2015) to deal with sample sets of over a hundred images per field campaign and image library libraries containing more than a thousand samples. APHIS uses two approaches for photo-matching, the Spot Pattern Matching (SPM) and the Image Template Matching (ITM). The former has been built on the already existing I^3S algorithm (Van Tienhoven et al. 2007) while the latter is a novel approach based on pixel matching that minimizes the user's pre-processing effort. ITM is a fast-running alternative to study species with clear or easily recognizable spots or coloured parts of the skin. The workflow and graphic interface of APHIS have been designed to reduce the time invested by the researcher in analytical tasks and to enhance user experience. An important feature in APHIS is the automatic creation of log files that register the score lists obtained at each comparison. It also produces a registry of the matches validated by the user, which will lead to an easy analysis of capture-recapture data. APHIS v.1.0 (freely available at http://www.imedea.uib-csic.es/bc/ecopob/) combines C++ and Java modules.

3) I^3S

This pattern-matching program was developed by Van Tienhoven et al. (2007) using Java 1.4.2 and C++, and requires the Java Run-time environment to run on personal computers. The entire code for the pattern-matching system, known as I^3S (Interactive Individual Identification System) is available at http://www.reijns.com/i3s. An image is opened in the application. Using a computer mouse, the user manually selects the three reference points to define the common reference area. The user then points out the most distinct pigment spots of each animal. Between 12 and 40 spots are selected within the reference area. The centre of each spot is marked and where spots overlap or join, the apparent centres of such spots are marked. The size of the spots is currently not considered important, whereas their relative position to one another is. The user can compare the image of the unknown individual with up to 50 possible matches provided in the list.

4) Wild-ID

Wild-ID was introduced by Bolger et al. (2012) and is available at http://envs.dartmouth.edu/people/douglas-thomas-bolger. Wild-ID processes the images in each dataset sequentially and calculates a similarity score for each pair of images. After all similarity scores are computed, Wild-ID presents the top-20 ranking images for each image for visual confirmation through the user interface. Consequently, the final decision for accepting or rejecting a match is left to the user.

5) Zoometrics

The prototype of this software was implemented in MATLAB 8.0 (The MathWorks, USA) and was described by Hoque et al. (2011). In order to find the similarity between two newts, the corresponding Region of Interests (ROIs) are compared. To compensate the misalignment due to flexible segmentation mechanism, a subsection of the ROI is isolated from one image that scans over the second image. A score is generated for each overlapping region and the overall comparison score is the one that shows maximum similarity.

To start with, images of the ventral side of individual newts were entered into the computer-assisted photo-identification software. Figure 3.4 shows the example of workflow of pattern extraction with Amphident software. Generally, the computer-assisted photo-identification involved the following steps:

(i) New images were downloaded into an image library;

(ii) The belly pattern is then extracted by first marking the area of the belly pattern, that is, defining the region of the belly pattern by the user;

(iii) The program then extracts this region from the original images into a black and white picture;

(iv) It is then further modified by a median filter to remove noise of edges and small spots;

(v) The obtained pattern is then compared with all existing patterns in the database;

(vi) For each pairwise comparison, a similarity value is determined by the number of matching pixels of both patterns;

(vii) The higher the number of matching pixels between shapes, that is, the more similar the patterns are, the higher the similarity score.

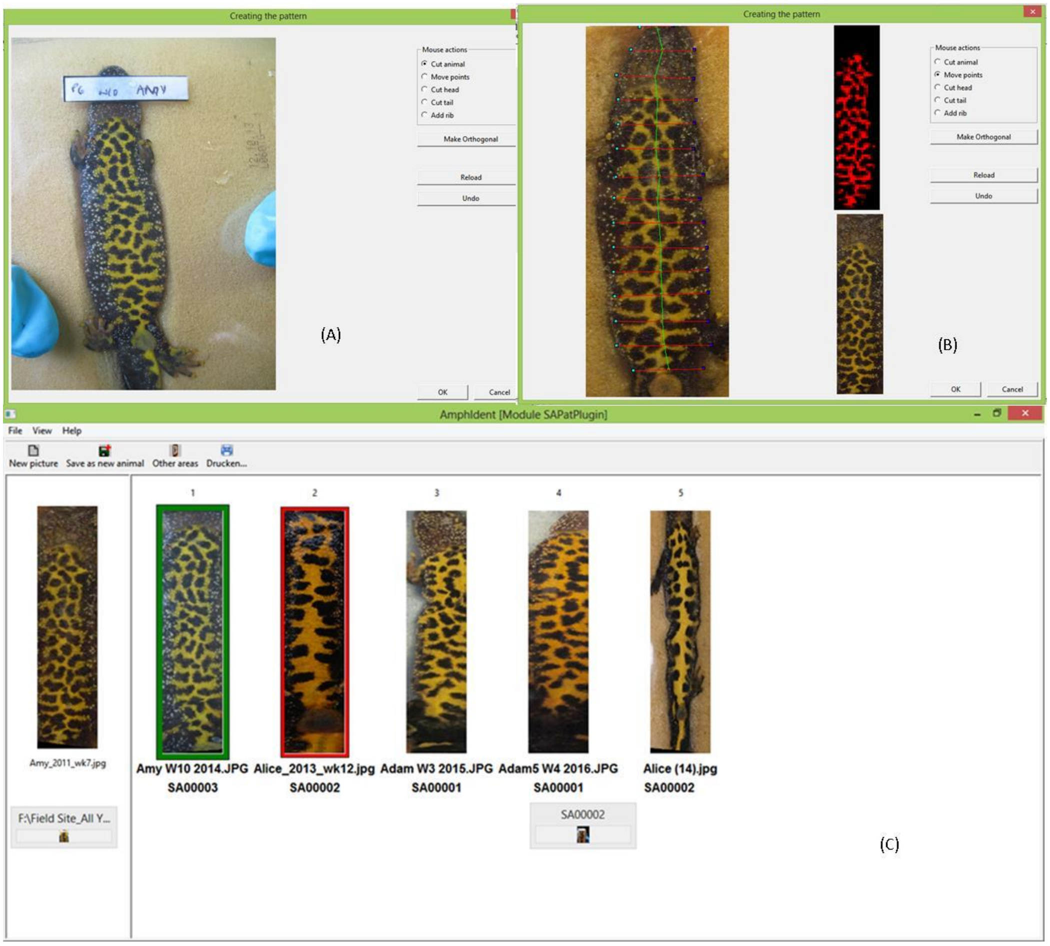


Figure 3.4 (A) The original, non-processed picture. (B) The pattern with superimposed grid and the preview to pattern selection in true and false colour. (C) The extracted pattern and the proposed matches (1–5) in order of likelihood supplemented by the original pictures of the extracted pattern and the selected one. Screenshot was taken from AMPHIDENT software

The performance of the programmes was compared in three ways:

1) Performance Over Time

Some animal natural marking patterns are known to change over time (Hastings et al. 2008). Therefore, whether the inter-year interval between captures influenced the accuracy scores in each image dataset were tested in this study. Subsampling procedures that selected a single photo per individual were used in this study because some individuals were recaptured on more than one occasion each year. Since many recaptures occur within one breeding season, this study selected only 50 pair matched-images (100 images) for each interval years in order to ensure longer periods were included in subsamples. Accuracy score of the matched images at the top 10 rankings were calculated to see if the scores were inversely related to the time interval between captures.

2) Number of reference images

The performance of the programmes were tested by randomly selecting between one and ten reference images per newt from the dataset and using all the remaining newts images as a test set. A total of 50 newts were used in this experiment. The number of times the correct newt was ranked in the Top 10 best matches was measured as a percentage. To correct for random effects, the same experiments were repeated 100 times and the averages were calculated over all results.

3) Image quality

Image quality can affect matching success (Kelly 2001). Therefore, images for matching were segregated into two groups based on their quality type: a ‘low quality’ group and a ‘good quality’ group images. The experiment was run on 50 different newts. The number of times the correct newt was ranked in the Top 10 best matches was measured as a percentage. To correct for random effects, the same experiments were repeated 100 times and the averages were calculated over all results.

3.2.4 Statistical Analysis

Scores from expert and non-expert respondents were divided into three categories: photo quality, pattern distinctiveness and consistency. To ascertain whether there was a difference in the performance of expert and non-expert respondents in using manual photo-identification methods, tests were run using Mann-Whitney U tests for the photo quality and pattern distinctiveness categories. Non-parametric tests were used for the photo quality and pattern distinctiveness statistical tests because the accuracy scores mean from these data were not normally distributed. Additionally, as the data were normally distributed, a parametric paired sample t-test was used to compare the accuracy score mean from first and second responses in sequential tests to evaluate the performance consistency in both respondent groups. To evaluate the software performance in identifying poor and good quality images, the accuracy scores for each of the packages was tested using a paired sample t-test. A two way ANOVA was performed to see whether accuracy score was influenced by the belly pattern stability across nine years of dataset. All statistical analysis was run through Minitab 17.

3.3 RESULTS

3.3.1 Manual Identification

It took an average of 64.5±24.4 seconds for an expert to identify a recaptured newt from the 136 photographs (Figure 3.5). It took a significantly longer time to determine that a new newt was a new individual, 207 ± 33 seconds ($t = 13.4$, d.f. = 28, $P < 0.0001$)

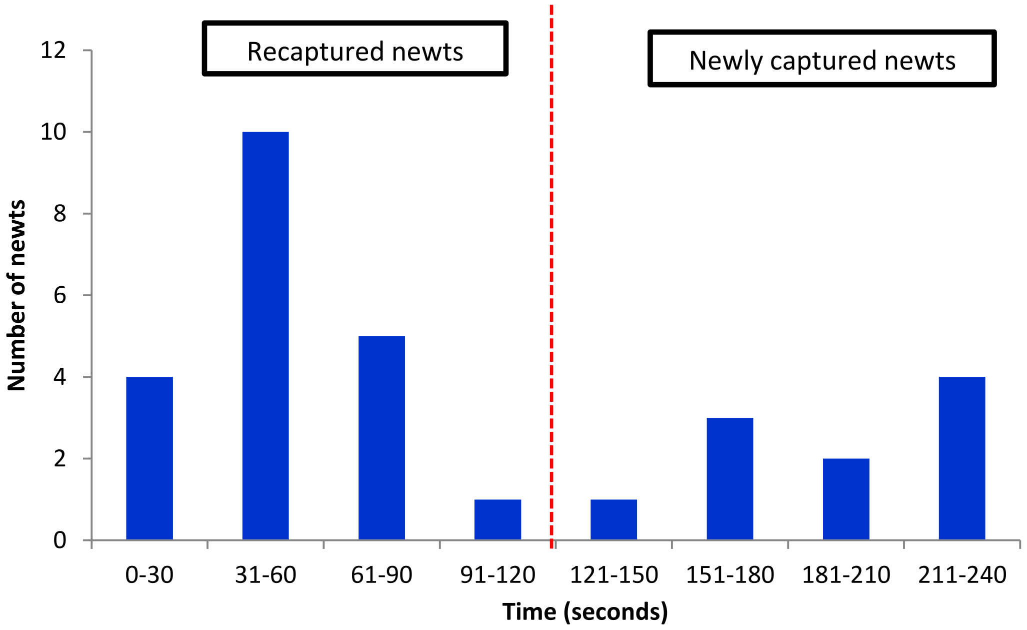


Figure 3.5 Time taken to identify newt individuals manually from 136 photographs. On the left side of red line were the recaptures and on the right were the newly captured newts

<u>Expert and non-expert performance in manual identification</u>

The experts recorded higher accuracy (score) in all three main tests than the non-expert group (Figure 3.6). The greatest accuracy from the expert group was achieved both by pattern distinctiveness and consistency, with on average 99.0% of matches made correctly. The lowest accuracy was recorded in terms of image quality (94%). The patterns for non-experts were similar, but on average they were 2-5% less accurate in their matchings than experts, but the differences between experts and non-experts were not always significant. For example, when image quality was considered, there was no difference between experts and non-experts in their accuracy ($U = 97.0$, $P = 0.239$). However, in terms of pattern distinctiveness the score in the expert group was higher than the non- expert group ($U = 56.5$, $p < 0.006$). Only the non-expert group recorded significant difference in accuracy scores between two sequential tests involving the same images of the same individual that were presented twice ($t = 2.3$, $p < 0.039$). This result indicates that non-experts were more inconsistent when presented with the same pair of images twice. On the contrary, the expert group displayed consistency over these two sequential tests because there was no significant difference in accuracy scores recorded between these two tests (t = 1.6, p = 0.134).

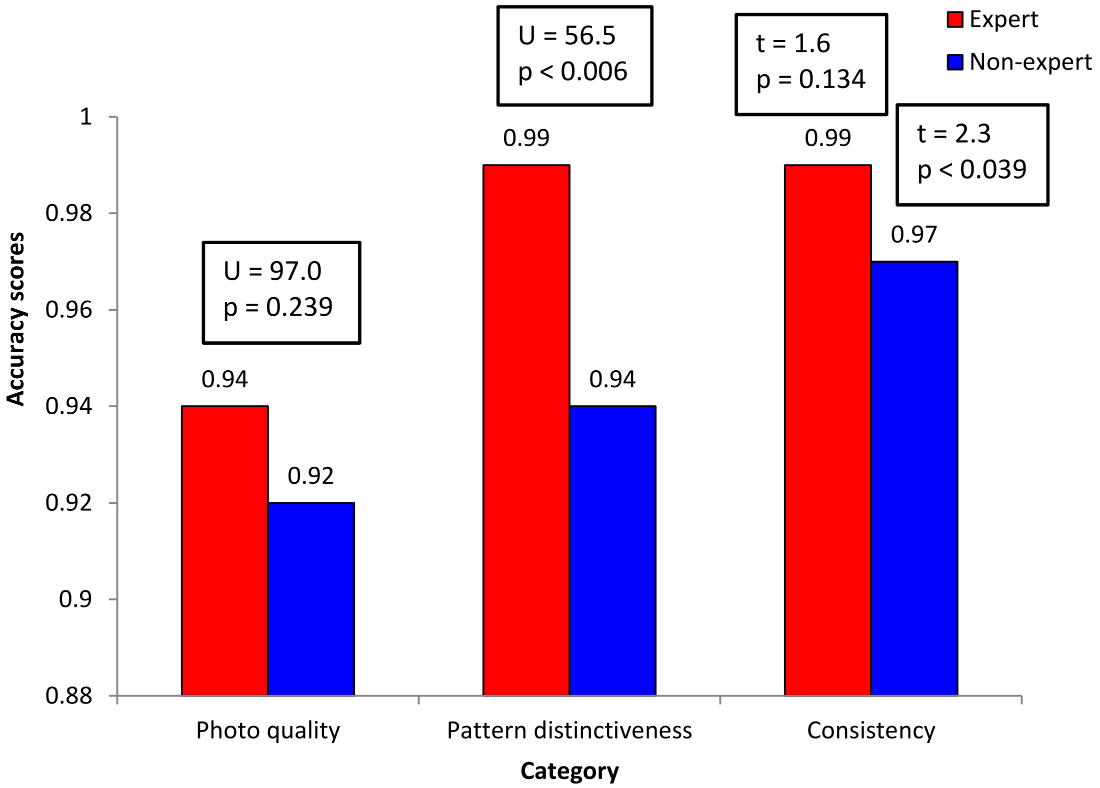


Figure 3.6 Accuracy scores of each test category from two groups; expert and non-expert. Photo quality test, Pattern distinctiveness test, and Consistency test

3.3.2 Software Evaluation

A total of 3127 adult great crested newt were captured from 154 individuals during the 17-year period. The vast majority of these individuals (n = 120) were captured more than twice and there were only 34 images for which individuals that were captured only once. It was evident that a high proportion of previously recorded individuals were recaptured every year. On average, almost 0.82% of animals were recaptured at least once in earlier years. More detailed analysis of individually recognized newts indicated that most animals probably return to the Field Site annually (see Chapter 5).

Inspection of all within-year and between-year recaptures revealed that the belly patterns of individual newts change to some extent. The changes normally involved the belly spots growing in size, often with nearby marks fusing. This therefore makes some of the belly patterns look dissimilar even when these are from the same newt (Figure 3.7). However, the belly patterns of individual newts in all datasets were sufficiently varied to allow for the unambiguous identification of all individuals in different years using the manual identification procedure. Thus, the continuous recapture of individuals enabled pattern changes to be tracked and all captured animals to be assigned as either recaptures or new captures with a high degree of certainty.

(a) (b)

Figure 3.7 Examples of belly pattern changes over > 6 yrs. Images (a) and (b) were taken from different newts

Image quality

The highest accuracy for the good quality images was achieved using Amphident software, which correctly identified 85% of the images in the Top 10 rankings. Wild- ID was second, with 79% accuracy, followed by I^3S, with 78% accuracy, Zoometrics (75%) and APHIS (66%). However, the accuracy scores for each software package gradually decreased with the low quality images. Figure 3.8 clearly shows that the accuracy scores of low quality images were nearly half that of the good quality images. Wild-ID software recorded the highest accuracy scores for the low quality images (56%) with Amphident yielding the lowest scores recorded (32%). From the paired t-tests, there was a significant difference in accuracy scores between good and low quality images for each of the software packages. Due to the direction of the t- value, this study concluded that there was a significant improvement in accuracy scores following the use of the good quality images.

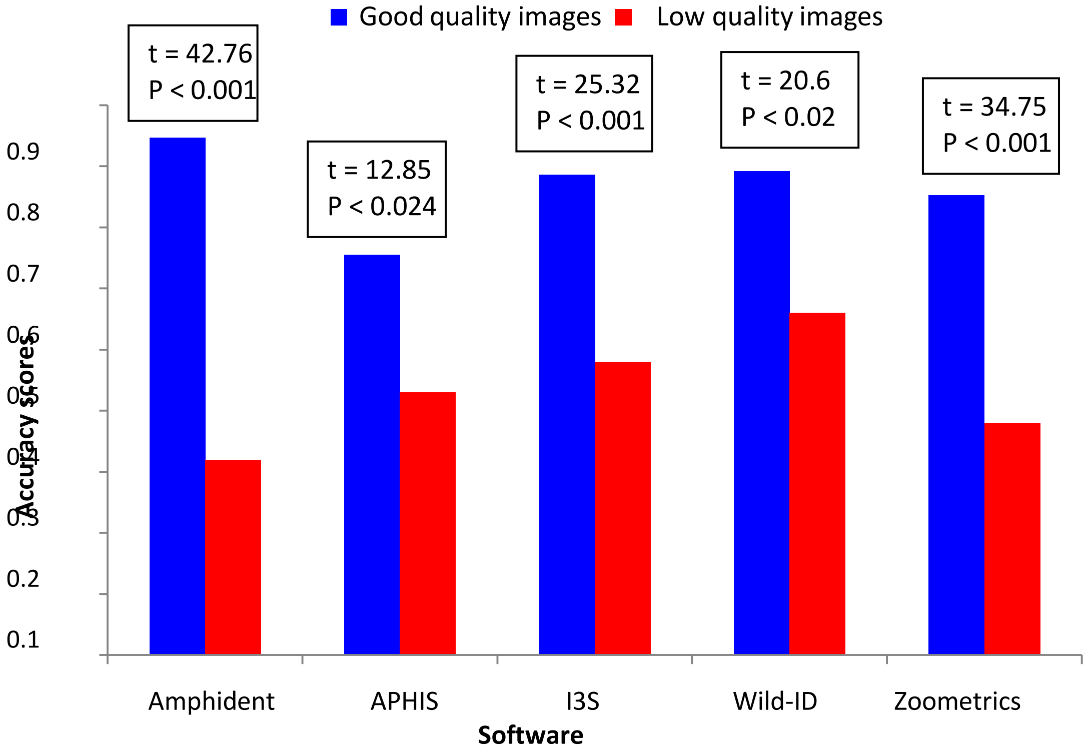


Figure 3.8 Summary of results from paired sample of t-test analyses for five different softwares packages that compare the accuracy scores of good and low quality images. The degree of freedom (d.f.) for each test was 49

Number of reference images

Considering multiple images per animal, accuracy scores increased nonlinearly as the number of total images available for matching increased (Figure 3.9). For example, accuracy increased from 76% using a single image to 92% when three reference images were available. Generally, an accuracy rate of ±75% required that at least three good quality images were available for an individual animal for accurate matching.

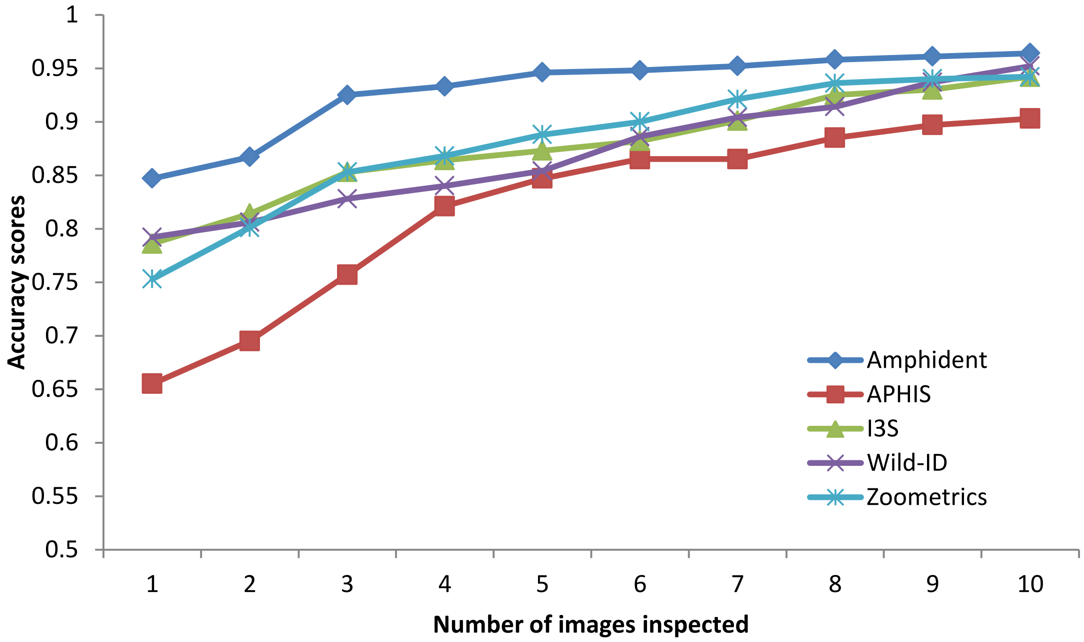


Figure 3.9 Effect of multiple images on visual matching success of computer-assisted image matching system. Accuracy scores clearly increase when more reference images are available

With only one reference image to test against, 65-85% of the images in the test database were correctly ranked as the correct choice (Top 10). Again, the greatest accuracy when multiple images were tested was by Amphident. Even if only one reference image was available for the comparison, it yielded an 84.7% chance of placing the image in the top 10. This recognition ability increased to an almost 93% chance of it being in the top 10 ranking using three reference images. APHIS software recorded the lowest accuracy scores (66%) when using only one reference image. However, it can be seen clearly from Figure 3.9 that these scores increased rapidly (84%) with the inclusion of four reference images of the same newt.

Pattern stability

Considering the reliability of reference images from the same newts captured in different years, the accuracy scores did not decrease as newts get older (Figure 3.10). A subsample of 50 newts recaptured over nine years showed the consistency of characteristics belly patterns that were recognized by the programs. It should be noted here that only the ‘year of capture’ data were available. Therefore, newts captured in the same calendar year are shown as having a 0 (zero) time interval. Overall, the variation in the accuracy scores over time did not vary between years for each of the software package. A two-way ANOVA analysis with the individual as a fixed factor and year as random factor was run on a sample of 50 newts to determine if there were differences in accuracy scores due to the time interval between captures. The results showed that the programmes performed well even when newts get older and the patterns potentially change (Figure 3.10).

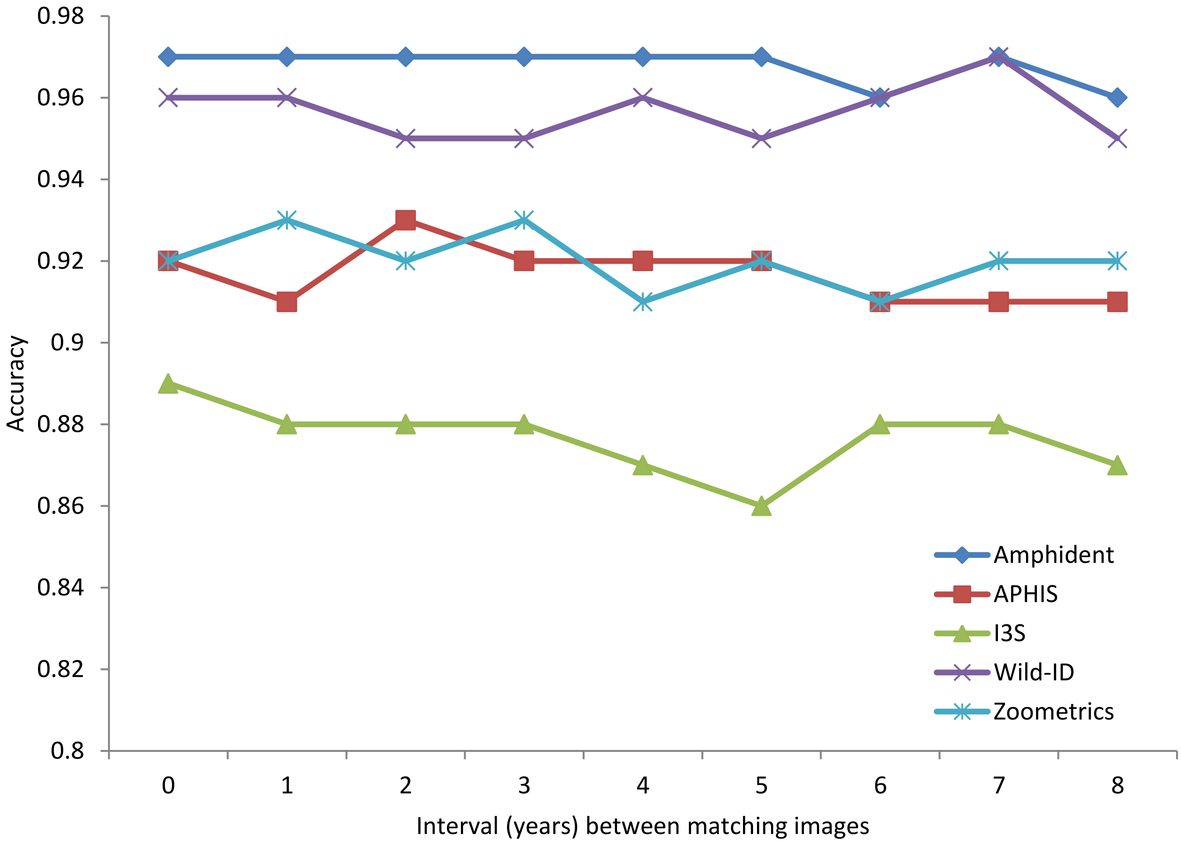


Figure 3.10 The accuracy scores in relation to time (years) between captures. The box shows a summary of results from two-way ANOVA comparing the accuracy scores of different packages. The degrees of freedom for each test was d.f.$_N$ = 8, d.f.$_D$ = 392

3.4 DISCUSSION

3.4.1 Manual Photo-identification

The manual photo-identification experiment allowed a comparison of performances between expert and non-expert groups while undertaking a photo identification test by using belly pattern images of the great crested newt. Generally, experts were more accurate identifying newt individuals. They were less influenced by pattern distinctiveness, and their performance was more consistent when identifying newts in two sequential tests. However, both experts and non-experts had difficulty in identifying individuals from poor quality images as the accuracy scores were the lowest in this category compared to the belly pattern distinctiveness and performance consistency categories. In spite of that, the ability to identify individuals from poor quality images appeared to not vary greatly between experts and non-experts. When accuracy scores from experts and non-experts were combined, low quality images were most inaccurately identified by the respondents (93%), followed by images with low pattern distinctiveness (97%) and consistency (98%). As the quality of the image decreases, the information in the natural markings of newts becomes obscured, and it becomes increasingly difficult to recognize the individual.

If mark-recapture studies do not take into account the potentially confounding effects of image quality, probability of recapture and error type/rate can be affected. Gowans and Whitehead (2001) found that the use of high-quality images for the identification of individual whales reduced the number of errors in photographic matching. In addition, digital photography has improved the image quality and increased the efficiency of analyses in the identification of several species of dolphins (Markowitz et al. 2003). In this study, high-quality images allowed respondents to better discriminate between spots on the newt’s belly, and enabled them to distinguish spots that were close together. The use of digital cameras increased the number of images that could be obtained in the field, thereby increasing the probability of obtaining good images. Digital images also increased the speed at which they could be loaded into a computer for analysis while preserving their quality.

The results of the pattern distinctiveness tests indicate that skill level was a predictor of performance in photo-identification methods. Pattern distinctiveness tests showed that experts more reliably distinguished similar newts than non-experts. On the other hand, the non-expert group had little difficulty differentiating two different newts that had similar belly patterns. This finding supports the notion that untrained volunteers are less accurate in their identifications (Alldredge et al. 2007). However, the current study suggests that if sufficiently trained, non-experts can perform as good as experts. Furthermore, the belly pattern technique is relatively easy to learn (Chase et al. 2015). Several studies have focused on factors that could affect non-experts' performance in identifying animals. Previous knowledge, prior experience, training, difficulty of task, commitment, physical fitness and familiarity with the environment, have all been found to have an effect on non-experts' ability to conduct identifications based on natural markings (Schmitt & Sullivan 1996; Foster-Smith & Evans 2003; Newman et al. 2004).

Before any identification system based on natural markings is developed, it is important to know whether the use of natural markings will be reliable (Pennycuick 1978). The success of the system relies on spot patterns that are variable enough to reliably identify individuals. The results from this test support the claim that newt belly patterns are unique and vary sufficiently to be used reliably to identify individuals. Indeed, 98.9% of matches by experts are correct when the patterns are similar. The results from the consistency test indicated that skill level again was a predictor in the respondents' performance. The expert group demonstrated similar accuracy scores in sequential tests where the same images of the same individual were presented twice. This finding indicates experts more consistently correctly identified newts compared to non-experts. Even though the performance of non-experts was inconsistent, they recorded slightly higher scores when answering the second sequential test. Non-experts improved their scores in the second set of questions (99%) compared to the first (94%). Non-experts therefore improve in their identifications as they gain experience in photo identification methods. Prior experience and knowledge of the subject do influence the ability of a person to identify great crested newts.

3.4.2 Computer-assisted Photo-identification

Computer-assisted photo-recognition produced high matching success particularly using good quality images. Although the accuracy scores for each program are generally good in all packages, AMPHIDENT recorded the highest scores. This study clearly demonstrates that the AMPHIDENT program is able to reliably identify individuals with a high accuracy for the high quality images and outperformed other programmes, although it was less reliable than other programmes with low quality images. AMPHIDENT has been developed specifically to identify individual great crested newts and fire-bellied toads (genus *Bombina*). This program applies the basis of a cross-correlation comparison technique to identify individuals based on unique colouration (Drechsler et al. 2014). Drechsler et al. (2014) found that of the 206 total recaptures identified, AMPHIDENT was able to correctly identify 78.86% and the false rejection rate (FRR) was only 2% and no false acceptances (FAR) were observed. They suggested that the high accuracy of AMPHIDENT program may be due to unusual areas of a shape pattern that makes the individual unique and are rarely form in other individuals which later can be marked as a distinctive character for identification.

In comparison, using Wild-ID Bendik et al. (2013) indicate a FRR of 0.76% for a large dataset of Jollyville Plateau salamanders (*Eurycea tonkwae*) using good quality images and 15.9% when using poor quality images, respectively. Until now, most of the computer-based individual pattern recognition approaches have been developed for a single or a group of target species and may have limited use for accurate identification of individuals of other species. However, WILD-ID is based on the scale-invariant feature transform (SIFT) algorithm, a method that extracts distinctive features independent of image scale and rotation (Lowe 2004): it has been developed by Bolger et al. (2012) as pattern matching software that has the potential for application to other species. AMPHIDENT presently has been introduced for shape pattern feature for several amphibian species. Drechsler et al. (2014) advise that the fundamental algorithm of AMPHIDENT offers an excellent recognition precision for individuals and performs at least as accurately as the WILD-ID software in large datasets.

The high matching accuracy attained with the WILD-ID software may be dissimilar for other forms of animal patterns. Morrison et al. (2011) applied the programme to patterns in wildebeest using search criteria that involved comparing the top 20 highest scoring candidate matches and achieved a False Rejection Rate (FRR) of 5.8%. However, Morrison et al. (2011) also demonstrate that these methods do not need to be free from error to sufficiently estimate population parameters. With ZOOMETRICS, this program is still being developed for the great crested newt and with the sophisticated classification scheme (e.g. sexes, stages and locations) should improve the error rates (Hoque et al. 2011). This technique could be applied to the standardized images taken of each newt. With optimization, its performance could be even further improved.

The accuracy of image matches decreases dramatically when using low quality images. The errors tend to occur when images contain external sources of variation (e.g. lighting, foreground objects, photographic perspective, mud or dust on fur etc.) that alter the visible pattern in the photograph so that either the computer algorithm or human observer cannot detect the true pattern (Morrison et al. 2011). The main source of low quality images in this study were from out of focus images, reflective camera flash, and debris cover. Bendik et al. (2013) using WILD-ID program observed error rates of 15.9% by using low quality images compared to only 0.76% when using high quality images. Hastings et al. (2008) using the same program noted that inclusion of low quality images reduced ranking success by 20% and increased additional visual matching error up to 20%. Thus, the advantage of computer-assisted pattern recognition diminishes when photo quality is poor, requiring visual inspection for more image pairs. Therefore, subjective grading of quality of images was required to control or account for misidentification of individuals. Sacchi et al. (2010) suggests that a strict standardization of the image collection process might further reduce the possibility of misclassifications. This has been done in other studies using image-matching of individuals (Friday et al. 2001; Kelly 2001).

This study indicates that pattern-matching accuracy improves if there are multiple reference images against which comparisons could be made. Morrison et al. (2011) recommended that researchers estimate the error rates prior to sampling so that they can select an appropriate sampling intensity. If the error rate is high and cannot be reduced by other measures, relatively high sampling intensity will be necessary to ensure that estimates based on available data have sufficient accuracy. Fortunately, photographic mark-recapture study for the great crested newt here is well suited for increasing sampling intensity and sample sizes because of the relative ease with which additional individuals can be photographed although generally recapture rates are low in most field studies. Photographing individuals twice or more in quick succession on the same day would be one possible way to do this as long as the two images were as challenging to match as ones taken on different days or years (Morrison et al. 2011). We recommend that the image repository should include at least three good quality images of each individual for reliable identification. In many historical studies of photo-identification, only the best quality images are retained as the reference material because of limited storage capacity for images (Van Tienhoven et al. 2007). The use of available pattern-recognition software may require a larger repository of images to be maintained, but a typical modern computer and large storage is now cost-effective to achieve.

One concern with using spot patterns as a mark-recapture technique is that an individual's spot pattern could change over time, rendering the spot pattern unreliable if there gaps of several years between recaptures (Chase et al. 2015). The stability in the belly pattern means that adults can be reliably identified for the rest of their lives when recaptured over several years using either manual or computer methods. Jonas et al. (2011) emphasize the importance of knowing whether or not body markings are variable before relying on these markings to identify individuals in a study. Qualitative observations suggest that belly patterns of the same great crested newt did not change much once they became an adult (> 2 years). Inspection of 450 captured newt images taken over nine years revealed that the spot patterns of adult great crested newts did not change much over time and are sufficiently varied to allow their individual identification using photo-recognition programs for the rest of their lives. However, a qualifier here is that is the case only for adults. Belly pattern may change between metamorphosis and adulthood, thus caution may be needed if sub-adults are included in any study. Some other species of pond breeding salamanders (e.g; *Ambystoma* spp.) can serve as model amphibians for long-term mark-recapture studies using pattern recognition because of their individually distinctive patterning, relatively low vagility, and predictable movement patterns associated with reproduction (Petranka 1998; Smlitsch 2008). Up until recently, and compared to other marking methods, pattern mapping was considered appropriate only for short studies of small populations (Arntzen et al. 2004). This study clearly shows that this is not the main concern anymore. Recent study by Mettouris et al. (2016) using Wild-ID program revealed that the spot patterns of individual alpine newts (*Ichthyosaura alpestris*) and smooth newts (*Lissotriton vulgaris*) did not change through time, and were sufficiently varied to allow their individual identification even in the larger datasets. Using ZOOMETRIC, Hoque et al (2011) observed that the variation of scores for the great crested newt over six years of study period was not significant. They suggested that a variable threshold may be used in the classification process to accommodate this phenomenon.

The errors in computer-assisted photo-identification in other species are more likely to occur due to pattern changes with age (Knox et al. 2013). Usually, spots grow in size, often nearby marks join. This change therefore makes the patterns look dissimilar even when these are from the same individual. Analysing *Eurycea* salamanders using Wild-ID, Bendik et al. (2013) suggest that photo-identification errors are mostly due to lost or evolving marks. Stebbins (1985) observed that tiger salamanders of all subspecies may darken with age. He therefore, suggests that age, not environment is a key factor in spot change. Reaser (1995) found that the spot pattern of adult California tiger salamanders (*Ambystoma californiense*) kept in captivity changed over time. Waye (2013) observed the spot patterns changing while the salamanders were in captivity and not in their natural habitat. However, the extent and especially the variety of changes in a relatively uniform and stable environment make it difficult to identify any particular factor directing those changes (Waye 2013). Therefore, the success and usefulness of photo-identification will largely be determined by how stable and easily distinguishable each animal's belly pattern is, and how many animals are involved. If there are strong similarities among individuals and high pattern complexity, the risk of misidentification increases. This can result in lower accuracy in identification and as a consequence, faulty estimates of population parameters. Bendik et al. (2013) suggests that species whose natural marks change quickly over time, either seasonally or annually will require relatively short intervals between capture periods or capture rates within periods to maintain sufficiently low error rates.

When comparing between manual and computer-assisted photo identification, the current study shows that experts can achieve nearly 100% accuracy when identifying good quality images and they take just 1-2 minutes to identify a newt based on 136 images taken from study site. In contrast, none of the computer packages get near this level of accuracy and it would take longer to identify one newt individual. However, if the number of reference images increases, manual identification will become impractical. According to Bolger et al. (2012), manual photo-identification has been applied mainly in the studies of relatively small animal populations and is less practicable for larger populations. Large images catalogues are bulky, offering the possibility of visual pattern-matching errors increase as the number of images increases (Gamble et al. 2008). Computer-assisted photo identification is vital for labour savings by minimises the total number of 'match' or 'no-match' judgements that have to be made by an observer (Bolger et al. 2012). In a catalogue of 2551 images of migratory wildebeest (*Connochaetes taurinus*), Morrison et al. (2011) estimated that computer-assisted photo- identification generate to a one third time savings comparative to a completely manual photo-identification. In a capture-mark-recapture study, labour savings by using computer-assisted photo-identification methods allows a larger number of images to be handled with a given level of work, which can lead into higher recapture rates (Bolger et al. 2012). An increase in recapture rates increases the power of population estimates and led to a bigger number of parameters to be assessed, such as animal movement or transition probabilities in multistate models (Williams et al. 2002). One potential constraint of computer-assisted photo-identification methods is that they might create misidentification errors that may bias mark-recapture population estimates (Yoshizaki et al. 2009). False rejection rates, FRR (failing to match two images of the same individual) can be high and may generate positive bias in population estimates and negative bias in survival estimates (Bolger et al. 2012). To overcome this problem, Hastings et al. (2008) suggest that misidentification error must be estimated when assessing the efficiency of computer- assisted photo-identification package.

In conclusion, no method of individual identification can be guaranteed to be completely error-free. The overall performance of each method and the impacts of different kinds and rates of error on inferences will depend on the organism, field conditions, dataset sizes, and study questions. Computer-assisted pattern-recognition methods appear to be less effective for low quality images. However, moderate quality images, for example if slightly out of focus or blurred because of movement could still be used as long as the arrangement of the spots, and their relative position to one another could be discerned. This study also proves that the misidentification errors are not likely to be happened in photo-identification of the great crested newt because the marking pattern is stable over time. Vincent et al. (2001) suggest that if marking patterns are adequately diversify between individuals, stable over time, and observers obtain adequate training, the frequency of false acceptance should be relatively rare. The important thing is the analytical approach need focuses on minimizing the effects of misidentification errors.

CHAPTER 4

METAPOPULATION DYNAMICS OF THE GREAT CRESTED NEWT IN AN AGRICULTURAL LANDSCAPE

ABSTRACT

Understanding the relationship between climate-driven habitat conditions and survival is key to preserving amphibian in the challenge of rapid climate change. This study describes how climatic factors influenced the dynamics of the great crested newt (*Triturus cristatus*) metapopulation over 19 years through interactions with adult survival. A capture-mark-recapture (CMR) study over nineteen years (1995 – 2013) was conducted within an agricultural site of Well Court farm, Canterbury. Model selection based on Akaike's Information Criterion indicated that apparent annual survival varied with year but did not differ between ponds or sex, and detection probabilities fluctuated widely between years and ponds. Estimates of metapopulation size varied considerably between pond and year. Further analysis of CMR with climatic covariates found that low annual survival of adult newts was related to mild, wet winters which impacted the metapopulations at the regional level. Therefore, survival varied between years but not between subpopulations. Only 1.4% inter-pond movements that were recorded indicate that metapopulation persistence is determined by larval recruitment that acts as a local factor in the ponds. This level of recruitment, adult survival and dispersal may not be enough to maintain metapopulation persistence by the rescue effect from source ponds in the future. Therefore management should aim to ensure aquatic habitat and terrestrial habitat are maintained to limit any extinction of any populations.

4.1 INTRODUCTION

Amphibians are currently experiencing global declines, the causes of which include habitat destruction and fragmentation, emerging diseases, the introduction of alien species and climate change (Blaustein & Kiesecker 2002; Beebee & Griffiths 2005; Cushman 2006). The most recent records from the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species™ estimated that nearly 40% of amphibian species are vulnerable to extinction, with the number of Threatened amphibian species are nearly the same as the total of Threatened species of birds and mammals combine together. Furthermore, although amphibians have survived multiple previous global mass extinctions, in the last 20-40 years rapid population declines have taken place on a scale not previously seen (Bishop et al. 2012).

Amphibians together with other taxa are likely to be enormously exposed to future climatic changes (Lemckert & Penman 2012). Amphibians are particularly susceptible to such environmental changes because of their ectothermic physiology and dependence on moisture (Wells 2007). As ectotherms, amphibians and other taxa in general are highly influenced by the physical environment, a number of these declines could be directly or indirectly linked to climate change (Blaustein et al. 2010). Climate, especially rainfall and temperature, directly affects many amphibians and other ectothermic animal through physiological limitations associated with species tolerances and the seasonal timing of activities such as growth and reproduction (Todd et al. 2010). Several studies have noticed earlier breeding in many amphibian species in regions that have experienced recent climate warming (Beebee 1995, 2002; Gibbs & Breisch 2001).

The global declines of many amphibian populations stress the need for long-term information on their demography and population dynamics (Blaustein et al. 1994). Issues relating to population assessment are therefore a key concern for those trying to conserve these groups, with detection and survival rates amongst the key factors. Amphibians are useful as a model system for studying the impacts of climate change because amphibian reproduction is strongly tied to water availability, water quality, and precipitation patterns. These three main factors are predicted to be extremely affected by climate change. Climatic factors may lead to a serious impact on amphibian populations and communities at various spatial and ecological scales (Griffiths et al. 2010). At the local landscape level, shifts in pond hydroperiods because of the climate influence can modify amphibian aquatic habitat (breeding site) heterogeneity, a requirement for persistence of diverse communities (Werner et al. 2009).

Many pond-breeding amphibian populations that exist as a metapopulation may rely upon the degree to which their habitat is fragmented (Marsh & Trenham 2001). The term ‘metapopulation’ was introduced by Levins (1969) to interpret a model of population dynamics of insect pests in agricultural areas, but the concept has been widely used to species that live in in artificially or naturally fragmented habitats. The Levins concept and model has become the classical metapopulation. More recently, Hanski and Gilpin (1991) interpreted a metapopulation as a “set of local populations which interact via individuals moving among populations”. In addition, Hanski and Simberloff (1997) defined a metapopulation “as a set of local populations within some larger area, where typically migration from one local population to at least some other patches is possible”.

Metapopulation ideas have frequently been applied to the conservation of amphibians because many species breed in discrete, patchily distributed water bodies (Griffiths et al. 2010). Amphibians are potentially attractive candidates for metapopulation studies, as they have highly patchy distribution patterns based on seasonal breeding aggregations at temporary ponds (Griffiths & Williams 2001). In this study, the term 'metapopulation' refers to a subdivided population that has a spatial structure and linked by dispersal. As the persistence of a metapopulation is ultimately determined by birth rates and death rates, the impact of climate on survival within different sub-populations is fundamental to understanding the dynamics and conservation of the system (Griffiths et al. 2010). These studies are valuable for conservation biology because they can provide an empirical basis for discovering the effect of habitat fragmentation and connectivity on local and regional population persistence (Marsh & Trenham 2001). They also provide a framework for predicting the consequences of future habitat loss and fragmentation on populations of interest (Marsh & Trenham 2001).

In this study, capture-mark-recapture data collected over 19 years (1995-2013) for a metapopulation of the great crested newts (*Triturus cristatus*) is examined to explore the impact of variation in survival on the metapopulation dynamics of the great crested newt. The study site was within an agricultural landscape near Canterbury, Kent, United Kingdom. In particular, the annual survival rates between subpopulations, between sexes and between years were investigated and were related to climatic factors. Additional information on the survival of great crested newts will be highly informative in highlighting possible effects on population dynamics.

4.2 STUDY SITE AND METHODS

4.2.1 Study Site

Well Court Farm, Blean

Refer to Chapter 2

4.2.2 Survey Methods

The chosen method for this study was trapping during the aquatic breeding season. Field surveys have been carried out annually from 1995 until 2013. My own data collection was conducted in 2013 only (permission for access was withdrawn after 2013), but analyses include the full data set. Traps were constructed from 1 litre plastic drink bottles, with the tapered end removed and inverted to create a funnel effect, through which a cane was passed and secured in the bottom of the pond (Griffiths 1985). Where ponds had synthetic liners (Garden Ponds), traps were secured to nearby vegetation or stones by means of string. The traps were spaced at approximately 2 metre intervals along the shoreline. Traps were numbered and their positions marked on a plan to allow for continuity of data between years.

A total of 108 traps were set each week, 26 at the Garden ponds and 16 at the Swimming pool and 33 each at the Snake and Pylon ponds (Figure 4.1). In most years two of the Garden Ponds dried up in early summer, reducing the number of traps set to 12. In some years the Pylon pond also dried up and if the pond desiccated, the number of traps was reduced accordingly, with a spacing of 1-2 m between traps had being maintained as far as possible.

Figure 4.1 Garden Ponds trapping plan with the number shows the trap position

Trapping started on the last Thursday in the month of February, and continued at weekly intervals until the end of the aquatic period (i.e. when no further newts were trapped), this usually occurred at the end of July. Traps were set between 2000 and 2200 hrs and were then checked and emptied between 0730 and 0900 hrs the next morning depending on the season. Any great crested newts captured were given a code that included details of the pond in which the capture occurred, the week of capture and the sex of the individual. The belly pattern of each great crested newt captured was photographed with the code visible.

4.2.3 Climatic Covariates

Refer to Chapter 2

4.2.4 Data Analysis

Refer to Chapter 2

4.3 RESULTS

4.3.1 Capture Rates

Over the 19 years of the study to the end of the 2013 season, 1,991 individuals were recorded from 3,522 captures. On average each individual was caught 1.77 times over the 19 breeding seasons. There was, however, high variation from year to year as well as between individuals. The actual number of newts captured in each year is shown in Figure 4.2 below in terms of the total number of captures and recaptures in each year for the whole metapopulation. Captures only are shown for 1995, the first year of the study, and then the data are split each subsequent year between new captures and recaptures of previously encountered individuals. The ratio of new individuals to recaptured individuals shows variation from year to year.

In most years the majority of the population was in the Garden Ponds, and it is here that the majority of successful breeding attempts occurred during the study period, as evidenced by the number of well-grown larvae and metamorphs found in traps at the end of each breeding season. Figure 4.3 demonstrates number of adult captures in each year for the four ponds (Well Court) and in terms of the proportion found in the main breeding site, Garden ponds. Larvae were only captured in the Snake Pond and Pylon Pond in years 2 and 12 respectively, but no larvae were ever captured in the Swimming Pool. If it occurred at all, recruitment from the Pylon, Snake and Swimming Pool sited was therefore likely to have been intermittent.

Declines in captures at the Garden ponds were followed by declines in the number of crested newts caught at all sites in subsequent years, with this pattern being particularly apparent in the major decline over the period 2000-2003. The more recent years of 2004-2013 showed counts fluctuating steadily but still lower than those experienced at the start of the survey period. Count data therefore suggest a population that is fluctuating from year to year.

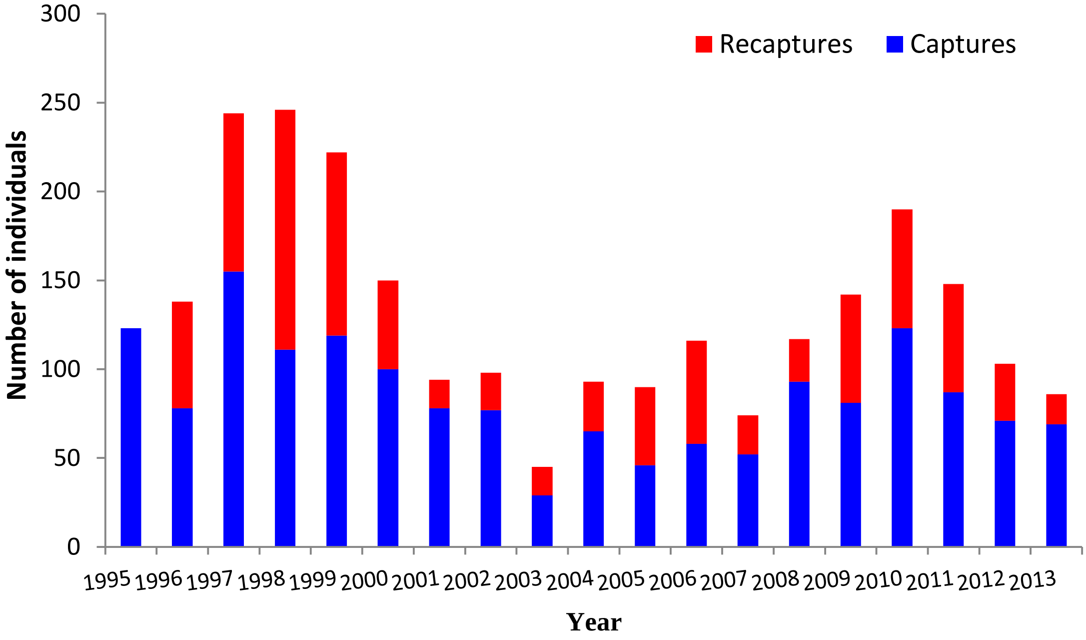


Figure 4.2 Total capture and recapture of individual adults by year

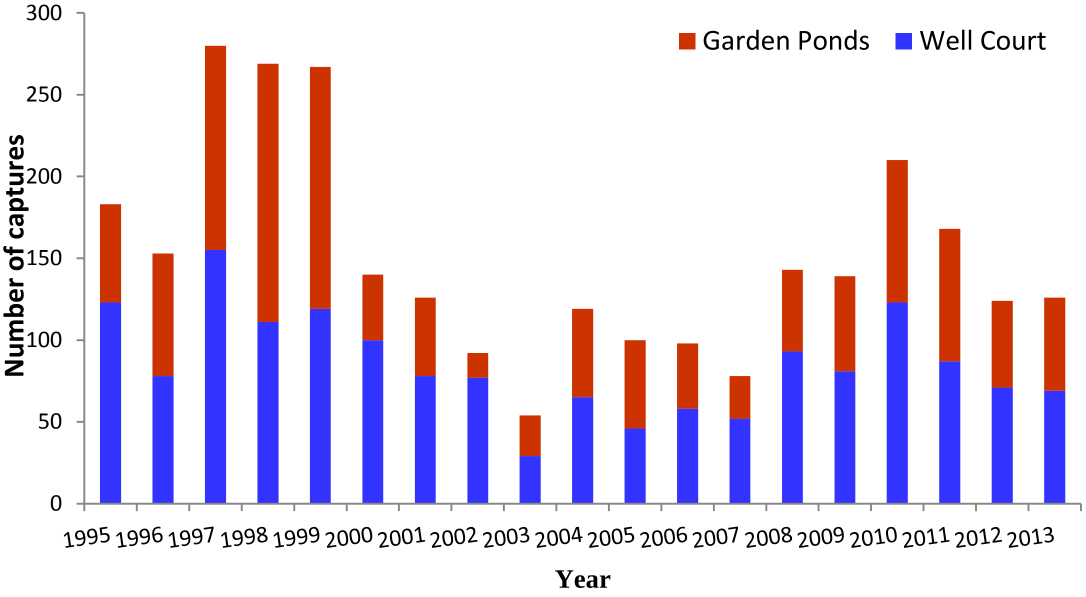


Figure 4.3 Total adult captures comparing Garden Ponds with total metapopulation

4.3.2 Movement between Ponds

Out of 3,522 captures by bottle trap over the period 1995-2013, only 30 captures involving 28 individuals were found to represent movement between ponds. This represented about 1.4% of the adults detected moving between ponds. There was a clear drop in the numbers of movements with increasing distance between ponds, with a total of 17 movements over 210 metres, dropping to nine over 250 metres, three at 310 metres, and one over 370 metres (Figure 4.4). Given that the next nearest colony is over 700 metres from the Well Court ponds, it would therefore appear that the Well Court metapopulation is isolated, at least as far as movements of adults are concerned. The analysis of detection and survival rates using the Cormack-Jolly-Seber method is therefore valid, as assumption on emigration has been met.

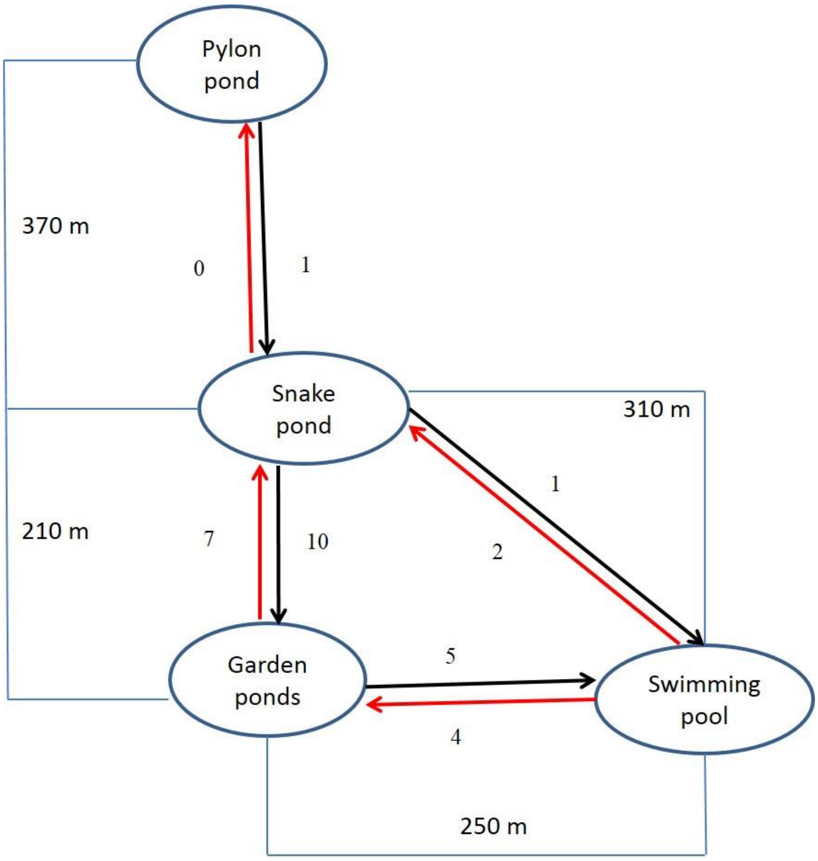


Figure 4.4 Distance and number of adult migrations between ponds within the Well Court metapopulation

4.3.3 Model without Climatic Covariates Used in the CJS Method in Program MARK

Data using the various group sizes were run and the most parsimonious model in each group noted, along with its deviance and *c-hat* (Table 4.1). Means were then taken for the 1000 bootstrap iterations run at each group size and *c-hat* adjustment calculated by deriving model deviance by bootstrap mean deviance and model *c-hat* by bootstrap mean *c-hat*. Cooch and White (2001) note that there is no clear view on which of these is the better approach and recommend using the higher of the two figures, on the basis that it is better to take a conservative view and consider the data a poorer fit (i.e. further from 1) than a better fit. In the case of the Well Court data this means that the *c-hat* adjustment has been based on deviance in all cases. Anderson et al. (1994) state that with real data *c-hat* should always be expected to be > 1, but should not generally exceed = 4. As the observed *c-hat* derived here is <4, and MARK allows for the simple alteration of the figure, it is well within acceptable parameters for a non-theoretical study. The effect of adjusting *c-hat* to the new value is to widen the confidence intervals for both detection and survival, but this should be viewed as a correction of the over-dispersion and nothing more.

Table 4.1 Initial model deviance and *c-hat* by groups, plus adjustments. The adjustment used for each group is highlighted in bold

No. of groups	Model deviance	Model c-hat	Bootstrap mean deviance	Bootstrap mean c-hat	Deviance based adjustment	C-hat based adjustment
8	703.69	4.75	478.67	3.49	1.47	**1.36**
4	608.99	4.55	448.74	5.68	1.36	**0.80**
2	703.69	4.75	549.86	3.96	1.28	**1.12**
1	517.99	5.29	374.05	3.86	1.38	**1.37**

Likelihood ratio tests (LRT) were carried out between first and second ranking models for four, two and one group analyses, but were not possible for the eight-group analysis, as the two models are not nested together. LRT test for single-group (χ^2 = 59.5, d.f. = 16, $p < 0.0001$) suggests that the most parsimonious model fits the data radically better than even the second best, with the two-group (χ^2 = 16.80, d.f. = 18, p = 0.5370) and four-group dataset (χ^2 = 16.51, d.f. = 13, p = 0.2224) the differences between first and second models are less clear.

Models were also compared by QAICc (Table 4.2), and these show very strong support for the most parsimonious model in all four cases. In the case of the eight-group analyses, time-dependent model, *Phi(t) p(t)* has a QAICc weight of 1, with all other models having a weight of 0. The most parsimonious models in the other three datasets are also very strong, the second most parsimonious in the four-group analysis being the strongest amongst the second placed, but the comparison with the most parsimonious model of 1/0.006 suggests that the most parsimonious model fits the data 166.7 times better than the second. Models other than the most parsimonious in each group size will therefore only be used with extreme caution. Where models have a similar AIC (or QAIC) weighting it is possible to run model averaging comparisons within MARK to determine the model that is the best fit, but given the differences in QAICc weightings here model averaging would not impact on any of the groups.

For the most parsimonious model, a survival rate varies between years in all four cases. In summary, the survival rates from the single-group model are 0.565 ± 0.012, two-group (0.5654 ± 0.014), four-group (0.574 ± 0.014) and eight-group (0.598 ± 0.0005). Detectability varied between years in all cases except for the four-group case. In this case, all four pond systems have a different detectability each year. The second most parsimonious model in each case showed a different result. Survival rate and detectability is constant each year for the eight and single-group, respectively. Meanwhile, for the four-group case, survival rate and detectability varies each year. Survival rate varies between male and female newts and detectability varies between years for the two-group case.

4.3.4 Model with Climatic Covariates

Two extra models which allow for an additive group and temporal effect (*g*t*) were fitted and compared to the other basic model (Table 4.3). The time-dependant model without covariates, *Phi(t) p(g+t)* emerged as the best model followed by Spring rainfall and Non-aquatic rainfall covariate-dependent model [*Phi(SR*NAR) p(g+t)*] as the second best model. Although the basic model had a smaller ΔQAICc value, the increase in fit was not substantial enough to indicate that the basic model was more parsimonious. Hence, those two best models are both important in this study. As the remaining models had a ΔQAICc value larger than 10, there was insufficient evidence to consider these models for further analysis.

Survival varied between 0.2 and 0.77 over the years from time dependent model, *Phi(t)* and, between 0.38 and 0.75 from covariate-dependent model, *Phi(SR*NAR)*. However, survival did not vary between ponds for both models. These indicate that only regional effects rather than local effects on all the ponds are affecting survival. Both model *Phi(t)* and *Phi(SR*NAR)* in Figure 4.5 indicated that inter-year survival remained high in the period 1995- 1998, before declining to a low in 2000-2001, after which there was a recovery. The *Phi(SR*NAR)* model pointed out that Non-aquatic rainfall (NAR) and Spring rainfall (SR) nicely explained the temporal

Table 4.2 Comparison of the two most parsimonious models in each group size for the Well Court data; *t* = time, and *g* = ponds

No. of groups	**Most parsimonious**	**QAICc weight**	**Model likelihood**	**2nd most parsimonious**	**QAICc weight**	**Model likelihood**
8	*Phi(t) p(t)*	1.000	1.000	*Phi(.) p(t)*	0.000	0.000
4	*Phi(t) p(g)*	1.000	1.000	*Phi(t) p(t)*	0.006	0.006
2	*Phi(t) p(t)*	1.000	1.000	*Phi(g*t) p(t)*	0.000	0.000
1	*Phi(t) p(t)*	1.000	1.000	*Phi(.) p(t)*	0.000	0.000

Table 4.3 CJS model selection based upon ΔQAICc in program MARK; *Phi* = survival, *p* = detectability, *t* = time, and *g* = ponds. Climatic variable; *SR* = spring rainfall, and *NAR* = non-aquatic rainfall. NP = number of parameters, and QAICc = quasi Akaike information criteria from adjusted data

Model	**QAICc**	**ΔQAICc**	**QAICc Weights**	**Model Likelihood**	**NP**	**QDeviance**
Phi(t) p(g+t)	2646.02	0.000	0.661	1.000	38	560.38
*Phi(SR*NAR) p(g+t)*	2647.35	1.338	0.339	0.512	25	588.55
Phi(t) p(g)	2669.63	23.612	0.000	0.000	22	616.97
Phi(t) p(t)	2679.26	33.245	0.000	0.000	35	599.85
Phi(g) p(t)	2680.59	34.579	0.000	0.000	22	627.94

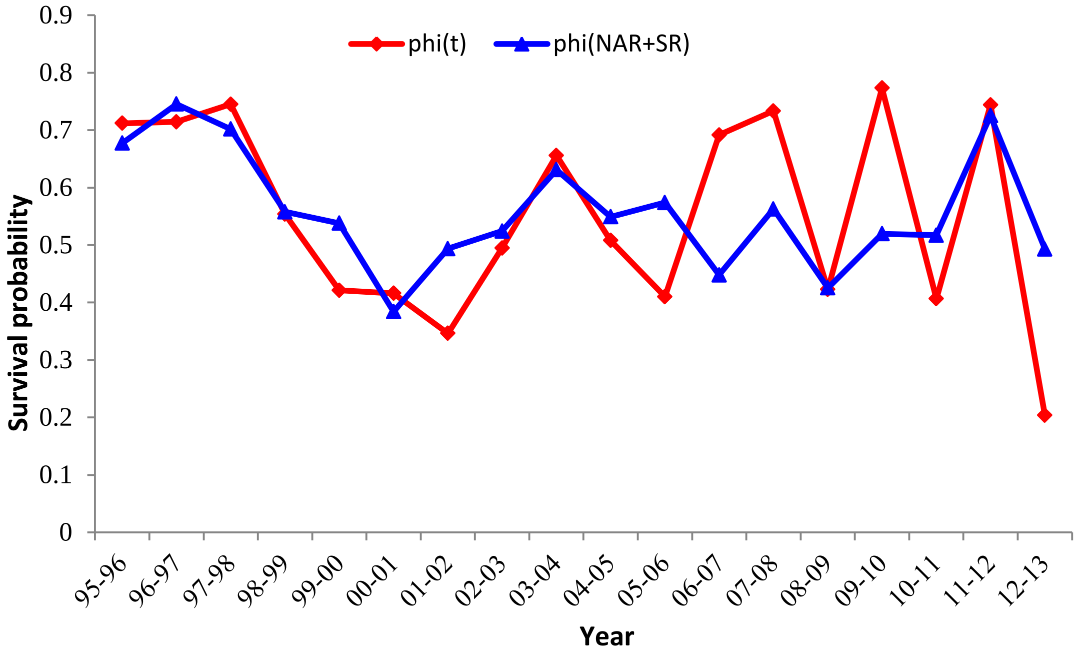
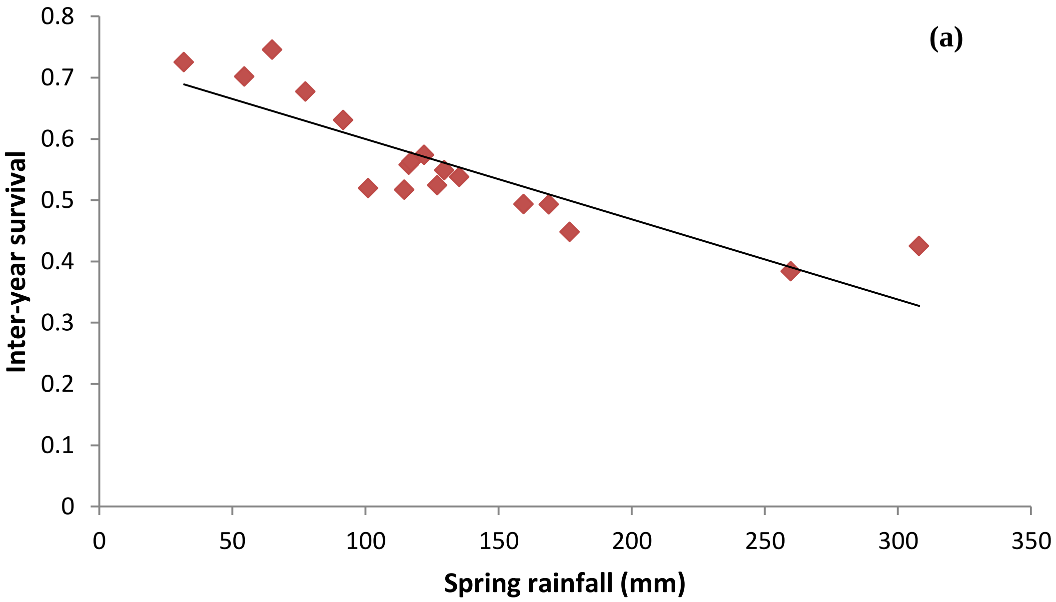


Figure 4.5 Time-dependent model parameter [*Phi(t)*], and covariate-dependent [*Phi(NAR+SR)*] parameter estimates

variation in the first years but then there was a drastic change where survival rate was very variable for a few years, before the two estimates start to coincide again starting from 2008 to 2013.

A multiple regression analysis of time-dependent model [*Phi(t)*] on SR and NAR confirmed a significant negative relationship between survival and total amount of seasonal rainfall ($R^2 = 0.82$, $F = 34.25$, $P < 0.001$, $y= 0.88 – 0.268x – 0.164x$). Partial regression coefficients indicated that both SR and NAR had a stronger relationship with the survival ($r^2 = - 0.76$, $t = 7.2$, $P < 0.001$) than NAR ($r^2 = 0.44$, $t = 3.6$, $P < 0.003$). The relationship between both SR and NAR with survival in Figure 4.6 suggests that for every increase of 100 mm of rainfall in the spring (March-May) and non-aquatic period (June-February) inter-year survival of adult great crested newts decreased by about 0.13 and 0.08.



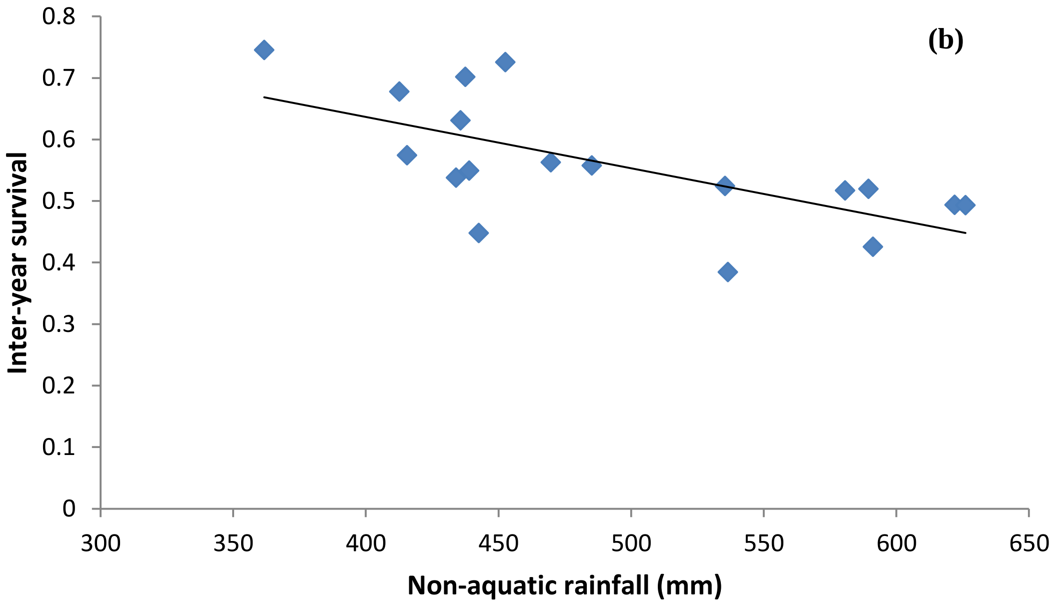


Figure 4.6 Relationship between spring period rainfall (SR) and inter-year survival (a), and non-aquatic period rainfall (NAR) and inter-year survival (b). Survival estimates are based on model *Phi(NAR+SR)*

4.3.5 Population Estimate

Detection rates at 0.66 (95% confidence interval 0.611 - 0.703) and 0.84 (0.737 – 0.911) were high in the Garden Ponds and Swimming pool respectively, but lower at 0.38 (0.313 – 0.441) in the Snake pond and also in the Pylon pond at 0.24 (0.115 – 0.423). The wide 95% confidence interval for the Pylon pond reflects the low and inconsistent capture rate at that site. There was a significant correlation between the inter-year survival rate in the Spring rainfall and Non-aquatic rainfall covariate-dependant model [*Phi(SR*NAR) p(g+t)*] and time-dependent model [*Phi(t) p(g+t)*] model (r = 0.63, d.f. = 18, P = 0.038). Thus, the population estimates for the Well Court metapopulation were prepared using the known number of captures by year multiplied by 1/detection rate for each pond from the time dependent model [*Phi(t) p(g+t)*]. Annual captures by pond are shown in Figure 4.7. The largest number of adult great crested newts was found in the Garden ponds (1826/4049), followed by Snake pond (1579/4049), Pylon pond (354/4049), and Swimming pool (290/4049).

Population sizes were generally high in the period 1997-2001, and then declined thereafter to almost half from the previous years until 2008. The contribution each pond made to the overall metapopulation size varied between years, as the detectability varied between ponds. There was a significant correlation between the population trends in the Garden Ponds and Swimming Pool ($r = 0.828$, d.f. = 18, $P < 0.001$) although the Garden Ponds – which was the smallest water body within the system - was the main source site of the metapopulation. Pairwise correlations between the trends in the other subpopulations were all non-significant. Fluctuations between different subpopulations therefore appear to be largely asynchronous.

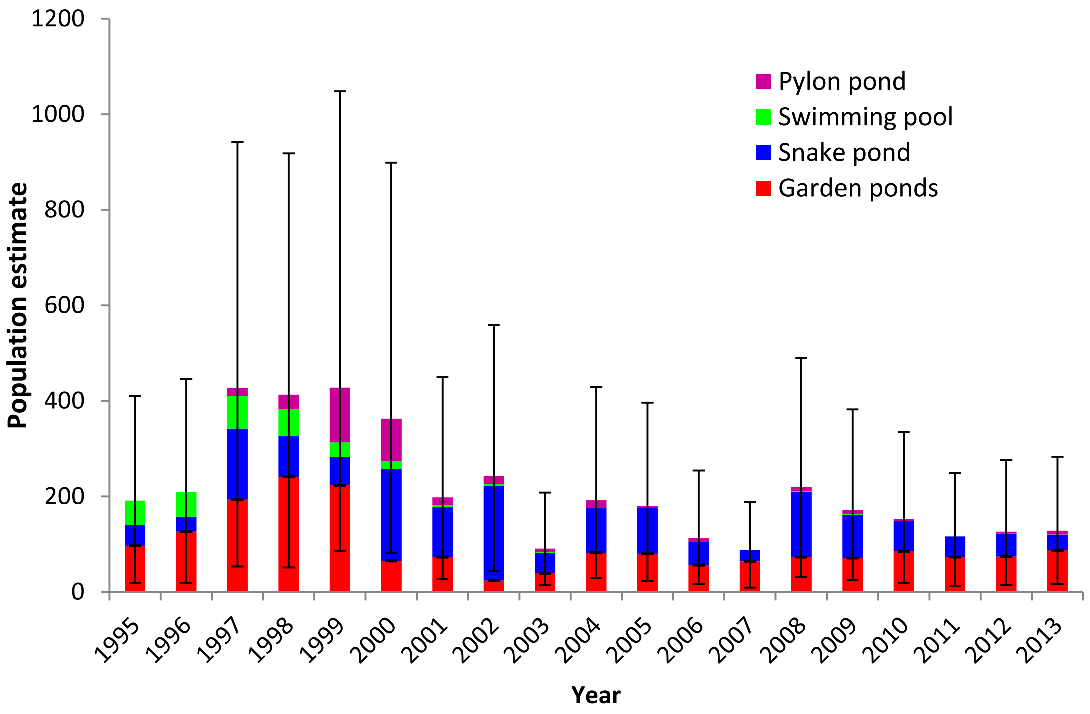


Figure 4.7 Population estimates for the total metapopulation by year, based on detection rate and individuals captured by pond using model *Phi(t) p(g+t)*. Vertical lines show 95% confidence intervals.

4.4 DISCUSSION

The study system comprised a subdivided population based on four breeding sites, and displayed some of the characteristics predicted by metapopulation theory. Regardless of how many groups were included in the dataset, with or without climatic covariates, the most parsimonious model in all cases shows survival rates to change over time rather than be affected by variation between ponds or between sexes. This is also the case in the second ranked covariate-dependent model, *Phi(SR*NAR) p(g+t)* which suggests that survival rates are closely linked to non-aquatic, NAR (January- February and June-December in each calendar year) and spring season rainfall, SR (Mac-May) rather than to conditions specific to an individual pond or sex group. This study indicates that survival ranged between 0.2

and 0.77 over the years from the top ranking time-dependent model, *Phi(t)* and, between 0.38 and 0.75 from second covariate-dependent model, *Phi(SR*NAR)*. However, survival is constant between ponds for both models. Previous population studies on amphibians reveal considerable variation in annual survival (Cooke & Arnold 2003, Gill 1978). Hagstrom (1979) estimated relatively high annual survival (78%) in a great crested newt population. In three separate studies, Baker (1999) found annual survival ranged from 31 to 100%, Arntzen & Teunis (1993) observed survival of between 33 and 57% and Griffiths et al. (2010) estimated values at between 25 and 80%.

Griffiths et al. (2010) also used the Well Court data set, albeit with fewer years (1995 – 2006), and found that variation in survival between years was inversely related to winter temperatures and winter rainfall. They discovered that the low annual survival of great crested newt was related to mild winters and heavy rainfall. This study has gone further by using the whole 19 years dataset (1995-2013) and found that only rainfall mainly affected newt survival in the additional years from 2006 – 2013. The importance of rainfall both prior to and after each aquatic season on survival rates the following year is intriguing. Like many other temperate amphibians, great crested newts usually hibernate on land, and gaseous exchange occurs by pumping air into the lungs as well as across the skin surface. In soil filled with water, newts may face respiratory challenges pumping air into the lungs. This limitation may lead to deficiency of oxygen reaching tissues, resulting in severe hypoxia (Shoemaker et al. 1992). This may be reflected in reduced survival.

Although the ponds differed in size and biological characteristics, the survival rate was found to be the same in all ponds, although varying over time. This may have implications for the management of populations as it suggests a relationship between rainfall with inter-year survival. A note of caution about this relationship must, however, be sounded. Other factors may cause poor survival, or apparent survival rates at individual ponds. An example is provided by the results for the 2006 survey in Figure 6. Here the evidence for the climatic variables suggests that survival from the previous year should have been high, whilst capture evidence showed that it declined. One possible explanation for the difference was that conditions in the largest of the Garden ponds were particularly poor in the 2006. During the summer of 2005 the Garden ponds were left unmanaged and contain submerged and overhanging vegetation, and this was not cleared until the following year (Griffiths et al. 2010). Poor aquatic habitat may therefore have affected survival in that year. Although management was carried out it was not completed until after the trapping season had ended. Captures were therefore very low during that year, but this is more likely to represent poor conditions in the trapping environment leading to low detection rather than a low survival rate. Use of climatic data to anticipate likely survival of amphibians between years is therefore more appropriate at the landscape level than for individual ponds.

The evidence presented here suggests that amphibians show short time delays in their response to climatic variation. In this study, it has been shown that rainfall is an important determinant of inter-year amphibian survival rate and short-term climatic fluctuations may be useful for monitoring the inter-year survival of amphibians. Projections for climate warming in the UK (Hulme 2002) suggest that by the 2080s annual temperatures may rise between 2.0 and 3.5 ^{0}C, whilst summer precipitation may decline by up to 50%. Conversely, heavy winter precipitation may increase with the overall precipitation levels remaining reasonably constant. In the recent report of IPCC (2013), climate change is projected to increase low-percentile winter temperatures than the mean, thereby decreasing temperature variability. Projecting data for individual years and locations into long-term climatic predictions is difficult, and the data presented here have represented year-to-year fluctuations rather than lasting trends. It does appear, however, that a succession of warm, wet winters could have major impacts on the survival of isolated metapopulations.

Population estimates have been based on detection rates and the actual number of individual crested newts caught in each year. The differences in overall population confidence intervals from year to year reflect the varying contribution of each pond within the metapopulation to the overall estimate for each year. The pond with the widest confidence interval in detection is the Pylon pond. This pond was not surveyed for the first two years of the study, and the absence of any data for these years and the consequent absence of any influence in the population estimate for those years have helped keep the confidence intervals for 1995 and 1996 narrow. In contrast, during those years when the capture rate in this pond was high, such as 1999 and 2000, the influence of the pond on the overall capture rate is very high and in extending the limits of the overall confidence intervals even higher. The Snake pond also has a low detection rate, but here the confidence intervals are narrower. Although there is fluctuation in catches from year to year, it is not as low as the Pylon pond capture rates. The Swimming pool represents a site that has a different pattern of captures to the Pylon pond. Detection rates were high at this site which was investigated from the very first year of the survey. That it made no contribution to the overall population estimate after 2002 is a function of the steady decline of the population from a peak in 1995 to extinction by 2003. This pond would therefore have contributed to narrow confidence intervals at the start of the study and would have influenced their widening as it became less important in the overall population.

The study of the four populations at Well Court was an investigation of metapopulation dynamics at a small spatial scale. The populations were assumed to form an isolated metapopulation during the 19 years for which they were studies. The dynamics of the Well Court populations were not influenced by dispersal from population outside of the Well Court study area and the dispersal was infrequent among the Well Court ponds (ca. 2.3% of individuals changing patch per season) which were separated by 200-800 m. Given the apparently low rate of adult dispersal between ponds in Well Court area, this observation supports the assumption that the dynamics of the Well Court populations were not influenced by dispersal from populations outside of the Well Court study area. The metapopulation described here shows some source/sink characteristics (Pulliam1988), with only two known breeding (= source) sites amongst the four sites monitored.

Pylon pond and Swimming pool may be termed sink ponds as adults have been caught in most or all years but there has been no evidence of successful breeding except in year 12 in Pylon pond. Newt larvae were never captured here, indicating repeated reproductive failure. A possible cause for unsuccessful juvenile recruitment at Pylon pond was the reduced water level in the summer months, and the consequent failure of newt larvae to complete metamorphosis. The pond drying process is not uncommon, leading to a loss of juvenile production in many amphibian species that breed in ponds (Kupfer & Kneitz 2000). At the Swimming pool, possible causes of recruitment failure may have been associated with high predation by numerous diving beetles (*Dytiscus marginalis*) and dragonfly larvae (*Aeshna cyanea*) as it never dries completely. Newt larvae are for the most part, solitary animals that live in aquatic vegetation to avoid predators but are nevertheless devoured by a wide range of aquatic invertebrates including dytiscid water beetles, backswimmer bugs (e.g. *Notonecta* species) and odonate larvae, as well as vertebrate such as fish, water birds, adult amphibian and amphibious reptiles (Beebee 1996). Even the larvae of crested newts which have powerful skin toxins as adults, are highly vulnerable to fish predation (Beebee 1996).

If those two ponds may be termed sinks, the Garden ponds are sources, for here regular breeding and recruitment into the metapopulation occurred. The Garden ponds were subject to immigration and emigration of both adults and juveniles. The findings contradict Oldham et al. (2000), who considered smaller ponds to be unsuitable for supporting viable breeding populations of great crested newts. However, Oldham et al. (2000) did note that intermittent drying of ponds at a rate of once per decade may have an overall beneficial effect, preventing the colonisation/ persistence of aquatic predators that rely on permanent water bodies more than newts. The Snake pond probably fluctuates between source and sink. In most years these ponds also represented the majority of the metapopulation in terms of captures and individuals. If reducing survival rates leads to smaller populations in all ponds, it appears likely that populations in the sink ponds will decline as surviving adults find themselves increasingly able to find space in the breeding pond. This may already be in progress, with populations at the Pylon pond and Swimming Pool declining to extinction with no evidence of recolonization. This study therefore suggests that increasingly poor survival rates as non-aquatic and spring season rainfall increases will affect metapopulations by reducing the number of ponds in use, leaving breeding ponds increasingly isolated and at high risk of extinction. There is already some evidence of this process. The population estimate shows high estimates for the years 1997, 1998, 1999 and 2000 and was low in other years. Evidence presented of inter-pond migrations shows that the majority of observed movements of adults (19 out of a total of 30) occurred in these same four years. Therefore, as the overall metapopulation decreases, so does gene flow between ponds.

The failure to move to other ponds may have been a function of the distance between ponds in relation to landscape structure. The ponds were located within an agricultural landscape at distances of not less than 250 m apart and the Garden ponds, which held the largest local population of the great crested newt was potentially isolated by a human settlement and frequently-used trail. Mullner (2001) discovered that the great crested newt only emigrated up to 50 m from the breeding ponds. Jehle and Arntzen (2000) pointed out low migration distances with an average of less than 20 m for radio-tracked newts. Nevertheless, Kupfer (1998) indicated an ability of the great crested newt to move up to several hundred meters to a breeding site. It must be concluded that the realised movement activity of newts from the pond depends on the habitat qualities of the surrounding environment (Mullner 2001). If local refuges and food are abundant in habitat adjacent to the pond, the newts would use them (Madison & Farrand 1998). The variation of known migration distances probably indicates an adaptive flexibility in response to local habitat conditions. However, this conclusion must be treated carefully as the main dispersal occurs in juveniles. Once adults return to a pond, they remain fairly faithful to that pond. Adults often show high breeding site fidelity and individuals frequently return to their natal site (Griffiths 1996). This is possibly due to their constraining demands for complex landscape structures with high connectivity (Marsh & Trenham 2001).

Populations are truly sub-divided when their dynamics are due mainly to within-patch recruitment rather than immigration (Harrison & Taylor 1997). This was true for all populations in the Well Court metapopulation. Productive populations (Garden ponds) were thought to function in the rescue of unproductive populations (Snake pond). Dispersal among populations, mainly juveniles was sufficiently frequent to enable the colonization of a new pond and was expected to lead to the ‘rescue’ of a declining population. Fluctuations in the size of each population were explained by fluctuations in within population rates of adult survival and recruitment, not by fluctuations in the dynamics of the other populations. The persistence of all populations therefore appeared to be governed mainly by within- population processes.

CHAPTER 5

COLONISATION AND POPULATION DYNAMICS OF THE GREAT CRESTED NEWT IN NEWLY CREATED PONDS

ABSTRACT

The declining amphibian problem can serve as a model for understanding the global biodiversity crisis in general. However, before this can happen more research is needed into amphibian population dynamics particularly their ability to colonise and re-colonise areas. This study investigates eight small ponds on the University of Kent campus that have contained a population of limited size since colonization in 2000. Capture-mark-recapture (CMR) data collected over a 16 year period was combined to explore the impact of variation in survival on the population dynamics of the great crested newt (*Triturus cristatus*). A total of 126 individuals were recorded from 430 captures in the breeding seasons 2000 to 2016. Model selection based on quasi-likelihood Akaike Information Criterion (QAICc) with climatic covariates indicated that apparent annual survival was high and did not vary between years despite mild, wet winter conditions. Splitting captures by gender suggested that males had a higher detection rate than females. Estimates of population size ranged from 13 to 57 individuals between years. Management practised through draining and refilling the ponds did have an apparent effect on the number of newts captured over subsequent years. Population increase could be due to the decrease in predatory invertebrates following pond desiccation and a subsequent increase in recruitment levels. Ultimately, conservation actions need to embrace management to reduce survival and enhance recruitment.

5.1 INTRODUCTION

Globally, 32% of amphibian species are threatened with extinction, more than in any other vertebrate class (Hof et al. 2011). The mechanisms that underlie amphibian declines are very complex and many causal factors are involved. Recent changes in global climate might impact adversely on amphibian populations (Beebee & Griffiths 2005). Global mean temperature rose by about 0.6°C over the past 100 years, which has had huge impacts on amphibians (Carey 2003). Various amphibian species at different spatial scales are apparently being affected by climate change and climate extremes (Shoo et al. 2011). The breeding phenology and distributions of various amphibian species are being shifted across the world (Blaustein et al. 2010). Parmesan (2007) confirmed that compared to any other functional/taxonomic group, amphibians have significantly started earlier their breeding activity, similar with changes in butterflies, birds, and trees. Mild winters have been linked with reduced fecundity in females and earlier breeding due to a lower body condition (Benard 2015). Although amphibian populations experience significant natural fluctuations, the impacts of unprecedented climate extremes and climate change are far less well understood (Scheele et al. 2012).

Climate change - including extremes in rainfall - is forecasted to be one of the most significant drivers of ecological change in the forthcoming century (Lawler et al. 2009). Although some researchers have related declines in amphibian populations to changes in rainfall (e.g. Pounds et al. 1999; Pounds 2001; McMenamin et al. 2008), the potential effects of variable patterns of seasonal rainfall have largely been overlooked in studies with temperate amphibians. Instead, investigations with amphibians have almost entirely focussed on the effects of increased temperature.

There is a need to test the hypothesis that seasonal rainfall events may become increasingly more influential on amphibians, especially in relation to the timing of reproduction. Examining how species react to shifts in climate may contribute an important early step in understanding and forecasting the effects of long-term climate change (Debinski et al. 2006).

Amphibians are effective as a model framework for investigating the impacts of a changing climate because their reproduction is rely closely to water availability, water quality, and patterns of rainfall, all of which are anticipated to be affected by climate change (NAST 2000). Hydroperiod - the period during which a waterbody holds water (Ryan & Winne 2001) - is an important pond feature that directly influences survivorship of pond-breeding amphibians (Pechmann et al. 1989). Short-term variations in rainfall can affect the hydroperiod of a temporary pond in an erratic way, and this may influence the survival of amphibians in a stochastic way (Griffiths et al. 2010). Moreover, recruitment seems to be extremely sensitive to rainfall as reproduction in amphibians is highly influenced by hydroperiod (Blaustein et al. 2010). Hydroperiods and pond features will probably be altered by climate change as seasonal hydrology shifts (Brooks 2009). The climate change model by Heisler-White et al. (2008) predict more variable patterns of rainfall, with extended droughts and higher (but less) rainfall occurrences, tallying with increased temperatures. Such disparity in the temporal distribution, rather than the total amount of rainfall per se, may be associated with population fluctuations in amphibians (Stewart 1995).

The great crested newt was chosen as a model species in this study because they may be sensitive to climate change and depend on small waterbodies that are liable to desiccation (Blaustein et al. 2010). Examining the impacts of climate change on a sensitive species such as the great crested newt could consequently lead to a wider understanding of the conservation needs of other amphibian species that are highly vulnerable to climate change. This study combined capture-mark-recapture data collected over a 16 year period to explore the impact of variation in survival on the population dynamics of the great crested newt (*Triturus cristatus*). This study examines eight small ponds on the north western boundary of the University of Kent campus that have contained a population of limited size since colonization in 2000. Unlike Well Court farm (Chapter 4), these ponds are located outside an agricultural area and are being well protected and managed with regular pond draining and relining. Thus, effects of disturbance and pond management on the dynamics of the newt populations can further be investigated and compared. Additionally, since these two study sites are located just three miles apart and feature same weather conditions every year, impacts of local and regional factors on newts population persistence can be analysed further. In particular, this study tests the hypotheses that: (1) annual survival varies between years; (2) annual survival is related to climatic factors and possibly climate change; and (3) males and females have an equal chance of survival and being detected on the trapping occasion and, (4) pond management enhance larval recruitment and survival of the great crested newt.

5.2 STUDY SITE AND METHODS

5.2.1 Study Site

Field Site

Refer to Chapter 2

5.2.2 Survey Methods

Field surveys have been carried out annually from 1999. My own data collections were conducted in 2013 - 2016 only, but analyses include the full data set. Newts were captured using plastic-funnel traps as described by Griffiths (1985). Traps consisted of 1L bottles of translucent plastic. These traps were made from a drinks bottle with the top cut off and inverted back into the bottles and traps were kept in place by a string attached to a stick on the edge of the pond (Figure 5.1).

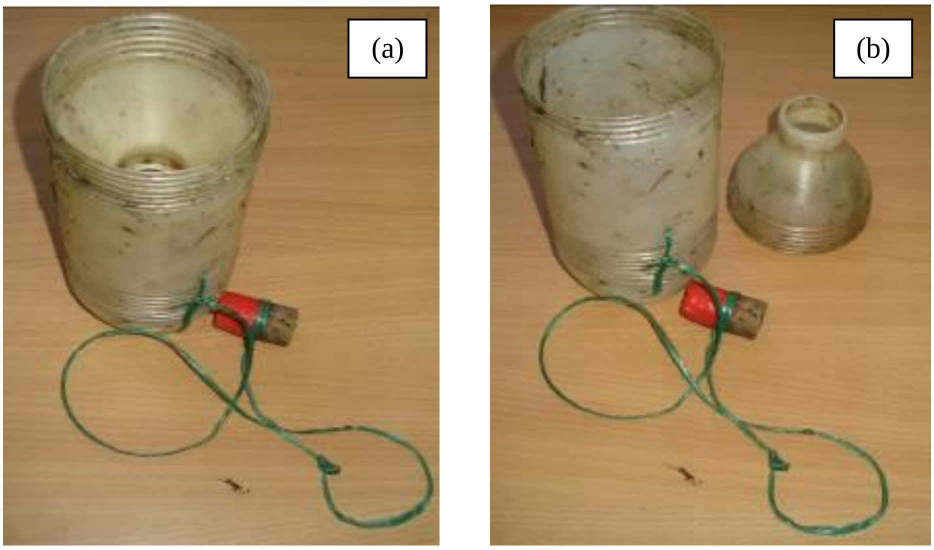


Figure 5.1 A bottle trap, (a) with and (b) without the funnel in position. Note the string used to attach the bottle to a stick on the bank of the pond to maintain the air bubble at the top of the bottle

Six traps were placed on the surface of the pond at c. 1 m intervals; one trap in each corner and two in the centre of the longer sides of the pond. An air bubble on the top of the traps is necessary to allow surfacing for air (Figure 5.2). In addition, until mid- or late April each year, two traps were also placed on the bottom at the deepest end of each pond (Trap 7 and 8) in all eight ponds. These were weighted using stones to ensure that they would sink to the bottom of the pond and remain situated there overnight. These traps were used only when the water temperature was low at the beginning of the season. After this the dissolved oxygen level in the water declined, especially at the bottom of the pond where decomposition decreases oxygen levels further and asphyxiation of newts in traps becomes a risk.

Figure 5.2 Bottle traps in position in one of the experimental ponds. Note the air bubble left in each bottle to allow surfacing for air, especially visible in the bottle on the top right corner. Two traps are also set on the bottom of the deep end of the pond until the third week of April

Trapping commenced on the last Thursday evening in February each year and continued at weekly intervals in each season. Trapping continued into July till August but usually trapping was stopped in mid-July because water levels were often dropping by then. A minimum of 21 trapping sessions were performed each year. Traps were set between 2000 and 2130 h and emptied between 0730 and 0830 h next morning depending on the season.

In December 2005 and 2014 all eight ponds were drained. The butyl liner in all four ponds was replaced and the ponds were refilled with tap water and allowed to re-colonise naturally. Any overwintering newt larvae were replaced in the ponds.

5.2.3 Newt Identification

Refer to Chapter 2

5.2.4 Weather Records

Refer to Chapter 2

5.2.5 Capture-mark-recapture Modelling

Because of the small size of the ponds, a weekly trapping programme captures most individuals in the ponds during most weeks of the breeding season, enabling detailed life histories to be built up. Refer to Chapter 2 for details of capture-mark-recapture analysis.

5.3 RESULTS

5.3.1 Adult Capture

Over the 16 years of the study to the end of the 2015 season, 430 captures of 126 individual newts had been made. A total of 64 and 62 individual males and females were captured respectively. The presence of larvae and metamorphs in the ponds in all years of the study indicated annual recruitment from this site. Details of the yearly males and females captured for the year 2000-2015 are shown in Figure 5.3. Great crested newts were detected in both the old ponds, created in 1998 and new ponds, created in 2009. The trends in the first eight years (2000-2007) were different from those in the second eight years (2008-2015). A rapid increase in the number of newts recorded was observed in 2008, two winters after draining and relining. The number of individuals captured rose again to 40 individual newts during 2010 surveys, two winters after four new ponds were created. This number remained constant in 2011 before declining to 29 in 2012. In 2013, the number of newts increased significantly to 52 before declining again to 45 in 2014 and rose again in 2015 after the ponds were drained and relined in the winter 2014.

Capture rates of males were consistently higher than those of females across the years. In some years, not all individuals known to be alive were caught. An independent t-test was used to compare mean of capture rates from 2000-2015 between male and female newts. This study found that males had significantly higher capture rates (102 ± 45) than females (78 ± 57), t = 1.253, d.f. = 30, p = 0.027.

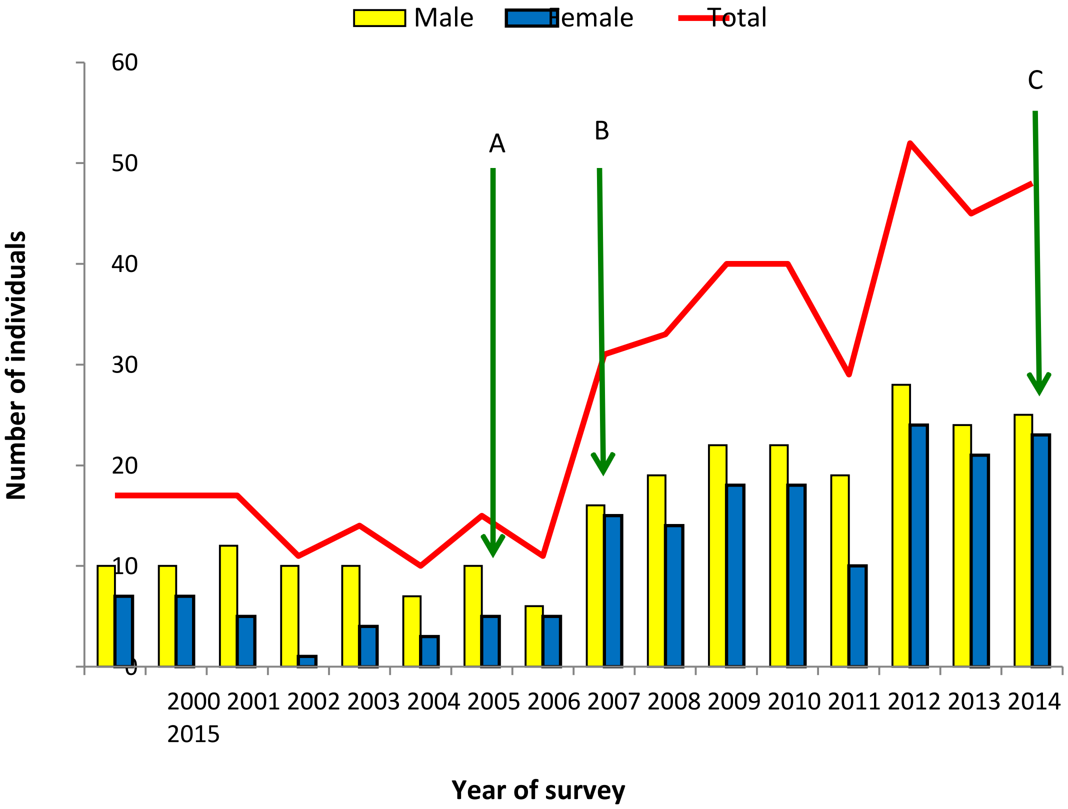


Figure 5.3 Total captures of individual adults by year and sex. A and C = pond drainage and relining; B = creation of 4 new ponds

5.3.1 Variation in Survival and Detectability between Sexes without Climatic Covariates

Models using males and females as a grouping factor (group = 2) were run and the most parsimonious model noted, along with its deviance and *c-hat* (Table 5.1). Mean deviance were then taken for the 1000 bootstrap iterations and *c-hat* adjustment calculated by dividing model deviance by bootstrap mean deviance and model *c-hat* by bootstrap mean *c-hat*. Cooch and White (2001) note that there is no clear view on which of these is the better approach and recommend using the higher of the two figures, on the basis that it is better to take a conservative view and consider the data a poorer fit (i.e. further from 1) than a better fit. In the case of the Field Site data, this means that the *c-hat* adjustment has been based on deviance in all cases. The results from 1000 bootstrap iterations showed that the data were over-dispersed and *c-hat* values were corrected to 1.03.

Table 5.1 Initial model deviance and *c-hat*, plus adjustments. The adjustment used is highlighted in bold

No of groups	Model deviance	Model c-hat	Bootstrap mean deviance	Bootstrap mean c-hat	Deviance based adjustment	C-hat based adjustment
2	258.5043	4.63	245.31	5.97	**1.03**	0.78

A summary of the most parsimonious models after *c-hat* adjustment is shown in Table 5.2, below. The model with constant survival but detection rates varying between males and females [*Phi(.) p(g)*] is the one best supported by the data, with an AICc weight of 0.574 that is only 1.545 times better than the next best supported model with an AICc weight of 0.371. However, the best model is 11.6 times better supported than the third best supported model. The fact that these models are the most parsimonious suggests that there is either relatively little variation in survival and detection rates between years at this site, or that there are currently too few years of data available for variation between years to be apparent.

Table 5.2 The most parsimonious models for *Triturus cristatus* at the Field Site. Model notation as described in the methods, *Phi* = survival, and *p* = detection rates. NP = number of parameters

Model	**AICc**	**ΔAICc**	**AICc Weight**	**Model Likelihood**	**NP**	**Deviance**
Phi(.) p(g)	588.22	0.000	0.573	1.00	3	297.99
Phi(g) p(g)	589.09	0.870	0.371	0.65	4	296.81
Phi(t) p(g)	593.07	4.840	0.051	0.09	17	273.21

Likelihood ratio tests were carried out between these three models and suggest that there is no clear difference between the first model - constant survival with detection varying between males and females [*Phi(.) p(g)*] and second model – both survival and detection varying between males and females [*Phi(g) p(g)*] (Chi^2 = 0.395, d.f. = 1, *p* = 0.530) (Table 5.3). It means that these two models do not fit the data radically better than each other. With the models *Phi(.) p(g)* and model *Phi(t) p(g)* - survival varies by time and detection rates vary between males and females; and models *Phi(g) p(g)* and *Phi(t) p(g)*, the differences between the first and second models are very clear and the first model fit the data radically better than the second model.

Table 5.3 Likelihood ratio tests for the three most parsimonious models with the ΔQAICc value below than 10.0

Reduced model	**General model**	χ^2	**d.f.**	***p***
Phi(.) p(g)	*Phi(g) p(g)*	0.395	1	0.5297
Phi(.) p(g)	*Phi(t) p(g)*	23.172	13	0.0397
Phi(g) p(g)	*Phi(t) p(g)*	22.777	12	0.0297

Output for the most parsimonious model of all, model *Phi(.) p(g)*, is shown below as Table 5.4. This model provides a fixed survival rate both over time and between the sexes, but separates out detection rates between male and female. This does not imply that survival rates are the same for both sexes, merely that there is insufficient data upon which to separate them.

Table 5.4 Survival and detection rates from model *Phi(.) p(g)*

Parameter	**Estimate**	**Standard error**	**95% confidence interval**
Survival (overall)	0.83	0.0195	0.7909-0.8675
Detection (male)	0.92	0.0226	0.8594-0.9503
Detection (female)	0.73	0.0443	0.6383-0.8109

In view of the close ranking of the models, model *Phi(g) p(g)* is also considered, although it must be noted that this is only considered to fit the data 0.87 times as well as the best fit model *Phi(.) p(g)*, above. Details for this model are shown below as Table 5.5. Both survival and detection rates are therefore higher for males than they are for females. In the case of detection, this is confirmed by both models used, in the case of survival it is confirmed by the second method only. Nevertheless, there is very little difference between the two top models in terms of parameter estimates.

Table 5.5 Survival and detection rates from model *Phi(g) p(g)*

Parameter	**Estimate**	**Standard error**	**95% confidence interval**
Survival (male)	0.85	0.0241	0.7964-0.8913
Survival (female)	0.81	0.0323	0.7354-0.8624
Detection (male)	0.91	0.0229	0.8570-0.9495
Detection (female)	0.74	0.0441	0.6468-0.8189

5.3.2 Variation in Survival and Detectability with the Climatic Covariates

A model that included climatic covariates as additive factors was fitted and compared to the other basic models (Table 5.6). The best model fit without covariates remained *Phi(.) p(sex)*, i.e. survival is constant between years, but detection rates varied between male and female. With the ΔQAICc below 2 as an evidence of substantial fit (Burnham & Anderson 2002), the first three models; *Phi(.) p(g)*, *Phi(g) p(g)* and, *Phi(NAR) p(t)* were regarded as important models in this study. Likelihood ratio tests were carried out between seven models that had ΔQAICc less than 10 and suggest that there are no clear differences between the first and six following models except between model *Phi(.) p(g)* and model *Phi(t) p(g)* (χ^2 = 24.775, d.f. = 14, p = 0.037) (Table 5.7). This means that the *Phi(.) p(g)* model fits the data radically better than the *Phi(t) p(g)* model. Although the model fit without covariates had a smaller QAICc value, the increase in fit was not substantial enough to indicate that the model fit without covariates was more parsimonious.

Survival between years was plotted from model *Phi(NAR) p(t)* output as in Figure 5.4. Inter-year survival was the highest in the period 2004-2005 and 2011-2012 with the value of 0.894 ± 0.03 and 0.896 ± 0.03, respectively. The lowest inter-year survival rate was recorded in 2013-2014 (0.76 ± 0.04). Exploratory correlations between annual survival and potential climatic predictors revealed a highly significant negative relationship with non-aquatic season rainfall (NAR) (Figure 5.5). From Figure 5.5, it can be seen clearly that the survival rate is decreasing if the NAR is increasing.

Table 5.6 CJS model selection based upon ΔQAICc in program MARK; *Phi* = survival and *p* = detection rates. Climatic variables; *NAR* = non-aquatic rainfall, *WT* = winter temperature, *SR* = spring rainfall, and *AF* = air frost. NP = number of parameters, and QAICc = quasi Akaike information criteria from data adjusted using *c-hat*

No	**Model**	**QAICc**	**ΔQAICc**	**AICc Weights**	**Model Likelihood**	**NP**	**QDeviance**
1	*Phi(.) p(g)*	588.22	0.00	0.3871	1.0000	3	297.99
2	*Phi(g) p(g)*	589.09	0.87	0.2505	0.6472	4	296.81
3	*Phi(NAR) p(t)*	589.83	1.61	0.1731	0.4471	4	297.55
4	*Phi(NAR + WT) p(t)*	590.96	2.74	0.0983	0.2540	6	294.57
5	*Phi(t) p(g)*	593.06	4.80	0.0344	0.0888	17	273.21
6	*Phi(NAR + SR) p(t)*	593.29	5.07	0.0307	0.0792	6	296.90
7	*Phi(NAR + WT + AF) p(t)*	594.40	6.18	0.0177	0.0456	8	293.84

Table 5.7 Likelihood ratio tests for the three most parsimonious models with the ΔQAICc value below than 10.0

No	**Reduced Model**	**General Model**	χ^2	**d.f.**	**Prob.**
1	*Phi(.) p(g)*	*Phi(g) p(g)*	1.172	1	0.279
2	*Phi(.) p(g)*	*Phi(NAR) p(t)*	0.433	1	0.511
3	*Phi(.) p(g)*	*Phi(NAR + WT) p(t)*	3.419	3	0.331
4	*Phi(.) p(g)*	*Phi(t) p(g)*	24.775	14	**0.037**
5	*Phi(.) p(g)*	*Phi(SR + NAR) p(t)*	1.090	3	0.780
6	*Phi(.) p(g)*	*Phi(NAR + WT + AF) p(t)*	4.147	5	0.528
7	*Phi(g) p(g)*	*Phi(NAR) p(t)*	-0.740	0	**0.000**
8	*Phi(g) p(g)*	*Phi(NAR + WT) p(t)*	2.247	2	0.325
9	*Phi(g) p(g)*	*Phi(t) p(g)*	23.602	13	**0.035**
10	*Phi(g) p(g)*	*Phi(SR + NAR) p(t)*	-0.083	2	**0.000**
11	*Phi(g) p(g)*	*Phi(NAR + WT + AF) p(t)*	2.975	4	0.562
12	*Phi(NAR) p(t)*	*Phi(NAR + WT + AF) p(t)*	2.987	2	0.225
13	*Phi(NAR) p(t)*	*Phi(t) p(g)*	24.342	13	**0.028**
14	*Phi(NAR) p(t)*	*Phi(SR + NAR) p(t)*	0.657	2	0.720
15	*Phi(NAR) p(t)*	*Phi(NAR + WT) p(t)*	3.714	4	0.446
16	*Phi(NAR + WT) p(t)*	*Phi(t) p(g)*	21.355	11	**0.030**
17	*Phi(NAR + WT) p(t)*	*Phi(SR + NAR) p(t)*	-2.330	0	**0.000**
18	*Phi(NAR + WT) p(t)*	*Phi(NAR + WT + AF) p(t)*	0.728	2	0.695
19	*Phi(SR + NAR) p(t)*	*Phi(t) p(g)*	23.685	11	**0.014**
20	*Phi(NAR + WT + AF) p(t)*	*Phi(t) p(g)*	20.628	9	**0.014**
21	*Phi(SR + NAR) p(t)*	*Phi(NAR + WT + AF) p(t)*	3.058	2	0.217

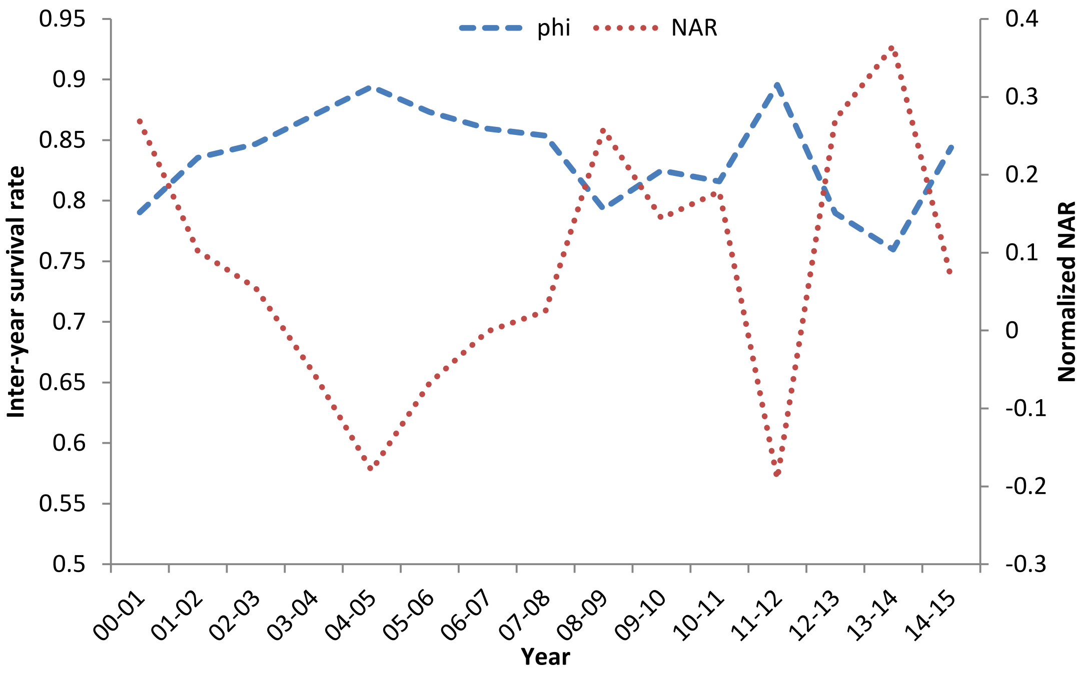


Figure 5.4 Inter-year survival rate showing variation over time and variation by climate where *phi* = survival, and NAR = normalized non-aquatic period rainfall

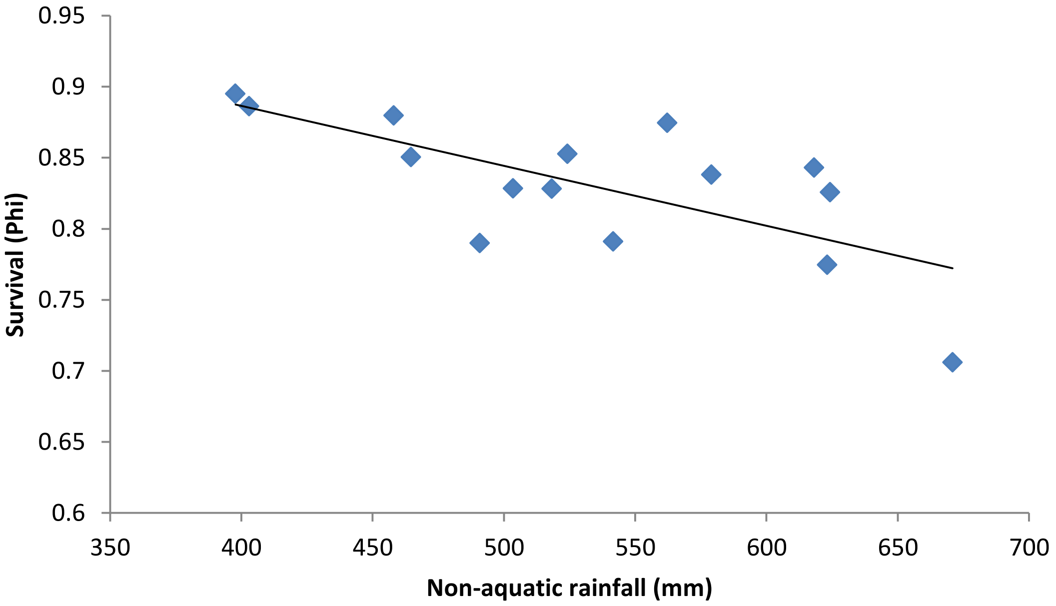


Figure 5.5 Relationship between non-aquatic period rainfall (NAR) and inter-year survival (r^2 = -0.484, $p < 0.004$, $N = 15$). Survival estimates are based on model *Phi(NAR)*

5.3.3 Detectability and Population Estimates

The analysis of the 16-year population trend at the Field Site revealed that overall detection rate was 0.84 ± 0.07 based on the third ranking model, *Phi(.) p(g)*. Initially, the detection rate was high in 2001, and then underwent a decline, before a gradual recovery after 2005 (Figure 5.6). The detection rate fell to its lowest point in 2012 (0.69 ± 0.06). Splitting captures by gender suggested that males (0.915 ± 0.02) had higher detection rate than females (0.833 ± 0.04). Population estimates were prepared using the known number of captures by year multiplied by 1/detection rate for year. Population sizes were generally low in the period 2001-2007, and then grew thereafter (Figure 5.7).

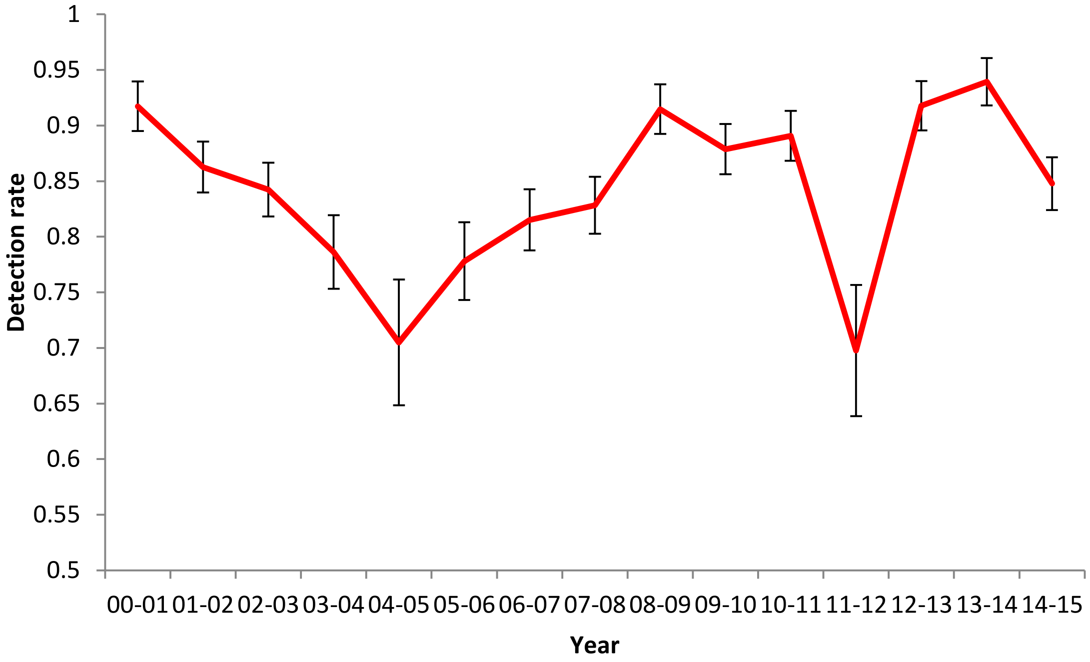


Figure 5.6 Detection rate by year for the Field Site ponds. Error bars denoted the standard error

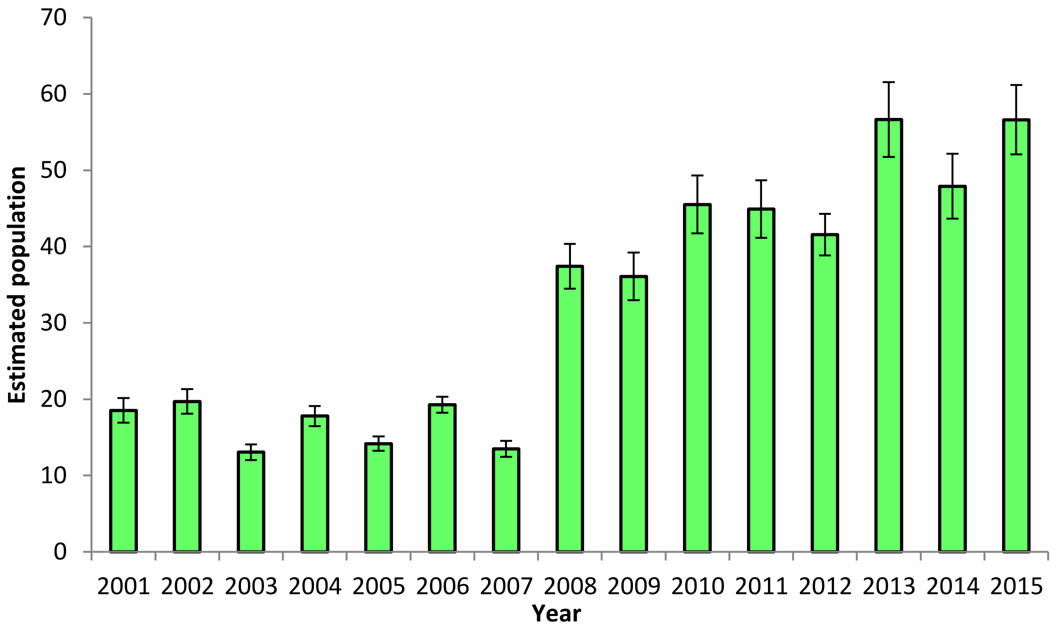


Figure 5.7 Population estimates for the total population by year, based on detection rate and individuals captured by year using model *Phi(NAR).* Vertical lines show 95% confidence intervals

5.4 DISCUSSION

It has been shown in this study that there was evidence of variation in detectability between the sexes. Males demonstrated a higher detection rates than females. This finding met Assumptions 1 and 2 of the Cormack Jolly-Seber model that animals have equal chances of both surviving to the next sampling occasion and, if they do, of then being detected. Analysis of the 16-year population trends reported that the adult survival was high with the males having higher survival rates than females. One of the best fitting models suggested that survival was negatively affected by the non-aquatic season rainfall.

The most likely explanation for lower female detection rates is that females may skip a breeding season and spend multiple years in the terrestrial habitat between breeding events. There have been several reported examples of females of closely related species skipping a breeding season. Jehle et al. (2005) recorded the behaviour in the Danube crested newt (*Triturus dobrogicus*), whilst Arntzen (2002) recorded it in marbled newts (*Triturus mamoratus*). Church et al. (2007) found that in tiger salamanders (*Ambystoma tigrinum*), <30% of females and <50% of males breed in a given year. This may account for the detection rates between years, and in particular the lower return rate in females who need to produce eggs and are thereby subjected to higher energetic costs. Even in species that breed every year, salamanders may skip a breeding season when environmental conditions are not favourable (Kinkead & Otis 2007). The majority of skipped season records in this study occur in 2003 (Sewell 2006), and it is possible that in this year breeding conditions were unsuitable for this species or conditions prior to the breeding season were unsuitable and females were unable to mature egg clutches.

One of the other possible explanations for higher detection rates in males is they have a higher probability of being captured in traps as they arrive early and spent a longer time in ponds during the breeding season, likely enhancing their chances for reproductive success (Jehle et al. 2011). In contrast, females in most years were first caught in weeks 4-5 (mid to late March). Last capture dates were over a similar short period in most years. Kupfer and Kneitz (2000) showed that males may visit the pond area on mild nights from the beginning of January and some males do not leave the breeding ponds until the autumn (Kupfer & Kneitz 2000). That males spent longer in the breeding ponds than females is unsurprising, and entirely consistent with other studies such as Hedlund & Robertson (1989), who attributed this behaviour to the lekking mate selection system of the species. This male-biased sex ratio operated through the breeding season. Males maximise their breeding chances by remaining in the pond until there are no more females available, whereas once a female has laid her eggs there is no breeding advantage to her in staying.

Population estimates have been based on detection rates and the actual number of individual crested newts caught in each year. The pond management that occurred in 2006 obviously had positive effects on the newt population (Wright 2009). There was a trend for numbers to increase after pond management. In 2008 and 2016, the numbers of new great crested newts captured increased and these new individuals were assumed to be first time breeders on the basis of their relatively small size. Great crested newts return to water to breed at 2-3 years old and the influx of new great crested juveniles in 2008 and 2016 was thought to be due to the high recruitment levels of 2005 and 2014 when early life stages benefited from a reduction in predatory species present in the ponds. When the ponds were drained invertebrate predator species were removed and as a result, recruitment the following year increased for the species. Therefore the new great crested newts arriving in 2008 were probably from the larval cohort of 2006 having returned to the ponds two years later to breed. The influx of new great crested juveniles in 2008 was thought to be due to the high recruitment levels of 2006 when early life stages benefited from a reduction in predatory species present in the eight ponds.

The mark-recapture analysis of the 16-year population trends revealed that adult survival at the Field Site was high, 0.83 ± 0.02 with the males having higher survival rates than females. Cummins and Swan (2000) found a survival rate of 0.85 (not broken down by sex), a similar rate to that given here, but surveyed in a larger and deeper pond than those at the Field Site. Arntzen and Teunis (1993) foun significant difference in survival rates between sexes, whilst other studies using capture-mark-recapture have suggested annual rates of between 0.50 - 0.78 (Hagstrom 1979; Hedlund 1990; Griffiths et al. 2010). Variation in survival rates between males and females is clear, since this emerged from the second best model in the CJS analysis in Mark. From both analyses with and without climatic covariates, males always have a higher survival rates than females. The difference in detectability between males and females is also clearly evident from the raw data which shows a higher capture rate of males when the individuals were 64 against 62.

Grayson et al. (2011) suggest that the majority of males are more likely to remain near the pond compared to females, to exploit their competitive breeding advantage. While males tend to remain near the breeding ponds, females generally engage in searching for food or dispersing and resting in a considerable distance from the breeding pool. When gravid females are ready to return to the breeding ponds again, they likely have a reduction in their movement. The high resting metabolic rates and reduced movement performance of gravid female salamanders compared to males may have important implications for the reproductive ecology and life history of salamanders (Finkler et al. 2003). Gravid females usually display a reduction in movement (Shine 1980; Seigel et al. 1987). Such reductions in performance may be based on physical hindrance of movements during locomotion (Sinervo et al. 1991), or physiological changes associated with carrying eggs (Olsson et al. 2000).

This reduction in movement may form a substantial component of the overall cost of reproduction. Gravid females with reduced movement may be more susceptible to predation or parasitism (Miles et al. 2000), thus reducing chances of survival to the next reproductive event. Additionally, gravid females may be less capable of foraging (Cooper et al. 1990), thus reducing energetic intake that would contribute to survivorship and future fecundity. These factors may in turn constitute selective pressures for behavioural and morphological characteristics of females that directly or indirectly reduce the cost of reproduction, such as changes in body size or in the relative size of morphological structures that influence movement performance (Shine et al. 1998) and alterations in habitat selection, feeding, and defensive behaviours (Brodie 1989; Cooper et al. 1990; Charland & Gregory 1995).

The third best fitting model, *Phi(NAR)* suggested that survival was adversely affected by non-aquatic season rainfall (January-February + June-December), and there was a strong negative relationship between survival and rainfall. A study near the same site in 1995-2006 by Griffiths et al. (2010) also demonstrated a variation in survival between years that was inversely related to rainfall. However, their study also identified that the low survival is related to the mild winters. In temperate ectotherms, continuous mild winter may cause depleting energy reserves of hibernating animals, while them being able to feed, resulting in poor breeding conditions the following spring (Reading 2007). However, this study suggests that high winter rainfall had a greater effect than mild winter temperature. In winter, great crested newts enter a period of low activity which usually begins in late September to mid-October and most animals are dormant by the end of November. While hibernating on land, gaseous exchange occurs by pumping air into the lungs as well as across the skin surface. In waterlogged soils, only gaseous exchange across the skin may be possible and this may substantially reduce the ability to meet certain respiratory challenges. Amphibians can suffer mass mortalities from anoxia when hibernating under waterlogged soils for unusually long periods as explained earlier in Chapter 4 (Shoemaker et al. 1992). This may be reflected in reduced survival.

Temperature and rainfall are two important climatic factors that are crucial to amphibian physiology and behaviour because of their part in reproductive migrations and gametogenesis (Beebee 1995; Todd & Winne 2006). Consequently mild winters may also result in reduced survival across the metapopulation (Griffiths et al. 2010). The present analyses suggest that high winter rainfall had a greater effect than mild winter temperatures. Globally, according to the Intergovernmental Panel on Climate Change (IPCC), the upcoming risk of both droughts and floods will increase in a warmer climate (Kundzewiczet al. 2007). Model predictions for the 2090s imply that globally, the proportion of the land surface in extreme drought events is projected to increase by 10 to 30 fold (Burke et al. 2006). At the same time, the episodes of heavy rainfall will possibly increase over many parts of the world in the 21st century (Seneviratne et al. 2012). Many amphibians have evolved in hibernation in response to cold temperatures and as a consequence, enabling survival under adverse environmental conditions play an important part in their life-history strategy (Wells 2007). However, markedly high temperatures during hibernation may also cause negative effects due to the increase in metabolic rate (Gilloly et al. 2001). Consequently, this can result in poor adult body condition after hibernation (Reading 2007), declines in reproductive investment (Jorgensen 1986), and increase in mortality (Corns & Muths 2002). Furthermore, Parmesan (2007) proposes that climate warming also make the hibernation period shorter. Shortening in hibernation period then might influence reproduction as since hibernation has been proposed to be a required life stage for the maturation of germ cells in some amphibians species (Brenner 1969). Climate change impacts on ecological aspects also comprise trophic mismatches (Gao et al. 2015). Shifts in breeding phenology caused by climate warming may cause temporal mismatches alter trophic relationships within food webs and affect secondary productivity (Gouveia 2013). Temperature-induced changes in phenology have driven birds to mislead peaks of food abundance and breeding activity (Van Der Jeugd et al. 2009). This situation can directly influence their individual body condition and population dynamics.

Furthermore, it has been suggested that the shift in rainfall patterns (Duellman & Trueb 1985) can alter the breeding phenology of amphibian species that reproduce in ponds. If ponds fill later in the season, then the shorter hydroperiod could lead to an increase in competition, higher predation rate and, lower survival. Meanwhile organisms are concentrated in the remaining ponds and they are more vulnerable to disease transmission and outbreaks. Shifts in breeding phenology of amphibian could therefore result in complex impacts on populations, altering the structure of population, and then likely causing declines of susceptible populations (Donnelly & Crump 1998).

Additionally, climatic changes can influence species distributions, normally through species-specific physiological thresholds of temperature and rainfall tolerance (Dervo et al. 2016). Amphibian migration between breeding and hibernation ranges in spring and autumn is a demanding stage with decreased survival, and potentially changes in this migration activity as a result of climate change could thus have implications at a population level. Normally newts initiate their migration in spring at the end of frost periods with increasing temperatures and when stimulated by rainfall (Malmgren 2007). Dervo et al. (2016) noted interspecific variation in migration pattern between the newts *L. vulgaris* and *T. cristatus*, as functions of temperature and rainfall. Rainfall was important for the migration pattern of both species. For *L. vulgaris*, numbers of migrators increased at lower temperatures but were dependent on higher rainfall levels as compared to *T. cristatus*. This could indicate that *L. vulgaris* are dependent on more stable temperatures compared to *T. cristatus* in order to sustain high numbers of migrators.

The observed decline in amphibian populations may directly or indirectly be linked to climate change as rainfall and temperature are key climate components acting directly on processes important for the population dynamics for amphibians (Dervo et al 2016). Predicted changes in survival rate and population size in relation to global warming concur with other studies of amphibians (e.g., Reading 2007) as well as species from other taxa such as birds (e.g., Both & Viser 2005). Very few studies, however, focus on the combined effect of multiple climate components. Given the strong support for interactive effects of both rainfall and temperature on the survival of the amphibians here, this study urges that both components should be considered in future studies of climate effects.

CHAPTER 6

COMPARISON OF BODY CONDITION INDEX AND THE SURVIVORSHIP OF THE GREAT CRESTED NEWT IN TWO POPULATIONS

ABSTRACT

Body condition has been related to fitness, and consequently survival of amphibians. It also may reflect variation in climatic conditions experienced previously. A ‘scaled mass index’ was used to estimate the body condition index (BCI) of male great crested newts in two separate populations in Canterbury, Kent. BCI were measured between 2002 and 2015 for 204 individual from four ponds in Well Court and 63 individuals from the Field Site. Annual survival was estimated from Cormack-Jolly-Seber capture-mark-recapture (CMR) models with climatic data and BCI as covariates. Model fit was determined using the quasi-likelihood Akaike Information Criterion (QAICc). Both study sites showed variation in body condition over the years. The BCI of each study site for each year was then compared over the 11 year period when they were monitored simultaneously. Higher body condition in Field Site ponds may be linked to higher habitat quality compared to the Well Court ponds that exist in agricultural areas. There was no effect of climatic conditions on the body condition at either of the populations studied. However, body condition was related to survival at one of the populations. Further analysis indicates that body condition was lower in ponds with high densities of newts. Generally, this study suggests that body condition is related to a local factor – population density, and habitat quality rather than regional or climatic factors. Agricultural activity may adversely affect the body condition of amphibians and pose a threat to their conservation.

6.1 INTRODUCTION

Body condition is defined as the relative size of energy stores (Jakob et al. 1996) and is assumed to be influenced by health and fitness of animals. Body condition provides information on the health of an individual and can affect a number of survival related behaviours (Hoey & McCormick 2004). Body condition is also important because it may be related to population and habitat quality parameters (Bancila et al. 2010). The body condition index is proposed as a simple and non-invasive way to measure an animal’s fitness. Generally, the body condition index (BCI) is determined by the relationship between body mass and body size, and is indicative of an animal's health or well-being (Sivan et al. 2014). The old method of estimating body condition is based on the direct measurement amount of fat deposits, but this technique is destructive for the studied animals (Blackwell 2002). Other alternative methods suggested such as total body electrical conductivity operating for estimating lipid and lean body mass in mammals and small birds have not proved very reliable (Bancila et al. 2010).

Considering that many species are fully protected by law, and many amphibian populations are declining (Stuart et al. 2004), non-destructive techniques are required to estimate body condition. Body condition indices (BCI) based on allometric relationships between length and body mass has been introduced as an alternative to the direct measurement of destructive techniques. Most commonly used body condition indices are several proportions between a linear measure of body size and body mass indices (BSI), or the use of residuals from a linear regression of body mass against BSI (Green 2001). Amphibians are very responsive to environmental quality and its change because of their complex life cycle that involves aquatic and terrestrial life stages (Bancila et al. 2010). Evaluating the body condition of amphibian populations is one of the options to test their responses to environmental stress. Body condition indices may indicate past foraging success, fighting ability, and the capacity to cope with environmental pressures, which may impact on reproductive success (Jakob & Marshall 1996).

Body condition and the climatic factors affecting it have gained much research interest recently, especially in the context of changing climate and its impact on amphibian populations (Reading 2007). According to Tomasevic et al. (2007), body condition is a crucial parameter related to survival and reproductive success. In temperate zone amphibians, body reserves are depleted during hibernation and breeding, and replenished during the following active period (Reading & Clarke 1995). Thus, inter-annual differences in body condition may reflect variation in climatic conditions experienced during the previous year, during hibernation, or immediately after emerging from hibernation (Tomasevic et al. 2007). Reading and Clarke (1995) found that change in body condition of female (*Bufo bufo*) was significantly correlated with temperature variation over an 11-year period. In a still longer-term study (23 years), Reading (2007) demonstrated clear relationships between increase in mean temperature, body condition decline, and decrease in fecundity. This may be because individuals use up more body fat reserves at higher temperatures due to increased metabolism (Reading 2007). This may result in increased susceptibility to disease, lower reproductive success, and subsequent population decline (Jarvis 2015).

Although several great crested newt population studies have been conducted (e.g. Baker 1999, Williams 1999, Jarvis 2010), few have examined factors affecting body condition indices and how this may impact population size (but see Jarvis 2015). Jarvis (2015) found that BCI varied significantly between years but there was no significant relationship between BCI scores and mean winter minimum air temperature. In the present study, the ‘scaled mass index’ (Peig & Green 2009, 2010) was used to estimate the body condition in two populations of the great crested newt. This study was based on a set of body length and body mass data collected from four sub-populations at Well Court (see Chapter 4) and a population at the Field Site (see Chapter 3).

The main aim of the present study is to examine the inter-annual post-hibernation body condition in male great crested newts from two study sites, and investigate the relationships between body condition and climatic conditions. In particular, this study tests the hypotheses that: (1) body condition varies between years and populations; (2) body condition is related to climatic factors; and (3) annual survival is linked to body condition.

6.2 STUDY SITE AND METHODS

6.2.1 Study Site

Two pond localities were the subjects of intensive investigation, and these are described separately within this chapter. The first of these groups was situated on Well Court Farm, and consisted of four pond systems (Garden ponds, Snake pond, Swimming pool and Pylon pond) where population studies were carried out over the period 2002-2013. The second group, known as the Field Site was situated on the north-west of the University of Kent Canterbury campus, and consisted of eight small ponds, supporting a small great crested newt population, where detailed individual morphometric and capture histories have been available since 2003. Details of the Well Court and Field Site study sites were described in Chapter 2, Chapter 4 and Chapter 5.

6.2.2 Materials and Methods

Annually, since 1995 for Well Court, and from 2003 for the Field Site, great crested newts arriving to breed at the study ponds have been captured and marked to denote the month and year of capture using a capture-mark-recapture method as described in Chapters 3 and 4. Captured male and female great crested newts were measured for snout-vent-length (SVL) and body mass (Ms). Body mass was recorded to the nearest 0.1 g and snout-vent length (SVL) was measured to 1 mm precision. Juveniles were excluded from this analysis because of uncertainty in tracking individuals through different age categories (see Chapters 3 and 4). A compact digital camera was used to take photographs of belly pattern markings to identify individuals. Once all measurements and data had been collected, all newts were released into the pond of capture.

As the species breeds over an extended period, and female body mass varies with the numbers of eggs laid, it was decided that an analysis of body condition index (BCI) would be using only males (Verrell & Halliday 1985). To avoid pseudo-replication (Luiselli et al. 2007), an average of SVL and mass of each individual during the peak seasons (March-May) every year was included once in the BCI calculation. SVL and mass for each individual were log transformed to normalise the data before BCI calculation were conducted. Different body condition indices (BCI) are available (Kuhn 1994; Jakob et al. 1996; Green 2001; Reyer & Battig 2004). However, there is debate as to which method is most suitable (Smith 2009).

One popular BCI is the residual from an ordinary least squares (OLS) regression of body mass (Y variable) against body size (X variable) (Jakob et al. 1996). However, Schulte-Hostedde et al. (2005) demonstrated that OLS residuals of mass on body size normally showed higher correlations with the absolute size of fat stores (in g) than residuals from an alternative regression technique (standardized major axis [SMA] regression). OLS residuals were also highly correlated with absolute values of lean dry mass (largely protein), and both results have been interpreted as BCIs (Schulte-Hostedde et al. 2005). However, Peig and Green (2009) question this reasoning because the absolute mass of fat or any other body component is not the most appropriate measure of 'true body condition' since it does not control for body size (a vital requirement for a BCI).

The SMA regression between body mass 'M' and a length measure 'L' is proposed because according to Warton et al. (2006):

(i) natural variability due to growth affects both size variables (M and L) within the structural relationship (i.e. both are indicators of body size);

(ii) M and L variables are not measured on comparable scales, thus the magnitude of natural variability and measurement error may vary between variables (a reason for preferring SMA to MA);

(iii) individuals have been randomly sampled, and the observed pairs of M-L data may deviate from the underlying true association between them;

(iv) the measurement error in L is unlikely to be negligible and may even be higher than that in M (Green 2001); and

(v) the aim of the regression is not to predict the value M of an unsampled individual given its L value, but to estimate the scaling exponent between two interdependent variables (Fairbairn 1997).

As an alternative, Peig and Green (2009) introduced the scaled mass index (SMI) that accounts for the allometric relationship between mass and a body structure measure and standardizes each measure so that direct comparisons among studies can be made. This method has been shown to be a better predictor of variations in fat and protein reserves than the ordinary least squares (OLS) residual method as well as five other methods (Peig & Green 2009, 2010). According to Peig and Green (2010), large and small individuals distribute resources differently. The proportion of stored energy therefore changes depending on the size of the animal so scaling is required to make meaningful comparisons. Hence, the scaling relationship between different size measures and components must be taken into account in order to produce a reliable BCI. The SMI standardizes body mass to a specific fixed value of a linear body measurement based on the scaling relationship between body mass and length. This allows for direct comparisons of body mass without the confounding effects of body size according to the equation (Peig & Green 2009):

Scaled mass index: $\acute{M}i = M_i \left[L_0 / L i \right]^{bSMA}$

Where,

M_i and L_i = body mass and the linear body measurement of individual *i*, respectively,

b_{SMA} = scaling exponent estimated by the standardised major axis (SMA) regression of M on L,

L_0 = arbitrary value of L (e.g. the arithmetic mean value for the study population) and,

$\acute{M}i$ = predicted body mass for individual *i* when the linear body measure is standardized to L_0.

Body condition indices (BCI) were calculated for 204 individual male newts captured from four ponds in March and April 2002 - 2013 in Well Court and 63 individuals from the Field Site (2003-2015). A two-way analysis of variance (ANOVA) was performed in Minitab to determine whether there was a difference in BCI between year and pond. Linear regression was used to determine whether there was a relationship between climatic variables, inter-year survival rate and population size with the BCI of males the following spring. Annual male survival was estimated from Cormack–Jolly–Seber (CJS) capture-mark-recapture (CMR) models using the software MARK (White & Burnham 1999). The details of the CMR analysis are provided in Chapters 3 and 4. In each of the remaining models, annual BCI's were included as covariates and additional to this, climatic covariates in the year preceding that of the BCI measurements (non-aquatic rainfall, spring rainfall, winter minimum temperature, and number of days of frost) that potentially affect male body condition in this study were included as additive effects.

The estimated male population for each year was calculated by multiplying the number of individuals caught in each pond by 1/detection probability for that pond (Griffiths et al. 2010). The size of the Well Court metapopulation was calculated by adding together the populations of the component ponds.

6.3 RESULTS

6.3.1 Well Court Metapopulation

Body size

A total of 204 male individual adults from 497 captured were recorded in the period 2002 to 2013 from four ponds, excluding the recaptures of the same individual each year (Figure 6.1). Figure 6.2 demonstrates the mean of annual SVL and body mass of males that were captured between 2002 and 2013. The SVL average among males was 71.53 ± 0.29 mm and the body mass mean was 9.44 ± 0.11 g. The intraspecific regressions of male ln Mass on ln SVL took the form of: ln Mass = 2.13 (± 0.048) ln SVL – 6.90 (± 0.204); $R^2 = 0.56$, $P < 0.001$ (Figure 6.3).

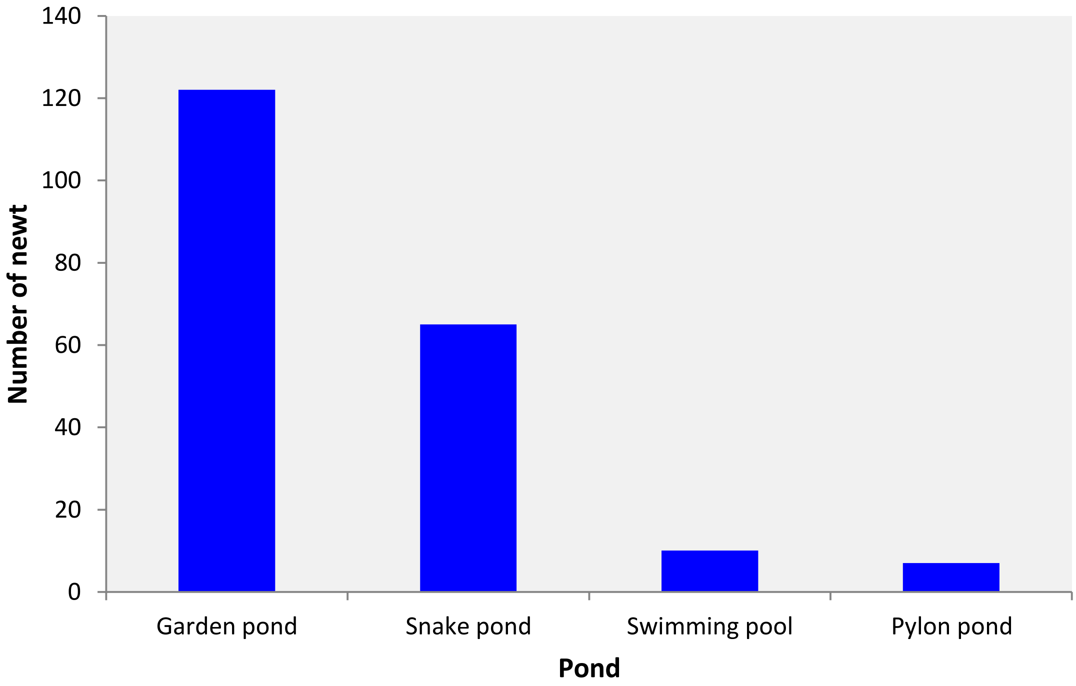


Figure 6.1 Number of adult males captured at each pond in 2002-2013

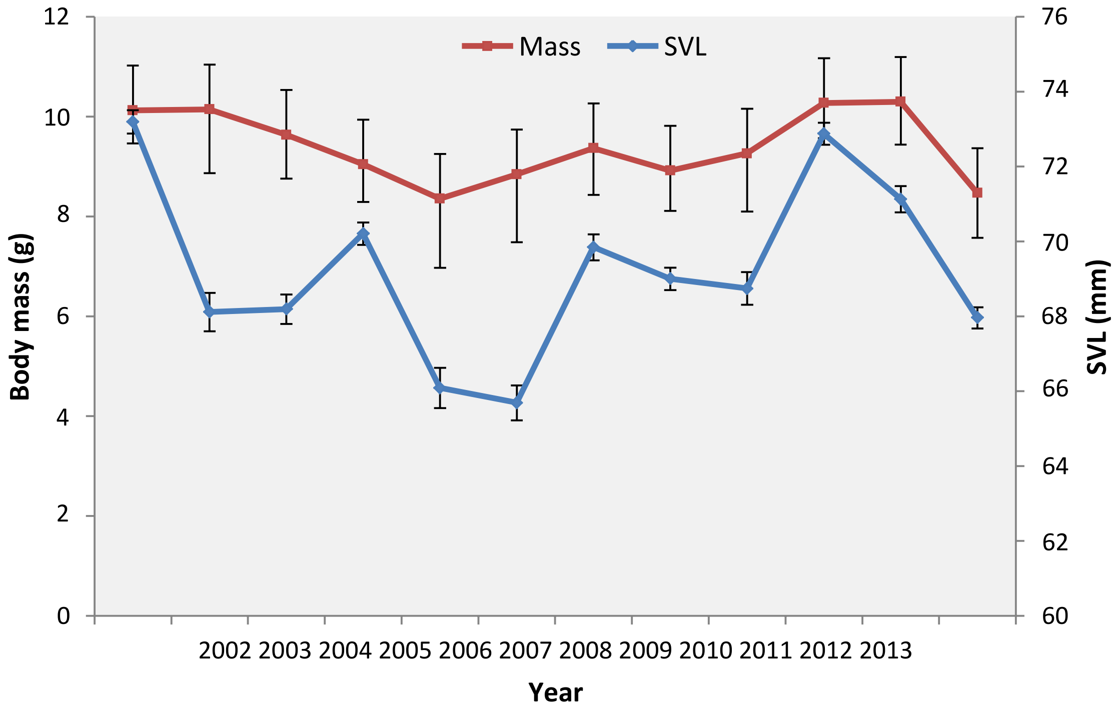


Figure 6.2 Well Court annual March and April average of mass (g) and snout vent length (SVL) for males. Error bars denote standard error

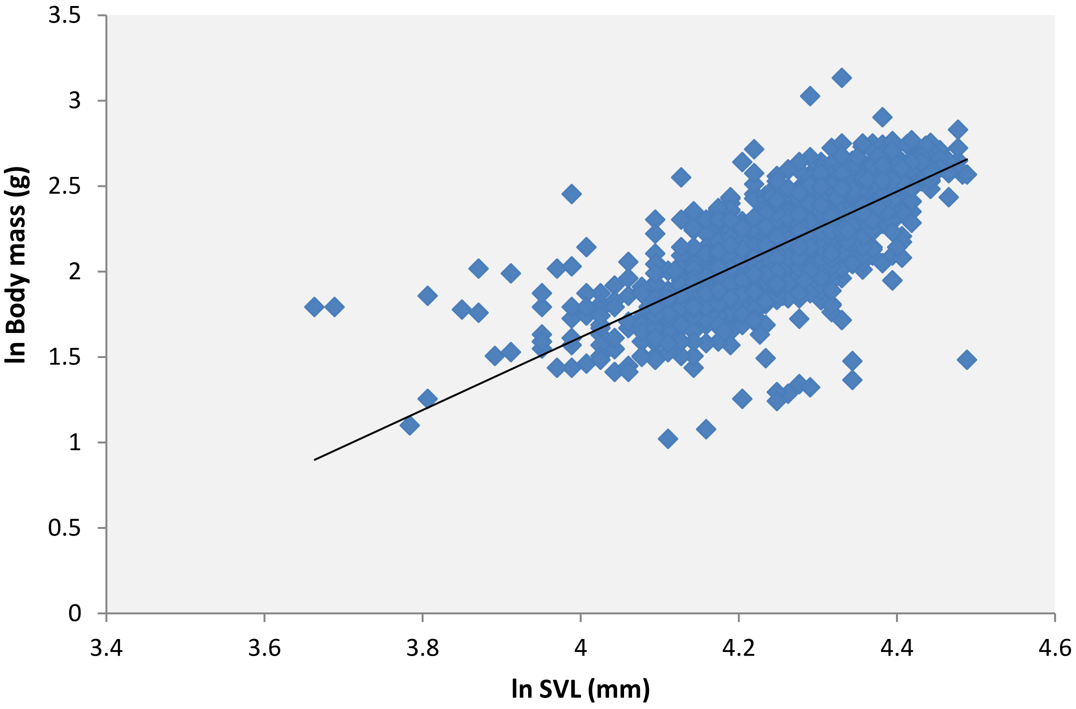


Figure 6.3 The intraspecific allometric regression of ln body mass (m; g) on ln snout-vent length (SVL; mm) in male newts at Well Court

Body condition

Interaction effects between year and ponds

Body condition of adult newts was assessed for the Well Court metapopulation and mean BCI of all males was 9.31 ± S.E. 0.18. Mean annual BCI varied between years with the lowest recorded in 2006 (8.21) and 2013 (8.39) and highest in 2011 (10.15) and 2012 (10.06). Table 6.1 shows the summary of ANOVA results for the interactions between years and ponds. Adult males showed significant difference in BCI scores between years with high standard error for each year indicating highly variable BCI through the year (Figure 6.4). A Tukey post-hoc test confirmed that the mean BCI scores were significantly lower in year 2006, 2007, 2009 and 2013 at 8.21 to 8.59, compared to a range of 9.0 to 10.05 in the period 2003 to 2005 and 10.15 to 10.06 in the period 2011 to 2012.

There was also a significant variation in male body condition between ponds (Figure 6.5). Garden ponds had the lowest mean BCI of 8.86 ± 0.09 compared to Pylon pond, which had the highest value of 11.61± 0.71. Although relatively few newts were captured, Swimming pool (10.17 ± 0.92) and Snake pond (9.96 ± 0.13) had mean BCI scores between those of the other two ponds. A Tukey test confirmed that Swimming pool and Pylon pond had a higher BCI than the Snake pond and Garden ponds, though this was based on a smaller sample size. Results for Swimming pool and Pylon pond are therefore likely to be less representative of the population.

Male BCI scores showed significant interaction between year and pond, indicating that the variation between years depends on the pond.

Table 6.1 Summary of results of analysis of variance using a two-way ANOVA, showing how body condition index of male great crested newts was affected by the factors of pond and year of sampling

	Sum of squares	**Mean square**	**F**	**d.f.$_{N}$**	**d.f.$_{D}$**	**P**
Year	44.707	10.14	4.53	11	193	0.0001
Pond	80.304	39.71	17.74	3	201	0.0001
Year x Pond	415.563	4.57	2.04	15	148	0.001

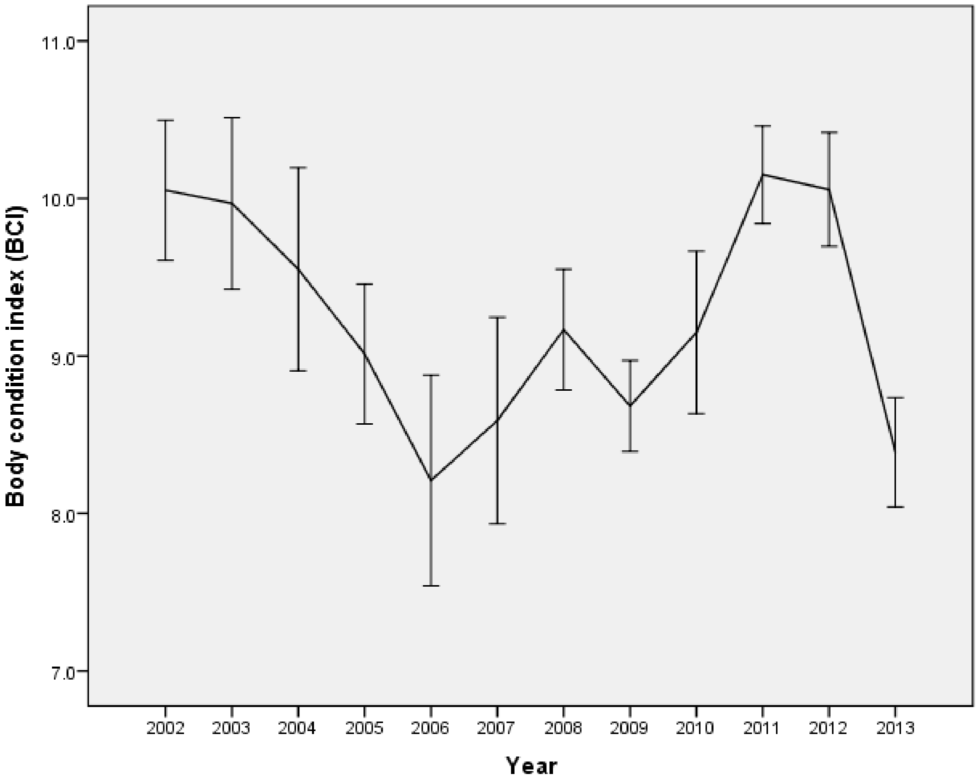


Figure 6.4 Mean of body condition index (BCI) of adult males in each study year, 2002–2013 at the Well Court. Error bars denote standard error

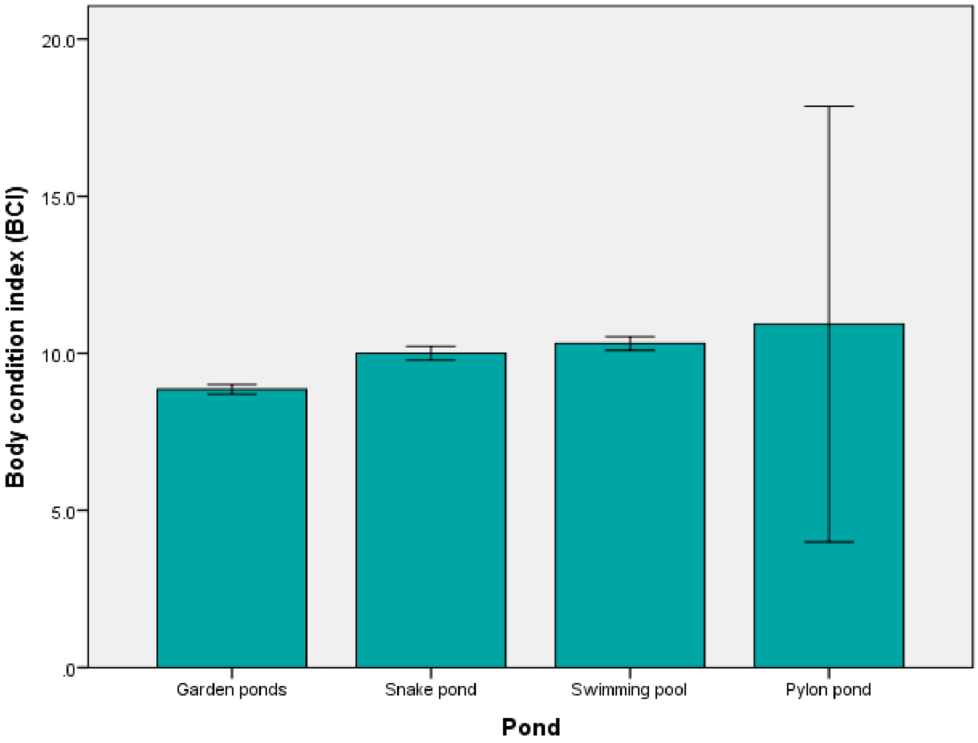


Figure 6.5 Mean of body condition index (BCI) of adult males in each study pond at Well Court over the period 2002-2013. Error bars denote standard error

Effects of body conditions on survival

The male recapture data (2002–2013) were over-dispersed and the *c-hat* value was adjusted to 1.52. The ΔQAICc weight values derived for each of the models showed that the *Phi(t)* model fitted the data best (Table 6.2). The model with male BCI as a covariate fitted the data second best, and following Burnham and Anderson (2002), this model indicated less support (ΔQAICc 3-7). There were three remaining models with ΔQAICc values within 3-7 which were *Phi(MaleBCI+NAR*), *Phi(Male BCI+SR)* and, *Phi(Male BCI+WT)*. There was no significant relationship between male BCI scores and non-aquatic rainfall (BCI= 0.0014 NAR + 8.4301; R^2 = 0.0178, N = 7, P = 0.775), spring rainfall (BCI= -0.0007 SR + 9.2682; R^2 = 0.0078, N = 7, P= 0.851), or mean minimum winter temperature (BCI= -0.0532 WT + 9.2955; R^2 =

0.0088, N = 7, P = 0.841). Therefore, body condition in spring was not affected by climatic variables over the previous months. A regression analysis of survival using model *Phi(Male BCI) p(t)* against male BCI (survival = 0.055 Male BCI + 0.1135 BCI, P = 0.679, R^2 = 0.037; N = 7) showed that there was no relationship between male BCI and survival.

Table 6.2 Model selection based on ΔQAICc values with the *c-hat* corrected as 1.52. *Phi* = survival, *p* = detection rate, *t* = time, *(.)* = constant, and *BCI* = body condition index. Climatic variable; *NAR* = non-aquatic rainfall, *SR* = spring rainfall, and *WT* = winter temperature. NP = number of parameters, and QAICc = quasi Akaike information criteria

Model	QAICc	ΔQAICc	AICc Weights	Model Likelihood	NP	QDeviance
Phi(t) p(t)	599.7267	0.0000	0.56556	1.0000	13	104.0596
Phi(BCI) p(t)	604.2161	4.4894	0.05993	0.1060	2	131.2679
Phi(BCI+NAR) p(t)	605.2667	5.5400	0.03544	0.0627	3	130.2945
Phi(BCI+SR) p(t)	605.7792	6.0525	0.02743	0.0485	3	130.8070
Phi(BCI+WT) p(t)	606.1014	6.3747	0.02335	0.0413	3	131.1292
Phi(.) p(t)	606.1189	6.3922	0.02314	0.0409	8	120.9037
Phi(t) p(.)	606.639	6.9123	0.01784	0.0315	8	121.4239
Phi(BCI+WT+NAR) p(t)	606.8292	7.1025	0.01623	0.0287	4	129.8248
Phi(BCI+NAR+SR) p(t)	607.2683	7.5416	0.01303	0.0230	4	130.2639

6.3.2 Field Site Population

Body size

A total number of 63 adult male great crested newts from 243 captures were identified in the Field Site from 2003-2015. The highest number of new individuals were recorded in 2013 (n = 28), followed by 2015 (n = 26) and 2014 (n=24). Figure 6.6 demonstrates the mean of annual SVL and body mass of males that were captured between 2003 and 2015. The SVL average among adult males was74.95 ± S.E. 0.22 g and the body mass was 11.53 ± S.E. 0.09 g. The intraspecific regressions of male ln Mass on ln SVL took the form of: ln Mass = 1.91 (± 0.061) ln SVL – 5.85 (± 0.261); $R^2 = 0.56$, $P < 0.0001$ (Figure 6.7).

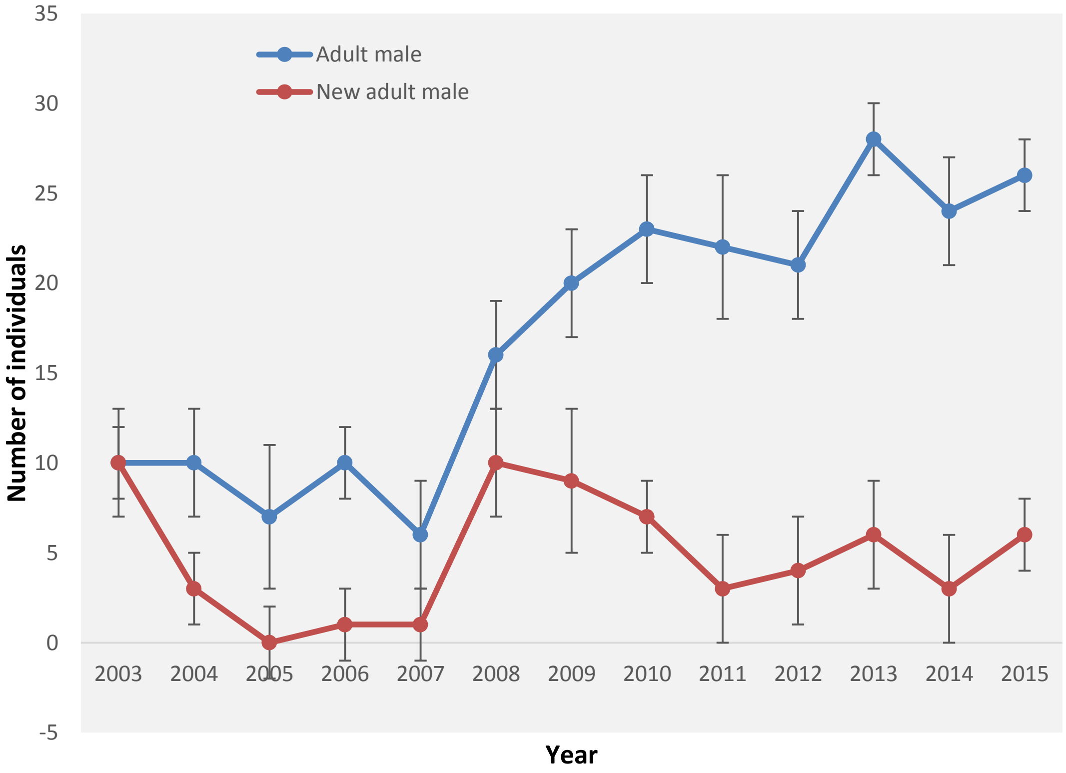


Figure 6.6 Field Site annual March and April average of mass (g) and snout vent length (SVL) for males. Error bars denote standard error

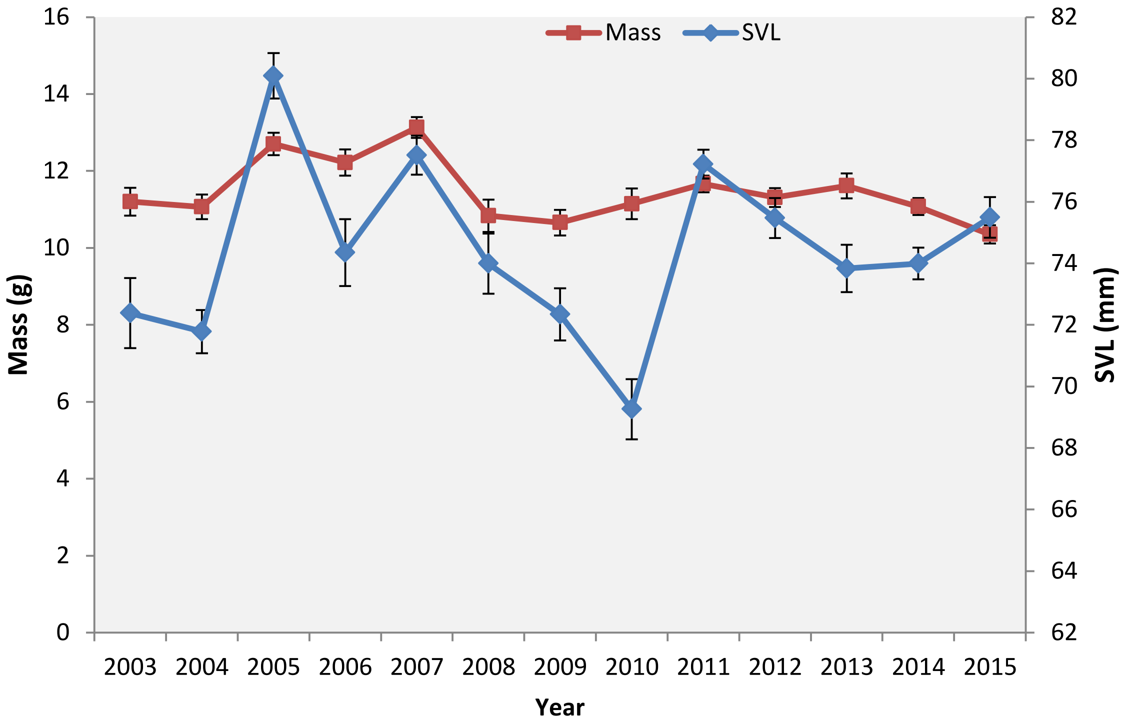


Figure 6.7 The intraspecific allometric regression of ln body mass (m; g) on ln snout-vent length (SVL; mm) in male newts at the Field Site. Error bars denote standard error

Body condition

Differences in body condition between years

A one-way ANOVA was conducted that examined the effect of year on the Field Site BCI. There was a significant difference between the annual BCI between years ($F_{12, 232}$ = 9.123, P < 0.0001). The BCI scores fluctuated over the years with the highest BCI scores in 2007 and the lowest in 2014 (Figure 6.8). The mean of the BCI scores for thirteen years were 11.61 ± 0.18. A Tukey post-hoc ANOVA revealed a significant difference in BCI scores between the years 2003 to 2007 and 2008 to 2015 (P < 0.001).

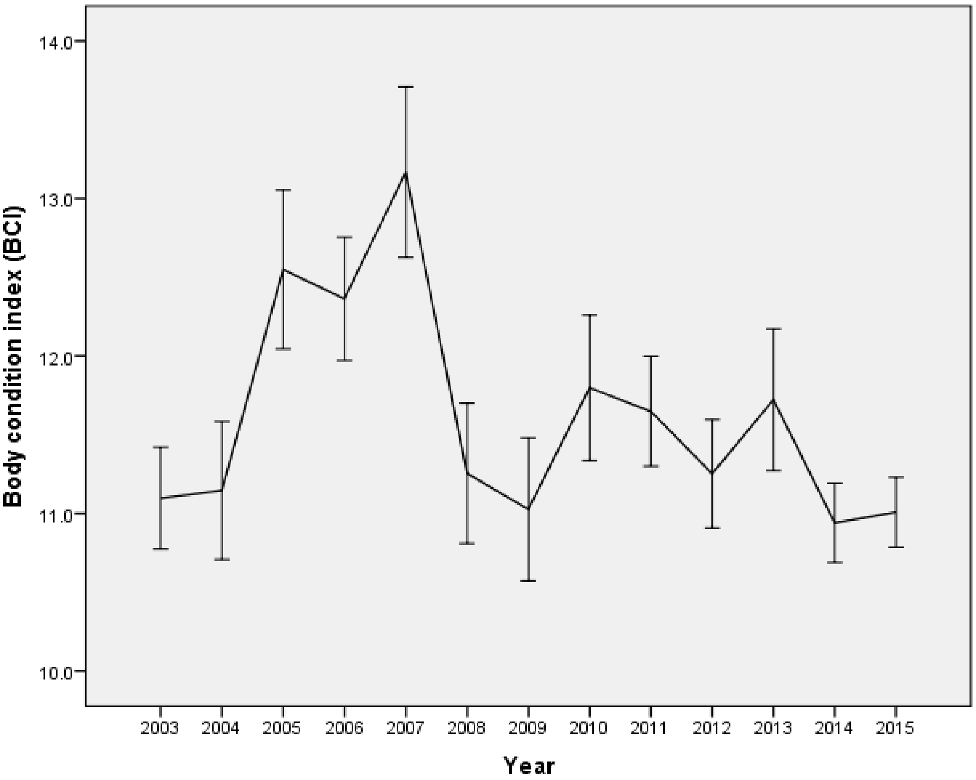


Figure 6.8 Mean of body condition index (BCI) of adult males in each study year, 2003–2015 at the Field Site. Error bars denote standard error

Effects of body conditions on survival

The male recapture data (2002–2015) at Field Site were over-dispersed and the *c-hat* value was adjusted to 1.21. The ΔQAICc weight values derived for each of the models showed that the model with a constant survival fitted the data best (Table 6.3). The model with the BCI as covariates fitted the data as the second best model and is considered important as the ΔQAICc was < 2. Between 2003 and 2015 annual survival and male BCI scores were positively related (Figure 6.9). None of the models with combinations of male BCI and climatic variables as a covariate fitted the data well in this study (ΔQAICc >2). Overall annual survival of adult males was estimated to be 0.87 ± 0.13 by using model *Phi(.) p(t)*.

Table 6.3 Model selection based on ΔQAICc values with the *c-hat* corrected as 1.21. *Phi* = survival, *p* = detection rate, *t* = time, *(.)* = constant, and *BCI* = body condition index. Climatic variable; *NAR* = non-aquatic rainfall, *SR* = spring rainfall, and *WT* = winter temperature. NP = number of parameters, and QAICc = quasi Akaike information criteria

Model	**QAICc**	**ΔQAICc**	**AICc Weights**	**Model Likelihood**	**NP**	**QDeviance**
Phi(.) p(.)	190.681	0.000	0.236	1.000	2	85.224
Phi(BCI) p(t)	192.322	1.641	0.104	0.440	2	86.865
Phi(BCI + WT) p(t)	192.790	2.109	0.082	0.348	3	85.273
Phi(BCI + NAR) p(t)	193.558	2.877	0.056	0.237	3	86.041
Phi(BCI + NAR + WT) p(t)	194.576	3.896	0.034	0.143	4	84.979
Phi(BCI +NAR + SR) p(t)	195.002	4.321	0.027	0.115	4	85.404
Phi(t) p(.)	207.652	16.972	0.000	0.000	14	76.044
Phi(.) p(t)	209.383	18.702	0.000	0.000	14	77.775
Phi(t) p(t)	226.660	35.979	0.000	0.000	25	68.000

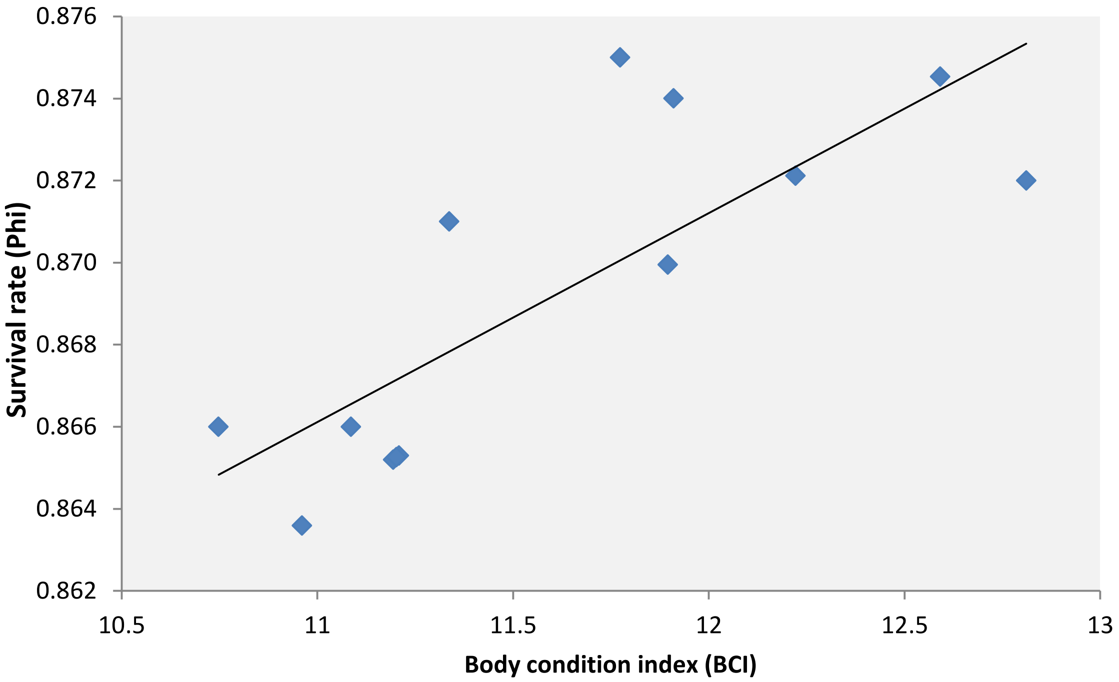


Figure 6.9 Relationship between annual male survival, and body condition index (BCI) in the previous year between 2003 and 2015 at the Field Site. The fitted line was derived from a regression of survival male against BCI ($F = 19.03$, $R^2 = 0.634$, $N = 13$, $P < 0.001$)

6.3.3 Interaction Effects of Pond and Year

BCI scores of male newts at the Well Court metapopulation and Field Site population were compared from 2003 till 2013, the years when both sites were monitored. A two-way ANOVA was conducted that examined the effect of pond and year on BCI scores. Male BCI scores did vary significantly between ponds ($F_{1,\ 739} = 6.85$, $P < 0.0001$). Male BCI scores also showed significant differences across year and pond ($F_{10,\ 739} = 11.92$, $P < 0.002$), suggesting that variation between years depends on BCI scores between ponds. The pattern of mean BCI scores was different to that exhibited by males from each pond. Males at the Field Site had a significantly higher BCI compared to the males at Well Court over the years ($F_{10,\ 739} = 4.59$, $P < 0.0001$) (Figure 6.10). The BCI scores of Well Court and Field Site were not significantly correlated although there appeared to be a slight negative relationship (r = -0.519, n = 11, p = 0.102).

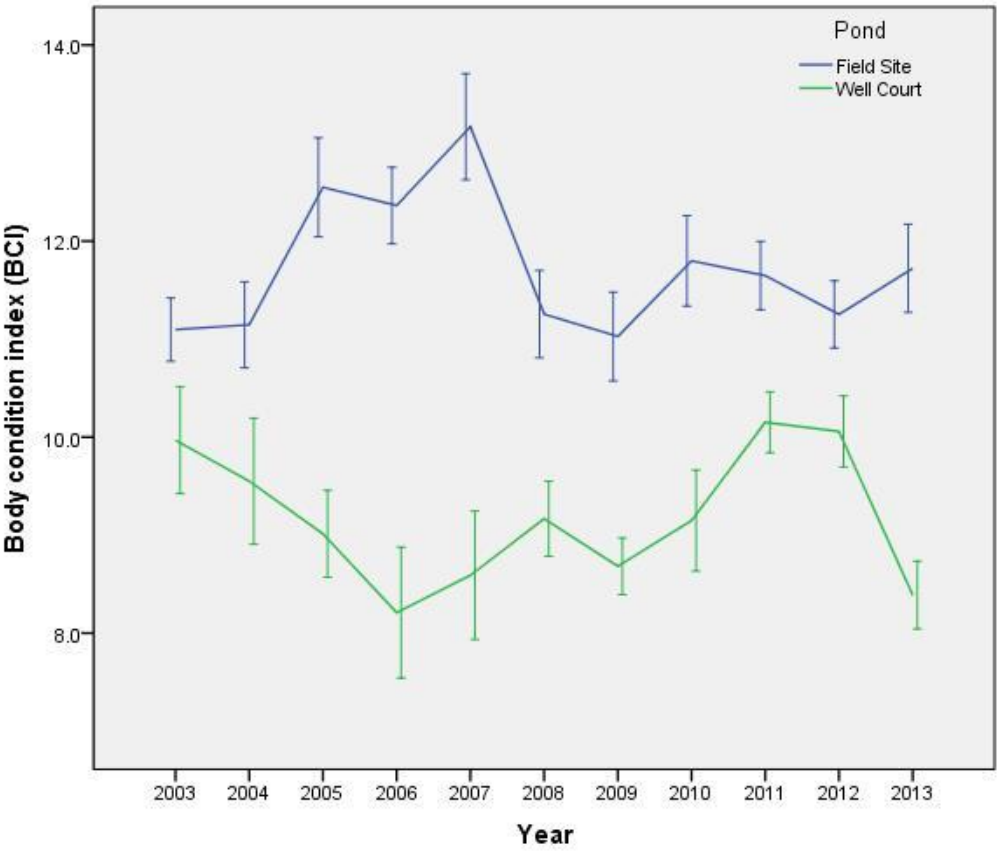


Figure 6.10 Comparison of male mean annual BCI scores at the Well Court metapopulation, and Field Site population. Errors bars denote the 95% confidence intervals

6.3.4 Interaction between Population and Body Condition

At Well Court, there was a non-significant relationship between male metapopulation sizes with the mean annual BCI. However, an analysis just based on male subpopulations at the Garden ponds indicated that the mean BCI increased as population decreased (Figure 6.11). A regression analysis confirmed that there was a negative significant relationship between male population size and the annual mean BCI at the Garden ponds (Figure 6.12). In general, the trend at the Field Site was towards an increase in number of adults after 2008. Male numbers at the Field Site ponds doubled after the addition of the new four ponds in 2008 (Figure 6.13). There was a negative significant relationship between male population size and BCI for the Field Site ponds (Figure 6.14). This finding suggests that male population size may explain BCI for the Field Site ponds and for the highest density subpopulation at Well Court. BCI at the Well Court ponds were only affected by male subpopulation at the Garden ponds over the duration of the study.

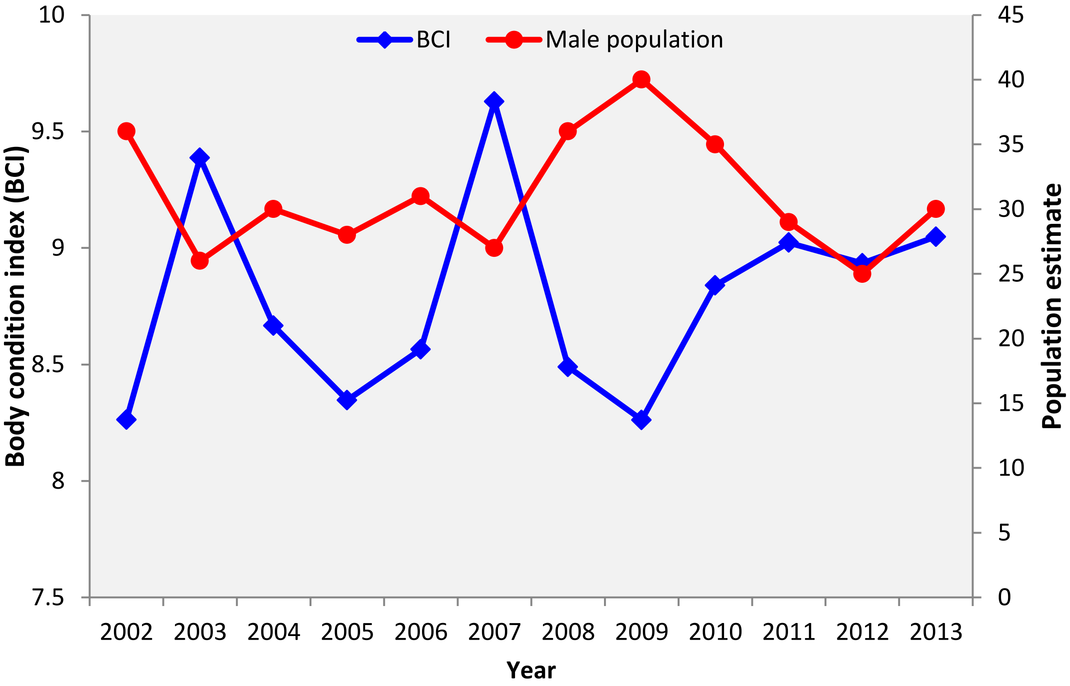


Figure 6.11 Population estimates for the Garden ponds population at Well Court by year, based on detection rate using model *Phi(t) p(t)*

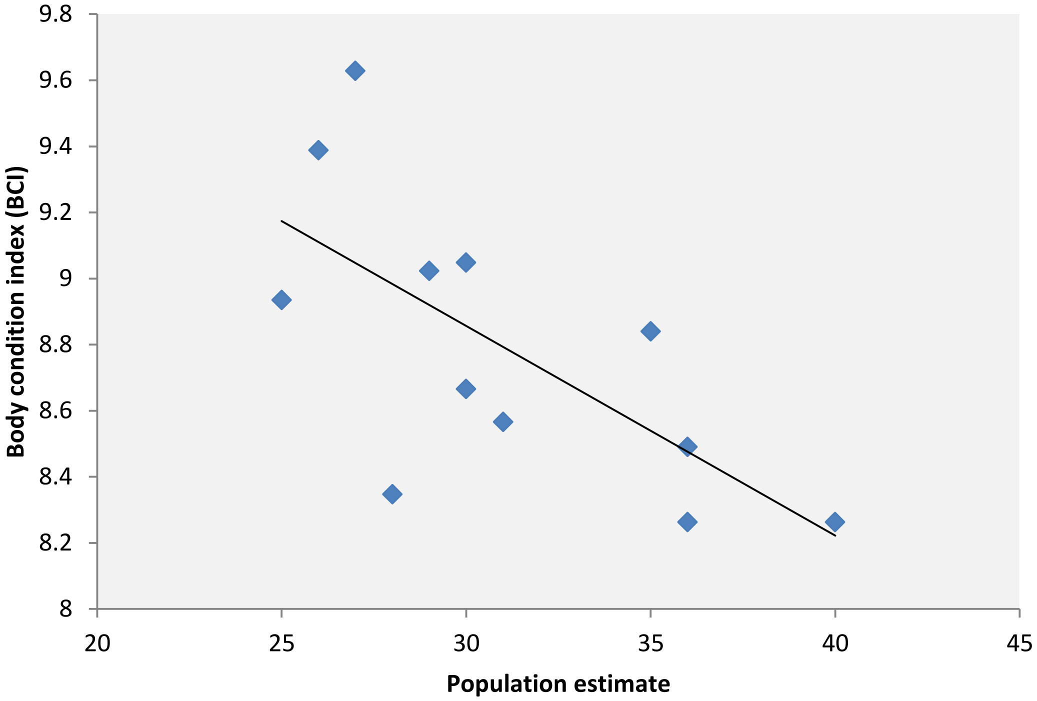


Figure 6.12 Linear regression analysis of body condition index (BCI) against male population size for Garden ponds at Well Court (F = 8.36, $R^2 = 0.456$, N = 12, P < 0.016)

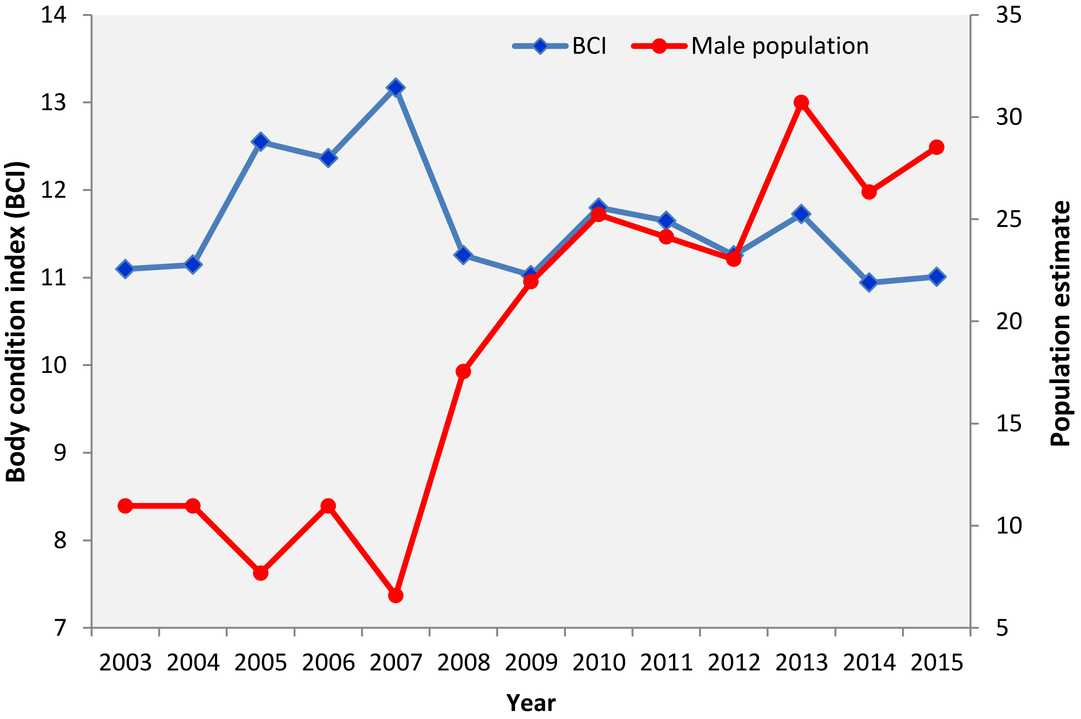


Figure 6.13 Population estimates for the Field Site population by year, based on detection rate using model *Phi(BCI) p(t)*

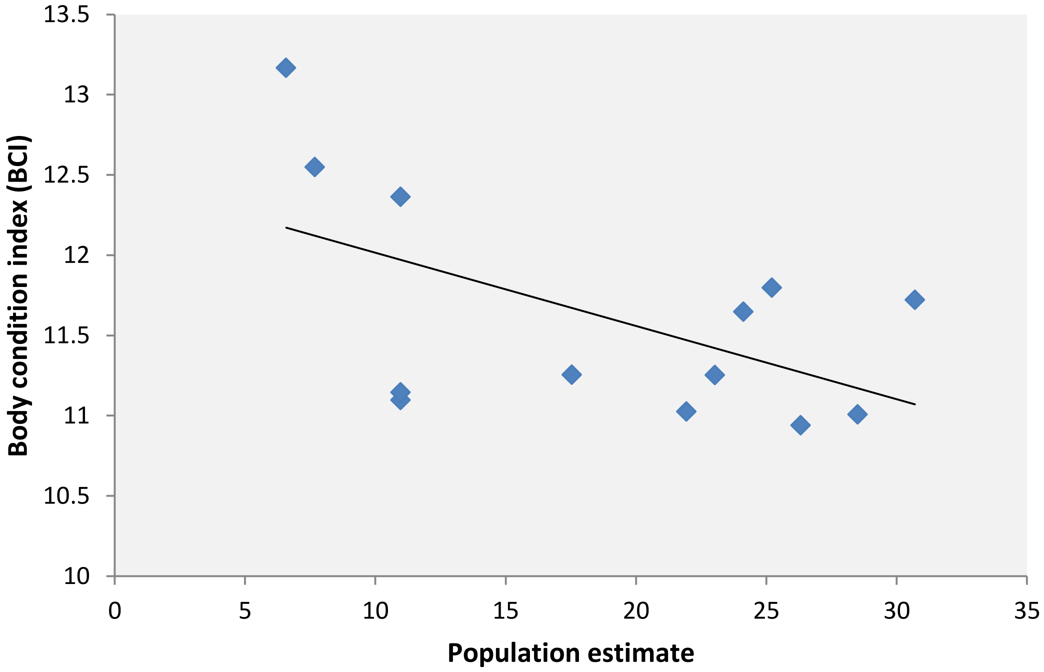


Figure 6.14 Linear regression analysis of body condition index (BCI) against male population for Field Site ponds (F = 12.47, $R^2 = 0.305$, N = 13, $P < 0.046$)

6.4 DISCUSSION

Both males at the Well Court and Field Site showed differences in body condition over the years. Other studies of amphibians have shown that body condition can vary over time (e.g. Wheeler et al. 2003; Bancila et al. 2010; Jarvis 2015) but few have documented the effects of climatic variables on body condition. This study revealed that there was no clear relationship between body condition of male crested newts and the climatic variations since 2002. However, the relationship between body condition with subsequent survival and population density of male newts was strong at the Field Site. Annual survival at the Well Court was found not to be influenced by body condition over the years and metapopulation density was not affecting the body condition here. Male newts from the Field Site population demonstrated a higher body condition compared to the metapopulation at the Well

Court.

Higher body condition in Field Site ponds was generally related to higher habitat quality compared to the Well Court ponds that exist in agricultural areas. The results of this study suggest that habitat attributes rather than climate may influence body condition because none of the models provided good fits with climatic variables at either site. This is supported by the fact that body condition differed between the two study sites and showed different patterns over an 11 year period when they were monitored simultaneously: this implies that local rather than regional factors are driving the patterns observed. An increase in foraging activity in good surrounding terrestrial habitat may lead to greater opportunities for the newts to feed and result in a higher body condition. Body condition in amphibians during early spring is remarkably associated with food intake during the preceding autumn (Sztatecsny & Schabetsberger 2005). Body condition in this study probably depended on resource intake during the terrestrial phase because newts were measured at the beginning of the breeding season (March-April). However, both terrestrial and aquatic feeding habitats need to be considered because newts do appear to take prey on arrival in the aquatic environment (Griffiths & Mylotte 1987).

According to Johnson (2007), body condition is one potentially useful index assessing habitat quality and its effects on individuals. Body condition may be a reflection of access to resources; individuals with abundant resources are expected to be in better condition than those occupying poor habitat. Janin et al. (2011) demonstrated that reduced body condition in common toad (*Bufo bufo*) populations was associated with decreased habitat suitability resulting from fragmentation. Similarly, Sztatecsny and Schabetsberger (2005) found that body condition of the common toad varied between sites and this was related to variation in habitat conditions that determine resource availability. Karraker and Welsh (2006) reported pronounced impacts of habitat degradation and loss on salamander body condition. According to Pope and Matthews (2002), the body condition of an animal mainly reflects food uptake in the previous year and is assumed to be indicative of habitat quality.

Well Court ponds exist in an agricultural area where food may be limited. Agricultural practices may affect the environment by reducing food availability of newts. These changes can limit the resource uptake of newts, reducing their body mass, which is reflected in a decrease in body condition (Brodeur et al. 2011; Zaya et al. 2011; Wagner et al. 2014). From previous studies, it is apparent that the majority of newts capture food prior to entering the hibernation period (e.g. Bogdan et al. 2011; Dimancea et al. 2011). Kuzmin (1995) lists earthworms, insects and their larvae, and slugs as their main prey on land. In agricultural areas, glyphosate is regularly applied as broad-spectrum systemic herbicide and crop desiccant. Verrell and Van Buskirk (2004) found that earthworms avoid soils contaminated by a glyphosate-based herbicide.

Higher body condition in newts from the Field Site ponds may also be related to the shorter distance of newts moving from the terrestrial sites to the breeding ponds after the winter hibernation. Newts that travel a shorter distance might utilize minimal body reserves compare to the newts that travel longer distance. The terrestrial habitat in the close vicinity of the Field Site ponds (< 200 m) usually consists of scrub, long grass and woodland area and offers plenty of hiding places in the form of logs and stones. It is noted that the habitats at this site changed little over the course of this study. The newts might use logs, old stumps and piles of old leaves surrounding the Field Site as these provide damp refugia during winter (Verrell 1985). A terrestrial over-wintering site close to the Field Site ponds might be an advantage particularly for males in the pond immediately at the onset of the reproductive season.

The fact that body condition decreased when the population density was high at the Field Site may be related to intra-specific competition. The same relationship also existed in the highest density sub-populations at Well Court, the Garden ponds. The high population density might increase intra-specific competition for food and resources and, consequently reduce the body condition of individuals. Remarkably, desiccation during the dry season may reduce the water level at the Garden ponds significantly, which may further intensify food and space competition, at least towards the end of the breeding season. The three other ponds at Well Court have been left largely unmanaged, resulting in advanced hydroseral succession. This process is eventually unfavourable to breeding newts as ponds become shaded, cool, lacking in macrophyte and invertebrate prey, and prone to early desiccation. According to Guerra & Aráoz (2015), it is possible that body condition is affected by population density through intraspecific competition and it decreases when amphibians increase. Hoare et al. (2006) found that low body condition in the tuatara (*Sphenodon guntheri*) population is interpreted as a response to resource competition associated with increased tuatara population.

Although male body condition and survival at the Field Site was higher than at Well Court, annual survival here was impacted by body condition. The only possible explanation of the relationship between the high body condition and reduced survival at the Field Site is that the Field Site demonstrated a high male population density. If body condition is the most important factor affecting their reproductive success, males may tend to invest resources towards maximising their body condition. One generally accepted assumption is that an increase in animal fitness is countered by a decrease in another life trait (Roff 1992).

On the contrary, Reading (2007) in his study of a common toad population showed that survival and body condition were positively related, with both variables declining in relation to increasing temperatures over a 27 year period. Abiotic factors (climate) may be only affecting the body condition of newts without any relationship with the inter-year survival rates. In the current study, climatic factors were not strong covariates of body condition at either Well Court or the Field Site. Catlin et al. (2014) found that body condition index does predict survival of pipping plover shorebird (*Charadrius melodus*) during particularly challenging times. For example, mass adjusted for structural body size predicted the survival of Florida scrub jays (*Aphelocoma coerulescens*) through a disease epidemic (Wilcoxen et al. 2010) and body condition predicted the survival of wintering Great tits (*Parus major*) when they were food limited (Gosler 1996). Also, stress physiology predicted the survival of marine iguanas during a starvation event (Romero & Wikelski 2010). During this period of study, such notable selective events were not observed at the Field Site.

However, Chapter 4 in this thesis has explained how the non-aquatic rainfall together with the spring rainfall affected the survival of newts at Well Court. Griffiths et al. (2010) in their study at Well Court between 1995 and 2006 confirmed that non-aquatic rainfall and winter minimum temperature impacted adult survival here. Although climatic conditions affected survival, this was not strongly reflected in body condition, suggesting that environmental conditions are not always ideal and that some years may indeed be challenging. Yet body condition indices failed to predict survival even during these years at the Well Court. Thus, the climatic conditions prior to the breeding season at this study site may be important for survival but not strong enough to impact the body condition of newts.

With respect to winter conditions, it appears that minimum winter temperatures and non-aquatic rainfall had a very weak impact on survival and body condition at the Field Site. Previous studies on amphibian populations suggest that mean winter temperatures have an effect on survival (Anholt et al. 2003; Araujo et al. 2006) with varying effects depending on location and species ecology. Several studies demonstrate a negative relationship between mean winter temperature and body condition the following spring. For example, Bancila et al. (2010) found that yellow-bellied toads (*Bombina variegata*) emerged from winter hibernation in higher body condition after colder winters. Earlier work by Reading and Clarke (1995) suggested that a mild winter and warm pre-spawning period had a negative effect on the body condition of hibernating anurans, through an elevated metabolic rate and the increase in energy reserve utilization. Thus, amphibians are more likely to emerge from hibernation in good condition after colder winters.

Apart from the colder winter, longer summers may increase body condition, because newts have more time to forage, grow and fatten up for the winter. Alternatively, hot dry summers may have negative impacts compared to hot wet summers (Reading & Clarke 1995). Amphibians in such circumstances are likely to enter hibernation in poor condition since high temperatures combined with low humidity increase the risk of desiccation, affecting their foraging and thus the rebuilding of body reserves (Tomasevic et al. 2007). The extreme environment may also result in catastrophic mortality of adult newts. However, this factor could not be the main reason in this study because survival rates do not suggest any catastrophic mortality.

In conclusion, the present study suggests that body condition is related to local rather than regional or climatic factors. The local factor here - population density - had a close relationship with body condition at both of the study sites. The study also indicates a close relationship between survival and body condition of newts on emergence from hibernation at the Field Site. Agricultural activity may be one of the factors that negatively influence the body conditions of newts and might represent an ongoing threat for its conservation.

CHAPTER 7

GENERAL DISCUSSION

7.1 POPULATION GROWTH AND REGULATION

The unprecedented long-term data of capture history and environmental variables on multiple study sites (Well Court and Field Site) enable this study to understand the relationship between local and regional factors on the population growth and regulations of the great crested newts. In conclusion, the persistence of the two populations relies on a combination of (1) local, population-specific factors - such as population density and pond desiccation, and (2) regional factors, such as climate that affect recruitment and survival from each pond. Firstly, this study is concerned with the impact of local-specific demographic rates on population regulation of newts at the Well Court metapopulation. Here, a small fraction of the individuals may occasionally occur in ‘sink’ ponds, where within-pond reproduction is insufficient to balance local mortality. Nevertheless, populations may persist in such ponds, being locally maintained by continued immigration from a more-productive ‘source’ pond nearby.

Survival of adult great crested newts at Well Court differed between years, but there was no variation in survival between subpopulations. Thus, asynchrony between subpopulations in their dynamics was more likely due to variation in juvenile dispersal and recruitment than variation in adult survival or dispersal. With only ca. 2% of adults moving between ponds, adult dispersal was too low to even out local population densities or synchronise their trends. Fluctuations in adult population sizes must therefore reflect increases in recruitment and possibly dispersal of juveniles - rather than dispersal of adults between subpopulations. The Well Court system seemed to be driven by regular recruitment only from the Garden ponds, supporting the idea of source-sink processes within the metapopulation system. Indeed, this suggests rescue-effect metapopulation system habitats that are not uniform and differ in quality (Pulliam 1988).

The other important local-specific factor may be density dependence. At Well Court, newts in the high-density source pond had lower body condition than newts in the other ponds. Intra-specific competition for resources in the source pond may therefore be intense. In a model suggested by Pulliam (1988), when the population density in the source is too high, it pays all surplus individuals - especially juveniles to emigrate because they can achieve a higher fitness by doing so. Nevertheless, individuals unable to occupy a source pond may emigrate to the sink because a poor quality breeding pond is better than none at all.

Variation in survival between years at the Well Court metapopulation was adversely linked to climatic factors that influence survival on a regional scale. Mild, wet winters may result in reduced adult survival and this reduction may reduce the number of breeding opportunities of newts at the Well Court metapopulation, and ultimately, population size. This study also suggests that dry summers may be an important factor that determines the pond hydroperiod and that will affect larval recruitment at the Well Court metapopulation. If a pond dries up, ecological succession is started anew (Griffiths 1997). Occasional desiccation eliminates predators and can have a positive impact on the larvae (Morin et al. 1990). The productive pond like the Garden ponds produce a large number of newt offspring every breeding season, providing a source for potentially increasing the number of individuals inhabiting a given pond network.

The Snake pond was very deep, and perhaps did not desiccate frequently enough to reduce predators (Hecnar & M'Closkey 1997). In addition, the Snake pond is over-shaded by trees, and may be colder and thereby retard amphibian larval growth, suggesting that thermal characteristics play an important role in amphibian development (Skelly et al. 2002). The Swimming pool and the Pylon pond are example of sink ponds where great crested newt larvae were never captured, indicating repeated reproductive failure. Infection of eggs by fungi, often linked to cool weather conditions, and predation of larvae are likely the major drivers of the reproductive failure in the Swimming pool. The Pylon pond on the other hand is too isolated from the other three ponds and frequently displays hydroseral succession. This process is eventually unfavourable to newts as ponds become shaded, cool, lacking in macrophytes and invertebrate prey, and prone to early desiccation.

At the Field Site, survival was constantly high every year with some effect of wet, mild winters. Pond management through periodic desiccation that removes invertebrate predators combined with topping up of ponds to prevent desiccation every year enhances recruitment and survival, and can offset potentially detrimental climatic influences, especially desiccation risk during the hot summers. Consequently, number of individuals increased significantly 2-3 years after ponds were drained or new ponds installed (Lewis 2012). However, the Field Site population still responds to a local-specific effect which is density dependence. The analysis on the effect of body condition on survival suggested that survival at the Field Site was related to decreasing body condition. However, there was no influence of any climatic variables on the body condition of the crested newts. Further analysis showed that population density was the main local factor influencing body condition.

At the Field Site, there may be increased competition for food that affects the body condition of newts (Baker 1992). Features relevant to adult survivorship are naturally selected on the basis of optimizing fitness/body condition, with a trade-off between resource allocation into somatic growth or into germ cells (Beebee 1996). Assuming the importance of adult survival as one of the drivers of crested newt population dynamics, these potential effects of body condition on survival have substantial effects for population dynamics (Stahl & Oli 2006). According to Rockwood (2006), population regulation usually does occur in the presence of density dependence.

7.2 CONCLUSION: CONSERVATION IMPLICATIONS

The study of the four populations at the Well Court farm was an example of how metapopulation dynamics can be driven by different factors at a small spatial scale. Amphibians often form metapopulations (Gill 1979), and in these cases conservation should occur at a landscape level, and extinction and re-colonisation dynamics may become an important characteristic of these populations (Sjogren-Gulve 1994). Well Court highlights the dynamic nature of population processes and illustrates how the elimination of a few core sub-populations or reducing the potential for migration could lead to the decline of a species over a much wider area. Sjogren (1991) showed that the probability of extinction was increased either by obvious deterministic factors (destruction of ponds) or just by distance from the nearest extant site. On the other hand, both source and sink populations can be important to the long-term survival of a source population, with sinks holding reservoirs of non-breeding adults. Reproductive surpluses from productive source ponds may maintain populations in sink ponds, where local reproductive success fails to keep pace with local mortality. For the sink ponds, if the source population is susceptible to chaotic behaviour, or shows great fluctuations such as drought or fire, the source population itself could be rescued if connected to a sink (Gyllenberg et al. 1993).

The rescue-effect is based on the idea that emigrants from source areas regularly supplement these small, extinction-prone populations. If the expected size of the small population is increased through this supplementation it becomes less prone to, or is rescued from extinction (Brown & Kodric Brown 1977). In a true sink habitat, a population would decline to extinction if cut off from its source population. The general trend within the Well Court metapopulation over a 19-year period was a slow decline after 2001 with the Swimming pool and possibly Pylon pond sub-populations going extinct (Griffiths et al. 2010). For the Snake pond, its function alternated between sink and source depending on the pond condition prior to breeding season such as pond desiccation. If adult survival in the metapopulation remains low - especially in the source ponds - there will be fewer breeding opportunities which can lead to greater vulnerability of repeated reproductive failure. One of the assumptions for long-term metapopulation persistence is that source ponds must produce large populations with positive growth rates, and are likely to be a source of emigrants. Other ponds are of low quality, have small populations, and consistently have negative growth rates (Pulliam 1988).

Metapopulation studies have traditionally highlighted the significance of habitat connectivity (Hanski & Ovaskainen 2000, Eycott et al. 2012, Van Buskirk 2012). Habitat connectivity is a conceptual framework that relates the spatial structure of a landscape and the responses of species to it (Vuorio et al. 2016). In amphibians, the enhancement of connectivity is of major importance because it assists recolonization if a pond dries out during summer as a consequence of dry weather conditions

(Vuorio et al. 2016). Terrestrial habitat surrounding breeding ponds is vital for an amphibian population to prosper. Connectivity of habitat patches is definitely important for such metapopulations to persist, and the value of suitable (especially woodland) between ponds, as well as the maintenance of ponds for long time periods was demonstrated in this study. Thus, effective management of a species requires an understanding of these metapopulation dynamics and a restoration of lost habitat and corridors/dispersal routes (Hanski et al. 1996). The emphasis should be on the creation of new ponds that are connected to occupied ponds by corridors of suitable dispersal habitat. In addition, ponds with existing populations should also be linked via habitat corridors, as to enable recolonization in the event of local extinction. On the larger scale, recently the prospect of climate change has added a second impetus for corridors because it may be that at some stage species will have to shift in respond to increased temperatures. In Britain, the trend towards earlier breeding in response to mild winters seen in several amphibian species over the past 20 years may be a sign of further changes yet to come (Beebee & Griffiths 2000).

Apart from terrestrial habitat, conservation management for pond breeding amphibian and great crested newts specifically also requires the maintenance of suitable ponds. Conservation actions at the local scale may offset reduced larval recruitment and adult survival at the regional scale. To manage ponds, over-deepening ponds should be avoided as larvae only prosper where competitors and invertebrate predator numbers are low. Apart from that, overhanging trees need to be cut back to increase exposure to sunshine, and when necessary parts are fenced off to prevent large predator access. Silt and debris should be removed from ponds to avoid hydroseral succession, and for artificial ponds, new liners of plastic or butyl rubber need to be replaced occasionally. To sum up, management is only worthwhile if both aquatic and terrestrial habitats are taken into account.

Research exploring possible effects of climate change on amphibians has remained surprisingly sparse (Uveges et al. 2016). As amphibians display complex life cycles which incorporate both aquatic and terrestrial stages (Wilbur 1980), further studies examining the general applicability of current findings across several amphibian life stages are urgently needed. For example, studies on possible effects of climate change on amphibians in most cases disregard effects on juveniles, despite the fact that good body condition and high survival rate of juveniles are crucial for population persistence (McCaffery & Maxell 2010). The present study suggests that the response of crested newts to climate change may be influenced by local pond conditions, stressing the importance of site-specific conditions for conservation strategies. Additionally, with projected alterations in water availability and changes in precipitation patterns globally, understanding how altered hydroperiods might affect amphibian survival and growth is possibly fundamental to amphibian conservation. Understanding the possible effects of future climate change on amphibians may inform the implementation of informed and effective conservation actions to protect this highly threatened group of animals.

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APPENDIX I

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OPEN

Seasonal variation in environmental DNA in relation to population size and environmental factors

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Analysing DNA that organisms release into the environment (environmental DNA, or eDNA) has enormous potential for assessing rare and cryptic species. At present the method is only reliably used to assess the presence-absence of species in natural environments, as seasonal influences on eDNA in relation to presence, abundance, life stages and seasonal behaviours are poorly understood. A naturally colonised, replicated pond system was used to show how seasonal changes in eDNA were influenced by abundance of adults and larvae of great crested newts (*Triturus cristatus*). Peaks in eDNA were observed in early June when adult breeding was coming to an end, and between mid-July and mid- August corresponding to a peak in newt larval abundance. Changes in adult body condition associated with reproduction also influenced eDNA concentrations, as did temperature (but not rainfall or UV). eDNA concentration fell rapidly as larvae metamorphosed and left the ponds. eDNA concentration may therefore reflect relative abundance in different ponds, although environmental factors can affect the concentrations observed. Nevertheless, eDNA surveys may still represent an improvement over unadjusted counts which are widely used in population assessments but have unreliable relationships with population size.

All living organisms continually expel DNA into the environment via faeces, urine, skin secretions, skin cells and gametes[1–3]. The emergence of techniques that are able to detect low levels of such environmental DNA (eDNA) has enormous potential to break new ground in areas such as invasive species research[4–6], pathogen detection[7], palaeoecology[8], and forensics and law enforcement[9]. The use of eDNA to survey rare and cryptic species that are difficult to detect using traditional methods also has wide implications for biodiversity assessment and the protection of species[10,11]. A relationship between the amount of eDNA present and measures of abundance has been demonstrated in both natural and mesocosm systems[6,12–18]. Although some studies suggest peaks in eDNA associated with breeding[16,19,20], the seasonal dynamics of eDNA in relation to population size are poorly understood. Consequently, eDNA is currently largely limited to surveys of presence and absence. Measures of abundance are more useful than presence-absence, but are often based on count data that are not adjusted for detection probability which can be misleading[21]. As such, producing reliable population, biomass or relative abundance estimates would be much more informative for conservation practitioners[10]. Before predicting abundance, the factors that influence eDNA concentration in relation to changes in population size and environmental factors need to be understood.

The concentration of eDNA at any point in time will depend on (1) the rate of production of eDNA by the species; and (2) how long eDNA persists in the environment[22]. eDNA release and accumulation rates depend on a number of factors including the density of individuals, their physiology, metabolism and temperature[23]. However, eDNA can be broken down by biotic and abiotic factors such as extracellular enzymes, high temperatures, UV, and chemicals[22,24–29]. In aquatic environments, eDNA can also become incorporated into sediment[17]. Persistence of eDNA in water after organisms are removed can range from less than one day[30], to over three weeks[22] depending on environmental conditions, whereas persistence in soil or sediment is likely to be much longer[31]. Despite this knowledge base, and the fact that eDNA concentration can vary seasonally[19,20], to our knowledge no studies

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have identified how seasonal population dynamics impact eDNA concentration in relation to other factors that influence DNA release and degradation. Therefore, whilst eDNA surveys promises to redefine how biodiversity is monitored in the future, there is considerable uncertainty about the relationship between eDNA concentrations and seasonal changes in population size, because of the influence of other environmental factors.

In this study we examined the relationship between eDNA and the seasonal population dynamics of great crested newts (*Triturus cristatus*) using a replicated but naturally colonised system of eight ponds. Adult great crested newts migrate into ponds to breed in the spring, with most returning to land in early summer. Breeding occurs in water with females laying eggs that hatch into aquatic larvae that metamorphose and emerge in the late summer or, occasionally, overwinter[32]. All of these stages may release eDNA into the water. As a European Protected Species, great crested newt eDNA surveys are currently being used to assess the presence-absence of species, but how eDNA fluctuates over this aquatic phase is unknown[33]. To fill this knowledge gap, adult and larval abundance, adult body condition and environmental factors including temperature, rainfall and UV, were used to evaluate their influences on eDNA concentrations throughout the aquatic period.

Results

Between 26 February 2015 and 29 October 2015, a total of 389 captures of 49 individuals were made across the eight ponds, with capture-mark-recapture models yielding an overall population size of between 53 and 60 individuals with a most likely population size of 57, although the numbers varied between ponds. Likewise, 408 larvae were captured between 28 May 2015 and 29 October 2015, with an estimated bottle trapping detectability of 0.39. Two distinct peaks were seen in eDNA concentration (Fig. 1). The first peak corresponded to the end of the adult breeding season in early June. The second peak was observed from mid-July to mid-August and corresponded with the peak in larval numbers. The influences on eDNA concentration over the breeding season (26 February to 18 June) were identified using the first set of models. The change in body condition measured by the Scaled Mass Index (SMI)[34] fell from a peak on 6 March through the breeding season and continued to fall into the post-breeding season, with most of the decline occurring from 9 April through to 4 June. Both sexes showed declines in SMI score with females showing a slightly greater decrease than males (Fig. 1). The sharpest decline in body condition for both males and females occurred in the key breeding months of April and May. During the same core period of April and May the mean eDNA concentration rose considerably but adult population changed very little, and larvae were first identified in the ponds at the beginning of June. As would be expected, temperature and UV both increased as the breeding season progressed, from early spring into early summer. This resulted in the model with the greatest AIC support (∆AIC to second model = 0.5) comprising adult abundance, larval abundance, temperature, and male and female body condition as predictors of eDNA concentration (Table 1). Three other models were shown to have strong support (∆AIC ≤ 2) also detailed in Table 1.

Further analysis was undertaken on AIC importance weights for individual predictors over the breeding season, with female body condition (cumulative AIC weight = 0.99), larval abundance (cumulative AIC weight = 0.797) and air temperature (cumulative AIC weight = 0.79) strongly supported by the analysis, while male body condition (cumulative AIC weight= 0.428) and adult abundance (cumulative AIC weight = 0.44) were only somewhat supported by the analysis.

Influences on eDNA concentration after adult newts had finished breeding were examined through the second set of models, which included potential predictors from 18 June to 22 October. eDNA concentration increased dramatically between 18 June and 30 July, corresponding with an increase in mean larval abundance. During the same period adult abundance nearly halved, indicating that the increase in eDNA was more likely due to larval than adult influences. Temperature also increased through this period from a mean weekly temperature of 15.9 °C to over 19 °C for all of July. eDNA concentration remained high until the middle of August when it fell by over 90% between 13 August and 27 August, and continued to fall into the autumn. Metamorphosis of larvae from the ponds resulted in larval abundance falling over the same period. Temperature remained above 15 °C through August but then fell to below 10 °C in October. The model with the greatest AIC support (∆AIC = 4.82) included larval abundance and air temperature (Table 2) as predictors of eDNA concentration. No other models were shown to have strong support (∆AIC ≤ 2), but one was shown to have limited support (∆AIC ≤ 7) also detailed in Table 2.

Further analysis was undertaken on AIC importance weights for individual predictors for the post-breeding season, with larval abundance (cumulative AIC weight= 0.998), and temperature (cumulative AIC weight= 1.0) strongly supported by the analysis; no other variables were found to be strongly supported by the analysis. Sample collection method was not found to be a significant predictor of eDNA in any of the models.

Discussion

Both laboratory and field studies have shown that an increase in abundance or density of target species can lead to an increase in either eDNA concentration[6,12,13,17,23,35] or eDNA detectability[36]. Our results take this further by showing that the eDNA contribution from different life stages of a semi-aquatic species varies seasonally. Although it was artificially created, our replicated pond system was ideal for this work, as it allowed for truly replicated samples to be taken, with robust population estimates of naturally colonising newts obtained. eDNA concentration within the breeding season increases as females lose body condition through reproductive behaviour and laying eggs. Male body condition and adult abundance also have some influence on eDNA concentration during the breeding season but not to the same extent as other variables. After adult breeding activity has finished, eDNA increases again as larval abundance increases, but with temperature also having an influence at this time.

The amount of eDNA in the environment depends on both DNA release from organisms and eDNA degradation rate[22]. These rates are likely to vary seasonally in response to environmental changes and the ecology of the species[17,25,37]. Strong temporal increases in eDNA during months associated with breeding have been observed in the Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*)[19] and Chinese and Japanese giant salamanders

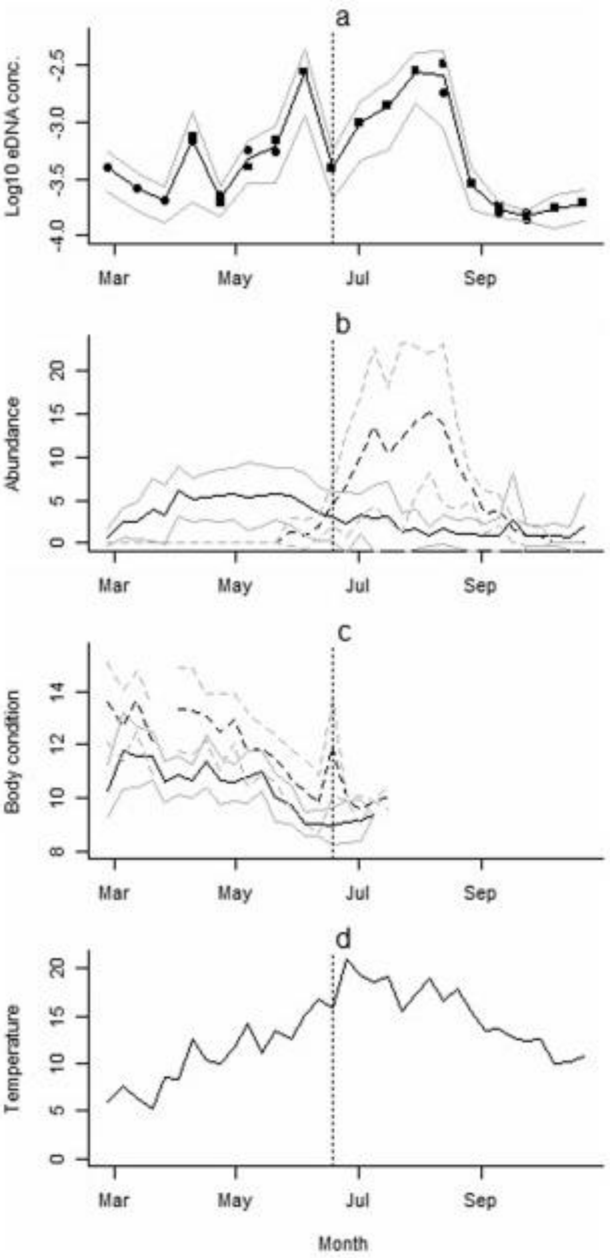

Figure 1. Seasonal variations in eDNA concentration, in relation to adult and larval population size, adult body condition and temperature. (**a**) Shows Log10 (x + 0.0001) of the mean eDNA concentration (ngμ L^{-1}), per pond (black line, solid circles collected using glass-microfiber filters, solid squares collected using precipitation in ethanol) with 95% confidence intervals (grey) across the eight ponds. (**b**) Shows the mean estimated population size per pond black (adults - solid line, larvae - broken line) with 95% confidence intervals (grey). (**c**) Shows mean body condition (males – solid line, females – dashed line) using the scaled mass index of adults caught each week throughout a survey season with 95% confidence intervals (grey). (**d**) Shows mean weekly temperatures in degrees Celsius through the study period. The vertical dotted line represents the end of the breeding season and the start of the post-breeding season, as related to the models described in Tables 1–2.

Predictor	Random	Value	SE	DF	t-value	p-value	AIC	AICc	ΔAIC	Weights
Adult Abundance	Pond	0.029	0.008	83	3.68	0.0004	97.91	99.6	0.00	0.263
Larval Abundance		0.043	0.021	83	2.01	0.0481				
Male Body Condition		−0.189	0.072	83	−2.61	0.0108				
Female Body Condition		−0.328	0.065	83	−5.02	<0.0001				
Temperature		−0.117	0.022	83	−5.32	<0.0001				
Larval Abundance	Pond	0.054	0.020	85	2.65	0.0095	99.13	100.1	0.50	0.204
Female Body Condition		−0.398	0.063	85	−6.31	<0.0001				
Temperature		−0.101	0.023	85	−4.33	<0.0001				
Adult Abundance	Pond	0.026	0.008	84	−4.99	<0.0001	99.13	100.4	0.83	0.174
Larval Abundance		0.066	0.020	84	3.32	0.0013				
Female Body Condition		−0.404	0.061	84	−6.66	<0.0001				
Temperature		−0.113	0.0223	84	3.223	0.0018				
Female Body Condition	Pond	−0.176	0.039	87	4.53	<0.0001	100.79	101.2	1.66	0.115

Table 1. Linear mixed effect models showing influences on eDNA concentration in the breeding season (26 February to 18 June). All models showing substantial support based on ΔAIC shown.

Predictor	Random	Value	SE	DF	t-value	p-value	AIC	AICc	ΔAIC	Weights
Larval Abundance	Pond	0.013	0.002	94	5.36	<0.0001	100.74	101.4	0.00	0.898
Temperature		0.056	0.014	94	6.13	<0.0001				
Collection Method	Pond	0.065	0.079	93	0.82	0.4166	105.31	106.2	4.82	0.081
Larval Abundance		0.012	0.002	93	5.29	<0.0001				
Temperature		0.088	0.014	93	6.16	<0.0001				

Table 2. Linear mixed effect models showing influences on eDNA concentration post-breeding season (18 June to 22 October). All models showing substantial or some support based on ΔAIC shown.

(*Andrias davidianus* and *A. japonicus* respectively)[20]. Doi *et al.*[16] found that seasonal variations in eDNA concentration were related to total biomass, rather than abundance or behaviour, in stream dwelling fish[16]. Our data support this with an increase in eDNA concentration associated with both peak breeding and peak larval abundance. Current eDNA survey protocols for great crested newts focus on the period adults are present in ponds[33].

In the past, positive great crested newt eDNA samples have been identified outside the breeding season[38]. We find a second period with high eDNA concentration at a time of year when adults are moving out of ponds into their terrestrial phase[39]. This post-breeding season spike can be attributed to other life stages, predominantly larvae, and the late August fall in eDNA, corresponds to the period larvae are metamorphosing and leaving the ponds[39]. Seasonal changes in eDNA therefore have implications for survey strategy. If the eDNA surveys are focused on assessing breeding rates, it may be more appropriate to attempt to target larvae by sampling over the post-breeding months. On the other hand, if surveys are aimed at determining occupancy by adults, this approach may be inappropriate. As with many other amphibians, great crested newts live in a metapopulations, where some ponds hold reservoirs of adults that are not breeding each year[40]. We have shown that one of the key influences on eDNA concentration after adults have finished breeding is larval abundance. Samples taken outside the core adult aquatic period may be useful in identifying successful breeding, due to the presence of larvae. However, in the cases of occupied but non-breeding ponds, samples in this period would likely return negative results, potentially missing important non-breeding sites for the species.

The relationship between eDNA water concentration and population size varies by season. For example, an increase in temperature is likely to influence both eDNA release, through higher activity levels[6], and breakdown rates, with an increase in DNA degradation[25,28]. We found that temperature had a significant influence on eDNA concentration during both breeding and non-breeding periods. During the breeding season, temperature increased as did eDNA concentration, while during the post-breeding season (late summer and autumn) both eDNA and temperature decreased. This suggests that the seasonal activity of newts outweighs any influence temperature has on DNA degradation. High levels of rainfall would potentially dilute ponds thereby reducing eDNA concentration. However, we found that rainfall had no influence on eDNA concentration in our system. Although UV has been found to influence DNA[41], its impact on degradation rates appears to be variable[3,28,42–45]. In the present study the correlation of UV with other potential environmental predictors means that separating its precise effects is confounded.

During the breeding season newts expend energy in courtship and reproduction, releasing pheromones[46,47], spermatophores and eggs into the environment, all potentially directly or indirectly releasing DNA with them. The release of these products into the environment will not only lead to an increase in eDNA but it will reduce the mass of an individual and lead to a reduction in body condition. We observed a fall in both male and female body condition through the breeding season both of which were shown to be a significant influence on eDNA concentration. Reductions in male body condition were not as pronounced as for females and are likely to come from the release of spermatophores and expenditure of energy during courtship. The greater decline in female body condition and influence on eDNA over that from males is likely to be related to the greater loss of body mass due to egg production and laying.

Great crested newt females lay between 200 and 400 eggs per year[48], which take between 15 and 20 days to develop[49]. However, this species suffers from a development arrest syndrome, with a chromosomal abnormality causing 50% of eggs to abort during the first two weeks of development[50]. As a result, this mortality is likely to release a large amount of eDNA into the water as eggs decompose. As egg production, egg abortion and hatching would be difficult to measure without destructive sampling, we believe that female body condition was a proxy measure for egg laying.

Can eDNA concentration be used as an index of relative abundance of target organisms rather than just presence or absence? Our analyses – which provide more accurate estimates of adult and larval numbers than widely used visual count or trap-based survey methods – demonstrate that factors other than newt abundance influence the amount of eDNA present seasonally. Using eDNA to map population trends would therefore be problematical, although a relative abundance estimate between similar ponds, sampled concurrently under the same environmental conditions may be possible. Current traditional count-based population assessments from visual or trapping surveys for amphibians or other aquatic organisms suffer from the same issues, as detection rates may have poorly understood relationships to total population sizes and vary according to environmental conditions[51]. For stream fish, predictive models incorporating eDNA concentration are developing to identify detection probabilities, abundance, as well as eDNA production and discharge[52]. To apply this to population assessments of lentic, semi-aquatic amphibians, models would need to include seasonally variable DNA release and degradation rates, as well as taking into account multiple life stages. As these relationships become clearer, the role of eDNA in assessing populations is likely to become an increasingly valuable and cost-effective tool in assessing and mitigating the challenging problem of global amphibian declines.

Methods

Study Site. The study site was located at the University of Kent campus in Canterbury, UK. The site consists of eight identical ponds measuring 1 m × 2 m × 0.7 m deep constructed using PVC liner and a water volume when full of 600 L. The eight ponds are arranged in a grid pattern with approximately 3 m between each pond. All eight ponds can be considered to experience the same environmental conditions. All eight ponds had been in place for a minimum of six years at the time of the study and were allowed to be colonised naturally by the three species newts in the area[53]. All species could freely move from one pond to another and to immigrate or emigrate. Over the winter prior to the study, all eight ponds were drained, liners replaced and filled with tap water so that all ponds were identical at the start of the study.

eDNA sampling. eDNA samples were collected from the eight ponds every 14 days from 26 February through to 22 October 2015. To avoid contamination, on each occasion eDNA samples were collected prior to the population monitoring. Two eDNA collection methods were used: (1) filtration of 1 L of sample water using a 0.7 µm glass-microfiber syringe filter (Sterlitech Corporation®, Kent Washington State, USA); and (2) precipitation of DNA from a 0.09 L sample volume in an ethanol, sodium acetate solution[54]. All field equipment was sterilised using 10% bleach, UV-Crosslinker or autoclave and sealed prior to transport to the study site, and a separate set of nitrile disposable gloves were used for each sample. Due to the small dimensions of each pond, a single 1 L surface sample, collected using a polypropylene wide mouth bottle, was deemed sufficient to provide a representative sample from each pond. The bottle was rinsed with pond water and used to stir the pond as suggested by Biggs *et al.*[33] prior to being filled.

Filtered samples were collected using a 100 mL syringe. The sample was removed from the collection bottle, and then drawn through a 0.7 µm glass microfiber syringe filter. The process was repeated with the sample homogenised before filling each syringe. The process was repeated until 1 L had been filtered or two filter units had become blocked. Residual water was removed from the filter unit by passing two syringes of air through each unit. Both filter units were then sealed in bags prior to transport to the laboratory where they were stored at −20 °C until extraction.

Samples collected using precipitation in ethanol consisted of six, sterile 50 mL centrifuge tubes containing 33 mL of absolute ethanol and 1.5 mL of 3 M sodium acetate solution. All six tubes were filled from the collection bottle to make the volume in each up to 50 mL, using a sterile disposable plastic pipette. This equates to a total volume per sample of approximately 90 mL. Each sample was placed in a sealable bag for transport to the laboratory, where they were stored at −20 °C until extraction.

Population assessments. The population in each pond was assessed using aquatic funnel traps[55]. Trapping commenced in the last week of February 2015 and continued weekly until the end of October 2015, encompassing the period adult and larval great crested newts are active[56]. Traps were left in place for between 11 and 12 hours overnight depending on the season. Ventral patterns of all adults caught were photographed and used for individual identification to allow for Capture-Mark-Recapture analysis to provide weekly detection probabilities[57]. Each adult was weighed on each capture event to the nearest 0.1 g, and snout-vent and tail length measured to the nearest 1 mm to assess body condition. To avoid contamination between ponds, surveyors wore disposable nitrile gloves that were changed between ponds. Additionally all bottle trapping equipment was sterilised at the start of the season with 10% bleach and dedicated equipment was used for sampling each of the eight ponds.

Torchlight counts of larvae were also conducted from the beginning of July onwards. This allowed calibration of the counts of larvae captured in the bottle traps at the same time. Torchlight counts involve shining a 1 million candle power torch through the surface of the water after dark. The light was moved systematically from one end of the pond to the other, counting all of individuals that could be seen within the water column. Due to the size of each of the study ponds and absence of vegetation, counts could be undertaken across the entire surface area and water column of each of the ponds.

Laboratory protocol. DNA extractions were conducted in a UV sterilisable work station in a laboratory with dedicated equipment. All extractions were based on the DNeasy Blood & Tissue Extraction kit (Qiagen®, Hilden, Germany) with amended protocols as outlined in the Supplementary Material. Periodic extraction blanks for both methods were undertake through the laboratory phase of the project to check for equipment contamination, and were all negative.

Real-Time qPCR was performed on all samples in a separate lab from DNA extraction and in a dedicated UV-sterilisable work station. qPCR was performed using previously published primers and hydrolysis probe[12] and qPCR assay and cycle condition[54] using a CFX Connect Real-Time PCR detection system (BIO-RAD® Hercules, California, USA). Eight qPCR replicates were performed per sample. qPCR standards were created from a serial dilution of a great crested newt tissue extract, quantified using a Qubit® 2.0 flurometer (Life Technologies™, Carlsbad, California, USA) with Double Stranded DNA High Sensitivity Kit following manufacturers' instructions, qPCR negative controls were also included in each run. The median value for the eight qPCR replicates was taken forward into the analysis for each sample. eDNA was found in all ponds, but not in each calendar week, with concentration varying between zero and 0.00845 ngμL^{-1}. The mean R-squared value of all qPCR standard curves was 0.99 and the efficiency was 90.3%.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated through qPCR from a serial dilution of a tissue extract from a great crested newt. The LOD related to the minimum concentration amplification was observed, while the LOQ was assigned to the minimum level that exhibited a high degree of conformity between qPCR replicates[24]. The LOD was found to be less than 10^{-7} ngμL^{-1}, with an LOQ of 10^{-5} ngμL^{-1}.

Great crested newt eDNA was detected in some or all ponds on each survey occasion. Eleven out of 200 eDNA samples analysed returned as negative. Negative results were split between both survey methods and were only found when eDNA concentrations were low either towards the start or end of the study.

Environmental Data. Mean temperature as well as UV levels for the 14 days between sampling were generated for the study site as a whole. Air temperature was recorded from the site hourly using a Tinitag® Plus 2 – TGP-4017 (Gemini Data Loggers, Chichester, UK) commencing on the 30 January. UV was recorded on a TR-74Ui – Illuminance UV Recorder (T&D Corporation® Nagano, Japan) at hourly intervals, from 17 February. An indication of the level of rainfall that occurred between each survey period was collected using a standard rain gauge, emptied at the time of the visual surveys.

Analysis. Losses of body mass during the breeding season are associated with egg deposition (females), spermatophore production (males) and utilization of fat reserves for breeding activity. Body condition estimates were generated using the Scaled Mass Index (SMI)[34]. The mean of the SMI values for all individuals caught each week were taken to produce each weekly value. SMI values could only be generated until the middle of July due to low adult numbers caught beyond that point. This was done for males and females separately as well as both sexes combined.

The Cormack-Jolly-Seber model and Program MARK[57] were used to generate a detection probability each week for adults captured in traps. The best fitting model was phi(.)p(t), or constant survival with variable detection probability. Detectability varied each week and ranged from 0 to 1 with the majority of results falling between 0.3 and 0.6, with outliers from this range only found in weeks when few individuals were caught. A single detection probability was generated for the larvae, using torchlight counts, as using capture mark recapture was not a viable option for larvae. Using ponds with high visibility, which allowed the entire pond to be observed, the number of larvae captured in traps was divided by the number of larvae counted in the torchlight surveys. This approach is appropriate in the case of this study due to the small size of the ponds allowing the entire pond to be searched by torchlight. A fixed detection probability of 0.39 was used in all weeks for two reasons. Firstly, the low number of individuals in the last few weeks of the study skewed detectability estimates. Secondly, torchlight counts only started on 9 June, after the first larvae were caught in traps, therefore no detection probability could be generated for the weeks before the introduction of torchlight counts. The population size for each pond in each week was estimated by multiplying the number of newts caught in traps by the reciprocal of the detection probability[21]. Population estimates and body condition scores are only included in the analysis for the weeks eDNA was collected.

Statistical analysis. eDNA concentrations were transformed prior to analysis using $y = \log10\,(x + 0.0001)$ to ensure normality. All statistics were conducted using linear mixed effect models (LMM)[58] using R version 3.1.3[59] and package nlme[60], LMM were chosen to account for the repeated measures on the same ponds through the season (treated as a random effect). Akaike's Information Criterion (AIC) was used to assess support for different models using package MuMIn[61]. Models with a ΔAIC of ≤ 2 were considered to have substantial support, while models with a ΔAIC of ≤ 7 were considered to have some support[62]. Using the full set of models, Akaike importance weights for predictors were calculated as measures of parameter importance, by summing the Akaike weights for each model containing that variable[62,63]. Parameters were classed as strongly supported by our models if they were significant in all strongly supported models (ΔAIC of ≤ 2) and had a cumulative Akaike weight of > 0.75[62]. Parameters were considered somewhat supported if they were significant in any of the strongly supported models (ΔAIC of ≤ 2) regardless of Akaike weight[62].

Two models were run, because different factors potentially influence eDNA concentration at different times of year: (1) a model encompassing the core adult aquatic period (26 February to 18 June); and (2) a model encompassing the post-breeding season when most adults will be on land (18 June to 22 October). A single model would be inappropriate because estimates for body condition were only available for those weeks when adults were in the ponds in high numbers, and would lead to a high degree of non-random missing data biasing the output. The first set of models therefore incorporated the breeding season (i.e. 26 February to 18 June), and comprised nine eDNA sampling occasions across 18 weeks. These models were constructed with "Pond" as the random variable to account for repeated sampling, and combinations of adult abundance, larval abundance, male body condition, female body condition, combined body condition, calendar week, collection method, air temperature, water temperature, rainfall and UV included as covariates. All variables were treated as continuous covariates with the exception of collection method which was nominal. Correlation coefficients were examined for covariates included in all strongly supported models (ΔAIC of ≤ 2), a pair of covariates were considered to be highly correlated with a correlation coefficient of >0.7[64]. This was found to be the case for UV and female body condition ($r = 0.868$ in the top model), and as a result UV was excluded from the analysis. No other pairs of covariates were found to be above this threshold.

The second set of models explored variation in eDNA concentration outside the breeding season (i.e. 18 June to the 22 October), including ten eDNA sampling occasions across 19 weeks, with "Pond" again used as the random variable to account for repeated sampling. Adult abundance, larval abundance, eDNA collection method, air temperature, rainfall, UV, and calendar week, were all included as covariates. All variables were treated as continuous covariates with the exception of collection method which was nominal. Correlation coefficients were examined for covariates included in all strongly supported models (ΔAIC of ≤ 2), a pair of covariates were considered to be highly correlated with a correlation coefficient of >0.7[64]. This was found to be the case for UV and calendar week ($r = 0.960$ in the top model), and as a result UV was excluded from the analysis, no other pairs of covariates were found to be above this threshold.

Collection method (i.e. ethanol precipitation versus glass-microfiber syringe filtration) was included as a variable in all of the models to check that there was no method-related bias. This was subsequently corroborated, with paired sample analysis showing no difference in eDNA extract concentration between the two methods (Buxton *et al.* in prep.).

Ethics Statement. The experimental procedure was approved by the School of Anthropology and Conservation (University of Kent) Research and Ethics Committee, with disturbance and handling of live animals undertaken under EPS Licence 2014-5025-CLS-CLS issued by Natural England, in accordance with the conditions of the licence.

Data accessibility statement. This statement confirms that, should the manuscript be accepted, then data supporting the results will be archived in the Kent Academic Repository.

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Author Contributions

A.B., R.G. – experimental design, A.B., N.Z., R.G. – traditional field work, A.B. - eDNA field work, A.B., J.G. laboratory design, A.B.–laboratory bench work, A.B., R.G. statistical design, A.B., N.Z. analysis, A.B. Core Text, R.G., J.G. text review and input.

Additional Information

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