

**Large-scale controls on the distribution and
breeding ecology of the common frog
(*Rana temporaria*) in an upland landscape**

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**A thesis submitted to University of Wales
for the higher degree of Doctor of Philosophy**

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Summary

There is limited research regarding amphibian distribution and status in rural upland habitats in Britain. Previous studies suggest potential influences on distribution and abundance arising from habitat modification and acidification. However, the extent to which these factors influence distribution at the landscape level is unclear. This study aimed to evaluate whether particular features of the aquatic and terrestrial habitat influence distribution and breeding ecology of common frogs (*Rana temporaria* L.) in rural upland areas. In particular, the extent to which aquatic and early terrestrial life-stages might be impacted upon by the quality of their respective habitats was investigated. Common frogs were found to be widespread and ubiquitous throughout the study area. No evidence was found to suggest that any specific biotic or abiotic feature of either the aquatic or terrestrial habitat significantly influenced distribution and relative abundance of breeding adults. However, reproductive success was found to be significantly impacted upon by acidity in coniferous and hilltop habitats. It is probable that juvenile recruitment at some ponds is chronically reduced and populations are maintained through immigration. Recruitment in improved habitats was highly variable between ponds and between years, although it was not possible to elucidate precise controls on larval populations in the natural habitats studied. Significant differences in physical condition at metamorphosis among wild populations were demonstrated. This may signify possible differences in future growth, life-fitness and dispersal ability between populations, or at least between natal ponds. Dispersal by new juveniles is potentially affected by the vegetation structure surrounding breeding ponds. Intra- and interspecific differences in behaviour regarding microhabitat use were demonstrated. Rural upland habitats can be valuable habitats for amphibians and the common frog in particular. However, further research is required to better understand rates of recruitment and immigration and how terrestrial habitats mediate juvenile survival and dispersal.

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Chapter 1

Introduction

1.0 Summary

Relatively little has been published regarding amphibian distribution and status in rural upland habitats. In central Wales, previous studies show variable pond occupancy. Presence in ponds in improved areas appeared to be associated with particular features of the surrounding terrestrial habitat suggesting that populations may be affected by rural upland land uses. Dependence upon both aquatic and terrestrial habitats means that potential influences on different life-stages need to be understood. Several major factors are thought to affect amphibian populations significantly and may have contributed to the worldwide decline in amphibians. Climate change, ultraviolet radiation, disease and alien introductions are unlikely to affect current upland distribution at the landscape level. Predation and environmental contaminants could potentially affect populations. Habitat modification by agriculture and forestry together with acidification probably warrant consideration for initial comprehensive examinations of distribution and breeding ecology in rural upland areas. Habitat modification has caused most of previous population declines nationally. Acidity is known to influence distribution and can impact negatively upon reproductive success. Some ponds in many upland areas are naturally acidic or became acidic following anthropogenic acidification. This PhD aims to evaluate whether particular features of the aquatic and terrestrial habitat influence distribution and relative abundance of common frogs (*Rana temporaria*, Linnaeus, 1758) in rural upland areas. It addresses to what extent aquatic and early terrestrial life-stages might be impacted upon by the quality of their respective habitats. Specifically, it examines whether habitats alter distribution and abundance patterns of breeding adults, reduce embryo and larval survival and juvenile recruitment, affect physical condition of new juveniles, which could have implications for future life fitness and affect survival at juvenile emergence and subsequent dispersal.

1.1 Amphibians in rural upland habitats

Compared to lowland areas there are relatively few published studies regarding distribution and status of amphibians in British upland habitats. These areas are often under-surveyed despite the fact that the areas' climate and geology favour a large number of water-bodies in western and upland Britain (Swan and Oldham, 1993). Consequently, it is not clear to what extent distribution and breeding ecology may be affected by differences in habitat quality in these areas. In central Wales, for instance, ponds vary considerably in size, hydroperiod (the length of time that the pond holds water), water chemistry and in the abundance and richness of invertebrates and vegetation (Slater, 1993). Terrestrial habitats include improved swards for sheep and cattle rearing, semi-improved rough grazing, unimproved grassland, heath or bog on the hilltops and commercial conifer forests. This diverse range of pond types and terrestrial habitats provide an opportunity for possible influences on distribution and abundance. There are, however, limited data regarding the distribution and breeding ecology of amphibians in the region. For example, the distribution of common frogs (*Rana temporaria*) was described as widespread and abundant in the mid-1980s (Griffiths, 1986) but there are no data available prior to this. Presence-absence surveys of ponds for common frogs show occupancy to vary between 18% and 70% (Knight, 1989; Williams, 1995, Slater, unpublished data). Williams (1995) proposed that frogs might be less abundant in areas of improved pasture and more abundant on semi-improved or unimproved upper slopes and hilltops. Where present in improved pasture, a positive association with adjacent woodland for frogs, smooth newts (*Triturus vulgaris*, Linnaeus, 1758) and palmate newts (*Triturus helveticus*, Razoumowsky, 1789) was suggested. The study focused on larval numbers which is not always the best indicator of frog presence or abundance (Griffiths and Raper, 1994). Dispersal, mortality and pond structure will all affect the capture rate. Similar differences in occupancy patterns between these rural habitat types were found by Swan and Oldham (1993), however. This could suggest that factors associated with some rural upland land uses might impact negatively upon amphibian populations.

1.2 Potential controls on amphibian populations

Amphibians are dependent upon both aquatic and terrestrial habitats so populations may be limited by factors affecting one or both environments. Therefore, it is

necessary to understand potential influences in both habitats on different life-stages. There are several major factors that are thought to have contributed significantly to worldwide amphibian population declines. These include habitat modification, predation, acidification and environmental contaminants, disease, increased ultraviolet radiation and climatic effects (reviewed by Alford and Richards, 1999; Beebee and Griffiths, 2005; Collins and Storfer, 2003). Each factor may operate singly or interact with others at each location. In Britain, all of the above mentioned controls may potentially affect populations. Some factors may have more relevance in the future, for example, climate change, while others such as acidification and habitat modification, will vary in significance according to the geographical area. Identification of possible controls on populations may be observed initially through effects on breeding ecology and distribution patterns.

It is likely that only a few of these factors would be significant influences upon current native amphibian populations at the landscape level. For instance, global temperatures have risen by about 0.6°C over the last 100 years and this is reflected in a rise of almost 1°C in central England. To date, notable climatic changes in Britain have been wetter winters and slightly drier summers (Hulme *et al.*, 2002). These trends are set to continue according to recent climate change predictions with proposed annual temperatures increases of 2 - 3.5°C by 2080. As a result, considerable regional and seasonal variation in the magnitude of temperature increases and precipitation changes over Britain is expected (Hulme *et al.*, 2002). Effects on breeding ecology and distribution are therefore likely to vary significantly according to species and location.

Effects might be particularly marked in southern England where population persistence will depend on the ability to tolerate and reproduce successfully under markedly altered temperature and moisture conditions. Along the English-Welsh border, predicted precipitation decreases in spring could be as great as 15% (Hulme *et al.*, 2002). Ponds in these warmer and/or drier areas may dry up faster, causing mass mortality of larvae and limiting recruitment. Even if ponds retain water, reduced water levels can affect food supply, larval density, predation risk and size at metamorphosis (Pearman, 1995). Size at metamorphosis has significant influence on future growth, survival and reproductive success (Berven, 1990; Werner, 1986). For terrestrial amphibians, hot, dry summers may potentially result in altered distribution and

abundance of invertebrate prey (e.g. Morecroft *et al.*, 2002) and type and structure of vegetative cover (e.g. Werkman and Callaghan, 2002). Additionally, rates of evaporative water loss experienced in such conditions may increase mortality directly or indirectly through limited activity, mobility, ability to evade predators and food supply (Carey and Alexander, 2003).

If conditions do not cause mortality they may still affect reproductive success. Dry summers that reduce foraging prior to hibernation and warm temperatures during hibernation can cause a loss of body condition which reduces female fecundity (Reading and Clarke, 1995). Warmer, wetter winters may provide increased foraging opportunities but only if plant species that provide foraging cover and prey species are also available at this time. Thus in the long term, species presence in an area will be affected by the spatial and temporal responses of other taxa (Harrington *et al.*, 1999). Predicting future distribution patterns will also require knowledge of local land use, habitat availability and dispersal ability into new habitat areas, as well as climatic requirements or tolerances (Berry *et al.*, 2002).

Although, climate change has been implicated in amphibian population declines, direct links have not been proved (Alexander and Eischeid, 2001; Pounds and Crump, 1994). Potential effects on British amphibians have received relatively little attention. There are, however, suggestions of altered breeding phenology, i.e. the initiation of the breeding season in relation to environmental cues. Early arrival at breeding ponds in southern England by all three *Triturus* species and early spawning by introduced edible frogs (*Rana kl. esculenta*, Linnaeus, 1758) and natterjack toads (*Bufo calamita*, Laurenti, 1768) corresponded significantly to a rise in early spring temperatures (Beebee, 1995). Chadwick (2003) also demonstrated a shift to earlier immigration dates for *T. helveticus* and *T. vulgaris* in central Wales. Again, this corresponded to increased average air temperatures immediately prior to the breeding season. In contrast, a lack of similar significant effect was demonstrated for *R. temporaria* by Beebee (1995) and for common toads (*Bufo bufo*, Linnaeus, 1758) by Reading (1998). Similar findings for temperate zone amphibians in North America (Blaustein *et al.*, 2001; Gibbs and Breisch, 2001) suggest that the claim of earlier breeding due to climate change is premature (Blaustein *et al.*, 2001). Climate predictions, however, suggest that the trend for earlier onset of spring is also to set to continue (Hulme *et al.*,

2002) and more long-term research will be required to determine more precisely climatic effects on breeding phenology.

Current climatic conditions are unlikely to have resulted in altered distribution and abundance to date. Evaluating the degree of any future changes will require knowledge of how present distribution and breeding ecology is affected by current aspects of aquatic and terrestrial habitats. For example, this might be a low pond density or a high proportion of shallow and/or late successional ponds which could result in reduced recruitment and/or poor juvenile condition from permanent ponds.

Levels of ultraviolet-B radiation (UV-B) (280-320 nm) at the earth's surface have increased in recent years (Kerr and McElroy, 1993; Middleton *et al.*, 2001). Whether UV-B significantly impacts upon amphibian populations is the subject of much scientific debate (Heyer, 2003). Research into effects of UV-B irradiation upon native species have also shown variable results (e.g. Hakkinen *et al.*, 2001; Pahkala *et al.*, 2000; 2003). The observed discrepancies between findings may reflect differences in experimental conditions and/or inter- and intraspecific variation in sensitivity (Pahkala *et al.*, 2003; Pahkala *et al.*, 2002). It is unlikely that ambient UV-B levels are harmful to British amphibians. The actual UV-B dose received by embryo and larval stages is dependent upon many variables. These include the amount of radiation incident to the water surface (which varies seasonally and annually), water chemistry, location of eggs and degree of vegetative shading (Corn and Muths, 2002). The characteristics of many natural waters mean that UV-B is rapidly attenuated and unlikely to pose a significant threat (Palen *et al.*, 2004). Additionally, levels of cloud cover associated with areas of high rainfall mean that current effects upon many upland populations are probably negligible.

Relatively few alien species that pose threats to native amphibians have been introduced to Britain. Non-native herpetofauna that predate or compete with native species presently exist in localised populations and include the African clawed toad (*Xenopus laevis*, Daudin, 1802), the alpine newt (*Triturus alpestris*, Laurenti, 1768) and the Italian crested newt (*Triturus carnifex*, Laurenti, 1768). Hybridisation occurs where *T. carnifex* is sympatric with *T. cristatus* and offspring have low viability. Although no significant impact on the native species has yet been observed, the

potential exists for significant reduction in *T. cristatus* reproductive success (Beebee and Griffiths, 2000; Swan and Oldham, 1993). *X. laevis* occurs in south Wales where a restricted growing season has so far limited reproduction and recruitment (Measey, 2001). Where conditions favour longer growth periods, *X. laevis* undergoes explosive reproduction (McCoid and Fritts, 1980, cited in Lobos and Jaksic, 2005). The species is also highly invasive, although impacts on native amphibians are unclear (Lobos and Jaksic, 2005). Climate changes may increase breeding success and dispersal resulting in increased competition and hybridisation with native species.

Predatory fish, may significantly affect reproductive success and distribution of some species. For example, *T. cristatus* larvae inhabit the open water column and are particularly vulnerable to predatory fish (Beebee and Griffiths, 2000; Cooke and Frazer, 1976). Similarly, *T. vulgaris* often occurs in the same habitats (Cooke and Frazer, 1976) and metamorphic success in fish ponds is strongly affected by presence of aquatic vegetation (Baker and Laverick, 1986). *R. temporaria* is less inhibited by fish but occupancy may be increased where adjacent ponds are fish-free (Baker and Laverick, 1986).

The incidence of reported 'red-leg' outbreaks among common frog populations is increasing, particularly in northern and western England in recent years, although reports from rural upland areas are few (<http://www.froglife.org/Disease.htm>). Dead and dying frogs typically present skin lesions, limb necrosis and/or haemorrhaging. Other life stages are also affected (Cunningham *et al.*, 1993). The disease was initially attributed to infection by the bacterium *Aeromonas hydrophila*. In 1993, a ranavirus (Iridoviridae) was isolated for the first time from diseased *R. temporaria*. The disease appears to result from a viral infection, often followed by secondary infection by *A. hydrophila* (Cunningham *et al.*, 1996). Reported outbreaks increased between 1985 and 1991. Most epidemics were concentrated in the southeast of England and mostly affected garden ponds (Cunningham *et al.*, 1993). It is not known how widespread the disease is across Britain but the incidence of reported outbreaks is increasing, particularly in northern and western England in recent years (<http://www.froglife.org/Disease.htm>). It is possible that the greater number of reports reflects increased public awareness. This would also explain the greater incidence at urban garden ponds. Alternatively, epidemics may be both higher in urban areas and

genuinely increasing. Outbreaks possibly result from environmental stress (Beebee, 1996a) and/or reduced immunity through population isolation. Although mortality during outbreaks can be high, populations appeared to recover quickly (Beebee, 1996a). It is unlikely that it has affected the distribution of wild populations to date although increasing incidence gives cause for concern.

Amphibians have demonstrated varying degrees of sensitivity to environmental contaminants (e.g. Calevro *et al.*, 1998; Hayes *et al.*, 2003). In agricultural areas, common contaminants are pesticide, herbicide and fertiliser mixtures but the effects of only a few of these toxicants on native species have been studied (e.g. Cooke, 1972a; Cooke, 1977; Cooke, 1981). Nitrate from fertilisers, but also potentially from anthropogenic deposition, could potentially affect rural upland populations. Determination of long-term effects on breeding ecology and distribution of wild populations will be difficult and compounded by interspecific differences in larval sensitivity (Baker and Waights, 1993; Johansson *et al.*, 2001). Toxicant concentrations can fluctuate significantly over very short-time periods due to run-off, for instance (Hayes *et al.*, 2003). Areas of high rainfall (or frequent irrigation) are likely to experience greater initial run-off which will vary in solutes concentrations according to soil type (Hornung, 1984). Once in water, ammonium nitrate concentrations can change with temperature, oxygen content and pH (de Solla *et al.*, 2002). The influence of agricultural inputs would, however, warrant closer investigation should presence and/or relative abundance be reduced in improved habitats.

The majority of declines of native amphibian species have largely been attributed to habitat modification (Beebee, 1975; Beebee, 1976; Cooke, 1972b; Cooke and Scorgie, 1983; Hilton-Brown and Oldham, 1991) and agriculture and afforestation are possibly major influences upon amphibians in rural upland areas. Acidification is another potentially significant factor affecting distribution in upland habitats. Although some ponds are naturally acidic, for example, those overlying peat, many additional parts of upland Britain became increasingly acidified during the twentieth century. Acidic atmospheric inputs arising from increased sulphur and nitrogen emissions were precipitated onto areas with poor buffering capacity. Habitat modification, particularly conifer afforestation, exacerbated the acidification process by increasing the level of pollutants leached into surface waters (Gee, 1990). Given that relatively little is known

the regarding distribution and breeding ecology in rural upland areas and there is already a suggestion of possible influence by habitat type, initial examination should consider the potential influences of these two factors on different life stages.

1.3 Habitat modification

Loss of wetland habitat, in particular, was a major cause of declines of native species. Breeding ponds disappeared through infilling, land drainage and neglect as agricultural practices changed and urbanisation increased (Cooke, 1972b; Cooke and Scorgie, 1983). Amphibian presence is, however, significantly influenced by the spatial distribution of ponds. As inter-pond distance decreases the likelihood of pond occupancy increases and colonisation is faster if adjacent ponds are already occupied (Marsh *et al.*, 1999). If pond density is at a level where inter-pond distances exceed dispersal capabilities, there may be a significant effect on distribution. Dispersal limits of British amphibians are unknown but some species are known to move as far as 1 km from the breeding site (Oldham and Swan, 1997 and references therein). An estimated 38% of 132 surveys conducted nationwide between 1982-1991 have a reported mean inter-pond distance >1 km. As only a proportion of ponds are typically occupied, actual distances between breeding ponds are likely to be considerably higher (Oldham and Swan, 1997). It is probable that the presence of several small ponds within close proximity may be more important in predicting species presence than the presence of a single larger water-body (Mann *et al.*, 1991).

Features of the ponds themselves also influence amphibian occupancy and abundance. These include pond size and depth (Kutka and Bachmann, 1990; Loman, 1988; Rowe and Dunson, 1993), hydroperiod (Brodman *et al.*, 2003; Eason and Fauth, 2001), shading (Loman, 1988; Sztatecsny *et al.*, 2004), presence of predatory fish (Baker and Halliday, 1999; Beebee, 1996b; Clemons, 1997; Hecnar and M'Closkey, 1997; Martinez-Solano *et al.*, 2003), water chemistry (Beebee, 1983; Rowe and Dunson, 1993), including acidity (Eason and Fauth, 2001; Leuven *et al.*, 1986) and vegetation cover (Cooke and Frazer, 1976; Hazell *et al.*, 2001; Sztatecsny *et al.*, 2004). It is, therefore, feasible that differences in pond occupancy or relative abundance in upland areas might be explained by biotic and/or abiotic features of the available aquatic habitats.

Many surveys only examine whether ponds are used by adults for reproduction but it is also important to assess how offspring survival might be affected by aspects of pond quality. All of the above factors could potentially influence juvenile recruitment either directly by reducing survival before metamorphosis or indirectly through effects on body size. Body size at metamorphosis has important implications for future amphibian life-history. As size increases, the likelihood of predation decreases (Werner, 1986), and fecundity (Gibbons and McCarthy, 1986; Reading and Clarke, 1995; Tejedo, 1992) and breeding success increase (Gibbons and McCarthy, 1986; Gittins *et al.*, 1980). Responses to parasitic infections, activity levels and feeding are all potentially affected by a reduction in body size (Goater *et al.*, 1993; Goater and Ward, 1992; Wheater, 1986; Wilbur, 1997). Survival rate, timing of metamorphosis and size at emergence are all influenced by interactions between environmental factors and development stage. For instance, changes in the amount or quality of available food, larval density, temperature, predator presence and acidity can all affect growth and development rate (Audo *et al.*, 1995; Brady and Griffiths, 1995; Kupferberg, 1997; Lardner, 2000; Newman, 1998; Skelly, 1992). Metamorphosis has received relatively little attention in field situations (Loman, 2002). Consequently, it is not generally clear to what extent particular field conditions actually induce significant differences in body size and 'condition' (i.e. length/weight ratio) and whether patterns of adult distribution reflect possible differences in recruitment.

Adults and juveniles are largely terrestrial and often only return to ponds to breed. Therefore, even if distances between suitable ponds are sufficiently short, the intervening terrestrial habitat has to permit movement between breeding ponds and be suitable for year-round habitation. Consequences of habitat modification are possible losses of cover, refuges and/or food availability which could result in absence or reduced abundance. Results from the National Amphibian Survey (Swan and Oldham, 1993) found that within improved grassland or arable areas, amphibians were generally more dependent on adjacent habitat features, such as patches of scrub, gardens and ditches. This is because modified areas are likely to be more impoverished in terms of available refuges and hibernation sites compared to intrinsically diverse woodland and rough grassland habitats (Swan and Oldham, 1993). Inimical habitat leads to isolation of amphibian populations. For example, Hitchings and Beebee (1997) found levels of marked genetic differentiation between urban *R. temporaria* populations despite their

close proximity. Lack of migration between urban populations resulted in genetic drift leading to a reduction in fitness and inbreeding depression (demonstrated by greater developmental abnormality and mortality of larvae). The degree of genetic relatedness between populations correlated closely with geographical patterns of development. Reduced diversity and fitness were also demonstrated for *B. bufo* in the same area (Hitchings and Beebee, 1998). Roads, in particular, may have significant impacts on distribution by reducing the probability of habitat occupancy (Vos and Chardon, 1998). Road traffic can be a major cause of mortality for migrating amphibians (Fahrig *et al.*, 1995) and may contribute to local population declines (Cooke and Sparks, 2004). Road density potentially has significant implications for genetic exchange between amphibian populations (Vos *et al.*, 2001). Reduced genetic diversity has occurred within *R. temporaria* populations isolated by motorways within relatively few generations (Reh and Seitz, 1990).

To date, surveys of native species have often related amphibian presence to the importance of terrestrial features within 100 m of the pond (Beebee, 1985; Marnell, 1998; Stumpel and van der Voet, 1998; Williams, 1995). However, dispersing juveniles often cover greater distances (Dole, 1971; Kupfer and Kneitz, 2000; Oldham, 1985) and common frog and common toad (*Bufo bufo*) adults are capable of moving up to nearly 1 km to reach other ponds (Baker and Halliday, 1999) or summer habitats (Heusser, 1969; Sinsch, 1988). Moreover, occupancy within 100 m of the pond can be highly seasonal and vary depending upon sex (Regosin *et al.*, 2003). Swan and Oldham (1993) evaluated terrestrial habitat to within 500m but received little data from surveyors regarding habitat at 1 km. Time and access difficulties probably hindered habitat recording at greater distances for many observers. Baker and Halliday (1999) assessed the number of 250 m grid squares containing buildings, woodland and riparian habitat within 1 km of agricultural ponds. More precise assessments of area cover and categorisation of woodland type or other vegetation were not made. Quantification of the total area covered by distinct habitat types surrounding ponds, such as deciduous, coniferous or mixed woodland, and improved, semi-improved or acid grassland could provide a better indication of potential influence on presence and relative abundance in rural upland areas. The proximity of particular habitats to breeding ponds may also be a potential indicator of the relative importance of particular features. As habitat up to at least 1 km away is likely to be ecologically

relevant, it would be useful to determine whether habitat assessments should consider the importance of features at distances greater than 100 m from the pond.

Evaluating the importance of particular habitat features also needs to consider whether there might be significant differences in terrestrial habitat requirements between adults and juveniles. This could arise due to possible differences in food requirements or risk of dehydration and predation, for example (Rothermel and Semlitsch, 2002). Juveniles are often the major dispersers in populations and are essential to the sustenance of local populations and metapopulations (Berven and Grudzien, 1990; Kupfer and Kneitz, 2000; Sinsch, 1992; 1997). Dispersal abilities of juveniles in different habitats are, however, generally poorly understood and considerably more research is required into dispersal capabilities and habitat use. It is expected that different habitat characteristics will have either a positive or negative effect on dispersal (Stevens *et al.*, 2004). Lack of protective cover in some habitats can significantly increase predation (Denton and Beebee, 1994) therefore, the type and composition of the terrestrial habitat surrounding breeding ponds may greatly affect mortality rates of emigrating juveniles. Rate of dispersal can be significantly affected by the type of terrain (Oldham, 1985; Stevens *et al.*, 2004). It is generally unclear whether certain habitats are actively avoided or selected by new juveniles, for example to reduce the risk of predation or dehydration. For reproductive output at ponds, i.e. successful metamorphosis, to effectively contribute to a population, dispersing juveniles need to be able to suffuse into surrounding habitats. To date, relatively few studies have examined the effects of terrestrial vegetation on emergence and early dispersal of juvenile amphibians, particularly native species. Moreover, little is known regarding potential differences in habitat use between different terrestrial life-stages.

1.4 Acidification

Acidity is known to influence amphibian distribution of British amphibians. For example, smooth newts (*Triturus vulgaris*) and great crested newts (*T. cristatus*) generally avoid low pH ponds whilst the palmate newt (*T. helveticus*) is more tolerant of soft water conditions (Cooke and Frazer, 1976; Denton, 1991; Yalden, 1986). As a result there can be marked differences in distribution in some areas which generally reflect geological changes. However, habitat use can vary across a species range and in

southern Norway *T. cristatus* does not avoid acidic ponds (Dolmen, 1980). Similarly, within Britain *R. temporaria* avoids low pH ponds in some lowland areas (Beebee, 1983) but breeds in acidic ponds in some upland areas (Aston *et al.*, 1987; Knight, 1989).

Acidified waters undergo significant chemical changes that broadly include reduced pH, conductivity and ionic strength with concomitant increases in aluminium, ammonium and sulphate (Leuven *et al.*, 1986). These changes in water chemistry are known to be toxic to aquatic stages of native species although the degree of toxicity is dependent upon complex interactions between water chemistry variables (Freda, 1991; Horne and Dunson, 1995) and is further influenced by stage of development (Beebee, 1986) and differences in acid tolerance (Glos *et al.*, 2003). Negative effects have been demonstrated on embryo survival, larval development, growth and behaviour of common frogs and smooth and palmate newts (Beattie *et al.*, 1991; Cummins, 1986; 1988; Griffiths *et al.*, 1993; Tyler-Jones *et al.*, 1989). Although, some amphibians do not necessarily avoid breeding in acidic habitats, distribution and/or abundance could be significantly affected through lack of recruitment as reproductive success is often impacted upon by low pH conditions (Bunnell and Zampella, 1999; Karns, 1992).

Agricultural improvement (and conifer afforestation) coupled with local variation in soil and bedrock results in local variation in acid-sensitivity (Hornung *et al.*, 1990) and a mix of acidic and non-acidic ponds within the landscape. Therefore, patterns of pond occupancy, relative abundance or recruitment might be expected to reflect water quality. Reduced abundance or recruitment might be particularly associated with presence of hilltop vegetation types such as acid grassland, acid heath and blanket bog, and/or conifer plantation. No other studies relating abundance or recruitment to acidic habitat features among native upland populations have been found. Abundance or recruitment may also be significantly affected by the mode of spawn deposition. It is possible that mortality may be lower within communal egg masses compared to isolated spawn clumps or spawn strings due to possible differences in buffering against external environmental conditions. Studies comparing acid-associated mortality for amphibian eggs deposited singly, within lone strands or clumps or in communal masses are unknown. Significant differences in mortality rates resulting from

spawning behaviour could have implications for inferring potential effects on recruitment and possible population declines to different species.

Following reduced sulphur deposition, some waters in Britain and Europe have shown signs of chemical reversal, that is increased pH (Evans and Jenkins, 2000; Folster and Wilander, 2002; Tipping *et al.*, 2000). However, there has only been a patchy recovery of biota to date (Soulsby *et al.*, 1997; Tipping *et al.*, 2002) suggesting that there may be a lag in biological recovery from acidification. To date, improved amphibian reproductive success corresponding to natural recovery from acidification does not appear to have been demonstrated. In light of chemical reversal in some areas, a reassessment of pond occupancy and reproductive success at current acidity levels is warranted.

1.5 Research aims

The overall objective of this PhD was to evaluate which biotic and abiotic factors within aquatic and terrestrial habitats may potentially control distribution of amphibians in rural upland landscapes. The common frog (*Rana temporaria*) was used as a model organism to test hypotheses that evaluated the effects of habitat quality on different life-stages. Chapter 2 tests whether abiotic and biotic features of both aquatic and terrestrial habitats can be related to the presence and relative abundance of breeding adults and if the surrounding terrestrial habitat varies in influence according to the scale of habitat assessment. Potential influences on aquatic and early terrestrial life-stages are investigated in Chapters 3, 4 and 5. Chapter 3 determines whether the current acidity levels negatively impact upon reproductive success in natural ponds. Specifically, the potential for a significant difference between embryo survival rates in acidic and circumneutral ponds is tested and the possibility of differences in acid-tolerance is assessed. In the absence of possible biological recovery and acid-tolerance, mortality rates are evaluated to determine if recruitment is potentially negatively affected by spawning behaviour and/or population size. The influence of aquatic habitats on juvenile recruitment is assessed under natural conditions in Chapter 4 through comparison of acidic and circumneutral ponds and between permanent ponds in general. Possible effects on the number and the physical condition of metamorphs are evaluated and related to differences in aspects of the aquatic environment with a

view to potential implications for future survival and fitness. Chapter 5 tests whether the terrestrial habitat could potentially limit survival and dispersal of new juveniles. Patterns of metamorph emergence are assessed in relation to type and structure of marginal and bankside vegetation. The null hypothesis being that the vegetation surrounding ponds has no significant influence on the direction of emigration. Potential influence of surrounding vegetation on direction and rate of early juvenile dispersal and microhabitat use, under contrasting microclimatic conditions, are examined under field and experimental conditions respectively. All chapters are self-contained as potential papers for submission to journals and have their own reference lists.

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Chapter 2

Factors affecting distribution and abundance of common frogs (*Rana temporaria*) in a rural upland landscape

2.0 Summary

Amphibian population declines worldwide have focused attention on the quality of both aquatic and terrestrial habitat and their effects upon distribution and abundance. Upland areas can comprise a diverse range of terrestrial and aquatic habitat types but little is known of how habitat quality affects amphibian populations across these landscapes. This study aimed to determine if the distribution and abundance of common frogs (*Rana temporaria*) is related to the aquatic and/or terrestrial habitat attributes in an upland area and if the importance of local terrestrial habitat is dependent upon the scale of assessment. Presence/absence and relative abundance were determined from breeding populations for ponds located within a $\approx 900\text{km}^2$ area of central Wales. Data for 66 ponds were related to pond quality (size, depth, vegetation cover, water chemistry, etc.) and land cover type within 250m, 500m, 750m and 1000m radii of the pond margin. *R. temporaria* was ubiquitous and abundant within the study area. Breeding ponds varied considerably in size, depth, vegetative cover and chemical composition, and were located within acid grassland, heath and bracken (hilltops and upper slopes), improved grassland (lower slopes) and conifer plantation. Some biotic and abiotic pond features were associated with the quality of adjacent terrestrial habitat. However, no significant relationships were detected with frog presence or abundance and aquatic or terrestrial features (at any scale). Populations in this landscape probably benefit from a relatively high pond density and a terrestrial habitat matrix that facilitates movement between breeding sites and summer habitats. More research is required to quantify i) the variation in potential recruitment at ponds of different type and ii) adult migration and juvenile dispersal through terrestrial habitats.

2.1 Introduction

Amphibians inhabit both aquatic and terrestrial habitat and populations can be affected by differences in quality of one or both habitat types. Features of aquatic habitats that can influence presence and/or abundance include pond size and depth (Kutka and Bachmann, 1990; Loman, 1988; Rowe and Dunson, 1993), hydroperiod (the length of time that the pond holds water) (Brodman *et al.*, 2003; Eason and Fauth, 2001), shading (Loman, 1988; Sztatecsny *et al.*, 2004), presence of predatory fish (Baker and Halliday, 1999; Beebee, 1996; Clemons, 1997; Hecnar and M'Closkey, 1997a; Martinez-Solano *et al.*, 2003), water chemistry (Beebee, 1983; Rowe and Dunson, 1993), including acidity (Eason and Fauth, 2001; Leuven *et al.*, 1986), and vegetation cover (Cooke and Frazer, 1976; Hazell *et al.*, 2001; Sztatecsny *et al.*, 2004). Important positive aspects of terrestrial habitat include woodland (Findlay *et al.*, 2001; Kolozsvary and Swihart, 1999; Laan and Verboom, 1990), adjacent wetlands (Baker and Halliday, 1999; Brodman *et al.*, 2003; Marsh *et al.*, 1999; Marsh *et al.*, 2000; Sjogren-Gulve, 1994), scrub (Beebee, 1977; Beebee, 1985) and terrestrial shelter, e.g. long grass or leaf litter (Hazell *et al.*, 2001; Loman, 1988; Marnell, 1998). Conversely, urban cover (Beebee, 1979; Knutson *et al.*, 1999; Rubbo and Kiesecker, 2005) and roads and/or railway lines (Findlay *et al.*, 2001; Reh and Seitz, 1990; Vos and Chardon, 1998) are likely to be negative. Fragmentation decreases species richness (Findlay *et al.*, 2001; Hecnar and M'Closkey, 1997a; Hecnar and M'Closkey, 1997b), abundance (Fellers and Drost, 1993; Mazerolle, 2003) and isolates populations genetically (Hitchings and Beebee, 1997; Hitchings and Beebee, 1998; Reh and Seitz, 1990).

Within Britain, habitat modification resulted in substantial population declines of several British amphibian species during the mid-twentieth century as increased human population densities and increased arable production led to loss and degradation of breeding ponds. Although the rate of decline slowed during the 1970s and 1980s, several species failed to recover significantly (Cooke and Scorgie, 1983; Hilton-Brown and Oldham, 1991). For example, the great crested newt (*Triturus cristatus*) favours relatively large, deep ponds and is less able to utilise smaller, newly created garden ponds. Conversely, common frogs (*Rana temporaria*) are more adaptable and will breed readily in such sites. Following these major declines, *R. temporaria* populations increased overall in Wales (Hilton-Brown and Oldham, 1991) but whether increases

(generally attributed to garden ponds) were uniform across urban, rural lowland and rural upland areas is unclear. In mid-Wales, for example, distribution was described as widespread and abundant in the mid-1980s (Griffiths, 1986) but there are no data available prior to this. Subsequent presence/absence surveys recorded frogs in 18-70% of sampled ponds (Knight, 1989; Williams, 1995, Slater, unpublished data) suggesting possible variability in habitat use. Generally, there are few published studies regarding amphibian distribution and status in British upland habitats, particularly in relation to aquatic or terrestrial habitat quality. Quantifying the effects of such factors in such areas is important given the diverse range of pond types and distinct terrestrial habitats. In central Wales, for instance, ponds vary considerably in size, hydroperiod, water chemistry and in the abundance and richness of invertebrates and vegetation (Slater, 1993). Terrestrial habitats include improved swards for sheep and cattle rearing, semi-improved rough grazing, unimproved grassland, heath or bog on the hilltops and commercial conifer forests. Williams (1995) proposed that frogs might be less abundant in areas of improved pasture and more abundant on semi-improved or unimproved upper slopes and hilltops. Where present in improved pasture, a positive association with adjacent woodland for frogs, smooth and palmate newts (*Triturus vulgaris* and *T. helveticus*, respectively) was predicted. Additionally, potentially positive and negative affects of water chemistry were also noted. However, the study focused on larval numbers which is not always the best indicator of frog presence or abundance (Griffiths and Raper, 1994) and only terrestrial habitat within 100 m of the pond was considered. Previous studies have also used a 100m radius when relating common frog presence to terrestrial habitat (Beebee, 1985; Marnell, 1998; Stumpel and van der Voet, 1998). Although a large proportion of adult amphibians may remain within 100m of a pond during the non-breeding season (Jehle, 2000; Jehle and Arntzen, 2000), dispersing juveniles often cover greater distances (Dole, 1971; Kupfer and Kneitz, 2000; Oldham, 1985), and common frog and common toad (*Bufo bufo*) adults are capable of moving up to nearly 1 km to reach other ponds (Baker and Halliday, 1999) or summer habitats (Heusser, 1969; Sinsch, 1988). Furthermore, occupancy within 100 m of the pond can be highly seasonal and vary depending upon sex (Regosin *et al.*, 2003). Therefore, evaluating terrestrial habitat at a range of scales within the known movement range of a species may improve understanding of distribution and abundance.

To gain better insight into potential factors controlling amphibian populations within a rural upland landscape this study aimed to establish the current status and distribution of *R. temporaria* in mid-Wales and determine i) if distribution and/or abundance of common frogs (*Rana temporaria*) is related to physical or chemical aquatic attributes and type of terrestrial cover surrounding ponds, and ii) if significance of terrestrial habitat is influenced by the scale at which the assessment is made.

2.2 Methods

2.2.1 Site selection

Data were gathered from ponds in a study area covering approximately 900 km² across parts of central Wales in 2002 and 2003 (*Figure 2.1*). Ponds were located within a typical range of regional habitat types (altitude range 150-515m) broadly comprising acid grassland, heath and bracken (hilltops and upper slopes), improved grassland and broadleaf woodland (lower slopes) and conifer plantation (*Plates 2.1, 2.2 and 2.3*). Ponds were selected both non-randomly, using local knowledge, and by random selection, the latter made from Ordnance Survey 1:25,000 maps using a random number protocol applied to 1 km² grid squares. Each selected grid square was visited and searched for ponds. Where squares held more than one pond, the one nearest to the SW corner of the square was used. No constraints were employed on, for example, pond size or permanence. Similarly, for ponds selected based on local knowledge only one pond per km² grid square was surveyed. During visits to 44 1 km² grid squares the total number of ponds still present or lost (filled in, dried up or lost to succession) were also recorded to determine actual pond density and gauge accuracy of maps.

2.2.2 Common frog spawning and frog spawn survey

R. temporaria has a short annual breeding season lasting a few days or weeks at each site (Wells, 1977). In mid-Wales spawning begins in February and is generally completed at all ponds within six to seven weeks (personal observation). Each female frog typically deposits a single clump comprising 1000-2000 eggs (Beebee and Griffiths, 2000). Freshly laid ova have a thin jelly coating that absorbs water and swells resulting in coalescence of neighbouring clumps and formation of spawn mats in one or more areas of the pond.



Figure 2.1 Study area in mid-Wales showing location of ponds surveyed for common frogs (*Rana temporaria*) and assessment of aquatic and terrestrial habitat in spring 2002 and 2003. Key: ● pond; ■ conifer plantation; ■ acid grassland/heath and bracken; ■ improved grassland and semi-natural broadleaf woodland.



Plate 2.1 Shallow, acidic *Rana temporaria* breeding ponds located in unimproved acid grassland in mid-Wales (SO 100 516).



Plate 2.2 *Rana temporaria* breeding pond located within commercial conifer forestry in mid-Wales (SN 851 417).



Plate 2.3 *Rana temporaria* breeding pond located within improved grassland in mid-Wales (SO 181 087).

In spring 2002, 53 ponds were inspected every few days for spawn and all clumps, either single or within mats, were recorded. In 2003, 43 sites were resurveyed together with an additional 30 ponds. Coalescence makes counting individual clumps difficult and time-consuming so the number of spawn clumps per pond (= number of breeding females per pond) was estimated using Griffiths *et al.*'s (1996) method using $y = 2.27 + 0.007x$, where y = number of spawn clumps and x = spawn mat surface area (cm^2) (Plates 2.4 and 2.5). This method was established using a relatively small number of spawn mats $<2.5 \text{ m}^2$. However, it was the most practical for estimating clumps in larger mats given the lack of a reliable proven alternative. Difficulty in distinguishing clumps due to coalescence and surface freezing meant verification of accuracy for larger mats in this study was impossible as repeated counts varied too widely. Counting was used only for easily distinguished clumps in small mats and for single clumps. To ensure all spawn was accounted for mat areas were measured at least twice, the second measurement taken seven to ten days after the first.



Plate 2.4 *Rana temporaria* spawn mat located on top of submerged/floating vegetation.

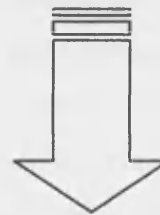


Plate 2.5 Dimensions of a spawn mat used to estimate numbers of breeding female *Rana temporaria*.

2.2.3 Aquatic habitat data

Survey methodology was adapted from Williams *et al.* (1998). Twelve physical habitat variables were measured for ponds in late winter/early spring prior to the first spawning period. Water area (m²) was estimated by transcribing length and width measurements and pond shape onto 1cm graph paper. Deep sediment and/or steep banks prevented measurement of maximum water and sediment depths at many ponds. Instead relative mean depths at 1m from the shoreline were obtained from measurements at regular intervals around the pond perimeter and uppermost sediment type noted. Ponds were recorded as seasonal if they were dry in summer. As *R. temporaria* typically spawns in shallow water (Cooke, 1975) the proportion of shoreline with a water depth 15-20cm at 20cm from the waters edge was also recorded. Shaded shoreline was taken as the proportion of shoreline covered by overhanging branches extending at least 0.5m over the water. Estimation of percentage macrophyte vegetation cover was done by eye and limited to submergent (rooted vegetation with/without leaves reaching the surface, including fully submerged grasses) and emergent (including grasses with leaves on/above the water surface) vegetation. Surface area covered by free-floating duckweed (*Lemna* spp.) was also recorded where present. General water quality of 66 ponds was assessed from 24 chemical variables obtained from 20ml water samples collected in March/April 2003. (Very low water levels prevented collection from remaining sites). Analysis was conducted by the Natural History Museum, London, using ion chromatography (high performance liquid chromatography) and inductively-coupled plasma atomic emission spectrometry.

2.2.4 Terrestrial habitat data

The terrestrial landscape surrounding the same 66 ponds was assessed from the Habitats of Wales dataset describing Phase 1 classification of vegetation cover (Countryside Council for Wales) using a GIS (geographical information system: ArcView 8.3, ESRI Inc.). Definitions of the major habitat classifications identified are contained in Appendix 1. Total area cover or total length was quantified for each habitat class/feature at four spatial levels (250m, 500m, 750m and 1000m radii from the pond margin) for each pond. Data obtained for 51 variables encompassed vegetation type, inland freshwaters, transport routes and buildings.

2.2.5 Statistical analysis

To prevent bias, random and non-randomly selected ponds were compared for differences in character. Physical attributes assessed for every pond were analysed using principal components analysis (PCA) performed on a correlation matrix. This effectively summarised pond type by separating different aspects of physical character onto axes sequentially. The first axis best explained variation within the dataset while each subsequent axis best explained variation with the proviso that variables that were uncorrelated to previous axes (thus confining any collinear variables to the same axis) (Jongman *et al.*, 1995). The scores of the first component were used as a single measure of character for each pond. Comparison of 'non-random character' scores with 'random character' scores demonstrated no significant difference between pond types (Kolmogorov-Smirnov 2-sample test: $d = 0.069$, $P > 0.10$), suggesting no significant difference between sample distributions. Subsequently, data from non-random and randomly selected ponds were combined for statistical analyses. Relationships of abundance with habitat variables were limited to ponds where both water chemistry and land cover data were available. Where chemical variables were below detectable limits half the detectable limit was used in statistical analyses (I. Jüttner, pers. comm.). Phosphate, arsenic, nickel and lead were not detected in any sample and were excluded from analyses. All habitat data were summarised as above using PCA performed separately on each aquatic and terrestrial dataset. The broken-stick criterion (Jackson, 1993) and scree plots were used for selection of significant principal components (PC). Significant aquatic and terrestrial habitat (1000m radius) PC scores were related using Spearman's rank correlations to assess the relationship between pond type and terrestrial habitat. Similar tests were performed relating abundance of breeding females to aquatic variables and to terrestrial variables at each spatial level. All analyses were performed in Minitab (Release 13.0)

2.3 Results

2.3.1 Pond density

The actual number of ponds in the landscape exceeded the estimate made from current Ordnance Survey maps (revised 1998). Estimated pond density was 0.4 ponds per km² and 34% of grid squares were expected to contain ponds. Searches revealed 63.6% of squares contained at least one pond and actual pond density was 1.1 ponds per km². Only two mapped ponds had been lost (filled in or dried up) and landowners reported no losses of unmapped ponds within the last 10-20 years.

2.3.2 Aquatic habitat

The aquatic characteristics of ponds were defined well by PCA with 52.7% of variation explained by the first four PCs. Water chemistry (*Table 2.1*) was captured by the first axis and reflected a transition between acidic and circumneutral ponds (*Table 2.2*). Acidic ponds possessed low pH, low ionic strength, decreased base cation concentration and increased metal concentrations in contrast to circumneutral sites where pH and conductivity were higher and coupled with raised calcium and magnesium levels. The second PC distinguished between very shallow seasonal ponds and deeper permanent ponds. An increase in submerged macrophyte cover with increasing pond depth was captured by the third PC and a general trend for increasing macrophyte cover with increasing pond size was depicted on the fourth axis.

Variable	Minimum	Median	Maximum
pH	4.2	7.2	9.9*
Conductivity	26	104	435
F	<0.01	0.02	0.07
Cl	1.47	8.67	45.30
NO ₃	<0.05	0.49	27.90
PO ₄	-	-	<0.05
SO ₄	0.46	4.77	23.67
Na	3.05	6.36	24.73
NH ₄	<0.05	0.24	6.69
K	0.14	1.05	19.74
Mg	0.40	2.17	12.12
Ca	1.50	34.50	69.00
Al	0.015	0.062	0.756
As	-	-	<0.005
Ba	0.001	0.008	0.065
Cu	<0.001	0.002	0.043
Fe	0.00	0.25	6.70
Mn	0.002	0.042	1.970
Ni	-	-	<0.005
P	0.005	0.029	0.447
Pb	-	-	0.005
Si	0.00	0.27	3.59
Sr	0.003	0.024	0.326
Zn	<0.002	0.005	0.035

Table 2.1 Range of chemical attributes obtained from 66 potential common frog (*Rana temporaria*) breeding ponds in mid-Wales. Samples were obtained in spring 2003, all measurements mg L⁻¹. One pond produced a very high pH reading although the reason for this is unclear.

	PC1	PC2	PC3	PC4
Eigenvalue	7.403	4.163	2.885	2.423
Percentage variance explained	23.1	13.0	9.0	7.6
Winter water area	n.s	0.263*	n.s	0.318*
Mean water depth	n.s	0.852***	0.485***	-0.288*
Mean silt depth	-0.287*	0.324*	0.567***	n.s
Mean total depth	n.s	0.813***	0.643***	-0.318*
Shallow shoreline	0.264*	-0.784***	-0.354**	n.s
Shaded shoreline	-0.494***	n.s	n.s	n.s
Permanence	-0.338***	0.609***	0.311*	n.s
Submergent vegetation cover	n.s	-0.263*	-0.584***	-0.615***
Emergent vegetation cover	n.s	n.s	0.396**	-0.289*
Total macrophyte cover	0.250*	-0.362**	-0.402**	-0.739***
Algal cover	n.s	n.s	n.s	n.s
Substrate	-0.378**	-0.378**	n.s	n.s
pH	-0.712***	n.s	-0.378**	n.s
Conductivity	-0.922***	n.s	n.s	n.s
F	-0.539***	-0.267*	-0.312*	n.s
Cl	-0.464***	n.s	n.s	-0.326**
NO3	-0.624***	n.s	n.s	n.s
SO4	-0.762***	n.s	n.s	n.s
Na	-0.564***	n.s	n.s	-0.341**
NH4	0.354**	n.s	n.s	n.s
K	-0.428***	-0.360**	n.s	n.s
Mg	-0.925***	n.s	n.s	n.s
Ca	-0.921***	n.s	n.s	n.s
Al	0.659***	-0.271*	n.s	n.s
Ba	-0.491***	-0.331**	n.s	n.s
Cu	n.s	-0.354**	n.s	n.s
Fe	n.s	n.s	0.311*	n.s
Mn	n.s	n.s	0.388**	n.s
P	n.s	-0.502***	n.s	n.s
Si	-0.560***	n.s	n.s	n.s
Sr	-0.880***	n.s	n.s	n.s
Zn	0.692***	n.s	n.s	n.s
Female abundance	n.s	n.s	n.s	n.s

Table 2.2 Spearman's rank correlations relating principal component analysis scores of biotic and abiotic pond variables from 66 ponds across mid-Wales to (i) the biotic and abiotic pond variables and (ii) the abundance of breeding female frogs (*Rana temporaria*). Significance of correlation coefficients is denoted as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and n.s. = not significant.

2.3.3 Terrestrial habitat

Improved land (grassland and conifer plantation) accounted for approximately two-thirds of the mean land cover surrounding surveyed ponds (*Figure 2.2*). Nearly a quarter comprised hilltop vegetation (heath, acid grassland, bog and bracken) whereas arable, buildings and gardens each contributed <0.05% to average land cover. The area covered by different land classes varied considerably between ponds depending on their general location (i.e. hilltop, conifer plantation, improved grazing) and their proximity to other land uses. The overall proportions of land cover type and the local variability in cover surrounding ponds was consistent across spatial levels. Consequently, PC scores for terrestrial cover at 250m, 500m, 750m and 1000m were often significantly intercorrelated (Spearman's rank correlations: $P < 0.05$). For example, habitat within 250m of a pond was best explained by the first PC (*Table 2.3*) describing the transition from hilltops and upper slopes (acid heath, acid grasslands, bracken, relatively more adjacent ponds and increased distance from roads and rivers/streams) down to the lower slopes and valley floor (improved grassland, semi-natural woodland, increased road length and river/stream length). This was similar for PCs at 500m and 750m except total river length and number of adjacent ponds were described better by the second PC with distance to railway lines by the third. Within a 1000m radius, length and distance to major roads were added to the second axis and bracken, dry acid heath, basin mire and river length added to the third (*Table 2.4*). Correlation of aquatic variables with PC scores for terrestrial cover within a 1000m radius demonstrated significant associations between pond character and the local landscape (*Figure 2.3*). Base cation concentration, pH and conductivity generally increased in areas of surrounding improved grassland (lower slopes) in contrast to lowered pH, a reduction in base cations and an increase in metal concentrations in ponds situated in acid grassland/heath (hilltops) (Spearman's rank correlation: $r_s = 0.512$, $P < 0.001$). As the area of improved grassland and broadleaf woodland on improved lower slopes increased there was a trend for an increase in macrophyte cover (Spearman's rank correlation: $r_s = 0.254$, $P = 0.044$). Deeper ponds had greater submergent macrophyte cover and ponds of moderate depth and vegetation cover coupled with increased adjacent coniferous woodland had fewer adjacent ponds in the local vicinity (Spearman's rank correlation: $r_s = 0.281$, $P = 0.025$).

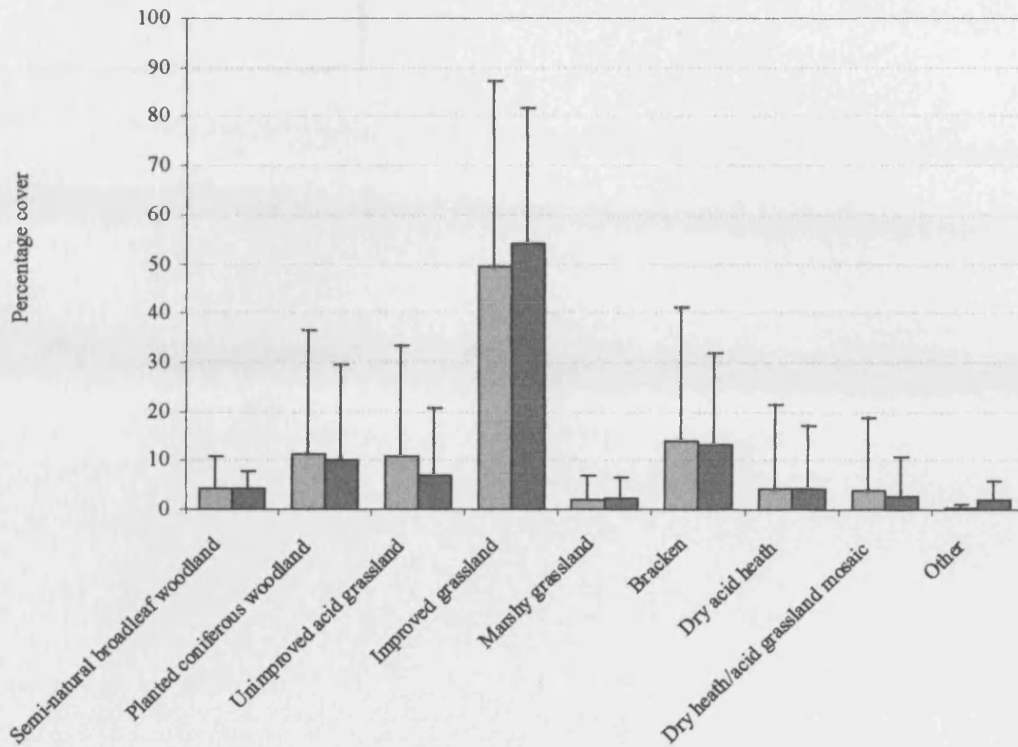


Figure 2.2 Mean percentage land cover (+ SD) within 250m (■) and 1000m (■) radii of the margins of 66 ponds surveyed for presence/absence and relative abundance of common frogs (*Rana temporaria*) in mid-Wales. Data derived from the Habitat Survey of Wales digitised dataset (Countryside Council for Wales).

Table 2.3

Table 2.4

	250m			500m		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	3.014	1.626	1.498	3.372	1.963	1.643
Percentage variation explained	20.1	10.8	10.0	21.1	12.3	10.3
Semi-natural broadleaved woodland	-0.609***	-0.305**	0.405**	0.522***	-0.445***	-0.272*
Planted coniferous woodland	-0.328**	n.s	n.s	0.409**	0.311*	n.s
Felled coniferous woodland	n.s	n.s	n.s	-	-	-
Unimproved acid grassland	0.430***	n.s	0.489***	-0.41**	n.s	n.s
Improved grassland	-0.632***	-0.455***	0.317**	0.640***	-0.306*	-0.317*
Marshy grassland	n.s	n.s	-0.399**	n.s	n.s	n.s
Bracken	0.40***	n.s	n.s	-0.576***	-0.371**	n.s
Dry acid heath	0.332**	n.s	n.s	-0.422***	n.s	n.s
Dry heath/acid grassland mosaic	0.440***	n.s	-0.262*	-0.438***	n.s	n.s
Acid/neutral scree	n.s	n.s	-0.365**	-	-	-
Not accessed	-	-	-	0.381**	n.s	n.s
Total minor road length	-0.701***	0.491***	n.s	0.591***	n.s	0.558***
Total river/stream length	-0.630***	n.s	-0.434***	-0.360**	-0.647***	0.259*
Number of adjacent ponds	-	-	-	-0.360**	-0.647***	0.259*
Distance to minor road	0.600***	-0.520***	n.s	-0.597***	n.s	-0.498***
Distance to river/stream	0.439***	0.366**	0.316*	-0.331**	0.276*	n.s
Distance to pond	n.s	0.433***	n.s	0.432***	0.626***	n.s
Distance to railway line	-	-	-	n.s	n.s	0.251*
Female abundance	n.s	n.s	n.s	n.s	n.s	n.s

Table 2.3 Spearman's rank correlations relating principal component analysis scores of terrestrial habitat surrounding 66 ponds in mid-Wales at radii of 250m and 500m to (i) the terrestrial habitat variables and (ii) the abundance of breeding female frogs (*Rana temporaria*). Significance of correlation coefficients is denoted as ***P<0.001, **P<0.01, *P<0.01 and n.s. = not significant.

	750m			1000m		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	3.366	2.509	1.873	3.939	2.811	2.366
Percentage explained	0.168	0.125	0.094	16.4	11.7	9.90
Semi-natural broadleaved woodland	0.598***	-0.296*	-0.263*	-0.669***	n.s	n.s
Planted coniferous woodland	0.387**	0.248*	n.s	-0.369**	0.405*	n.s
Unimproved acid grassland	-0.352**	0.288*	-0.278*	-	-	-
Semi-improved acid-grassland	n.s	0.431***	n.s	-	-	-
Improved grassland	0.558***	-0.441***	-0.305*	-0.637***	n.s	0.368**
Marshy grassland	n.s	n.s	n.s	n.s	n.s	n.s
Bracken	-0.348**	n.s	0.315*	n.s	-0.272*	-0.463***
Dry acid heath	-	-	-	n.s	n.s	-0.392**
Dry heath/acid grassland mosaic	0.531***	n.s	n.s	0.572***	n.s	n.s
Acid/neutral flush	-	-	-	0.423***	n.s	0.308*
Basin mire	-	-	-	0.252*	n.s	0.373**
Arable	n.s	n.s	-0.527***	n.s	n.s	n.s
Track	-	-	-	n.s	n.s	0.254*
Not accessed	0.390**	n.s	n.s	-0.422***	n.s	n.s
Total A road length	0.428***	-0.462***	0.358**	-0.435***	-0.451***	n.s
Total B road length	-	-	-	-0.445***	-0.474***	-0.321**
Total minor road length	0.751***	0.27*	n.s	-0.612***	0.347**	-0.487***
Total river/stream length	0.270*	-0.448***	-0.330**	-0.343**	n.s	0.667***
Number of adjacent ponds	-0.432***	-0.543***	n.s	0.375**	-0.695***	n.s
Distance to A road	-0.409**	0.457***	-0.411**	0.409**	0.541***	0.303*
Distance to B road	n.s	0.360**	n.s	0.409**	0.541***	0.303*
Distance to minor road	-0.698***	-0.266*	n.s	0.557***	-0.386**	n.s
Distance to river/stream	n.s	n.s	n.s	n.s	n.s	n.s
Distance to pond	0.467***	0.436***	n.s	-0.327**	0.560***	n.s
Distance to railway line	n.s	n.s	0.361**	n.s	n.s	-0.382**
Female abundance	n.s	n.s	n.s	n.s	n.s	n.s

Table 2.4 Spearman's rank correlations relating principal component analysis scores of terrestrial habitat surrounding 66 ponds in mid-Wales at radii of 750m and 1000m to (i) the terrestrial habitat variables and (ii) the abundance of breeding female frogs (*Rana temporaria*). Significance of correlation coefficients is denoted as ***P<0.001, **P<0.01, *P<0.01 and n.s. = not significant.

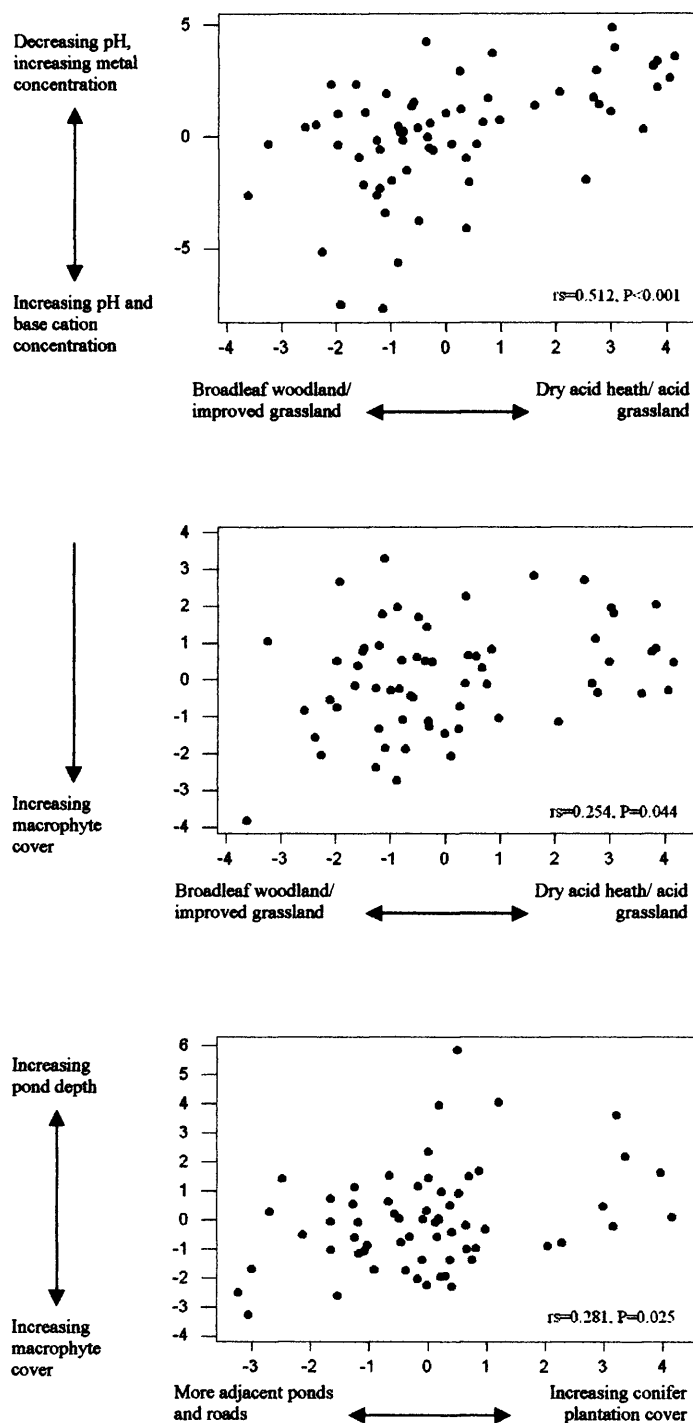


Figure 2.3 Significant positive relationships of pond habitat variables and surrounding terrestrial habitat within 1000m radii of pond margins. Spearman's rank correlations performed on principal component analysis scores. Top: Ponds in improved land (lower slopes) are generally circumneutral and rich in base cations contrasting with ponds in acid grassland/heath (hilltops) which have low pH, are base poor and richer in metals. Middle: Macrophyte cover increases in ponds where improved land cover is greater. Bottom: Deeper ponds generally have more submergent macrophytes. Ponds located where coniferous cover is greater are of moderate depth and vegetation cover with fewer adjacent ponds.

2.3.5 Pond occupancy and relationships between breeding frogs and habitat variables

The proportion of ponds used by breeding frogs was high. Frogs spawned in 90.6% and 93.1% of ponds in 2002 and 2003, respectively. The total estimated number of breeding females fell in 2003 but not significantly (Mann-Whitney test: $W = 1934.5$, $P > 0.05$, $n = 43$). On a pond by pond basis the differences between years achieved borderline significance (Wilcoxon's signed rank test: $W = 581.5$, $P = 0.051$) suggesting a slight decrease across the majority of ponds. Spawn was found in ponds at least once and only two ponds never contained spawn. The number of breeding females was not significantly associated with any of the aquatic variables (Spearman's rank correlations: all tests $P > 0.05$). Frogs spawned in ponds where water chemistry varied widely, e.g. from acidic to circumneutral to alkaline: pH 4.2–pH 9.9. Considerable variations were found in base cation and metal concentrations and, as a result, in ionic strength. For instance, calcium and magnesium levels ranged between 1.5 - 69.0 mg L⁻¹ and 0.39–12.12 mg L⁻¹, respectively, concentrations for aluminium and zinc between 0.01–0.75 mg L⁻¹ and <0.002–0.03 mg L⁻¹, respectively, and conductivity between 26–435 µS cm⁻¹. Both temporary and permanent ponds were utilised. Relative water depths were recorded from 10 cm to >1 m and ponds were used where as little as 5% of the margin was considered as an ideal depth for spawning, the frogs often spawning on top of submerged vegetation in such cases. Whilst some ponds were used where vegetation cover was nearly 100%, vegetation was absent in other breeding sites. Ponds with clay/mud and peat substrates were used equally. There were no significant associations with female abundance and any terrestrial variables immediately adjacent to pond margins, i.e. within a 250m radius (Spearman's rank correlations: all tests $P > 0.05$). No significant relationships were found between abundance of breeding females and terrestrial variables at increasing scales (Spearman's rank correlations on coverage within 750m, 500m and 1000m: all tests $P > 0.05$) suggesting no effect of scale where area cover of a variable increases with an overall increase in area. Breeding populations were found across all major terrestrial habitat types; on unimproved hilltops with adjacent acid grassland, heath and bracken, in grazing pasture surrounded by improved swards, scrub and broadleaf woodland and within commercial coniferous woodland.

2.4 Discussion

In mid-Wales, *R. temporaria* is currently ubiquitous and abundant. This suggests little change in general distribution within the last two decades (Griffiths, 1986). However, current pond occupancy appears to be greater than previous surveys suggest. Presence/absence surveys conducted on 143 ponds in 1983-4 found common frog presence to be 53%; breeding occurring in two-thirds of occupied ponds (Slater, unpublished data). A second survey of 85 ponds in 1987 detected tadpoles in only 18% of ponds (Knight, 1989) but larvae were found in 70% of 40 ponds sampled in 1995 (Williams, 1995). This may be due to both timing and sampling methodology. All three earlier studies involved surveying for tadpoles in early summer using bottle traps and/or hand nets. After hatching, tadpole numbers may be relatively low because of high spawn mortality due to predation (Cooke, 1975) or fungal infection (Blaustein *et al.*, 1994). Moreover, during the latter part of the aquatic phase chances of detecting larvae are lower. Tadpoles will be widely dispersed (Griffiths and Mylotte, 1986) and numbers may be significantly reduced by, for example, predation (Calef, 1973; Cecil and Just, 1979; Loman, 2002), and as metamorphosis and emergence onto land commences (metamorphs were observed at least once in Knight's study). Increased macrophyte cover hinders capture rates of amphibian larvae in traps (Oldham *et al.*, 2000) and using bottle traps as a survey technique for frogs is not recommended (Griffiths and Raper, 1994). Furthermore, hand netting can fail to detect tadpoles in some confirmed breeding ponds (personal observation). Therefore, presence of frogs was potentially under-recorded in all previous surveys. In contrast, spawn clumps are highly visible and allow a more accurate estimation of adult population size (Griffiths and Raper, 1994). However, in this study 26% of mats exceeded $>2.5 \text{ m}^2$ and there is a need to verify the accuracy of using mat surface area to estimate numbers of breeding females for these larger spawn mats.

The absence of comparable long-term data makes it impossible to determine real changes in distribution. Female breeding population sizes fluctuate significantly from year to year and significant changes in distribution and abundance will be evident only after several years of continuous surveys (Meyer *et al.*, 1998; Pechmann *et al.*, 1991). In year two of this study, female numbers fell slightly in the same ponds between years. Breeding population size has been related to recruitment of metamorphs in previous years (Beebee *et al.*, 1996; Berven, 1990; Loman, 2002). The slight

widespread decline might suggest a stochastic regional effect, such as an historic reduction in juvenile output due to a previous dry summer, or perhaps a widespread loss of breeding adults. Alternatively, poor recruitment or extinction at the local, i.e. pond level, could result from deterministic effects such as disease, predation, desiccation or pollution, for example. Several effects might operate simultaneously to different degrees within a pond network. Significantly reduced recruitment at some ponds could be masked by movements of breeding adults between neighbouring ponds and is only evident as an overall reduction in numbers.

The use of fewer ponds in the first survey year might be due to an abundance of other available sites. At one unused site, frogs spawned in another pond <5m away. Frogs will also spawn in puddles, tractor ruts and flooded fields (Cooke, 1975; Cooke, 1985) and were observed doing so during this study. The second unused pond was acidic, fed by runoff from the surrounding conifer plantation. It is possible that recruitment of breeding adults is lowered significantly by increased spawn mortality following *Saprolegnia* infection in low pH conditions (Banks and Beebee, 1988; Beattie *et al.*, 1991). Continued recruitment losses could theoretically lead to local extinction and so presence may represent immigration. Colonisation (or recolonisation) is affected by proximity to adjacent ponds (Baker and Halliday, 1999; Marsh *et al.*, 1999; Marsh *et al.*, 2000). Frogs have been proven to reach new ponds at least 1 km away (Baker and Halliday, 1999) establishing breeding colonies relatively quickly (Beebee, 1997; Stumpel and van der Voet, 1998) and pond density within the study area is moderately high. At least one pond is estimated to be present within every 1 km² for approximately two-thirds of the landscape providing a mean pond density of 1.1 ponds per km². This mirrors densities of 1.4 per km² in lowland Wales (Williams *et al.*, 1998) and is higher than 0.4-0.8 per km² estimated in mid-Wales during the 1920s by Rackham (1986). This lower figure might be due to map inaccuracy, the recent creation of new ponds (Gee *et al.*, 1994) and/or fewer losses to land use changes as reported for other parts of Britain. Interestingly, five of the six native amphibian species are present to some degree in mid-Wales. Providing ponds and surrounding habitat are suitable for each, the present pond density and relatively short inter-pond distances (the majority of study ponds had at least one pond within 250m) would permit occupancy by all five species (Oldham and Swan, 1997).

Frogs used ponds with a diverse range of physical and chemical attributes spread across very different terrestrial habitats. The high pond occupancy rate ($\geq 90\%$) and lack of significant correlation between habitat variables and abundance supports the general opinion that frogs inhabit a wide range of habitats and are not particularly selective in their choice of breeding sites (Beebee and Griffiths, 2000; Marnell, 1998). Similar results were obtained in another study relating rural terrestrial features to presence of *R. temporaria* (Baker and Halliday, 1999). However, failure to detect significant effects might result from inaccuracies in mapped data due to recent land use changes or lack of sufficient detail in the data gathered (Baker and Halliday, 1999; Knutson *et al.*, 1999). Terrestrial microhabitat features were not measured here and it is possible that number and proximity of both summer and winter terrestrial cover might significantly affect abundance and year to year presence/absence. Patches of long grass and/or scrub as cover are significant microhabitat determinants for *R. temporaria* (Marnell, 1998; Strijbosch, 1979a) and a link has been shown between presence of breeding frogs and distance to summer grassland habitats (Loman, 1988). Marnell's study also highlighted the importance of terrestrial refugia (log piles and tree stumps) in predicting frog presence/absence. Had parametric techniques been appropriate, analytical sensitivity might have been adversely affected by separate treatment of aquatic and terrestrial variables and not treating the landscape as a whole (Pope *et al.*, 2000). Knutson *et al.* (1999) described several studies where either landscape or aquatic variables examined in isolation explained relatively little variation within datasets. More detailed surveys of both aquatic and terrestrial habitats at many sites seem to be required to provide the best indication of amphibian habitat requirements. Alternatively, the mixture of habitat types around ponds might be sufficient to support populations adequately. In general, open areas are seen as poor amphibian habitat and a combination of woodland, pasture and scrub is more favourable (Beebee, 1981). Use of woodland and scrub adjacent to wetlands has been demonstrated for several species (Findlay *et al.*, 2001; Jehle and Arntzen, 2000; Knutson *et al.*, 1999; Laan and Verboom, 1990; Malmgren, 2002). Swan and Oldham (1993) found rough grassland, woodland and moorland to be positive influences on common frog occupancy. Improved grassland accounts for half the land cover in Wales (Howe *et al.*, 2005) and accounts for a similar proportion in this study area because the major land use is sheep and cattle farming. However, much of the pasture observed around ponds exists as relatively small fields frequently bordered by hedges

and/or ditches with areas of mixed or broadleaf woodland and scrub within close proximity. Proportions of different land cover types at 250m were not markedly dissimilar from those at 1000m. Significant correlations of land cover with scale suggest that for each pond a similar mixture of habitat types extended some way from the pond margin. Again, lack of recording detail may have hindered detection of the scale at which any ecologically relevant features become important. The small amount of area covered by buildings suggests little impact arising from urban development. Similarly, while traffic density has been implicated in contributing to population declines (Cooke and Sparks, 2004; Fahrig *et al.*, 1995) the number of major roads in the area is relatively low.

This study also showed that moorland and coniferous habitats were not avoided. These sites are typified by acidic ponds with low ionic strength. Breeding in such ponds has been documented previously within the study area (Knight, 1989) and in English upland ponds (Aston *et al.*, 1987), although other studies propose avoidance of low pH, low conductivity sites (Beebee, 1983; Strijbosch, 1979b). Stumpel and Voet (1998) suggested a negative relationship between ionic status and presence of frogs in older ponds (but not newer ones). The complex chemistry of different water bodies makes it difficult to isolate precise factors relating to *R. temporaria* distribution. Many of the measured variables are inter-correlated and it was not possible to determine any subtle influences in this study. Also, single water samples obtained once during the embryonic or larval period will not reflect daily or seasonal changes in chemistry which might affect populations. As mentioned above, embryo survival and subsequent recruitment may be affected adversely within acidic ponds. To understand fully the effect of water quality on distribution and abundance, recruitment to the local breeding population needs to be quantified and distinguished from immigrants breeding in these sites. Water chemistry has been related to distribution of newts in Britain (Cooke and Frazer, 1976; Denton, 1991; Yalden, 1986) generally through geological changes. However, it is likely that if geology is relatively homogeneous distributions will be unrelated to water chemistry (Baker and Halliday, 1999). Solid geology within the study area is relatively homogeneous (comprising Silurian rocks) but drift geology varies locally.

The importance of aquatic vegetation cover for common frogs is ambiguous. In new ponds, vegetation has been associated with frog presence in one case (Baker and Halliday, 1999) but not in a second (Stumpel and van der Voet, 1998). Swan and Oldham (1993) found frequency of occurrence to be significantly reduced in the complete absence of emergent and submergent vegetation. However, frogs will certainly spawn in non-vegetated sites (personal observation) and vegetation provides tadpoles with a potential refuge from predatory fish (Baker and Halliday, 1999). In contrast, newts show a strong preference for vegetated ponds (Cooke and Frazer, 1976) as it provides food sources for their prey, protection from predators and a substrate for egg attachment (Oldham *et al.*, 2000). Fish presence was not included in the analysis although they are predators of frog tadpoles. Only four ponds had confirmed fish presence (brown trout *Salmo trutta* (Linnaeus, 1758) and common carp *Cyprinus carpio* (Linnaeus, 1758), one site; three-spined stickleback *Gasterosteus aculeatus* (Linnaeus, 1758), one site; and goldfish *Crassius auratus* (Linnaeus, 1758), two sites). Frogs do not avoid sites containing fish (Beebee, 1979; Marnell, 1998) and spawned in all four ponds in both years. Again, knowledge of long term impacts on recruitment would be required to identify any potentially significant affects on distribution and abundance. Other factors such as pond age were excluded because landowners' estimates were not always reliable. Age is not thought to influence frog presence (Beebee, 1979) although breeding populations might be larger in younger ponds (Stumpel and van der Voet, 1998) before predators become established.

The lack of significant associations found between frogs and their environment in this study does not preclude either pond or landscape quality from having a significant effect on the dynamics of distributions and abundance. Local factors at ponds can significantly affect recruitment (Banks and Beebee, 1988; Kupfer and Kneitz, 2000) and poor recruitment in the long term may mean some ponds are incapable of sustaining a native population. However, amphibians routinely move between aquatic and terrestrial habitats (Kupfer and Kneitz, 2000; Loman, 1994; Perret *et al.*, 2003; Sinsch, 1987) and amphibian populations are often viewed as metapopulations where subpopulations at ponds undergo extinction followed by recolonisation through migrant exchange from adjacent subpopulations (Marsh and Trenham, 2001). Therefore, to understand better the causes underlying distribution and abundance, discrimination is necessary between effects due to inter-pond variation in recruitment

and those due to movement of frogs within the landscape (Marsh and Trenham, 2001; Skelly, 2001). Further studies into mechanisms controlling *R. temporaria* distribution in mid-Wales will focus on i) the degree to which inter-pond variation affects potential recruitment and ii) the emigration and dispersal of juveniles into the local landscape.

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Chapter 3

Embryo mortality in common frog (*Rana temporaria*) spawn in acidified Welsh ponds

3.0 Summary

Increased anthropogenic acidic deposition and conifer afforestation during the last century resulted in increasing acidification of many surface waters in central Wales. Local populations of *Rana temporaria* often breed in low pH ponds despite a proven reduction in reproductive success in acidic conditions. Although pH has increased in some acidified UK waters corresponding to reduced sulphur deposition, biological recovery has been patchy and intermittent. The purpose of this study was to determine any significant differences in embryo mortality between acidic and circumneutral ponds. Differences in mortality between ponds due to potential variation in acid-tolerance were accounted for using a translocation experiment. Effects of spawn deposition (isolated clumps vs. communal mat) on mortality rates were also evaluated. Embryo survival was consistently high in circumneutral sites but lower and highly variable in acidic ponds. There was no evidence to suggest increased acid tolerance of embryos in acidic ponds. Translocation results show that early exposure to low pH resulted in high mortality. Conversely, early development in circumneutral ponds contributed to higher, but variable, survival rates after transfer to low pH conditions. Mean embryo survival per clump was significantly greater for clumps developing within spawn mats than for lone clumps suggesting greater potential impact of acidification on small populations. Survival increased with increasing calcium concentration and rate of increase was similar for both lone clumps and for clumps within mats. These results suggest that amphibian reproductive success is still significantly impacted upon by acidity in mid-Wales. Long-term monitoring of populations in conjunction with studies of chronic and episodic acid inputs is required to determine whether acidity is likely to contribute towards population declines through reduced juvenile recruitment.

3.1 Introduction

During the 20th century, poorly buffered lakes and streams in parts of Britain became acidified due to acidic deposition exacerbated locally by land-use change, typically conifer afforestation (Gee, 1990). In acid-sensitive areas, this impacted directly and indirectly upon the distribution and abundance of several taxa (Ormerod and Wade, 1990). In Wales, for example, negative effects on aquatic and riparian biota occurred along more than half of the 24,000 km of total river length (Agren, 2002). Although evidence of recent, sudden amphibian population declines due to anthropogenic acidification is generally lacking (Alford and Richards, 1999; Vertucci and Corn, 1996), acidification has almost certainly contributed to some population declines. In Britain, some natterjack toad (*Bufo calamita*) breeding ponds became increasingly acidified during the last century following acidic atmospheric inputs and habitat modification (Beebee *et al.*, 1990). *B. calamita* generally avoids acidic ponds and reproductive success is negatively affected in low pH conditions (Banks and Beebee, 1988; Beebee, 1986). As a result, acidification has almost certainly contributed to losses in some heathland areas (Beebee *et al.*, 1990). It is also well known that distribution and abundance of amphibian species is influenced by pond acidity (Beebee, 1983; Bunnell and Zampella, 1999; Eason and Fauth, 2001; Leuven *et al.*, 1986; Strijbosch, 1979). Acidified ponds are characterised by low pH, low conductivity and ionic strength and relatively high levels of metals, such as aluminium, and ammonium and sulphate which tend to increase as pH decreases whereas alkalinity, conductivity, base cations and anions, and dissolved organic carbon all decrease (Leuven *et al.*, 1986). Generally, low pH has significant toxic effects on embryo survival and hatching success, and larval development, growth and behaviour (Beattie *et al.*, 1991; Clarke and LaZerte, 1985; Cummins, 1986; Cummins, 1988; Freda, 1991; Griffiths *et al.*, 1993; Padhye and Ghate, 1988; Tyler-Jones *et al.*, 1989), although interactions of the inter-correlated variables, in conjunction with development stage and species, affect degree of toxicity (Freda, 1991). This may explain why avoidance of acidic ponds is not always universal. For example, common frogs (*Rana temporaria*) and great crested newts (*Triturus cristatus*) avoid low pH ponds in some regions (Beebee, 1983; Denton, 1991; Strijbosch, 1979) but not in others (Aston *et al.*, 1987; Dolmen, 1980). Alternatively, this may also reflect differences in acid tolerance between populations. Several studies have suggested that populations originating in acidic environments have greater embryonic survival under low pH conditions

compared to circumneutral populations (Glos *et al.*, 2003; Merila *et al.*, 2004; Rasanen *et al.*, 2003). However, this tolerance may not be as evident in natural conditions because of the variable effects of other water chemistry parameters (Glos *et al.*, 2003).

The results from Chapter 2 show that within central Wales, common frogs are widespread and abundant and often breed in acidic ponds located within coniferous and moorland habitats. However, during that study it was noticed that mortality within some spawn clumps appeared to be higher than in clumps developing in circumneutral ponds. Previous studies of upland *R. temporaria* populations in England and Scotland have shown that although frogs breed in acidic ponds (Aston *et al.*, 1987), acid toxicity significantly reduces embryo survival (Beattie *et al.*, 1991; Cummins, 1986). Mortality in low pH conditions is exacerbated by low temperatures, which are typical of upland ponds in early spring (Beattie, 1987), and elevated aluminium and zinc concentrations (Beattie *et al.*, 1991; Cummins, 1986; Leuven *et al.*, 1986; Tyler-Jones *et al.*, 1989). However, calcium levels may ameliorate toxicity and reduce mortality in some acid-sensitive ponds (Cummins, 1988). In addition, the mode of spawn deposition may also play a significant part in affecting embryo survival rates. Common frogs typically deposit their eggs in a single clump which often coalesces with neighbouring clumps to form a larger 'spawn mat' (Beebee and Griffiths, 2000), although clumps can be deposited in isolation (personal observation). However, previous research assessing acid-toxicity has been restricted to whole or sub-divided clumps. Studies comparing acid-associated embryo mortality for amphibian eggs deposited singly, within lone strands or clumps or in communal masses are unknown. It is possible that mortality may be lower within communal masses due to potential buffering against external environmental conditions. Conversely, mortality may be higher amongst communal clumps due to increased transmission of the fungus *Saprolegnia* spp. (Kiesecker and Blaustein, 1997) which commonly infects spawn in cold, acidic ponds (Beattie *et al.*, 1991).

In recent years, some acid waters in Britain and Europe have begun to show signs of chemical recovery (i.e. increasing pH) coinciding with reduced sulphur deposition (Evans and Jenkins, 2000; Fölster and Wilander, 2002; Tipping *et al.*, 2000). However, evidence for biological recovery corresponding to signs of chemical reversal is patchy and intermittent (Soulsby *et al.*, 1997; Tipping *et al.*, 2002). Given the impact of acid

conditions on reproductive success, a reduction in acidity would be expected to have significant positive effects on amphibian reproductive success and, in turn, on sustaining future populations. Specifically, reduced acidity would be expected to result in increased rates of embryo survival coupled with reduced fungal infection relative to circumneutral ponds. Any differences in embryo survival between lone egg clumps and communal clumps would be similar in acidic and circumneutral ponds, although similarity in survival rates between pond types may reflect increased acid-tolerance of frogs breeding in acidic ponds and not a reduction in acidity. To date, improved amphibian reproductive success corresponding to natural recovery from acidification does not appear to have been demonstrated. The aims of this study were i) to determine if current acidity levels significantly affect embryo survival relative to circumneutral ponds, ii) to determine if there are differences in acid-tolerance between populations under natural conditions and iii) in the absence of potential biological recovery or increased tolerance, to determine if future recruitment potentially may be more negatively affected through increased embryo mortality in isolated clumps relative to communal masses.

3.2 Methods

3.2.1 Study area

The study area in central Wales overlies Ordovician and Silurian shales and mudstones of low to moderate buffering capacity coupled with a mixture of acid soils (podzols, brown podzolic soils, stagnohumic gleys) and non-acid soils (brown earths) (Hornung *et al.*, 1990). Annual rainfall is high, ranging between 871-1690 mm (<http://www.met-office.gov.uk/climate/uk/averages/19712000/rr/17.gif>), and acidic (mean pH 4.89) with high annual sulphur and nitrogen deposits of 10-20 kg ha⁻¹ year⁻¹ and 15-25 kg ha⁻¹ year⁻¹ respectively (Reynolds *et al.*, 1999). Agricultural improvement (and conifer afforestation) coupled with local variation in soil and bedrock results in local variation in acid-sensitivity (Hornung *et al.*, 1990) and a mix of acidic and circumneutral ponds within the landscape. Generally, pH<6.0 ponds are located within conifer plantation and on hilltops dominated by acid grassland, acid heath and blanket bog. In contrast, circumneutral ponds are located within improved or semi-improved pasture. Five acidic ponds and five circumneutral ponds were used in this study. Four acidic ponds were located within coniferous forest (altitude 320-460 m) and the fifth was situated on moorland hilltop (altitude 510 m). Shallow water depths, non-permanence of ponds and vandalism of equipment meant it was not possible to use ponds within one habitat type. All five circumneutral ponds were situated within semi-improved sheep pasture (altitude 270-350m). Ponds were searched for presence of spawn every few days in February and March 2004. Embryo survival was assessed for single clumps and clumps within spawn mats in both pond types. Translocations of eggs between pond types were used to assess acid-tolerance of embryos originating from acidic and circumneutral ponds.

3.2.2 Embryo survival in single clumps

To measure embryo survival within isolated clumps five 2-3 day old spawn clumps were randomly selected from the spawn mat at each pond. Lone clumps were not always present so all clumps were obtained from mats in order to be comparable. Each was checked to confirm fertilisation had occurred before being placed into separate submerged baskets and allowed to develop normally. Baskets comprised an upright cylinder of plastic mesh 25 cm long and 20 cm in diameter. Fine netting (≈0.5 mm mesh) covered the sides and bottom of the cylinder allowing water to circulate freely but excluding predators. Five baskets were secured to floating polystyrene blocks. The

array was partially submerged to a depth of 15-20 cm with the basket tops remaining above the water surface (*Plate 3.1*). Once all the clumps had hatched all free-swimming tadpoles and dead eggs/embryos were counted to assess embryo survival.



Plate 3.1 Basket arrays constructed to hold single *Rana temporaria* spawn clumps and groups of translocated eggs.

3.2.3 Embryo survival within spawn mats

Embryo survival in mats was assessed by estimating the number of eggs infected by fungus (Kiesecker and Blaustein, 1997). Fungal infestation (typically *Saprolegnia* spp.) is commonly associated with mortality of common frog eggs in acidic ponds (Beattie *et al.*, 1991; Strijbosch, 1979) and spreads across a spawn mass within a few days (Blaustein *et al.*, 1994) (*Plate 3.2*). Affected eggs are rapidly covered by an opaque white mass of hyphae which renders them easily visible (Kiesecker and Blaustein, 1997). Because infected eggs are often distributed unevenly across a spawn mat (personal observation) a stratified semi-random sampling approach was used to measure infection rates. Areas of infected eggs were marked onto a scale drawing of the mat according to infection levels of <25%, 25-50%, 50-75% and >75%. Counts

were made of infected and uninfected eggs visible in a 5 x 5 cm square, the number of counts being proportionate to the infected area. Squares were placed randomly but always >10 cm away from previous samples to prevent repeated measurement of the same clump. Where only a few infected eggs were present the eggs were simply counted. The proportion of uninfected embryos was averaged for each measured area of spawn mat and multiplied by the number of clumps in each area to provide an estimate of mean proportion of clump surviving per area. Clump number per area was estimated as in Chapter 2, using *Griffiths et al.*'s (1996) equation. The area data were combined to provide a mean estimate of embryo survival per clump for the whole mat.

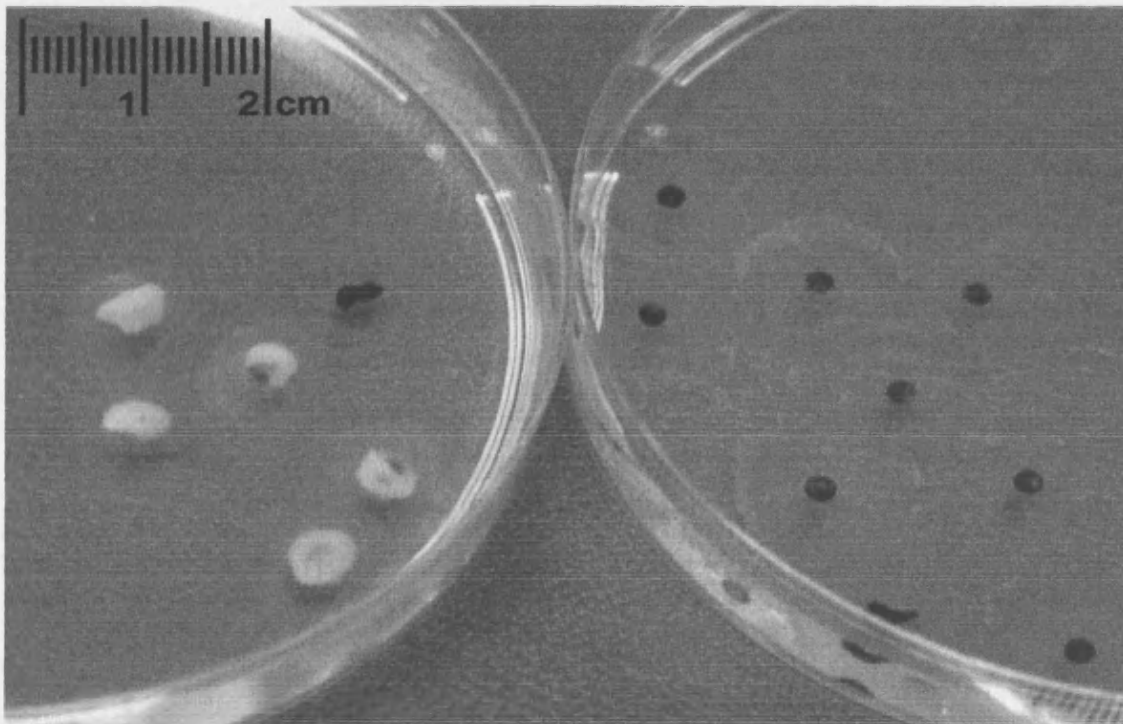


Plate 3.2 Embryos of *Rana temporaria* from an acidic hilltop pond infected with *Saprolegnia* sp. fungus (left) and embryos from a circumneutral study pond (right).

3.2.4 Survival of translocated embryos

One fresh spawn clump was selected from a spawn mat at each acidic pond. Six groups of 20 eggs were gently detached and inspected for signs of fertilisation. All eggs used were at development stages 1-9 (Gosner, 1960) with no obvious abnormalities. One group was retained within the natal pond while the remaining groups were transferred to the five circumneutral ponds with one group per site. The process was repeated for

spawn clumps selected from circumneutral ponds and translocated to acidic ponds. Native and translocated egg groups were kept separately in small baskets in ≈ 8 cm of water. Baskets were smaller versions of the ones described above with a length and diameter of 10 cm and 8 cm respectively. Six baskets were secured inside a square plastic frame attached to polystyrene floats. The array was then positioned close to the spawn mat.

3.2.5 *Water chemistry*

Differences in acid-base status between acidic and circumneutral ponds were confirmed from water samples taken from each pond in March 2003. Analysis of 22 chemical variables was conducted by the Natural History Museum, London, using ion chromatography (high performance liquid chromatography) and inductively-coupled plasma atomic emission spectrometry. Minimum and maximum water temperature, taken at a depth of ≈ 5 cm, and pH were recorded weekly during the course of the experiment (*Tables 3.1 and 3.2*).

3.2.6 *Statistical analysis*

Differences between acidic and circumneutral breeding populations, that is numbers of breeding females and clump sizes, were examined using two-sample t-tests to compare number of clumps and eggs respectively. Relating amphibian distribution to acid-base status is affected by significant inter-correlation of many acid-base variables (pH, Ca, Al and organic acids) (Freda, 1991). In this study, calcium concentration was chosen as a syntopic measure of both acidity and acid-sensitivity against which to compare embryo survival. ANCOVA was applied to examine survival differences in relation to pond acidity and location of clump deposition (i.e. lone or communal). Mean proportion of spawn surviving was subjected to logit transformation ($\ln y = y/(1-y)$) prior to analysis. To test for possible differences in acid tolerance of embryos from acid and circumneutral populations a Kruskal-Wallis analysis was performed on the proportions of eggs surviving after translocation. Subsequent pairwise comparisons were conducted using Mann-Whitney tests. All analyses were performed in Minitab (Release 13.0)

CIRCUMNEUTRAL SITES

Pond	1	2	3	4	5
National grid reference	SO 008 618	SO 101 563	SO 178 572	SO 030 642	SN 974 582
Altitude (m)	270	300	310	350	320
Mean pH	5.9±0.2	6.2±0.2	6.5±0.3	6.3±0.4	5.4±0.6
Mean conductivity	97.1±6.1	150.3±10.8	107.9±10.4	70.6±10.4	46.4±4.8
Minimum temperature	4.8±2.8	3.1±2.1	5.5±2.7	2.5±2.0	4.0±1.6
Maximum temperature	14.3±5.2	12.3±3.5	12.1±3.8	12.5±2.7	13.8±1.9
F	0.015	0.037	0.027	0.27	0.006
Cl	9.09	12.979	21.861	8.921	5.82
NO ₃	15.392	3.003	0.573	3.663	<0.05
PO ₄	<0.05	<0.05	<0.05	<0.05	<0.05
SO ₄	5.074	7.360	4.774	5.780	2.583
Na	6.071	8.365	15.004	5.825	4.957
NH ₄	0.933	<0.05	0.293	0.274	<0.05
K	0.741	3.038	0.756	1.248	0.290
Mg	2.820	3.379	1.972	2.991	1.190
Ca	9.181	9.272	6.395	6.333	4.939
Al	0.056	0.060	0.025	0.146	0.043
As	<0.005	<0.005	<0.005	<0.005	<0.005
Ba	0.001	0.013	0.008	0.006	0.003
Cu	0.002	0.002	0.001	0.011	0.018
Fe	0.043	0.160	0.310	0.424	0.150
Mn	0.011	0.013	0.014	0.196	0.043
Ni	<0.005	<0.005	<0.005	<0.005	<0.005
P	0.010	0.020	0.011	0.010	0.034
Pb	<0.005	<0.005	<0.005	<0.005	<0.005
Si	0.233	0.290	1.222	0.254	0.038
Sr	0.032	0.029	0.019	0.021	0.023
Zn	0.004	0.002	0.002	0.005	0.005

Table 3.1 Altitude (metres above sea level) and physico-chemical attributes of five circumneutral study ponds detailing mean weekly measurements (\pm SD) of pH, conductivity ($\mu\text{S}/\text{cm}^{-1}$) and minimum and maximum water temperatures ($^{\circ}\text{C}$) together with results for 22 variables from water samples taken in spring 2003 (all mg L^{-1}).

ACIDIC SITES

Pond	6	7	8	9	10
National grid reference	SN 851 417	SO 022 768	SN 863 516	SN 963 597	SO 052 774
Altitude (m)	355	320	455	510	460
Mean pH	4.8±0.2	5.5±0.2	4.3±1.2	3.4±0.2	4.6±0.1
Mean conductivity	40.9±5.2	50.3±6.7	29.3±2.8	38.9±1.6	73.9±4.9
Minimum temperature	5.2±2.6	3.7±1.7	2.0±1.8	2.1±1.3	2.7±1.7
Maximum temperature	10.9±2.3	13.7±1.4	10.9±3.5	11.7±3.6	9.5±0.8
F	0.011	<0.01	0.011	0.006	0.022
Cl	7.654	8.428	5.845	5.757	11.793
NO ₃	1.48	0.361	<0.05	<0.05	0.196
PO ₄	<0.05	<0.05	<0.05	<0.05	<0.05
SO ₄	3.878	1.504	2.197	1.119	7.295
Na	5.540	6.839	4.263	4.308	7.932
NH ₄	0.287	0.631	<0.05	0.123	<0.05
K	0.143	0.327	0.180	0.170	0.215
Mg	1.164	1.373	0.532	0.516	1.711
Ca	1.794	1.870	0.105	0.511	2.771
Al	0.120	0.041	0.198	0.067	0.197
As	<0.005	<0.005	<0.005	<0.005	<0.005
Ba	0.005	0.005	0.006	0.002	0.015
Cu	0.006	<0.001	0.010	0.002	<0.001
Fe	0.208	0.489	0.251	0.408	0.254
Mn	0.107	0.434	0.506	0.031	0.300
Ni	<0.005	<0.005	<0.005	<0.005	<0.005
P	0.019	0.013	0.013	0.023	<0.01
Pb	<0.005	<0.005	<0.005	<0.005	<0.005
Si	0.216	0.278	<0.002	0.004	0.023
Sr	0.012	0.007	0.004	0.004	0.014
Zn	0.031	0.005	0.023	0.016	0.009

Table 3.2 Altitude (metres above sea level) and physico-chemical attributes of five acidic study ponds detailing mean weekly measurements (\pm SD) of pH, conductivity ($\mu\text{S}/\text{cm}^{-1}$) and minimum and maximum water temperatures ($^{\circ}\text{C}$) together with results for 22 variables from water samples taken in spring 2003 (all mg L^{-1}).

3.3 Results

3.3.1 Breeding populations

Breeding population sizes did not differ between acidic and circumneutral ponds. Similar numbers of females were found in both water types (Two-sample T-test: $t = 1.16$, d.f. = 8, $P = 0.281$) and their clumps comprised similar numbers of eggs (Two-sample T-test: $t = 0.43$, d.f. = 48, $P = 0.666$).

3.3.2 Embryo survival and effect of spawn location

The mean estimated numbers of tadpoles hatching per clump from spawn mats was much greater than the average numbers hatching in lone clumps (ANCOVA: $F = 5.74$, d.f. = 1:20, $P = 0.026$) (Figure 3.1). The proportion of embryos surviving in circumneutral ponds was consistently high but varied considerably in acidic ponds. For example, for single clumps proportion of eggs hatched per clump was 0.98 ± 0.02 in circumneutral ponds compared to 0.38 ± 0.34 in acidic ponds. For both single clumps and mats embryo survival increased with increasing calcium concentration (ANCOVA: $F = 12.99$, d.f. = 1:20, $P = 0.002$), although rate of increase did not differ according to whether eggs were developing within a single clump or within a mat (ANCOVA: $F = 0.32$, d.f. = 1:20, $P = 0.578$). Fungal infection was present to some degree in all unhatched eggs from single clumps in acidic ponds. In circumneutral ponds a few undeveloped eggs appeared grey and cloudy rather than black but fungal infection was not obvious. Accounting for expected development failure arising from abnormally shaped eggs counted at the start of the experiment (<1% of clumps, both pond types), an average 1.4% of circumneutral clumps failed to develop past stages 12-13 for reasons other than obvious fungal infection.

3.3.3 Stage of developmental arrest and tadpole abnormalities

Cursory examination of infected eggs in single clumps showed that development had probably not surpassed stages 15-16 in nearly all cases. The exception occurred in two acidic ponds (Ponds 7 and 8) where a certain proportion of one clump reached stages 17-18 (Gosner, 1960) before dying. All embryos developing beyond this stage successfully completed hatching. Amongst hatched tadpoles in all ponds, several types of physical abnormalities were apparent although there was no significant indication that abnormalities were associated with acid or circumneutral pond type (Mann-Whitney test: $W = 639.5$, $P = 0.235$). Deformities comprised fluid-filled pockets

visible under the skin surface, protrusion of the guts through the abdomen wall, a kinked or stunted tail or a very small body size. Generally, occurrences of such abnormalities were low (*Table 3.3*). The largest percentage of total abnormalities in tadpoles from any one clump was 1.5% and 1.4% for circumneutral and acidic ponds, respectively.

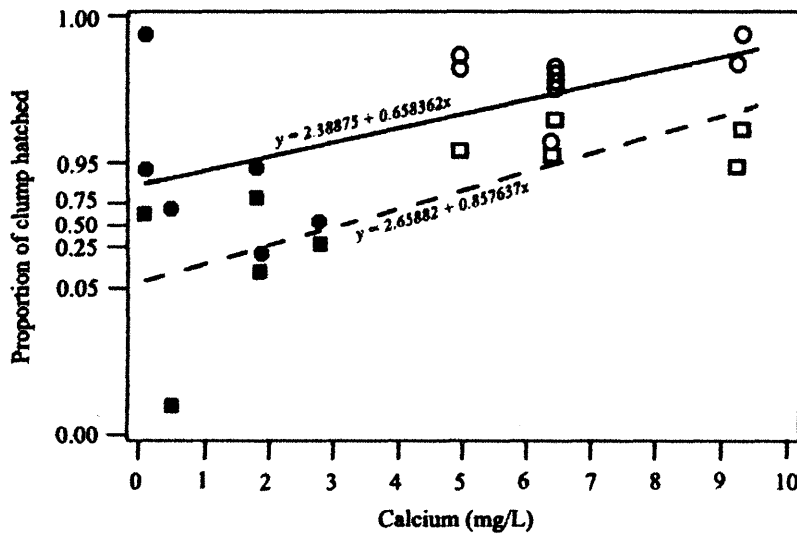


Figure 3.1 The relationships between the estimated mean proportions of eggs hatching from single clumps and clumps within mats (logit scale) and acidity measured in terms of calcium concentration (mg L^{-1}). Solid line and dotted line denote fitted regression lines for clumps within mats ($R\text{-}Sq = 35.0\%$, $F = 6.998$, $P = 0.02$) and single clumps ($R\text{-}Sq = 51.0\%$, $F = 8.342$, $P = 0.02$) respectively. (■ = single clumps in acidic ponds and □ = single clumps in circumneutral ponds, ● = clumps within spawn mats in acidic ponds; ○ = clumps within spawn mats in circumneutral ponds.)

	Site	Fluid-filled pockets	Extruded guts	Small body size	Abnormal tail	Not specified	Total
Circumneutral	1	7	2	4	0	7	20
	2	1	1	1	0	0	3
	3	12	1	0	1	0	14
	4	0	6	3	0	2	11
	5	0	0	0	0	0	0
Acidic	6	0	0	0	2	1	3
	7	0	0	0	0	0	0
	8	3	2	2	0	4	11
	9	0	0	0	0	0	0
	10	0	0	0	0	1	1

Table 3.3 Type of abnormality and numbers of affected tadpoles observed in five spawn clutches isolated and monitored at acidic and circumneutral ponds in spring 2004

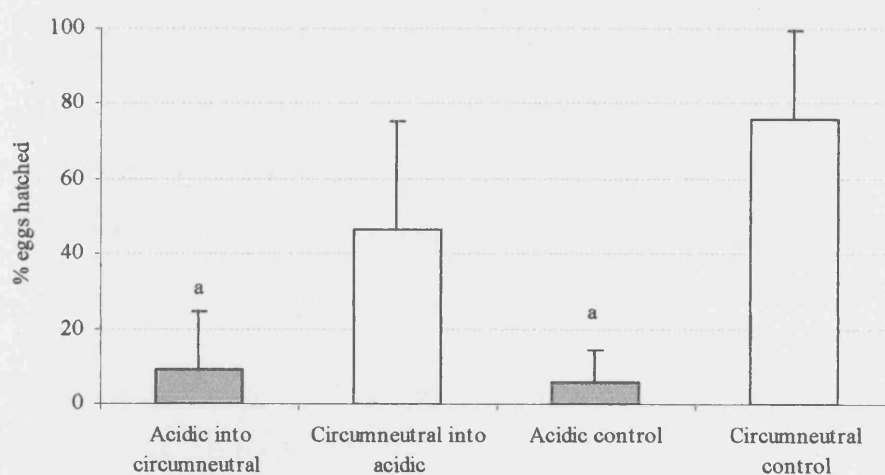


Figure 3.2 Percentage hatching (mean \pm SD) following translocation of eggs into ponds of contrasting pH and of eggs left to develop in their natal ponds. Identical letters denote non-significant ($P > 0.05$) pairwise comparison following a Kruskal-Wallis test.

3.3.4 Survival of translocated embryos

Survival rates of eggs differed significantly between the four experimental groups (Kruskal-Wallis test: $H = 33.17$, d.f. = 3, $P < 0.001$). Highest embryo mortality (85-100%) occurred in eggs deposited and reared in acidic ponds but translocation to circumneutral water early in development did not significantly improve survival (Mann-Whitney test: $W = 387.5$, $P = 1.000$) (Figure 3.2). By contrast, most eggs deposited and reared in circumneutral water experienced low mortality (10-20%). Eggs transferred from circumneutral ponds into acidic ones demonstrated variable survival rates (10-90%). This was not significantly different from eggs remaining in their natal ponds (Mann-Whitney test: $W = 348.0$, $P = 0.029$) but the poor survival of eggs in one circumneutral pond (35%) may have influenced the result.

3.4 Discussion

This study and others suggest that common frogs in Britain will breed in acidic waters (Aston *et al.*, 1987; Knight, 1989). However, previous research has documented the significant impact that acidity has on the survival and development of *R. temporaria* embryos and larvae (Beattie *et al.*, 1991; Beattie *et al.*, 1993; Beattie *et al.*, 1992; Cummins, 1986; Cummins, 1988; Cummins, 1989; Tyler-Jones *et al.*, 1989). The results of this study confirm that populations in mid-Wales are similarly susceptible to reduced reproductive success in acidic ponds and at current acidity levels there may be a potentially significant impact on recruitment. Although, pH increases are apparent in some UK waters, to date there is a lack of chemical evidence for de-acidification in Wales, possibly due to ineffective reductions in sulphur deposition and/or ineffective pH change (Buckton *et al.*, 1998). This suggests that reproductive success in terms of embryo survival is unlikely to improve in acidic ponds in the immediate future. In order for embryo survival to increase, pH would have to undergo a sustained increase and episodic depressions of pH to be of insufficient strength to cause mortality. Moreover, biological recovery of taxa is likely to depend on the reversal of multiple chemical and biotic parameters, not just increased pH (Buckton *et al.*, 1998; Masters, 2002).

In the absence of other causes of catastrophic failure (e.g. complete freezing or desiccation) the method used for estimating reproductive failure due to a fungal pathogen seems a reasonable way to estimate failure due to acidity. Only a very small proportion of eggs appeared not to develop in non-acid conditions and virtually all failed eggs were infected with fungus. To what degree the fungus directly contributes to mortality is unclear (Beattie *et al.*, 1991). Dead *R. temporaria* embryos are colonised more readily than live ones and live embryos are more susceptible to infection in earlier stages (pre stage 16) than in later stages (Robinson *et al.*, 2003). Blaustein *et al.* (1994) similarly reported invasion of living *Bufo boreas* (Stejneger, 1893) embryos by the fungus which resulted in death by development stages 14-16 (Gosner, 1960). Only in cases where infection occurred after this stage did afflicted larvae survive. Acidity was not mentioned as a co-factor influencing infection. In the absence of *Saprolegnia*, the natural conditions in acidic upland ponds (low pH, low temperatures, increased aluminium levels) are sufficient to reduce survival of *R. temporaria* embryos by a similar development stage (Beattie *et al.*, 1993; Beattie *et al.*,

1992). Amphibian embryos and larvae appear to be particularly susceptible when pH falls below <4.0-4.5 (Albers and Prouty, 1987; Beattie *et al.*, 1992; Beebee and Griffin, 1977; Clarke and LaZerte, 1985; Cummins, 1988; Olsson *et al.*, 1987) and this situation occurred at least once in all but one of the acidic ponds. Signs of delayed infection were not evident in this study and mortality is likely to have resulted from acidic conditions either directly or indirectly as infection took hold in embryos already subjected to acid stress.

There is also evidence that mortality is affected by spawning behaviour, a factor that has not been previously investigated in relation to acidity. In ponds where i) spawn clumps are few and deposited far apart, ii) many clumps form only loose aggregations or iii) few clumps form very small mats, the mortality rate could be significantly greater than where many clumps develop en masse. The increased maximum temperatures found within the centre of mats compared to the mat periphery and to single clumps promotes faster embryonic development (Beattie, 1987; Hakansson and Loman, 2004) thus reducing exposure to unfavourable water conditions. As *Saprolegnia* infection is notably greater at lower temperatures (Banks and Beebee, 1988) a protective effect against infection may exist for clumps in the centre. This was only truly evident at one acidic pond (Pond 9) where nearly 100% mortality occurred in the numerous single clumps and at the edges of the spawn mat but many tadpoles successfully hatched from central clumps. At other ponds fungal infection was patchy and not always restricted to outer clumps. Pattern of infection is contrary to that described in other species by Kiesecker and Blaustein (1997) where mortality was much greater amongst communally developing eggs compared to non-communal eggs, probably due to mode and distance of pathogen transmission. However, minimum temperatures also tend to be lower in mat centres and although this may not affect overall development rate (Hakansson and Loman, 2004) it may be sufficient to facilitate infection. If only a small degree of environmental stress (low temperature and/or acidity) is required to lower resistance to fungal invasion and the pathogen is present throughout the pond then this would explain greater infection in single and more central clumps.

Notable differences in pH tolerance have been found between amphibian species (Karns, 1992). Intra-specific regional differences have also been observed (Karns,

1992; Merila *et al.*, 2004) including in *R. temporaria* (Glos *et al.*, 2003; Tyler-Jones *et al.*, 1989), but indications of local-scale adaptation between populations were not apparent in this study. The translocation experiment failed to show increased survivorship of embryos originating from acidic ponds compared to those from circumneutral ponds. Conversely, embryos experiencing the start of development in neutral pH waters before transfer fared significantly better than eggs transferred from acidic to circumneutral ponds. This suggests that the lethal effects of acidity are more significant in the earlier stages of development, a result that concurs with studies on English populations where embryo death occurred by stages 7-12 (late cleavage – gastrulation) (Beattie *et al.*, 1991; Beattie *et al.*, 1992). However, Olsson (1987) found no difference in mortality attributable to development stage when embryos were exposed to short-term depressions in pH.

The variable tolerance of circumneutral embryos to low pH also concurs with other results demonstrating a facility for the species to withstand a dynamic environment (Beattie *et al.*, 1992). Additionally, there was considerable variation in survival between acidic ponds which makes effects on recruitment across several ponds difficult to determine, even without the future complication of interacting factors such as larval density, food and predation. It is probable that the complex toxicology of acidic water means that measured chemical conditions are not accurate predictors of successful breeding (Freda, 1991). Certainly, in the absence of repeated water chemistry measurements and the lack of data regarding aluminium speciation and dissolved organic carbon (DOC) the variation in this study cannot be fully explained.

It is not possible to tell what long term effect acidity has had on frog populations in Britain. Hypothetically, in isolated acidic ponds a chronic reduction in recruitment could occur until numbers of returning adults, and spawn clumps, falls below a critical threshold and the rate of decline becomes more acute and ultimately the population becomes unsustainable. Acidification resulted in an aging *B. bufo* population and the complete disappearance of *R. temporaria* within a few years at one Swedish lake (Hagstrom, 1977; Hagstrom 1980, cited in Leuven *et al.*, 1986). In reality, it is likely that potential sink populations at acidic ponds are supplemented to some degree through immigration from neighbouring circumneutral source ponds. In this study area, pond density is relatively high in neighbouring agricultural land (see Chapter 2)

and *R. temporaria* is capable of dispersing at least 1 km to colonise ponds (Baker and Halliday, 1999). If regular immigration is occurring then there also would be no reason to expect evidence of increased pH tolerance at acidic ponds. Currently, breeding population sizes in the two pond types are similar although there has been a failure to breed at two acidic ponds in previous years. However, amphibian populations fluctuate markedly from year to year (Pechmann *et al.*, 1991). This effect needs to be distinguished from recruitment fluctuations due to both chronic and episodic acid input. The two incidences of mortality at later development stages might be evidence of episodic input. Even small or short-term depressions in pH caused, for example, by snowmelt (Hagen and Langeland, 1973), can have a detrimental effect on survival and population persistence (Cummins, 1988; Freda, 1991; Olsson *et al.*, 1987) and snowmelt may have had an effect at some ponds in this study. Furthermore, the acidity of waters within conifer forest tends to be greater than on acid moorland (Bird *et al.*, 1990) so episodic inputs will differ in severity according to the surrounding habitat. Immigration rates and the potentially complex source-sink dynamics resulting from migration between habitats of different quality (Watkinson and Sutherland, 1995) also require study to truly understand what effect acidity has on amphibian distribution and abundance.

In addition to common frogs, other native amphibian populations may suffer impacts from acidity. In Britain, Natterjack toads (*Bufo calamita*) (not found in mid-Wales) strongly avoid acidic ponds and select sites with a neutral pH for breeding, whereas common frogs (*Rana temporaria*) and common toads (*Bufo bufo*) appear to be more tolerant of similarly acid conditions (Beebee and Griffin, 1977). The effect of acidity on *B. bufo* embryos is not known but because *B. bufo* lays its eggs in strings it may be more susceptible to acid toxicity and/or to *Saprolegnia* infection. At least one local population of a circumneutral pond has declined following catastrophic loss of embryos associated with the fungus (F. M. Slater, pers. comm.). Male or juvenile toads have been observed at three of the acidic ponds, although there was no evidence of successful breeding (tadpoles or metamorphs) following surveys in 2004. Smooth newts (*Triturus vulgaris*) and great crested newts (*T. cristatus*) are at the edge of their distribution ranges in mid-Wales (Griffiths, 1986). Both species typically avoid ponds with pH <6.0 (Cooke and Frazer, 1976; Denton, 1991; Knight, 1989; Yalden, 1986). *T. cristatus* does inhabit dystrophic, acid ponds in Norway (Dolmen, 1980) although

embryos may be unable to survive in ponds with $\text{pH} < 4.5$ (Griffiths and de Wijer, 1994). *T. cristatus* was not detected at any acidic ponds following surveys in 2002 and 2004, although it is present at two of the circumneutral sites. The palmate newt (*T. helveticus*) is more acid tolerant (Cooke and Frazer, 1976; Denton, 1991; Knight, 1989; Yalden, 1986) and its embryos are more tolerant of low pH than either of the other two species (Griffiths and de Wijer, 1994). *T. helveticus* bred successfully in one acidic pond and was the dominant urodele in both pond types. The susceptibility of newt eggs to acidity and *Saprolegnia* infection is not well known and requires further study. Their eggs are typically laid singly and wrapped within a leaf but it is not clear whether this affords protection from fungal infection (Green, 1999; Marco *et al.*, 2001) or whether acidity is associated with increased infection.

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Chapter 4

Variation in recruitment of juvenile *Rana temporaria* from permanent ponds

4.0 Summary

Persistence of adult breeding populations depends on successful metamorphosis in preceding years. Number and size of emerging metamorphs can have significant implications for future breeding population size and individual survival and reproductive success. Survival and size are significantly influenced by multiple interactions of environmental factors during various stages of larval growth and development. There are, however, relatively few studies of variation among metamorphs in natural populations. Total numbers of metamorphs and their condition, i.e. body size, were compared on emergence from 19 fish-free permanent ponds, including several acidic ponds. Tadpole and metamorph density and survival were related to summarised physical and chemical characteristics of ponds (e.g. size, vegetation cover, acid-base status), initial spawn amount, number and type of predators, number of potential competitors, and metamorph length and weight. There was considerable variation in numbers of surviving tadpoles and metamorphs between ponds and variation between years. Tadpole survival, but not metamorph survival, was negatively related to initial spawn amount suggesting significant early predation. Metamorph survival was positively correlated with tadpole survival. Increased survival was associated with a decrease in length. There was variation in length-weight ratio between several ponds and differences in length were not always accompanied by expected differences in weight. There was no significant difference between median number and size of metamorphs from acidic and circumneutral ponds suggesting that differences in environmental conditions during larval development are compensated for, or cancelled out, by the time of metamorphosis. Long-term research is needed to assess whether recruitment at acidic ponds fluctuates yearly, as may be the case in circumneutral ponds, or is continually chronically depressed at some ponds. More research is also required to assess the effects of body size on juvenile dispersal and growth in contrasting terrestrial habitats.

4.1 Introduction

For amphibian populations to persist a significant proportion of the larval population must complete metamorphosis and survive to maturity at least once per generation. Breeding population sizes can be related to the number of juveniles produced in preceding years (Beebee *et al.*, 1996; Loman, 2002a). Survival rate, timing of metamorphosis and size at emergence are all influenced by interactions between environmental factors and development stage. For instance, reduced food levels during the first half of development can prolong development time but not affect size at metamorphosis. Reductions during latter stages do not alter development time but do reduce size at metamorphosis and increase mortality (Audo *et al.*, 1995). The same effects are additionally influenced by larval density and temperature (Newman, 1998). Furthermore, differences in food quality, particularly protein content, affect growth and development rates (Kupferberg, 1997). Predator presence can reduce growth or development rate and mass at metamorphosis (Lardner, 2000; Skelly, 1992) probably through reduced activity levels (Hokit and Blaustein, 1995; Kupferberg, 1997; Laurila, 2000; Skelly, 1992) possibly resulting in changes in food selectivity (Kupferberg, 1997). Conversely, individuals can increase growth rate when released from competition by predation (Wilbur, 1997) or prolong the larval period to achieve metamorphosis without a loss in size (Laurila and Kujasalo, 1999). Acidic conditions incur reduced growth (Brady and Griffiths, 1995; Griffiths *et al.*, 1993a; Warner *et al.*, 1991) and reduced size at metamorphosis which may be compounded by high density and limited food (Warner *et al.*, 1991), or impaired feeding behaviour (Brady and Griffiths, 1995; Griffiths, 1993).

Such effects on body size have important implications for future amphibian life-history. As size increases, the likelihood of predation decreases (Werner, 1986) and fecundity (Gibbons and McCarthy, 1986; Reading and Clarke, 1995; Tejedo, 1992), and breeding success increase (Gibbons and McCarthy, 1986; Gittins *et al.*, 1980). For *Rana* spp. average size at metamorphosis is positively related to adult size (Werner, 1986) and larger size at metamorphosis leads to increased survival, earlier maturation and larger adult size (Berven, 1990). In addition to increased predation risk, smaller juveniles can suffer greater initial mortality following parasite infections (Goater and Ward, 1992). Small size induces mechanical and physiological constraints (Taigen and Pough (1981) and Pough and Kamel (1984) cited in Lardner, 2000). Shorter limbs may

reduce activity (Wilbur, 1997) and reduced stamina may constrain dispersal, foraging and predator evasion for a considerable period after metamorphosis (Goater *et al.*, 1993). For small females, energy reserves might be targeted towards growth rather than reproduction. Later breeding could result in lower metamorphic success for species that breed in temporary ponds (Tejedo, 1992). Anurans are gape limited meaning prey size is determined by mouth width (Toft, 1980) and smaller animals are thus limited to taking more small, potentially less nutritious, prey items (Wheater, 1986).

Metamorphosis has received relatively little attention in field situations and the relevance of experimental studies is not well known (Loman, 2002a). Recent studies in Swedish ponds found evidence for density-dependent effects on metamorph size, a negative association with survival and predator numbers and that metamorph survival was correlated with future breeding population size (Loman, 2002a; Loman, 2002b). Differences in recruitment from temporary and permanent ponds were also highlighted (Loman, 2002a). Predation strongly affected recruitment in permanent ponds. Conversely, absence of established predators in temporary ponds meant that in years where water was retained until metamorphosis, juvenile recruitment exceeded recruitment in permanent ponds. However, failure to metamorphose before desiccation resulted in little or no recruitment (Loman, 2002a). In future years, recruitment in some regions may increasingly depend on output from permanent ponds. Climate change resulting in reduced or unpredictable precipitation has been implicated as a contributory factor in some population declines (Fellers and Drost, 1993). In some areas temporary ponds are expected to dry up sooner and remain dry for longer periods adversely affecting successful reproduction and isolating remaining productive ponds (Brooks, 2004). Temporary ponds lost to habitat modification, specifically by land drainage, would also place more importance on recruitment from remaining permanent ponds. Findings from Chapter 2 show that adult frogs do not appear to select breeding sites on the basis of water chemistry. However, in Chapter 3 it was shown that embryo survival is significantly reduced in acidic ponds within the study area. Thus, it would be expected that recruitment in these ponds would be lower than in circumneutral ponds. However, it is not clear if metamorph condition would also differ significantly between pond types. No other field studies relating to metamorphosis from acidic ponds have been found. Understanding factors that affect variability in number and

quality of juveniles from permanent ponds may therefore play an important role in identifying possible sink habitats.

The aims of this study were to i) evaluate the variability in total numbers emerging from permanent ponds in a rural upland habitat, ii) evaluate the variability in 'condition', i.e. body size between ponds and iii) assess which aspects of the natural larval environment potentially influence metamorph output.

4.2 Methods

19 permanent breeding ponds situated in grazing pasture, conifer plantation and moorland hilltop within central Wales were evaluated in terms of general physical and chemical habitat characteristics. (Ponds were among those studied in Chapter 2. See *Figure 2.1* for study area) Initial spawn density was subsequently recorded in early spring 2004 and ponds were later sampled to assess numbers of *R. temporaria* tadpoles. Common toad (*Bufo bufo*) tadpoles were also counted as they may compete potentially with *R. temporaria* for food resources. Amphibian larvae and/or eggs are predated by aquatic invertebrates, fish and adult newts (*Triturus* spp.) (Cooke, 1975; Loman, 2002a; Reading, 1990; Rowe and Beebee, 2005; Wilbur, 1997). Fish were not present in any of the study ponds but numbers of invertebrate predators and newts were recorded. All recorded factors were then used to assess potential effects of the pond environment on number and size of emerging metamorphs.

4.2.1 Pond habitat

Twelve physical variables were measured for each pond: water area (m²), sediment depth, sediment type (clay/silt or peat), water depth (winter and spring), total depth (winter water and silt depths), proportion of shallow shoreline, proportion of shaded shoreline, percentage submergent, emergent and total macrophyte vegetation cover and percentage algal cover. Measurements for sediment and water depths were relative and obtained 1 m from the bank at perimeter intervals. Shaded shoreline was taken as the proportion of shoreline covered by overhanging branches extending at least 0.5 m over the water. Acid-base status was assessed from single 20ml water samples collected in the previous spring. Sample analysis was conducted by the Natural History Museum, London, using ion chromatography (high performance liquid chromatography) and inductively-coupled plasma atomic emission spectrometry.

4.2.2 Spawn density

In early spring, ponds were searched for the presence of spawn every few days. Lone clumps were counted easily but coalescence of multiple clumps made counting difficult. Therefore, clump numbers within spawn mats were estimated using Griffiths *et al.*'s (1996) equation (described in Chapter 2). To ensure all spawn was accounted for, counts and mat area measurements were done twice, the second measurement taken seven to ten days after the first. The majority of study ponds are circumneutral

but five were acidic (<pH 6.0). A previous study revealed significant variability in egg hatching rates due to *Saprolegnia* infection within these particular ponds (see Chapter 3). Conversely, estimated hatching success within five circumneutral (\approx pH 7.0) ponds used in this study was highly consistent (0.9984 ± 0.003). To compensate for hatching differences, the total number of hatched clumps was estimated using hatching rates previously calculated for these ten ponds. *Saprolegnia* was not evident at any of the nine remaining circumneutral ponds so hatching rate was estimated using mean hatch rate from known circumneutral ponds.

4.2.3 Predation pressure and tadpole survival

Four to six weeks after hatching, hand-nets were used to collect tadpoles and predators. Water and/or silt depth restricted netting to the pond perimeter so measures of animal density per unit bottom area sampled were not possible (Loman, 2002a). Instead, total numbers of tadpoles and predators captured per pond were used as measures of tadpole and predator density. To be comparable, data were adjusted for differences in sampling intensity due to, for example, dense vegetation impeding netting. In addition to *R. temporaria* and *B. bufo* tadpoles, the following invertebrates were recorded: Odonata larvae, *Dytiscus* spp. larvae and adults, *Notonecta* spp., and *Ilyocoris cimicoides* (all ≥ 1 cm). The newts *Triturus helveticus* and *T. vulgaris* were the only vertebrate predators encountered and recorded. Tadpole survival was assessed using an index derived from the number of tadpoles divided by the number of spawn clumps (Loman, 2002a). Indices were calculated for both total spawn and hatched spawn.

4.2.4 Metamorph survival

A standardised trapping method using pitfall traps was employed to determine numbers of emerging metamorphs. Pitfall traps most often comprise a pot buried in the ground so that the open top is flush with ground level, effectively a vertically buried tube. Traps comprising horizontal tubes or boxes have previously been used successfully (Harrison, 1985; Hayward *et al.*, 2000; Paull *et al.*, 1981) and are suited to sampling a given length of pond perimeter without the need to erect drift fences. Traps were made of 50 cm lengths of plastic drainage pipe, 12 cm diameter, with a ≈ 2.5 cm wide slit along the length. Each was sunk into the ground 30-50 cm from the water, parallel to the waters edge and 2-3 metres apart around the entire perimeter. Convex internal sides prevented animals escaping and removal of soil immediately behind the

trap ensured only animals moving away from the water were captured. The ends of each tube were covered by fabric or thin plastic secured with elastic bands. Vegetation immediately surrounding traps was left relatively undisturbed but any falling directly across trap openings was removed. Counts of captured animals were made every 48 hours as it was not possible to reach every trap within 24 hours. However, wet earth or clay left at the bottom of traps provided a source of moisture during warm weather and successfully prevented mortality from desiccation. The narrow trap openings prevented the capture of non-target animals such as small mammals. Metamorphs were distinguished from juveniles by the presence of a residual tail and/or darker pigmentation around the urostyle not present in juveniles after full resorption of the tail (personal observation). Prior to analysis of metamorph density, total metamorph catches were adjusted for differences in the proportion of the perimeters sampled. Metamorph survival was assessed using an index derived from the number of metamorphs divided by the number of spawn clumps (Loman, 2002a). Again, indices were calculated for total spawn and hatched spawn. As capture efficiency of traps was not evaluated in the previously mentioned studies, experimental trials were used to evaluate the effectiveness of the trapping method and permit better estimation of juvenile recruitment. A trap was set in each of six enclosures. Enclosure width was equal to trap length so trap ends fitted against enclosure walls and prevented metamorphs from circumventing the traps. Thirty groups of 15-20 metamorphs were released \approx 50 cm in front of the traps. Each group was used only once. Percentage capture rate was determined from the number of captured animals and the number of animals that crossed traps without being captured.

4.2.5 Metamorph size

Where total captures per visit were less than 30 all animals were retained temporarily for measurement. Where capture rates were higher, 30 animals were randomly selected for measurement. All animals were released 3-4m behind the traps. To evaluate size, body length (snout-urostyle length) was measured to the nearest 0.01mm and weight measured to 0.01g. Length measurements were made on animals with tails \leq 0.5mm. To minimise weight differences due to differences in hydration all animals were placed first in shallow water for 30 minutes.

4.2.6 Statistical analysis

Physical and chemical variables for ponds were summarised using principal components analysis (PCA). Different aspects of physical and chemical character were separated onto axes sequentially. The first axis best explained variation within the dataset while each subsequent axis best explained variation with the caveat that variables that were uncorrelated to previous axes (Jongman *et al.*, 1995). Interpretable principal components (PC) were selected using the broken-stick criterion (Jackson, 1993) and the relevant PC scores used as measures of habitat character for individual ponds. Predator, tadpole and metamorph numbers and tadpole and metamorph indices were related to PC scores and to each other using Pearsons' correlations following log transformation of raw data to achieve normality. Non-parametric tests were used to compare metamorph body lengths and weights between ponds. Assessment of body condition using length-weight relationships (e.g. Jorgensen and Wind-Larsen, 1987; Reading and Clarke, 1995) will be skewed by variation in length or weight frequency distributions between ponds (Chadwick, 2003). Instead, body condition was assessed by comparing weights between ponds for a given length (Chadwick, 2003). Spearman's rank correlations related metamorph length and weight to pond habitat and numbers of tadpoles, predators and metamorphs and to tadpole and metamorph survival indices. Where multiple tests were used Bonferroni corrections were applied to reduce the probability of making a Type I error (Sokal and Rohlf, 1995). All analyses were performed in Minitab (Release 13.0)

4.3 Results

4.3.1 Estimating juvenile recruitment

Capture rates within trial enclosures varied considerably from 20-100% with a mean (\pm SD) capture rate of $63.4\% \pm 19.7\%$ (Figure 4.1). However, comparison of small and large sized groups of animals (small: 9.00 -13.50 mm, mean 10.60 -11.67 mm; large: 13.51-16.95 mm, mean 14.32 -15.31 mm) showed capture rate to be unaffected by size (Two-sample T-test: $t = 0.29$, d.f. = 13, $P = 0.774$). In two tests conducted using metamorphs with longer tail lengths (≥ 5.00 mm) capture rates were 83% and 67%. Capture variability meant that estimating total metamorph output for field ponds was unfeasible.

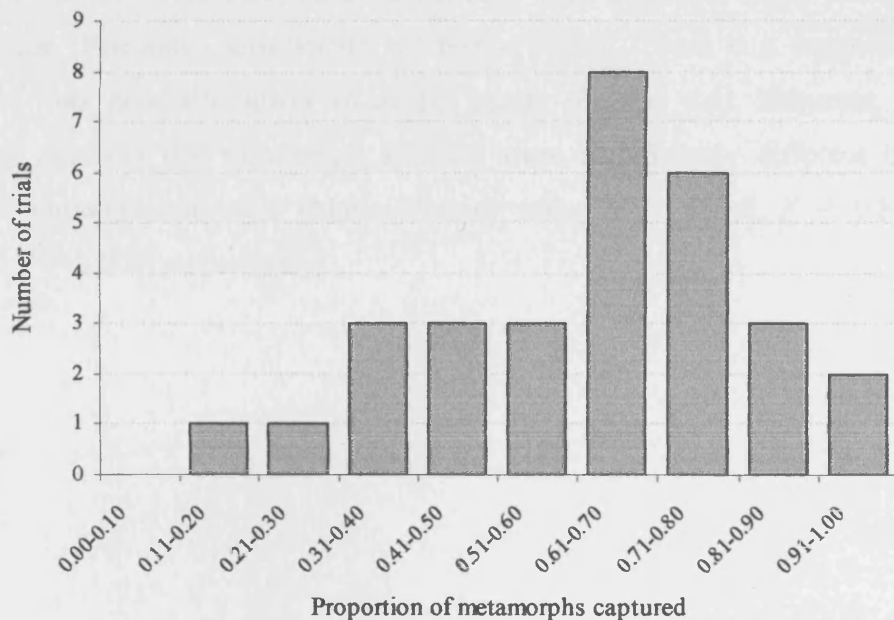


Figure 4.1 Proportion of *Rana temporaria* metamorphs caught in tube-style pitfall traps. Thirty trials were conducted within artificial enclosures to assess the proportion of animals that were captured as they crossed the traps.

4.3.2 Variation in juvenile recruitment between ponds and years

PCA separated different aspects of pond physical structure and acid-base status reasonably well. The first three axes accounted for 52% of variation in the dataset (Table 4.1). Acidic ponds (low pH, low conductivity, low base cation concentrations and higher zinc levels) and circumneutral ponds (pH \approx 7.0, higher conductivity and base cation levels and lower zinc levels) were distinguished on the first PC. The second PC depicted a slight trend for decreasing vegetation cover. A decrease in shaded shoreline accompanying a slight increase in ammonium and nitrate was evident on the third PC. Considerable variability in tadpole and metamorph density were obvious between circumneutral ponds and between acidic and circumneutral ponds (Figure 4.2 and Figure 4.3). However, there were no significant correlations with either tadpole or metamorph density and any pond habitat variables, including acid-base status (Pearsons' correlations: all tests $P > 0.05$). Similarly, neither tadpole nor metamorph survival were correlated significantly with acid-base status or any other pond variable (Pearsons' correlations: all tests $P > 0.05$). There is a suggestion that recruitment was generally lower in acidic ponds (Figure 4.4). However, neither metamorph numbers nor metamorph survival were significantly different between acidic and circumneutral sites (Mann-Whitney tests: $W = 143.0$, $P = 0.812$ and $W = 140.0$, $P = 1.000$, respectively).

PC1 26.3%	PC2 13.7%	PC3 12.0%
- Ca*** - Conductivity*** - Mg*** - pH** - Na** - Sr** - K** - SO4** - Ba** - Cl**	- Fe*** - Submergent macrophyte cover*** - Total macrophyte cover*** - Emergent macrophyte cover*** - P* - Silt depth* - Sr*	- Cu*** - Shallow shoreline** - Shaded shoreline** + NO ₃ ** + NH ₄ **
+ Total macrophyte cover* + Emergent macrophyte cover* + Submergent macrophyte cover* + Al* + Summer water depth* + Zn**		

Table 4.1 Attributes of pond habitat as described by principal components analysis on data from 19 ponds in mid-Wales. Percentage variation explained by each principal component (PC) is shown. + and - signify direction of change for each variable on each PC. Significance of Pearson product-moment correlations relating variables to each PC is denoted as *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.

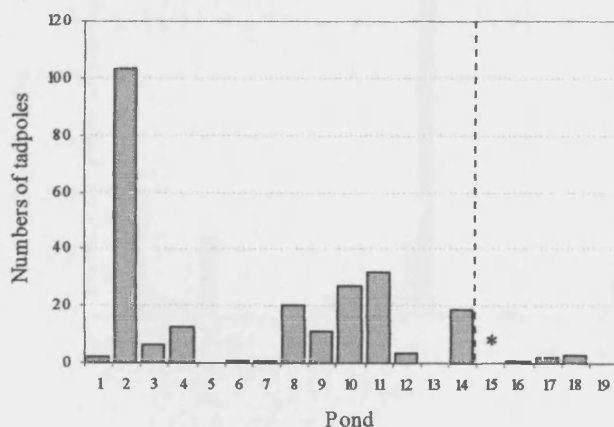


Figure 4.2 Numbers of *Rana temporaria* tadpoles caught by hand-net around the perimeter of 19 ponds in mid-Wales. Numbers were adjusted to compensate for differences in sampling intensity so that each bar represents captures along 45% of the perimeter. Bars to the left of the vertical dashed line are numbers captured in circumneutral ponds (\approx pH 7.0) and bars to the right are captures in acidic ponds ($<$ pH 6.0). *pond not netted due to dense emergent vegetation.

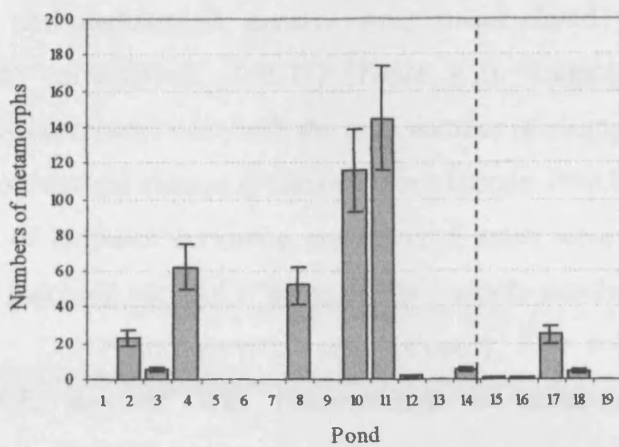


Figure 4.3 Numbers of emerging *Rana temporaria* metamorphs caught in pitfall traps placed around the perimeter of 19 ponds in mid-Wales. Numbers were adjusted to compensate for differences in trapping density so that each bar represents captures along 10% of the perimeter. Bars represent variation in numbers emerging based on a mean (\pm SD) capture rate of 64.3% \pm 19.7%. Ponds to the left of the vertical dashed line are captures in circumneutral ponds (\approx pH 7.0) and ponds to the right are captures in acidic ponds ($<$ pH 6.0).

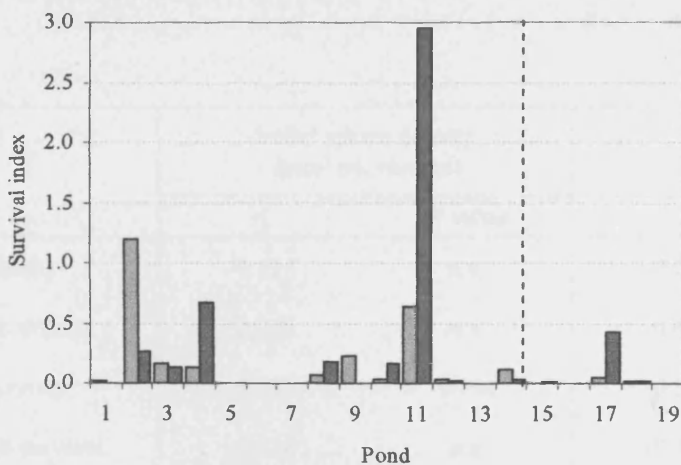


Figure 4.4 Survival indices of *Rana temporaria* tadpoles (■) and emerging metamorphs (■) captured in 19 ponds in mid-Wales. Indices are derived from the number of tadpoles or metamorphs divided by the total number of spawn clumps initially deposited within the pond. Captures were corrected for differences in sampling intensity between ponds. Bars to the left of the vertical dashed line are captures within circumneutral ponds (\approx pH 7.0) and bars to the right are captures within acidic ponds ($<$ pH 6.0).

4.3.3 Spawn density and survival to metamorphosis

Tadpole and metamorph density were uncorrelated with initial spawn densities (Pearsons' correlations: $P > 0.05$) (Table 4.2). Tadpole survival was significantly negatively correlated with both the total number of clumps deposited and the estimated number of hatched clumps (Pearsons' correlations: $P < 0.05$). ANCOVA confirmed that numbers of tadpoles surviving and survival rates were not significantly affected by different hatching success in some ponds (tadpole numbers (intercept): $F = 0.01$, d.f = 1:26, $P = 0.911$ and survival rates (slopes): $F = 0.01$, d.f = 1:26, $P = 0.911$). Metamorph survival was uncorrelated to initial spawn densities (Pearsons' correlations: $P > 0.05$) but was significantly correlated with tadpole survival (Pearsons' correlation: $r = 0.756$, $P = 0.007$). Data from six of the study ponds surveyed in the previous year demonstrated variation in metamorph numbers between years (Table 4.3). In the second year, recruitment and survival remained low in one pond (pond 16, acidic), decreased at one pond (pond 2) and either failed completely or was extremely low at two others (ponds 9 and 13). Numbers remained the same at pond 4, although survival was greater in the second year. Most notably, at pond 13, no metamorphs were captured during this study. However, in the previous year 2921 animals were captured, the highest recruitment at any pond in any year.

	Initial spawn density (total no. clumps)		Initial spawn density (hatched no. clumps)	
	r_s	P value	r_s	P value
Tadpole density	-0.124	n.s.	-0.080	n.s.
Metamorph density	0.298	n.s.	0.444	n.s.
Tadpole survival	-0.533	0.041	-0.536	0.039
Metamorph survival	-0.218	n.s.	-0.351	n.s.

Figure 4.2 Results of Pearsons' correlations relating density and survival of *Rana temporaria* tadpoles and metamorphs to the initial amount of spawn found in 19 ponds in mid-Wales.

Pond	Year 1		Year 2	
	No. metamorphs (\pm SD)	Survival index	No. metamorphs (\pm SD)	Survival index
2	5 \pm 1	-	23 \pm 5	0.27
3	26 \pm 5	0.42	5 \pm 1	0.14
4	61 \pm 12	0.42	63 \pm 12	0.67
9	18 \pm 3	0.38	0 \pm 0	0.00
13	2921 \pm 575	22.47	0 \pm 0	0.00
16*	1 \pm 0.2	0.001	1 \pm 0.2	0.001

Table 4.3 Differences in numbers (\pm SD) of emerging *Rana temporaria* metamorphs caught in pitfall traps placed around the perimeter of six ponds in mid-Wales. Numbers are based on a mean capture rate of 64.3% \pm 19.7% and represent captures along 10% of the perimeter. *denotes acidic pond, pH <6.0. Survival indices are derived from the number of captured metamorphs divided by the total number of spawn clumps initially deposited within the pond.

4.3.4 Predators, potential competitors and survival to metamorphosis

No significant correlations with any predator and pond feature, including acid-base status, were found (Pearsons' correlations: all tests $P > 0.05$). Numbers of individual predators and total predators were unrelated to both tadpole and metamorph densities (Pearsons' correlations: all tests $P > 0.05$) and tadpole and metamorph survival (Pearsons' correlations: all tests $P > 0.05$). Both tadpole and metamorph density were unrelated to numbers of toad tadpoles (Pearsons' correlations: $r = -0.114$, $P = 0.856$ and $r = -0.471$, $P = 0.529$ respectively). Toad tadpoles also had no significant effect on tadpole or metamorph survival (Pearsons' correlations: $r = 0.000$, $P = 0.999$ and $r = 0.051$, $P = 0.949$).

4.3.5 Metamorph size

Measurements per pond varied between $n = 1$ and $n = 226$. To compare length differences between pond cohorts, measurements for all 20 individuals (two ponds) were used plus randomly selected samples of measurements for 30 individuals (six ponds). Weight comparisons were made between the same ponds using weights for individuals measuring 12.00-12.99 mm selected from each entire cohort (sample sizes > 10). Comparisons showed a significant difference in median body length between ponds (Kruskal-Wallis test: $H = 71.90$, $d.f = 7$, $P < 0.001$) (*Table 4.4*). Closer examination revealed metamorphs from two ponds (4 and 11) to be significantly



smaller than animals from the other ponds (Mann-Whitney test: $P < 0.001$ all tests, significant after Bonferroni correction for twelve tests, $\alpha = 0.05$) (Table 4.5). Median body length between ponds 4 and 11 did not differ significantly (Mann-Whitney test: $W = 951.5$, $P = 0.594$). No significant differences in length were found between the six remaining ponds (Mann-Whitney test: all tests $P > 0.05$).

Comparison of median body weights revealed no significant difference in weight between the two small cohorts (Mann-Whitney test: $W = 606.0$, $P = 0.739$) (Table 4.6). Metamorphs from ponds 2, 8, 10 and 17 were significantly heavier, as well as longer, than animals from ponds 4 and 11 (Mann-Whitney tests: $P < 0.001$ all tests). Comparisons between animals from remaining ponds showed significant differences in weight despite similar body lengths. Metamorphs from ponds 3 and 17 were of similar length but those from pond 17 were significantly heavier than those from pond 3 (Mann-Whitney test: $W = 107.0$, $P < 0.001$, significant after Bonferroni correction for seventeen tests, $\alpha = 0.05$). Conversely, despite having greater body length, metamorphs from ponds 3 and 12 did not differ significantly in weight from the shorter animals from ponds 4 and 11 (not significant after Bonferroni correction for seventeen tests, $\alpha = 0.05$).

There were no significant correlations between body length or body weight and pond quality (Spearman's rank correlations: all tests $P > 0.05$). Length and weight were uncorrelated with both tadpole density (Spearman's rank correlations: $r_s = -0.571$, $P = 0.139$ (length) and $r_s = -0.405$, $P = 0.320$ (weight)) and metamorph density (Spearman's rank correlations: $r_s = -0.548$, $P = 0.168$ (length) and $r_s = -0.619$, $P = 0.102$ (weight)). However, metamorph length was significantly negatively correlated with tadpole survival (Spearman's rank correlations: $r_s = -0.786$, $P = 0.021$) and metamorph survival (Spearman's rank correlation: $r_s = -0.714$, $P = 0.047$). Weight was unrelated to survival of either tadpoles (Spearman's rank correlation: $r_s = -0.595$, $P = 0.120$) or metamorphs (Spearman's rank correlation: $r_s = -0.595$, $P = 0.120$). Metamorph length was also significantly positively correlated with the number of newts present (Spearman's rank correlation: $r_s = 0.732$, $P = 0.039$) but not with any other individual predator or total predators (Spearman's rank correlations: all tests $P > 0.05$). Predator density also had an insignificant effect on metamorph weight (Spearman's rank correlations: all tests $P > 0.05$). Toad tadpole numbers had no

discernible effect on body size (Spearman's rank correlations: $r_s = 0.342$, $P = 0.406$ (length) and $r_s = -0.619$, $P = 0.102$ (weight)).

Pond	Length (mm)			Weight (g)		
	Minimum	Median	Maximum	Minimum	Median	Maximum
2	11.160	12.770	15.290	0.200	0.240	0.320
3	11.210	12.800	14.510	0.170	0.200	0.310
4	9.910	11.495	13.750	0.130	0.180	0.250
8	11.530	13.050	15.030	0.160	0.230	0.360
17*	11.500	12.900	14.750	0.190	0.250	0.560
10	9.720	13.115	15.040	0.110	0.225	0.610
11	9.200	11.345	13.540	0.130	0.180	0.250
12	9.670	13.120	16.220	0.210	0.235	0.340

Table 4.4 Minimum, median and maximum snout-urostyle lengths (mm) and minimum, median and maximum weights (g) taken from *Rana temporaria* metamorphs captured on emergence from eight ponds in mid-Wales. Weights represent individuals 12.00-12.99 mm in length. Figures in bold represent metamorphs from two 'small' cohorts (ponds 3 and 7) that do not differ from each other in median length or weight. *denotes acidic pond, pH<6.0.

Pond	2	3	4	8	17 [†]	10	11	12
2	-	n.s.	<0.001*	n.s.	n.s.	n.s.	<0.001*	n.s.
3		-	<0.001*	n.s.	n.s.	n.s.	<0.001*	n.s.
4			-	<0.001*	<0.001*	<0.001*	n.s.	<0.001*
8				-	n.s.	n.s.	<0.001*	n.s.
17 [†]					-	n.s.	<0.001*	n.s.
10						-	<0.001*	n.s.
11							-	<0.001*
12								-

Table 4.5 P-values resulting from Mann-Whitney tests for differences in median snout-urostyle length (mm) of *Rana temporaria* metamorphs captured on emergence from eight ponds in mid-Wales. *denotes significance at the Bonferroni adjusted significance level (12 tests, $\alpha = 0.05$), n.s = not significant, [†]acidic pond, pH<6.0.

Pond	2	3	4	8	17*	10	11	12
2	-	0.007	<0.001*	n.s.	n.s.	n.s.	<0.001*	n.s.
3		-	0.023	0.049	0.001*	n.s.	0.042	0.045
4			-	<0.001*	<0.001*	<0.001*	n.s.	0.004
8				-	n.s.	n.s.	<0.001*	n.s.
17 [†]					-	<0.001*	<0.001*	n.s.
10						-	<0.001*	n.s.
11							-	0.006
12								-

Table 4.6 P-values resulting from Mann-Whitney tests for differences in median weight (g) of *Rana temporaria* metamorphs (12.00-12.99 mm length) captured on emergence from eight ponds in mid-Wales. *denotes significance at the Bonferroni adjusted significance level (17 tests, $\alpha = 0.05$), n.s = not significant, [†]acidic pond, pH<6.0.

4.4 Discussion

The results of the experimental trials measuring trap capture success meant that more precise estimates of actual recruitment could not be made reliably. Experimental design probably accounted for a considerable part of the variability in capture rate. When animals were released in front of the traps many dispersed towards the sides of the enclosure, not just straight ahead. On reaching the sides metamorphs moved along the walls and so crossed the trap at its very ends. The opening at the last 1-2 cm of each end was covered where the material ends were secured, effectively forming a bridge over the trap. This effect was not accounted for and numbers crossing at the ends varied between trials. To improve estimates of juvenile recruitment, it would be better to monitor dispersal directions directly from the water's edge at a range of margins, (e.g. vegetated and unvegetated) and just count individuals crossing the open part of the trap. Additionally, animals in the field were not marked so the rate of recaptures is unknown. Recaptures would, however, have been reduced in most instances by placement of traps within 50 cm of water's edge as the majority of animals will disperse well beyond this distance into the surrounding area. Also, tail resorption and colour changes distinguished newly emerged animals from those which had remained close to the pond and been recaptured.

Even accounting for overall average capture variability, this study has shown that within permanent ponds there is considerable variation in juvenile recruitment within ponds of broadly similar type (circumneutral or acidic). The lack of significant difference detected between acid and circumneutral ponds in terms of metamorph output might be affected by the small sample size; only a limited number of acid sites were accessible for trapping. Acidity during the larval phase reduces survival (Rasanen *et al.*, 2002), slows growth rates and results in smaller size at metamorphosis (Cummins, 1986). Feeding ability can be negatively affected (Brady and Griffiths, 1995; Griffiths, 1993) which may result in deformities due to dietary deficiencies (Cummins, 1989). However, it is entirely feasible that juvenile recruitment in acidic ponds is not negatively affected relative to circumneutral ponds. *Rana temporaria* tadpoles can survive prolonged exposure to low pH (Cummins, 1986; Glos *et al.*, 2003) possibly as a result of an acclimation effect during the embryonic stage (Rasanen *et al.*, 2002). Also, reduced growth early in development can be compensated for by the time metamorphosis is reached if acidity is reduced (Rasanen *et al.*, 2002).

Significant interactions between density and pH mean that tadpoles growing at high density in circumneutral ponds can be significantly smaller at metamorphosis (e.g. Pond 4) than tadpoles at high density in acidic ponds (Cummins, 1989). Perhaps reduced larval density and competition following high rates of embryo mortality (see Chapter 3) allow for increased growth and off-set reductions caused by acidity. No obvious abnormalities were observed during this study and although only one acidic pond provided sufficient data for statistical analysis, there was no suggestion of reduced length or weight. Metamorph lengths for three other acidic ponds were all within the size ranges recorded at circumneutral sites. However, comparisons of metamorph numbers between years suggest that in circumneutral ponds lower juvenile recruitment in one year may be offset by higher recruitment in the preceding or following year but this may not be the case in some acidic ponds. Long term studies are required to assess whether recruitment is chronically depressed at acidic ponds, fluctuates from year to year or varies from pond to pond. Even if juvenile recruitment at acidic ponds is relatively low, the overall effect on recruitment to the adult breeding population may not be as marked once differences in the carrying capacity of the terrestrial habitats are taken into account (Cummins, 1989).

Fluctuations in survival and size at metamorphosis between many ponds are probably due to differences in interactions between predator numbers, larval densities and food quality and/or abundance. The significant negative association between tadpole survival and initial spawn amount indicates that mortality rate increases with increasing numbers of eggs or hatched larvae. However, lack of association of spawn clump number with metamorph survival suggests that this mortality rate is not sustained throughout the larval period. Predator numbers have been negatively associated with tadpole and metamorph survival in other studies (Banks and Beebee, 1988; Loman, 2002a; Rowe and Beebee, 2005). High initial mortality is probably due to predation of young larvae which falls once older larvae reach a certain size refuge (Baker and Beebee, 1997; Brodie and Formanowicz, 1983; Cooke, 1974; Formanowicz, 1986). Greater body length of metamorphs in ponds with higher newt numbers would support this, although other unmeasured factors correlated to both newt presence and tadpole growth cannot be ruled out. In addition to young larvae, newts will also predate *R. temporaria* eggs (personal observation and Cooke, 1975). Early predation would explain a lack of significant association when the entire larval

period is considered, i.e. there is no association with metamorph survival. Reduced predation would also account for the positive correlation of tadpole survival with metamorph survival. The lack of association between tadpole survival and predator density might be due to predation by mallard ducks (*Anas platyrhynchos*). These were present at some ponds and can consume large numbers of newly hatched larvae (F. M. Slater, pers. comm.) but their effects were not measured.

Mallards may also have contributed to the dramatic difference in recruitment between years at Pond 13, although they were present in both years. In excess of 2000 juveniles were caught in the year preceding this study but no metamorphs or tadpoles were captured in the current year, despite similar amounts of spawn in both years. *Saprolegnia* infection was not evident, although other pathogens cannot be ruled out. Invertebrate and newt numbers were not high compared to other ponds so predation is unlikely to be solely responsible. Canada geese (*Branta canadensis*) were not observed at any study ponds during frequent visits in previous years but pairs arrived and reared young at this and another pond (where no metamorphs were caught) during the study. As terrestrial herbivores they are not considered predators of amphibian larvae but they may have affected larval survival through pollution of the water. At Pond 13, large amounts of faeces were present around the nesting area at the water margin. Accumulated droppings may cause nutrient enrichment, algal blooms and a reduction in dissolved oxygen (Stevens, 2005). High nitrate concentrations, however, do not necessarily increase mortality of *R. temporaria* larvae and it is likely that pollutant exposure would have to be considerable and very acute for the more toxic ammonia and nitrite to have a significant effect (Johansson *et al.*, 2001). Also, no change in algal cover was observed.

It is perhaps not surprising that numbers of *B. bufo* tadpoles had no significant effect on *R. temporaria* size or survival. Frogs commonly breed a few weeks before toads (Cooke, 1975) giving frog tadpoles a growth advantage. Although both species feed on filamentous algae, diatoms, and other micro-organisms, the two species can occupy separate feeding niches within the same pond (Harrison, 1987). Additionally, *R. temporaria* tadpoles can reduce growth of *Bufo calamita* larvae in laboratory experiments, possibly mediated by the alga *Prototheca richardsii* (Griffiths *et al.*,

1993b) although it is unclear whether such interference competition occurs in the field (Baker and Beebee, 1997). No reports were found of *B. bufo* inhibiting *R. temporaria*.

Ponds 4 and 11 produced juveniles significantly smaller than those from other ponds. Survival rates (and larval densities) were relatively high suggesting a possible density-dependent effect on size at these ponds but no association with density and survival was found. This may be because estimations of density were based on numbers per given length of perimeter and ideally should have been related to pond area. *R. temporaria* metamorph body length can vary between ponds in any year by up to 4 mm and between year lengths for a given pond typically vary by <2mm (Loman, 2002b). Comparison of mean (or absolute) lengths from all 19 ponds revealed a similar size range as that found by Loman across his 22 study ponds (including temporary ponds). Miaud *et al.* (1999) recorded metamorph length at one pond at high altitude (2300m) to be slightly larger on average (14-18 mm). This may be a reflection of slower growth rates (through possible over wintering of tadpoles in colder ponds) which then continue into the terrestrial phase resulting in larger adults in high altitude populations compared to lowland populations (Miaud *et al.*, 1999; reviewed by Morrison and Hero, 2003). Altitudinal gradient is slight in the study area (120-510m) and not sufficient to produce related differences in length or weight. Loman (2002b) also observed that regardless of factors affecting development rate in any year, tadpoles reached a size typical for each pond which supported Werner's model that each environment has an optimal size for metamorphosis. Although length data were only available for one year, it was noted that metamorphs collected from Pond 4 in the previous year were of a similar size (i.e. small) to those caught during this study year. Adults from this pond also appear smaller on average compared to adults at other ponds (personal observation).

Size differences at maturity may often reflect differences in growth during the terrestrial phase presumably due to differences in foraging conditions (Semlitsch, 1988), such as predation risk, availability and quality of food and microhabitat. Additionally, differences in body condition, i.e. weight to length ratio may also relate significantly to future fitness (i.e. reproductive success) but does not seem to be evaluated very often in juveniles. This may be because weight differences are considered too small to make a substantial difference in the long term. Also, observed

differences could be artefacts of different hydration states. However, in this study any effect was minimised by maximising hydration state prior to measuring. The observed differences in weight for a given body length suggest differences in body condition, possibly in fat reserves or muscle mass. Individuals with a greater weight to length ratio, that is heavier for a given length, may have a slight advantage over other individuals with a low weight to length ratio. Greater energy reserves could offset some of the disadvantages of smaller size, such as a prolonged dispersal period compensating for reduced locomotory ability. Many amphibians undergo most growth in the terrestrial phase (Werner, 1986) often prior to reaching sexual maturity (Miaud *et al.*, 1999; Reading, 1988). Initial growth may be enhanced by a greater initial mass. Alternatively, negative impacts on growth may be reduced where a short-term decline in habitat quality occurs, such as a drop in prey availability. Differences in conditions within the terrestrial habitat have previously been suggested as the cause for differential growth (Reading, 1988; Semlitsch, 1988). Interestingly, although metamorphs from two ponds were significantly shorter than those from other ponds they were of similar mass to some longer animals. It is possible that larvae put growth effort into achieving greater length at the expense of fat reserve or muscle mass, perhaps to reduce predation risk. If food quality or abundance was also poorer in the pond with longer individuals this may exaggerate the weight difference, or there may be genetic differences between ponds. It would be interesting to see how differences in weight to length ratio were transferred into the juvenile growth phase. It is also worth noting that individuals from the acidic pond were relatively heavier on average than animals from at least one circumneutral pond.

Berven (1990) demonstrated increased survival of larger, earlier emerging *Rana sylvatica* juveniles compared to later emerging individuals of both large and small size. In this study, there was no indication that the earliest emerging metamorphs were larger. Analysis of body lengths at the start, middle and end of the emigration period within ponds revealed no predictable pattern in emergence sizes. There was also considerable overlap in the emergence periods at all ponds. However, the three ponds with the highest numbers of metamorphs (Ponds 4, 10 and 11) started emergence ≥ 1 week before other ponds and included the two smaller cohorts. These ponds also had the longest emigration periods of 46-47 days. The shortest emigration period lasted for 30 days (Pond 3). Earlier emergence of the smallest individuals may partially negate

the effect of smaller size by lengthening the growth and dispersal period for a certain proportion of the juvenile population. Additionally, prolonged emergence reduces competition for resources in the area immediately around the pond benefiting all individuals. However, it is not clear how significant such effects would actually be on survival or fitness.

The many interactions between factors affecting tadpole growth and development mean that correlative studies such as these cannot identify the causative agents regulating juvenile recruitment (Loman, 2002b). Furthermore, expected significant correlations may be insignificant if the natural variation of a factor within ponds is small, although this may mean that it has less influence in natural conditions (Loman, 2002b). Therefore, experiments conducted under natural conditions will be the best method of determining ecologically relevant factors in natural populations. Unfortunately, concurrent experimental studies designed to examine effects of density, competition and predation on metamorph size in these ponds failed due to high tadpole mortality in most of the replicates. However, significant variation in juvenile recruitment exists between permanent ponds within a given year in terms of both number and size of emerging metamorphs. There is also evidence to show that variation occurs between years. More research is needed to quantify long-term juvenile recruitment patterns at acidic ponds. However, it is not immediately apparent from differences in body size that juveniles start terrestrial life at a disadvantage compared to animals from circumneutral ponds. Differences in size are not restricted just to body length but also to the weight-length ratio. As numbers of surviving metamorphs are significantly related to future adult breeding population size (Beebee *et al.*, 1996; Loman, 2002a) and size at metamorphosis is significantly related to survival and future fitness (i.e. reproductive success) (Berven, 1990), more research is required to assess how body size at metamorphosis affects juvenile dispersal and survival in terrestrial habitats of different quality. Such research would also highlight potential carry-over effects from the larval environment that are not immediately apparent at emergence.

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Chapter 5

Emergence, dispersal and microhabitat use by juvenile *Rana temporaria*

5.0 Summary

Many amphibians spend the greater part of their life in terrestrial habitats and habitat quality has potentially significant influences upon dispersing individuals. In many amphibian species, juveniles are the major dispersers and population persistence depends on exchanges of these individuals, especially between source and sink habitats. However, the dispersal capabilities of juveniles within different habitats are generally poorly understood. For common frogs (*Rana temporaria*) which typically inhabit areas with moist, vegetative cover the abundance of, and selection for, certain types of vegetation around breeding ponds may have significant implications for initial dispersal. The aims of this study were to determine under natural conditions what effect, if any, marginal and bankside vegetation has on emergence onto land and how vegetation type, cover and height might influence dispersal into the surrounding area. Differences in selection of specific microhabitats typical of the study area were also examined under experimental conditions. Pitfall traps were used to evaluate differences in emergence relative to presence of emergent marginal and bankside vegetation around the perimeters of seven ponds. Additional traps were placed around four ponds at distances of 25 m and 50 m from the pond margin along ten randomly placed transects to capture dispersing animals. Vegetation was assessed from quadrat measurements of vegetation type, percentage total cover and mean height at six locations along each transect. Selection of preferred microhabitat was assessed by releasing juveniles into four blocks comprising replicate areas of short grass, deciduous leaf litter, coniferous leaf litter and bare earth. Selection was assessed under both wet and dry conditions to examine effects of substrate moisture. New frogs demonstrated no preference for presence of emergent, marginal vegetation or type of bankside vegetation upon emergence. Emergence was not generally orientated in any particular direction although very boggy, waterlogged areas were avoided. Higher than expected numbers emerged in one area at one pond but the reason for this was not clear. Vegetation cover was high at all ponds at all measured distances and mainly

comprised grass and herb. Scrubby vegetation was restricted to the pond margin. Vegetation height varied between ponds and around individual ponds and tallest vegetation occurred around the pond banks. Dispersal of juvenile frogs and toads appeared to be unrelated to vegetation height and animals moved across areas of short grass with little cover. In contrast, juvenile newts showed a significant preference for transects with taller grass and herb. There was a suggestion that dispersal may not be immediate but once underway distances are covered quickly. It was not possible to tell whether dispersal was random or orientated towards adjacent woodland. In experimental plots there was a highly significant preference for short grass under both wet and dry conditions and all open areas were generally avoided. This contrasted with some field observations. It was concluded that dispersing juveniles will cross areas with reduced and/or drier vegetation cover but will probably select moist vegetated areas typical of older life-stages when not actively dispersing. Larger expanses of non-vegetated habitats such as roads and mature conifer plantations may potentially inhibit dispersal within the study area. However, considerably more detailed research is needed to assess dispersal ranges across such habitats and to determine at what size habitat areas may become limiting to dispersal.

5.1 Introduction

After metamorphosis, many amphibians often only return to ponds in order to breed and so conduct the longest part of their life-span in terrestrial habitats. Terrestrial habitat quality potentially has significant effects on populations. Survival, growth, distribution and abundance are dependent on the carrying capacity of the terrestrial habitat, specifically availability of food and shelter (summer and winter) and predation pressure. For instance, many anurans achieve $\geq 80\%$ of the adult size through growth after metamorphosis (Werner, 1986) but variable foraging conditions within the terrestrial habitat during this time potentially affect growth rates and ultimately lifetime fitness (Semlitsch, 1988). Presence of suitable shelter may significantly influence presence and abundance within a given area (Denton and Beebee, 1993; Kleeberger, 1985; Marnell, 1998) and species forced to use atypical terrestrial habitats can suffer increased predation, reduced foraging ability and loss of condition (Denton and Beebee, 1994). Areas of unsuitable habitats also significantly affect migration and dispersal (Reh and Seitz, 1990). Amongst amphibians, juveniles are often the major dispersers and are essential to the sustenance of local populations and metapopulations (Berven and Grudzien, 1990; Kupfer and Kneitz, 2000; Sinsch, 1992; Sinsch, 1997). This is because many species, including *R. temporaria*, typically form fairly closed populations within a geographically limited area around the breeding pond (Sinsch, 1992). Although, some adults disperse locally to form adjacent sub-populations (Reading *et al.*, 1991), there is generally strong site fidelity to breeding and terrestrial habitats (Loman, 1994; Reading *et al.*, 1991; Sinsch, 1992). The quality of the terrestrial habitat must therefore be considered in terms of potential effects upon juvenile dispersal and survival in addition to its capacity to support a more spatially restricted adult population.

The importance of terrestrial habitat quality to juvenile stages potentially begins around the pond margin at metamorphosis. Morphological changes reducing locomotive ability mean metamorphosing amphibians are vulnerable to both aquatic and terrestrial predators present along the shoreline (Hazell *et al.*, 2001; Rowe and Beebee, 2005). Once on land, there may be a delay for physiological changes to be completed before dispersal is possible (Cohen and Alford, 1993). Presence of emergent marginal and bankside vegetation would therefore reduce predation risk (Hazell *et al.*, 2001). In addition, increased humidity levels within vegetation will reduce evaporative

water loss preventing dehydration of animals with a large area to volume ratio. Recent evidence shows that newt metamorphs leave ponds in a non-random fashion, possibly in response to chemical cues left by adults, and potentially gain an advantage in finding suitable terrestrial habitat quickly (Hayward *et al.*, 2000). For many anuran metamorphs, including common frogs (*Rana temporaria*), this advantage does not exist as adults return to land immediately after breeding. Therefore, emergence will be entirely random or possibly influenced by the marginal or bankside habitat, in particular the presence and/or type of vegetation. If frogs do not actively select vegetated areas at emergence then juvenile survival is likely to be reduced at ponds where margins are exposed, for example, where banks are heavily grazed or trampled by livestock, or banks lie adjacent to roads or tracks. If frogs select habitats on emergence that potentially increase their survival, the numbers dispersing would be potentially much higher. If there is strong habitat selection then this may also have implications for future directions of dispersal.

In some populations, the majority of juveniles will disperse rapidly after emergence from the natal pond (Oldham, 1985; Schroeder, 1976) and can reach distances of up to ≈ 5 km (Dole, 1971; Schroeder, 1976). Dispersal rates are such that relatively small juveniles can reach neighbouring habitat patches several hundreds of metres away within a few weeks (Sinsch, 1997). Patterns of metamorph emergence and subsequent dispersal and habitat use are, however, generally poorly understood. Within heterogeneous landscapes one would expect different habitat characteristics to have either positive or negative effects on dispersal (Stevens *et al.*, 2004). It does appear that rate of dispersal is significantly affected by the type of terrain (Oldham, 1985; Stevens *et al.*, 2004) but it is generally unclear whether certain habitats are actively avoided or selected by new juveniles, for example to reduce the risk of predation or dehydration. The widespread distribution of frogs in the study area (see Chapter 2) suggests that terrestrial habitats favour adult frogs. However, for reproductive output at ponds (i.e. successful metamorphosis) to contribute effectively to the population dispersing juveniles need to be able to adequately permeate surrounding habitats. It is, therefore, necessary to identify features of terrestrial habitats that are important for juvenile stages and to understand where juveniles may differ from adults in microhabitat requirements.

The present study was conducted to evaluate the effects of microhabitat on emergence and early dispersal of new juveniles under natural and experimental conditions. The specific aims were i) to determine whether emergence of *Rana temporaria* metamorphs onto land was random or related to the marginal or bankside habitat, ii) to determine if juvenile dispersal into the surrounding habitat was related to habitat structure (vegetation cover and height) and iii) to determine which, if any, habitat types may potentially enhance or inhibit juvenile dispersal.

5.2 Methods

Field observations were conducted between June and August 2004 at eight ponds situated within two major land use types; improved grazing pasture and commercial conifer plantation. Pitfall traps around the pond perimeter were used to assess sites of metamorph emergence. Patterns of dispersion into the surrounding area were evaluated using traps at distances of 25 m and 50 m from the pond margin. Use of four microhabitat types common to the study area (bare earth, short grass, deciduous leaf litter and coniferous leaf litter) was investigated under wet and dry conditions in replicated experimental plots.

5.2.1 *Metamorph emergence patterns*

Sites of emergence around the perimeter of eight ponds were evaluated using tube-style pitfall traps as described in Chapter 4 (Harrison, 1985; Hayward *et al.*, 2000; Paull *et al.*, 1981). Vegetation obscuring the trap opening was removed but care was taken to leave surrounding vegetation undisturbed. Counts of captured animals were made every 2-3 days (with the provisos described in Chapter 4) once emergence began. The location of marginal, emergent vegetation (mostly grasses, *Juncus* spp. and marsh cinquefoil *Potentilla palustris*) at the water's edge, together with trap positions, was recorded onto a drawing of each pond. Also recorded was type and extent of the bank vegetation which was categorised as short grass/herb (height ≤ 5 cm), long grass/herb (height ≥ 5 cm, including *Juncus* spp.), moss or bare earth.

5.2.2 *Direction and rate of dispersal*

Dispersal into the surrounding habitat was monitored at four ponds. Because of the difficulties of placing and accessing traps under densely planted conifers, efforts were concentrated on dispersal behaviour from ponds within grazing pasture. Pitfall traps were placed at distances of 25 m and 50 m along a transect extending at 90° from the water's edge. Locations for ten transects at each pond were selected at random with the number of transects being in proportion to the amount of surrounding habitat type, e.g. pasture or woodland. Traps were checked at the same time as perimeter traps and every 2-3 days after the emergence period had ended for approximately four-six weeks. Vegetation structure along each transect was assessed from percentage total cover and mean height measured within 0.25 m² quadrats. Mean height was determined from nine measurements at three locations (left, middle, right) across the top half of the

quadrat, across the centre and across the lower half. Six quadrats were placed at 10 m intervals along the transect starting from the pond margin (0 m) up to the traps at 50 m. Cover was categorised as bare earth, debris (leaf litter, sticks, dead grass, animal droppings), grass, herb (including common nettle *Urtica dioica*, dock *Rumex* spp., daisy *Bellis perennis*, and clover *Trifolium* spp.), bramble (*Rubus* spp.), sapling (ash *Fraxinus excelsior*) and bracken (*Pteridium aquilinum*). Where multiple vegetation layers were present, for example a bracken canopy occluded a ground cover of grass and herb, cover and height for each layer was recorded.

5.2.3 Habitat utilisation by new juveniles

To determine if emigrating juveniles actively select or avoid particular microhabitats, four experimental blocks were constructed within a greenhouse. A greenhouse was used in preference to an outdoor site to allow better control of experimental conditions, i.e. dry or wet. Each experimental block contained four microhabitat types: bare earth, short grass (height ≈ 5 cm), deciduous leaf litter (mostly oak *Quercus* spp. and beech *Fagus sylvatica*) and conifer needles (Sitka spruce *Picea sitchensis*). Because of potential differences in temperature and shade within the greenhouse, each microhabitat type was replicated four times within a block using a 4 x 4 Latin square design. Each microhabitat plot measured approximately 0.5 m x 0.5 m. Four blocks were used with dissimilar arrangements of replicates, two separate blocks on each side of the greenhouse (Figure 5.1). Surface temperatures were recorded within each microhabitat type on both sides of the greenhouse using dataloggers. Two probes placed in a grass plot and a bare earth plot at ≈ 5 cm above ground level allowed a general comparison of relative humidity to be made. Plots were left for several days to ensure dry soil in all treatments. 50 *R. temporaria* juveniles originally collected upon emergence from the study ponds were released into the centre of each block. After 24 hours the number of juveniles per microhabitat replicate was recorded. As animals were easily disturbed on approaching and searching the blocks, a Perspex frame isolating each replicate and preventing movement into neighbouring replicates was used. The frame was suspended ≈ 10 cm above the surface prior to the start of the experiment and slowly lowered just before counting. After counting, soil moisture¹ (m.m³) was measured in each replicate in each block. Three measurements were taken

¹ *Theta-probe soil moisture sensor*. Delta-T Devices. 128 Low Road, Burwell, Cambridge, CB5 0EJ, United Kingdom.

within each replicate at the top left corner, centre and bottom right corner, and the mean used as a measure of soil moisture for the microhabitat type. In order to increase replication, the experiment was repeated. A limited number of test animals meant it was not possible to use inexperienced juveniles. However, animals were released into the neighbouring block so the layout of microhabitats differed from before. The experiment was also conducted under wet conditions to assess the effect of substrate moisture on microhabitat use. Each replicate was soaked with water as evenly as possible 24 hours before the animals were released and this was repeated for the second trial. Fresh animals were used for the first trial but again released into neighbouring blocks for the second.

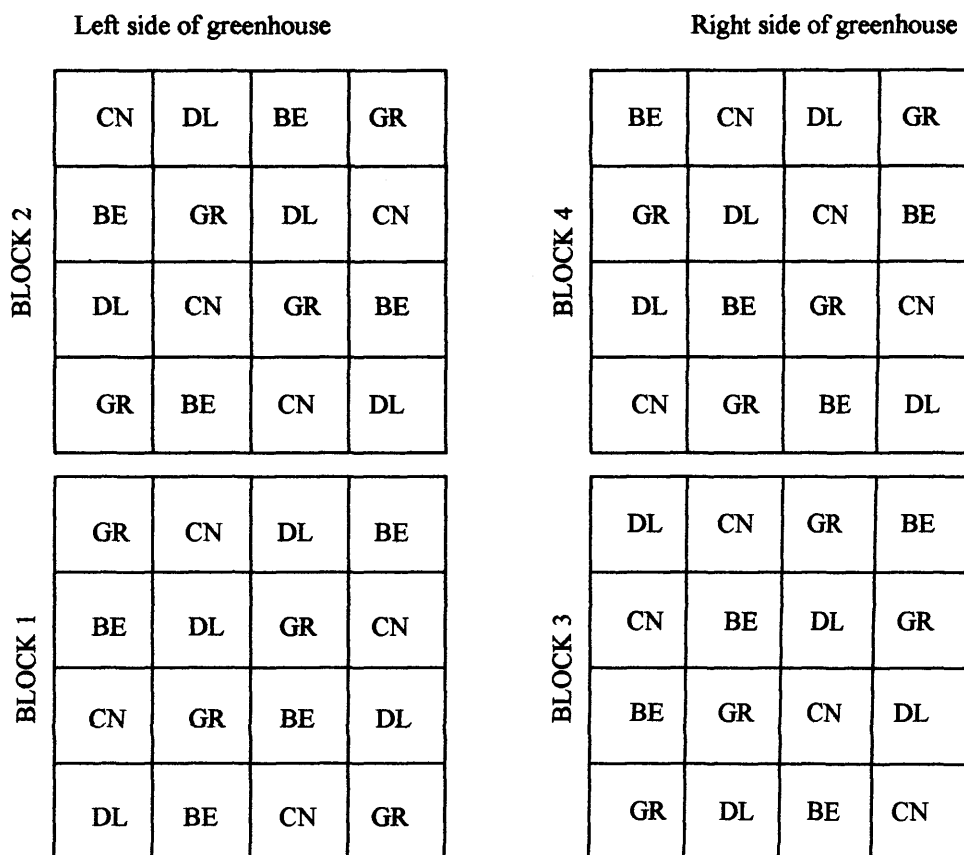


Figure 5.1 Design of experimental plots to examine microhabitat use by new juvenile *Rana temporaria*. GR = short grass; CN = conifer needles; DL = deciduous leaves; BE = bare earth. The microhabitat plots within a block each measured approximately 0.5 m x 0.5 m.

5.2.4 Statistical analysis

The emergence behaviour of metamorphs at each pond was assessed using χ^2 goodness of fit tests to determine if variation between emergence frequencies was related to marginal or bankside vegetation, or orientated in a general direction to the north, south, east or west. A Scheirer-Ray-Hare test was used to test for differences between ponds in overall vegetation height and cover measured within a 50 m radius of the pond margin. Significant differences in overall height and height at fixed distances from the pond margin were determined with Mann-Whitney tests. To examine variation in vegetation height around individual ponds, two-way ANOVAs were used following log transformation of raw data to achieve normality. Friedmans' test was used where suitable transformation was not achieved. Variation in type of vegetation cover around individual ponds was analysed using Scheirer-Ray-Hare tests. T-tests were used to compare vegetation height at locations where juveniles were caught with locations where no juveniles were caught. Raw data were log transformed prior to analysis to achieve equality of variance. To examine microhabitat use by juveniles, χ^2 goodness of fit tests were used. Soil moisture differed significantly between the first and second 'wet' experiments and there was a potential temperature difference between the two sides of the greenhouse. Therefore, the four experiments were tested individually and for each experiment Blocks 1 and 2 were combined and tested separately from Blocks 3 and 4 (also combined). ANOVAs were used to test for differences in soil moisture between microhabitat types and *a posteriori* Tukey-Kramer pairwise comparisons of group means identified which microhabitat types differed significantly from each other. Bonferroni corrections were applied where multiple tests were used to reduce to the probability of making a Type I error (Sokal and Rohlf, 1995). All analyses were performed in Minitab (Release 13.0)

5.3 Results

5.3.1 Metamorph emergence patterns

Pattern of metamorph emergence was not significantly related to the presence or absence of marginal vegetation at five out of seven ponds (χ^2 goodness of fit tests: $P > 0.05$ all tests). Emergence at Pond 8 was not analysed because more than one category comprised < 5 animals. However, at Ponds 3 and 4 frequencies of emerging metamorphs were significantly different from expected values (χ^2 goodness of fit tests: $\chi^2 = 49.634$, d.f. = 1, $P < 0.001$ Pond 3, and $\chi^2 = 8.986$, d.f. = 1, $P < 0.01$ Pond 4) (Figure 5.2). In both cases, fewer animals than expected emerged where marginal vegetation was present. Conversely, numbers leaving the ponds where vegetation was absent was higher than expected at both ponds. There was no significant difference between observed and expected frequencies of metamorph emergence in relation to vegetation on the bank at any pond (χ^2 goodness of fit tests: $P > 0.05$ all tests). Similar tests were performed to see if emergence was generally orientated in any particular direction (north, south, east or west). Pond 2 was not analysed as numbers were < 5 in more than one category. At Pond 1 observed and expected frequencies differed significantly (χ^2 goodness of fit test: $\chi^2 = 15.946$, d.f. = 3, $P < 0.01$) (Figure 5.3). Higher than expected numbers of metamorphs exited the pond on the eastern side while fewer than expected exited at the southern end. There was also a highly significant shift in the expected emergence pattern at Pond 3 (χ^2 goodness of fit test: $\chi^2 = 30.284$, d.f. = 3, $P < 0.001$). A decrease in the number expected to emerge along the western edge of the pond coincided with raised numbers emerging towards the south. Observed emergence patterns did not differ from expected patterns at any of the remaining ponds (χ^2 goodness of fit tests: $P > 0.05$ all tests).

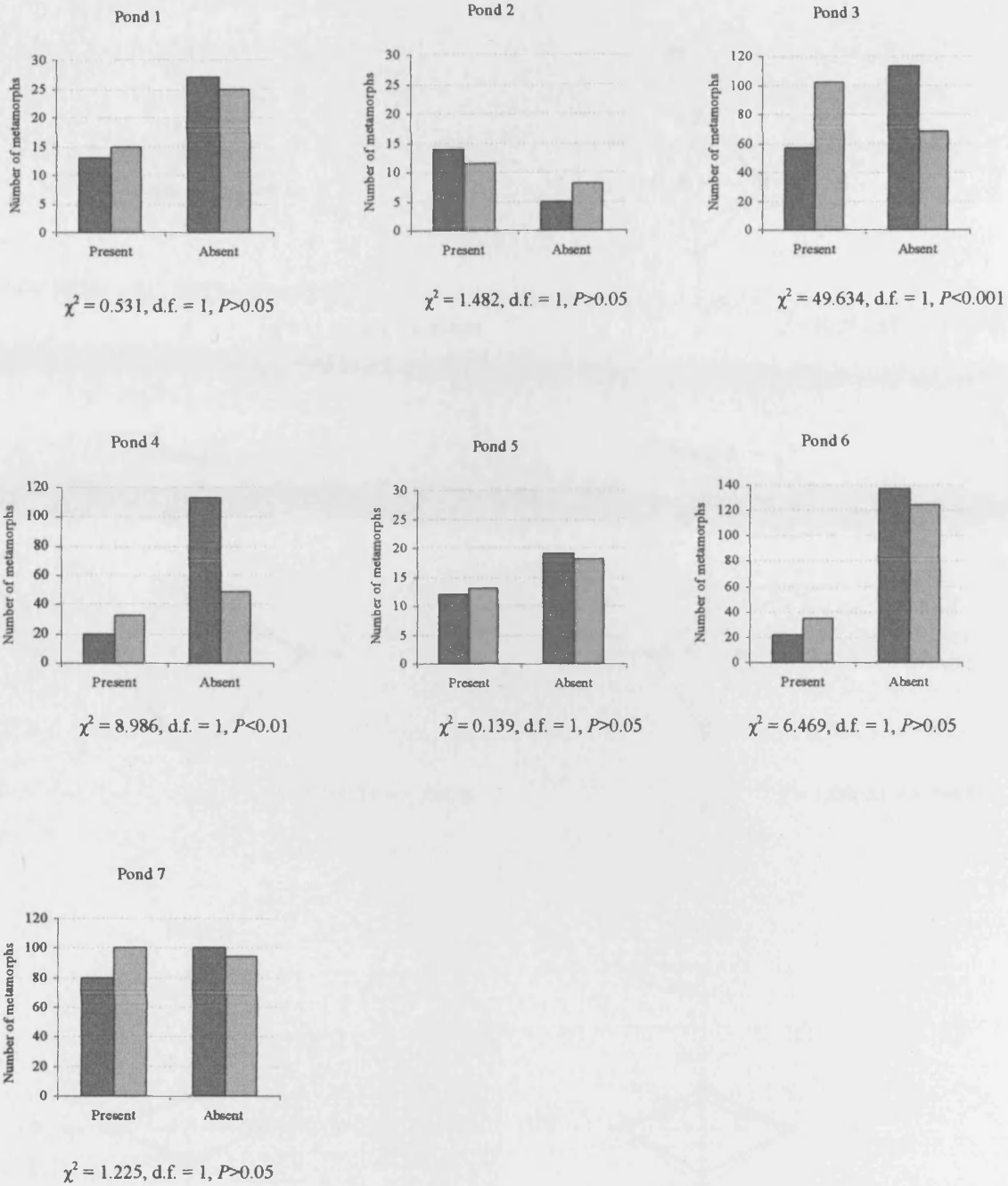


Figure 5.2 Observed (■) and expected (▨) frequencies of emerging *Rana temporaria* metamorphs captured in pitfall traps around the perimeter of seven ponds in mid-Wales. Site of emergence is categorised as occurring where aquatic marginal vegetation is present and where aquatic marginal vegetation is absent. Results of χ^2 goodness of fit tests comparing observed and expected frequencies are shown.

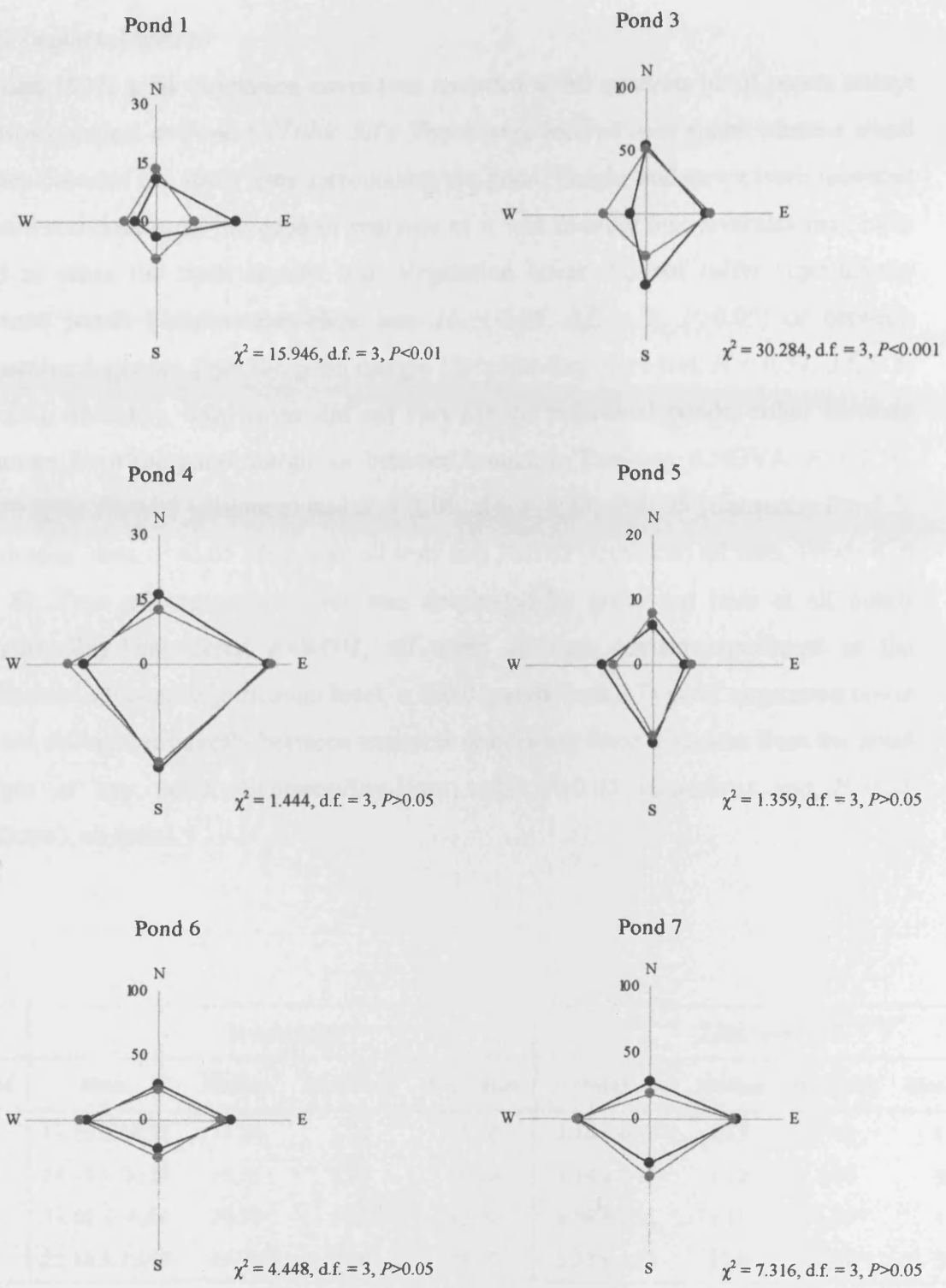


Figure 5.3 Observed (●) and expected (●) frequencies of emerging *Rana temporaria* metamorphs captured in pitfall traps around the perimeter of six ponds in mid-Wales. Direction of emergence is categorised as being towards the north (N), east (E), south (S) or west (W). Results of χ^2 goodness of fit tests comparing observed and expected frequencies are shown.

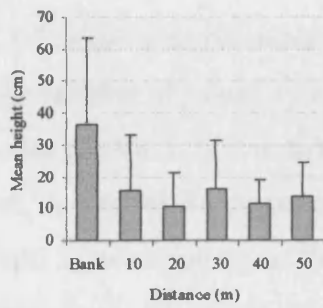
5.3.2 Dispersal habitat

At least 100% total vegetation cover was recorded in all quadrats at all ponds except for two quadrats at Pond 6 (Table 5.1). These were located over water where a small stream bisected the study area surrounding the pond. Height and cover were recorded as zero and data were included in analyses as it was feasible that juveniles may have tried to cross the open stream bed. Vegetation cover did not differ significantly between ponds (Scheirer-Ray-Hare test: $H = 7.05$, d.f. = 3, $P > 0.05$) or between successive distances from the pond margin (Scheirer-Ray-Hare test: $H = 0.57$, d.f. = 5, $P > 0.05$). Similarly, total cover did not vary around individual ponds, either between distances from the pond margin or between transects (Two-way ANOVA: $F = 0.56$, d.f. = 5,45, $P > 0.05$ (distance) and $F = 1.09$, d.f. = 9,45, $P > 0.05$ (transects) Pond 3; Friedmans' tests: $P > 0.05$ (distance) all tests and $P > 0.05$ (transects) all tests, Ponds 4, 6 and 8). Type of vegetation cover was dominated by grass and herb at all ponds (Scheirer-Ray-Hare tests: $P < 0.001$, all tests; all tests remain significant at the Bonferroni adjusted significance level, $\alpha = 0.01$, eight tests). Type of vegetation cover did not differ significantly between transects or between fixed distances from the pond margin at any pond (Scheirer-Ray-Hare tests: $P > 0.05$ (transects) and $P > 0.05$ (distance), all tests).

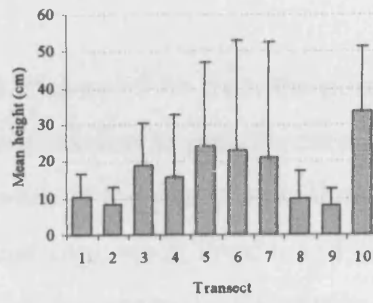
Pond	Height (cm)				Total cover (%)			
	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum
3	17.26 ± 18.57	11.20	1.00	85.00	1.16 ± 0.11	1.15	1.00	1.41
4	24.75 ± 20.21	16.65	5.40	76.20	1.14 ± 0.13	1.12	1.00	2.00 [†]
6	11.02 ± 6.89	10.50	1.50*	47.90	1.08 ± 0.21	1.11	1.00*	1.30
8	22.36 ± 15.58	16.95	4.00	76.30	1.23 ± 0.22	1.20	1.02	2.14 [†]

Table 5.1 Vegetation height (cm) and total vegetation cover (%) within a 50 m radius of the margin of four *Rana temporaria* breeding ponds in mid-Wales. Summary data were obtained from 60 quadrats placed around each pond (six quadrats placed along ten transects). *zero height and cover recorded in two quadrats located in a small stream, [†]multiple vegetation layers.

Mean vegetation height within 50 m radius of the pond margin differed significantly between ponds (Scheirer-Ray-Hare test: $H = 26.31$, d.f. = 3, $P < 0.001$). Height also differed according to distance from the pond margin (Scheirer-Ray-Hare test: $H = 34.19$, d.f. = 4, $P < 0.001$) but differences were independent of the study pond (Scheirer-Ray-Hare test: $H = 12.00$, d.f. = 4, $P > 0.05$). Generally, vegetation was shorter at ponds 3 and 6 than at Ponds 4 and 8 (Mann-Whitney tests: $W = 3156.5$, $P = 0.013$ (Pond 3 vs. Pond 4); $W = 3026.5$, $P = 0.0016$ (Pond 3 vs. Pond 8); $W = 2951$, $P = 0.0004$ (Pond 6 vs. Pond 4); $W = 2715.5$, $P < 0.0001$ (Pond 6 vs. Pond 8), all tests remain significant at the Bonferroni adjusted significance level, $\alpha = 0.05$, four tests). Vegetation height did not differ significantly between Ponds 3 and 6 (Mann-Whitney test: $W = 3810.0$, $P > 0.05$) or between Ponds 4 and 8 (Mann-Whitney tests: $W = 3596.0$, $P > 0.05$). There was no significant difference in height between the bank at the pond margin and at 10 m from the margin (Mann-Whitney test: $W = 1808.5$, $P > 0.05$). Vegetation was generally taller on the bank than at 20 m - 50 m (Mann-Whitney tests: $P \leq 0.001$ all tests) and taller at 10 m than at 20 m (Mann-Whitney test: $W = 1895.0$, $P = 0.0083$). Significant differences in height between 10 m and 30 m, 40 m, and 50 m (Mann-Whitney tests: $P \leq 0.02$ all tests) were considered insignificant at the Bonferroni adjusted significance level, $\alpha = 0.05$, seven tests. Height differences between 20 m - 50 m were insignificant (Mann-Whitney tests: $P > 0.05$, all tests). Vegetation height also varied significantly around individual ponds (*Figure 5.4*). At Pond 3 height varied between distances but not between transects (Two-way ANOVA: $F = 3.26$, d. f. = 5,45, $P = 0.014$ (distance) and $F = 1.86$, d. f. = 9,45, $P > 0.05$ (transect)). Conversely, height varied between transects at Pond 4 (Friedmans' test: $S = 23.11$, d.f. = 9, $P = 0.006$) but not between distances (Friedmans' test: $S = 5.60$, d.f. = 5, $P > 0.05$). Height differed significantly between both distances and transects at Pond 6 (Friedmans' test: $S = 17.12$, d.f. = 5, $P = 0.004$ (distance) and $S = 16.93$, d.f. = 9, $P = 0.050$ (transect)) and at Pond 8 (Two-way ANOVA: $F = 11.90$, d. f. = 5,45, $P < 0.001$ (distance) and $F = 2.60$, d. f. = 9,45, $P = 0.017$ (transect)).

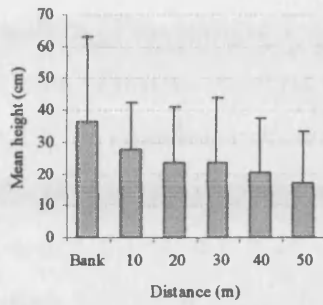


$F = 3.26, d. f. = 5, P = 0.014$

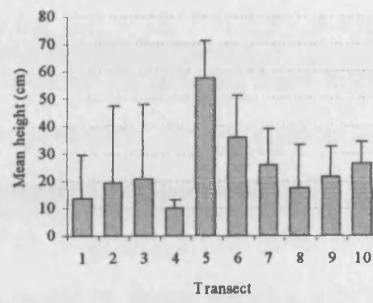


$F = 1.86, d. f. = 9, P > 0.05$

Pond 3

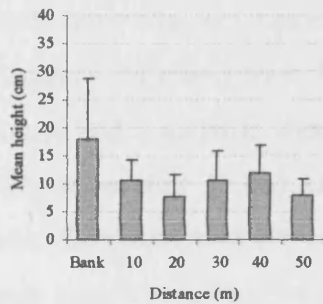


$S = 5.60, d. f. = 5, P > 0.05$

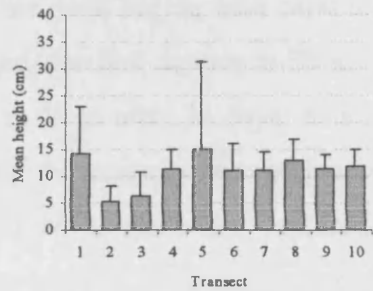


$S = 23.11, d. f. = 9, P = 0.006$

Pond 4

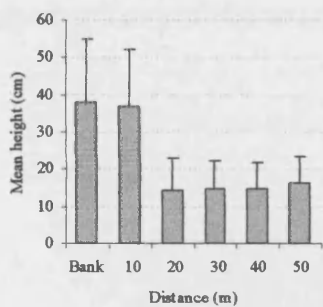


$S = 17.12, d. f. = 5, P = 0.004$

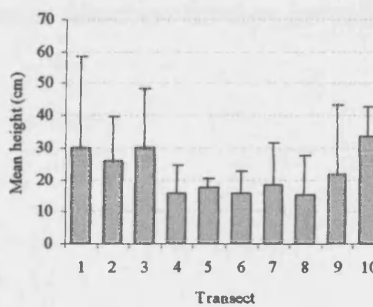


$S = 16.93, d. f. = 9, P = 0.050$

Pond 6



$F = 11.90, d. f. = 5, P < 0.001$



$F = 2.60, d. f. = 9, P = 0.017$

Pond 8

Figure 5.4 Mean (+ SD) vegetation height measured around four *Rana temporaria* breeding ponds in mid-Wales. Data were obtained from quadrats placed along 10 random transects extending outwards from the pond margin. Starting on the bank at the pond margin, six quadrats were placed at 10 m intervals along each transect. Results of Two-way ANOVA and Friedmans' tests comparing heights between distances and between transects at each pond are shown.

5.3.3 Dispersing juveniles

The number of juveniles caught at 25m and 50m from the pond margin was low at all ponds (Table 5.2). Animals were not marked so possible recaptures could not be ruled out. A total of 21 captures were made at the four ponds; three captures were made at Pond 3, two captures at Pond 4, four captures at Pond 6 and three captures at Pond 8. Data from all ponds were pooled for analysis. Vegetation height did not differ significantly between transects where captures were made and transects with no captures (Two-sample T-test: $t = 0.68$, d.f. = 38, $P > 0.05$). A total of 23 juvenile newts, or efts, (*Triturus vulgaris* and/or *Triturus helveticus*) were also captured at Ponds 4, 6 and 8. In contrast to *R. temporaria* juveniles, the vegetation height of transects where efts were captured differed significantly from transects with no captures (Two-sample T-test: $t = 3.14$, d.f. = 28, $P = 0.004$). Vegetation was taller in transects containing efts (mean 26.22 cm \pm 12.79 cm) compared to those without efts (mean 15.42 cm \pm 6.63 cm). Juvenile frogs at Ponds 3 and 8 were captured in traps at 25 m from the pond 24-28 days after emergence from the pond began, with captures at 50 m occurring 39-51 days after emergence. In contrast, the first capture at 25 m at Pond 4 occurred after 8 days and a juvenile was caught at 50 m after 24 days. At Pond 6, juveniles reached 50 m traps after 29 and 31 days but the majority of captures were at 25 m after 30-33 days. Common toad (*Bufo bufo*) juveniles were also captured in traps at Pond 8 and were caught simultaneously in four traps at 25 m and two at 50 m 24 days after first emergence. Although data were insufficient to analyse statistically, dispersal did not appear to be confined to any particular direction for frog juveniles.

Pond	<i>Rana temporaria</i>		<i>Bufo bufo</i>		<i>Triturus</i> spp.	
	25 m	50 m	25 m	50 m	25 m	50 m
3	2	2	-	-	0	0
4	2	1	-	-	3	5
6	5	2	-	-	1	0
8	6	1	13	11	10	4
Total	15	6	13	11	14	9

Table 5.2 Total numbers of juvenile *Rana temporaria*, *Bufo bufo* and *Triturus* spp. captured in pitfall traps within a 50 m radius of four breeding ponds in mid-Wales. Traps at each pond were located along ten random transects at 25m and 50 from the pond margin.

5.3.4 Habitat utilisation by new juveniles

Juveniles demonstrated a strong preference for microhabitat choice (*Figure 5.5*). Grass was favoured over conifer needles, bare earth and deciduous leaf litter under both wet and dry conditions (χ^2 goodness of fit tests: $P < 0.001$ all tests, all tests remain significant at the Bonferroni adjusted significance level, $\alpha = 0.01$, eight tests). Soil moisture differed significantly between microhabitat types in all four experiments (ANOVA: $P \leq 0.03$) (*Table 5.3*). *A posteriori* comparisons of group means showed that the moisture content in soil under grass was significantly higher (at $\alpha = 0.05$ level) than in all other microhabitat types in both wet and dry experiments. Additionally, both deciduous and coniferous leaf litter had soil moisture levels equal to or less than bare earth in both wet and dry conditions.

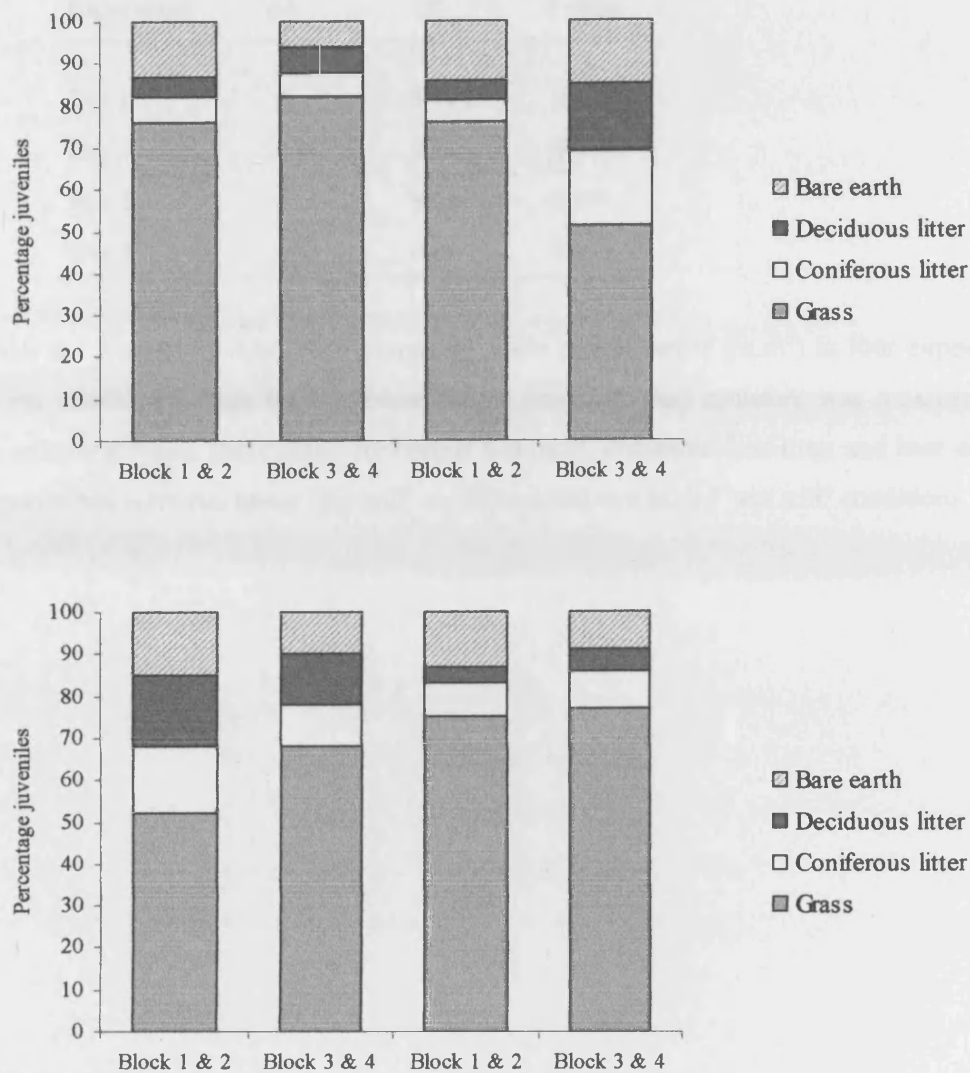


Figure 5.5 Percentage of *Rana temporaria* juveniles located in four microhabitat types; short grass, coniferous leaf litter, deciduous leaf litter and bare earth under dry soil conditions (top graph) and wet soil conditions (bottom graph). Microhabitats were replicated four times each within four experimental blocks. The experiment was conducted twice for both dry and wet conditions. The experimental blocks on the left and right sides of the greenhouse were analysed separately (Blocks 1& 2 and Blocks 3 & 4, respectively) due to differences in soil moisture and temperature (χ^2 goodness of fit tests: $P < 0.001$, all tests significant at the Bonferroni adjusted significance level, $\alpha = 0.01$).

Experiment	d.f.	<i>F</i>	<i>P</i> value
Dry 1	3	5.12	0.003
Dry 2	3	3.32	0.026
Wet 1	3	9.26	<0.001
Wet 2	3	6.84	<0.001

Table 5.3 Results of ANOVAs comparing mean soil moisture (m.m³) in four experiments to assess microhabitat use by *Rana temporaria* juveniles. Soil moisture was measured in four microhabitat types: short grass, coniferous leaf litter, deciduous leaf litter and bare earth. Two experiments were run under 'dry soil' conditions and two under 'wet soil' conditions.

5.4 Discussion

Rana temporaria juveniles and adults are known to inhabit similar microhabitats, e.g. long grass and woodlands (Loman, 1980; Loman, 1994; Savage, 1961). However, there is little or no information on microhabitat use by newly emerged juveniles undergoing early dispersal. The results of the field study show that during this stage, juvenile frogs do not actively select vegetation cover during emergence and will cross areas of very short grass and small areas void of vegetative cover during preliminary dispersal. Similar findings have been observed for common toads where new juveniles dispersed through relatively open cereal fields as well as through more dense vegetation (Oldham, 1985). This is in contrast to adult toads which, like adult common frogs, typically avoid semi-open habitats in favour of areas with more vegetative cover (Denton and Beebee, 1994).

Although juveniles generally showed no preference for presence of aquatic emergent vegetation around the pond margin or on the bank there was a distinct avoidance of areas with marginal vegetation at two ponds. At both of these ponds the depth of marginal vegetation was extensive and there was no marked transition between pond and 'dry' land. Instead the adjacent area was waterlogged and generally boggy. If *R. temporaria* fail to leave the water at metamorphosis they 'drown' (Savage, 1961 and personal observation) and this may be why these areas were avoided. This would also explain why emergence along the eastern side of the pond was lower than expected at Pond 3. There must be another explanation for the changes in predicted emergence at Pond 1. Emergence along the eastern edge of the pond was favoured over emergence to the south. Increased numbers of juveniles have been observed leaving ponds at the site of spawn deposition (Paull *et al.*, 1981). Spawn in Pond 1 was located at the southern edge. Along part of the eastern edge the bank was slightly steeper which may have funnelled animals to the area where most individuals were caught. Here the shoreline was shallow with a mixture of herb vegetation >30 cm tall and semi-exposed mud. There is no obvious reason why animals would not have followed the bank in the other direction to the south. This area also comprised semi-exposed wet mud interspersed with yellow iris (*Iris pseudacorus*). It is not clear why animals used one area more than the other.

Around the ponds studied for dispersal behaviour, the only areas lacking in vegetation cover were the stream bed at Pond 6 and a ≈ 2.5 m wide mud track parallel to the northern side of Pond 3 at a distance of ≈ 5 m away. Juveniles were captured beyond this so it did not appear to affect dispersal. Other studies have shown that some dispersing juveniles will cross non-vegetated areas and that these are generally traversed more quickly than vegetated areas (Oldham, 1985; Stevens *et al.*, 2004). The risk of dehydration can be reduced if juveniles wait until conditions are favourable, i.e. after rainfall, before crossing areas such as roads (Oldham, 1985; Paull *et al.*, 1981), although there is still the risk of predation and mortality from traffic. The terrestrial habitat analysis undertaken in Chapter 2 demonstrated that there are few major roads within the study area but Pond 8 lies 265 m from the major road linking north and south Wales and Pond 6 is within 450 m of a road, although traffic density here is much lower. Roads are known to be effective barriers to amphibian movements (Reh and Seitz, 1990). Mortality increases with increasing traffic density (Fahrig *et al.*, 1995) and mortality of migrating adults is a potential factor contributing to some local population declines (Cooke and Sparks, 2004). Migration may also be affected by other factors such as run-off, exhaust emissions, vibration and noise (Buchanan, 1993 cited in Fahrig *et al.*, 1995). Hels and Buchwald (2001) estimated that up to 10% of a *R. temporaria* population were killed annually by traffic at one site. However, Cooke and Sparks (2004) propose that common frog populations are less likely to be impacted upon by roads than toad populations because adult frogs do not migrate as far as adult toads, they move faster across roads and generally avoid such open areas. Although mortality may be reduced by not crossing roads, there still might be significant effects upon populations. Reh and Seitz (1990) found that major roads impede transfer of individuals and cause genetic isolation of populations within a relatively short length of time. Road mortality studies have traditionally focused upon migrations of breeding adults but given the greater distances moved by juveniles research is needed to assess exactly how roads affect juvenile dispersal. For example, it is not clear that juvenile common frogs avoid crossing roads, particularly in wet weather. They will also cross more slowly than adults so mortality rates may be high, particularly where traffic volumes are high. Tunnels built under roads to conserve migrating populations of breeding adults may also increase survival of emigrating juveniles but there are no known studies of their effectiveness for juveniles.

Similarly, it is not known to what extent rivers affect dispersal. No animals were captured in traps beyond the stream at Pond 6 (water channel width $\approx 1 - 4$ m) but the generally low capture rates mean it is not possible to determine whether dispersal was affected. In summer, flow rates are generally lower which would aid crossing and it is possible that some dispersal may occur as a result of animals being swept downstream. Although adults would have little difficulty in crossing relatively small rivers, it would be interesting to see at what particular width and/or flow rates dispersal by juveniles is inhibited. Additionally, predation by fish such as trout (*Salmo trutta*), could perhaps limit dispersal in some areas.

In this study, the majority of habitat immediately surrounding ponds had consistently high vegetation cover and excessive trampling by livestock was not evident. Grass and herb accounted for the majority of cover. Cover by brambles, bracken and moss was largely restricted to the area around the pond banks. In contrast, vegetation height was much more variable both between ponds and around individual ponds. Generally, vegetation tended to be tallest on the banks around the margin where there was less impact from grazing. Many animals emerging from the pond were still in the latter stages of metamorphosis and residual tails were still visible. Dispersal may not be possible until the considerable physical and physiological changes that occur at metamorphosis have been completed and/or size has increased to reduce mortality from evaporative water loss (Cohen and Alford, 1993). Vegetation in this area will be important in providing juveniles with a suitable microclimate and protection from predators. At two ponds, vegetation was shorter and as low as 1 - 1.5 cm in some quadrats compared to minimum heights of 4 - 5.4 cm at the other two sites. However, the lack of significant difference in vegetation height between areas where juveniles were caught and areas where they were not caught suggests that very young frogs do not necessarily avoid areas with short grass, i.e. areas that are heavily grazed. The same appeared to be true for juvenile *B. bufo* although sample sizes were too small to analyse statistically. Conversely, *T. vulgaris*/*T. helveticus* juveniles appeared to favour areas with taller grass/herb cover. Adults of these two species typically inhabit scrubby areas close to woodland and generally avoid open pasture (Beebee, 1980; Beebee, 1981). Marnell (1998) found that long grass cover around breeding ponds was favoured as a microhabitat by adult frogs but it negatively influenced the presence of adult *T. vulgaris*. However, long grass may significantly reduce mortality of juvenile

newts forced to cross open fields during dispersal. Rothermel and Semlitsch (2002) found that increased mortality was the most probable explanation for differences in salamander capture rates between open fields and woodlands. Efts may not be able to move through vegetation as quickly as anuran juveniles and so may be more prone to dehydration and predation in short grass. Areas of taller vegetation may therefore connect and prevent isolation of breeding ponds and preferred terrestrial habitats. This confirms the importance of evaluating habitat use for all life-stages as microhabitats used by adults and juveniles can differ (e.g. Rothermel and Semlitsch, 2002; Sjogren-Gulve, 1998). Moreover, studies need to be detailed and ideally replicated to reflect differences between superficially similar habitats as dispersal may potentially vary considerably within a general habitat type.

Although juvenile frogs (and toads) disperse across areas of shorter vegetation, it is not known to what extent survival may be reduced compared to those within taller or more dense vegetation. Oldham (1985) found no evidence for reduced survival of *B. bufo* juveniles in arable fields compared to those dispersing through scrub. As vegetation height increases relative to body length, speed of movement and linearity of movement decrease (Stevens *et al.*, 2004). Therefore, movement through short vegetation can be faster and so off-set some of the risks associated with exposure in more open areas. Body size also has an effect on locomotive ability and larger juveniles can move more quickly and for longer periods (Goater *et al.*, 1993; Stevens *et al.*, 2004). In this study, data were insufficient to assess dispersal rates, although there appeared to be a delay of about 3 weeks before animals were detected at 25 m and then they were found at 50 m almost simultaneously. This may suggest that once dispersal was underway distances of tens of metres can be covered relatively quickly and this appeared to be the case for both frog and toad juveniles. Such fast dispersal rates have been observed for other anuran species (Dole, 1971; Oldham, 1985; Sinsch, 1997). One frog juvenile took a maximum of eight days to cover 25 m (assuming it was one of the first to emerge from the pond) averaging approximately 3 m per day. This also suggests that some individuals are capable of dispersal very soon after emergence. Local environmental factors, e.g. substrate moisture, may have favoured immediate dispersal at this pond. Vegetation was taller and some of the surrounding area was damp and marshy so the warm, dry weather that possibly delayed the start of dispersal at other ponds might have had a less negative effect here. At other ponds, many animals might have resided

in the longer vegetation nearer to the pond until cooler, wetter weather favoured migration. Smaller individuals in particular may remain close to the pond during extremely warm conditions to avoid dehydration (Cohen and Alford, 1993). Dole (1971) found that the most significant dispersals of leopard frog juveniles (*Rana pipiens*, Schreber, 1782) occurred following rain. Also, as already mentioned many individuals may not have been in a physiological or physical condition to begin dispersing straight after emergence. The differences in metamorph body size found between ponds in Chapter 4 means that the amount of vegetation cover around pond margins may be relatively more important at ponds where smaller animals emerge.

Failure to quantify precise dispersal times and rates is due in part to the low capture rate. In this study, 20 traps per pond were used (not including traps assessing emergence at the pond margin). This meant that at a radius of 50 m from the pond margin only 1-2% of the imaginary circumference was covered which obviously would explain the low capture rates. Pitfall traps have been used previously to assess dispersal of juveniles after emergence (Oldham, 1985). To be effective, traps need to be at sufficient density to realistically portray abundance and dispersal rates within different habitats. This is labour intensive and expensive, particularly where multiple ponds are trapped and as trap damage due to farm machinery and livestock can be high (Oldham, 1985 and this study). At Pond 6, however, capture rate was still low even though four transects were separated by only 3 - 4 m (a coverage of $\approx 17\%$) and emergence was relatively high (see Pond 10, Chapter 4). Many juveniles were observed in the long grass at the pond margin for several weeks which seems to suggest that initial dispersal rates were too low to be detected, even at a higher trapping density, and that the main dispersal phase had possibly not begun by the time the study ended.

The low capture rates also mean that it is not possible to tell if dispersal was focused in any particular direction. Initial observations suggest that dispersal was probably random as animals were captured in transects that were orientated away from each other by $\geq 90^\circ$. However, dispersal direction can be radial or highly directional and can change between years (Dole, 1971; Sinsch, 1997), probably depending upon changes in topography and/or microclimatic conditions. If dispersal is highly directional to begin with, once the habitat becomes more suitable (e.g. more cover and/or a wetter

substrate) then dispersal patterns may change and become more random. Random dispersal in multiple directions is more advantageous as it reduces densities and ensures that all neighbouring habitat patches and sink populations are reached (Sinsch, 1997). Ideally, dispersal studies should be conducted for longer periods and repeated between years in order to detect such changes. Unfortunately, long-term studies have been prevented to date by the lack of a suitable tracking technique. Radio-telemetry studies are prohibited by small body sizes preventing use of traditional transmitters (Heyer *et al.*, 1994). High mortality rates in the first few days or weeks after metamorphosis (Goater, 1994; Savage, 1961) might mean that large numbers of animals would have to be fitted with transmitters initially to obtain sufficient data. For current studies, care is needed when inferring the relative importance of habitats and identifying potentially isolated or fragmented habitats as what appears to be an unsuitable habitat in a short-term study may not actually be so.

On closer examination of two ponds, all captures were within transects extending towards trees and woodland cover but this may be coincidental. Juvenile wood frogs (*Rana sylvatica*, LeConte, 1825) show a preference for woodland habitat and the presence of understorey vegetation immediately upon emergence (de Maynadier and Hunter, 1999). Sjogren-Gulve (1998) found that vegetation had no effect in determining dispersal directions of adult and older juvenile *R. temporaria*. However, juveniles demonstrated significant target-orientated movement to and from nearby woodland. Woodland habitats provide cooler, more stable microclimates that may be preferred by common frogs (Sjogren-Gulve, 1998).

In the experiments examining specific microhabitat use there was a strong avoidance of both deciduous and coniferous leaf litter, as well as bare earth, and grass was preferred in all instances. Soil moisture levels were significantly higher in grass plots in both wet and dry conditions and, surprisingly, moisture levels were sometimes slightly higher in bare earth plots than under leaf litter. Bare earth soil may have been slightly wetter than litter soils due to a leak in the greenhouse roof sometime before the experiment began, although all blocks were covered prior to and during the experiments. In the second 'wet' experiment water may also have evaporated from the litter surface before it could soak down fully into the soil layer, although replicates were still wetter than in the previous 'dry' experiments. Adult *R. temporaria* exhibit

behavioural thermoregulation by moving between cooler and warmer areas and have a preferred summer temperature range of 10-20°C (Sinsch, 1984). Temperatures at the conclusion of the four experiments ranged between 15.3 -22.3°C. (Unfortunately, it was not possible to compare temperatures between microhabitats due to datalogger failures.) Thermal preferences of small juveniles are unknown but relatively higher moisture levels in the grass plots would help prevent dehydration under very warm conditions. However, relative humidity in bare earth plots in the 'wet' experiments appeared to be similar to levels in grass plots in the 'dry' experiments suggesting that soil moisture in non-vegetated areas in the 'wet' experiment was probably sufficient to prevent dehydration. In addition, juveniles in the field were found in areas much drier than in the experimental blocks suggesting that the experimental conditions could be easily tolerated. Grass plots might also be preferentially selected as they afford potentially greater protection from some predators. Although animals crossed areas of very short grass and dirt tracks in the field and crossed open plots to reach the vegetated plots in the greenhouse, young *R. temporaria* juveniles are diurnal (Loman, 1980) and in natural conditions they probably minimise exposure by remaining in suitable cover once they find it. It is also possible that although drier conditions may be well tolerated, juveniles will choose a wetter substrate if available when not actively dispersing. For example, leopard frogs typically inhabit wet, marshy grassland and wet woodlands, and both adults and juveniles often only move significantly following rainfall (Dole, 1965; Dole, 1971). However, juveniles can travel through much drier areas during dispersal (Dole, 1971) and it is known that adult frogs usually select very wet soil surfaces even though they are capable of tolerating much drier ones (Tracy, 1976).

This study has shown that at emergence and during early dispersal juvenile frogs may utilise habitats that adult, and older juveniles, are generally considered to avoid and will disperse across at least small areas with little or no vegetative cover. However, there may be a strong aversion to remaining in such areas and/or that survival in such areas may be compromised even under superficially suitable microclimatic conditions. In the absence of more detailed knowledge regarding mortality and dispersal ranges within different habitats under different microclimatic conditions, maintaining the presence of frequent patches of 'traditional' cover is recommended. This may also benefit dispersing juveniles of other species about which relatively little is known.

This study was conducted within a rural upland area where grazing pasture forms a significant part of the landscape. However, large expanses of heavily grazed pasture are rare as there are numerous patches of long grass, scrub, woodlands and hedgerows within close proximity of many ponds and between ponds. Another potentially limiting habitat, however, might be the extensive plantations of coniferous trees. The density of commercially planted trees means that in mature stands there is no grass or herb understorey and in warm, dry weather dispersal may be limited by the lack of moist cover. Consequently, predation by birds and small mammals, for instance, may also be higher. More research is required to assess longer-range dispersal ability and long-term survival rates within different habitats of different size. Without the ability to conduct long-range surveys it is difficult to quantify the relative importance of different habitats to juveniles and to identify the critical size at which some habitats become barriers to movement. Ideally, landscapes would comprise a mosaic of habitat patches of small enough size to prevent fragmentation but without detailed knowledge of dispersal capacity and mortality rates within different habitat types (which will vary from species to species) it is difficult to gauge ideal patch sizes. However, small scale studies can still offer insight into whether unused habitats are genuinely barriers to movement and also how habitats used by adults and dispersing juveniles may differ (e.g. Marsh *et al.*, 2004; Sjogren-Gulve, 1998).

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Chapter 6

Discussion and conclusions

6.0. Thesis background

Rural upland areas are relatively poorly studied in terms of amphibian distribution and published information regarding regulation of amphibian populations can be lacking. In central Wales, for example, the available data show highly variable pond occupancy rates for common frogs (*Rana temporaria*) (Knight, 1989; Williams, 1995, Slater, unpublished data). It appears that amphibian distribution may be influenced by features of aquatic and terrestrial habitats (Williams, 1995). However, the extent of influence that is evident at the landscape level is not understood. Factors thought to have contributed to amphibian population declines globally may also affect native populations. Closer examination of the literature shows that several of these are unlikely to impact significantly upon current distribution and abundance, although there may be effects in the future as habitats are altered by changes in climate. Environmental contaminants arising from agricultural and forestry land uses and predation by fish could be potential influences. These factors are generally secondary influences accompanying habitat modification by agriculture and conifer afforestation together with acidification, and would require closer investigation should initial patterns of distribution suggest reduced presence or abundance in improved areas. The most significant and large-scale influences on amphibians in rural upland areas will probably arise from habitat modification by agriculture and conifer afforestation, and acidification. Potential influences could affect different life-stages through altered distribution and abundance patterns of breeding adults and/or reduced embryo and larval survival resulting in reduced juvenile recruitment. Number and physical condition of new juveniles may be affected by the aquatic environment, which could have implications for future life fitness and abundance. The surrounding terrestrial habitat may also affect survival at juvenile emergence and affect subsequent dispersal potentially restricting distribution.

Habitat type has already been implicated as a potential influence upon amphibian populations in central Wales. The above hypotheses were therefore investigated across

the same region using *R. temporaria* as a model organism. Possible patterns of pond occupancy and relative abundance of breeding females were evaluated in relation to biotic and abiotic features of aquatic and surrounding terrestrial habitats at the landscape scale. Possible influences on aquatic and early terrestrial life-stages were subsequently assessed in more detail with replicated studies performed at a more restricted scale.

6.1 Synthesis of findings

The present study found a very high occupancy rate ($\geq 90\%$) of breeding ponds in two concurrent years demonstrating that common frogs are currently ubiquitous and abundant in rural upland areas of mid-Wales. Furthermore, *R. temporaria* was found to exploit a diverse range of aquatic habitats for breeding and was found to breed in acidic and non-acidic ponds located in unimproved hilltop grassland, conifer plantation and improved pasture. There was no evidence to suggest that distribution or abundance were limited by land-use type. These results were contrary to earlier assessments of frog presence/absence in the area which found lower occupancy rates, possibly influenced by habitat type (Knight, 1989; Williams, 1995 and Slater, unpublished data). In particular, the present study provided no evidence to suggest that frogs were less abundant in improved pasture relative to unimproved and semi-improved upper slopes and hilltops as suggested by Williams (1995). Differences in timing and methodology of previous surveys and population fluctuations between years (Meyer *et al.*, 1998) would account for the variable occupancy rates found in previous studies. The lack of continuous data prevents speculation regarding actual changes in status and distribution.

The prevalence of frogs in many ponds contributed to the lack of significant association with aspects of aquatic and terrestrial habitat. Land cover type measured at four successive distances around the pond was highly correlated suggesting no significant changes in habitat cover within a 1 km radius of the pond margin. The habitat requirements of British species have been described generally from their distribution and abundance in a variety of different native habitats (Beebee, 1977; Beebee, 1979; Beebee, 1980; Beebee, 1981; Beebee, 1983) and non-native habitats (Dolmen, 1980; Laan and Verboom, 1990; Strijbosch, 1979a; Strijbosch, 1979b).

Discriminant analysis and regression techniques have also been applied to identify features of aquatic and terrestrial habitats that may potentially explain presence or absence of species (Baker and Halliday, 1999; Beebee, 1985; Marnell, 1998; Scribner *et al.*, 2001). Therefore, there is generally a good understanding of the types of habitats in non-upland areas that are required to sustain populations and the types of habitats that are likely to exert a negative influence on distribution. This study found numerous areas of suitable habitat near to most ponds, specifically woodland, scrub and grassland, which would facilitate a widespread distribution. Similarly, pond density was relatively high which, in conjunction with suitable terrestrial habitat, increases chances of pond occupancy (Mann *et al.*, 1991; Marsh *et al.*, 1999). Animals are able to move between nearby ponds and can colonise adjacent areas relatively quickly. Indications of juvenile dispersal rates and microhabitat use in this study suggest that movement between ponds is unlikely to be impeded enough to limit distribution and abundance.

Future climate changes may result in altered distribution and reduced abundance of one or more amphibian species. Warmer and/or drier conditions might lead to the total loss of some breeding ponds and a reduced pond density. Remaining ponds may have a notably shortened hydroperiod which could affect recruitment through increased mortality or a loss of body condition ultimately leading to smaller body size and possibly reduced fecundity (Berven, 1990; Pearman, 1995). Significantly, warmer or drier terrestrial conditions may also affect the dispersal of metamorphs. At some ponds in this study there appeared to be a delay in dispersal, perhaps because animals were waiting for more favourable climatic conditions (i.e. a period of rainfall). Prolonged dry spells and a reduction in the number of moist areas, such as long wet grass for example, could increase mortality and/or limit the direction of dispersal. Conversely, areas which become wetter may see an increase in suitable wetland habitats that provide additional breeding sites and more moist terrestrial habitats, thereby facilitating a wider distribution and greater abundance of amphibians.

Rates of movement between sites coupled with lack of 'choosiness' by females selecting spawning sites could mask potential limits on distribution arising from significantly reduced reproductive success in acidic ponds. Embryos appeared to be particularly sensitive to acidic conditions in the earlier stages of development and

embryo mortality was often high in acidic ponds. There was no evidence to suggest that frogs breeding in acidic ponds produced more acid-tolerant offspring than frogs spawning in circumneutral ponds. This may reflect use of acidic ponds by adults originating from circumneutral areas. Poor reproductive success suggests that current acidity levels within the study area could potentially affect distribution of common frogs and possibly affect reproductive success of other species, such as common toads (*Bufo bufo*) and palmate newts (*Triturus helveticus*). Studies of other taxa within central Wales suggest little evidence for chemical reversal of acid conditions and sustained biological recovery is likely to require significant changes in several abiotic and biotic factors (Buckton *et al.*, 1998; Masters, 2002). This study is the first to suggest a possible change (acceleration) in rate of decline once population size has decreased to a critical size. This is likely to occur because large spawn mats have greater survival rates than small ones or isolated clumps. Variability in hatching success between acidic ponds, however, makes it difficult to assess what proportion of ponds in the landscape are genuinely sink habitats. Detecting rates of potential decline will be hindered by unknown immigration rates from neighbouring areas and natural fluctuations in population size. The long term effects of acidity on frog populations are unknown within the study area but it is probable that populations are maintained through immigration.

Unsurprisingly, recruitment of juveniles at acidic ponds was low but similar levels of recruitment were also found in several circumneutral ponds. Metamorph production was highly variable both between ponds and between years in circumneutral ponds. In contrast, it is possible that recruitment at acidic ponds is low in the majority of years and in the majority of ponds. Observed fluctuations in recruitment at circumneutral ponds were possibly due to predation, although this was difficult to ascertain without a targeted experimental approach. A significant negative association between tadpole survival and initial spawn amount indicated that mortality rate increased with increasing numbers of eggs or hatched larvae. Lack of association of spawn clump number with metamorph survival suggested that this mortality rate was not sustained throughout the larval period. High initial mortality was probably due to predation of young larvae which fell once older larvae reached a certain size refuge (Baker and Beebee, 1997; Brodie and Formanowicz, 1983; Cooke, 1974; Formanowicz, 1986).

As well as differences in metamorph numbers, there were also significant differences in body condition, i.e. weight/length relationship. Metamorphs from some ponds were significantly smaller in terms of length and/or weight and as a result may face increased mortality and a reduction in future reproductive fitness (Berven, 1990; Werner, 1986). It is also possible that some ponds consistently produce small juveniles and this may affect their relative contribution to the adult population. Comparisons of condition, as opposed to just differences in length, have not previously been conducted between metamorphs in wild populations. Interestingly, there is a suggestion that despite low numbers, juveniles from acidic ponds are of similar or larger physical size to those from circumneutral ponds. Therefore, embryos that do continue to develop in acidic ponds may not necessarily produce juveniles with comparatively reduced chances of survival and growth or poorer dispersal ability.

Although this study did not permit evaluation of differences in dispersal rates in relation to size and condition, it did suggest that particular aspects of the terrestrial habitat could influence dispersal. For example, the amount and structure of vegetation on the banks around the pond was considered to be important in providing a suitable micro-climate and protection from predators until dispersal began. Additionally, juveniles were found to utilise habitats that older juveniles and adults are generally considered to avoid. Once dispersal was underway, new frogs moved quickly and often crossed open ground with little or no vegetative cover. Adults are generally thought to avoid exposed areas such as roads, for example (Cooke and Sparks, 2004). However, during non-dispersing periods open areas are probably avoided in favour of vegetative cover. Juveniles demonstrated a significant preference for vegetated experimental plots under both wet and dry conditions although dispersal occurred over much drier, less vegetated terrain in the field. This may reflect selection of a more suitable microclimate but is also probably a significant predator avoidance strategy. There was also a suggestion that dispersal may have been orientated towards adjacent woodland but this could not be confirmed. New toads appeared to share similar dispersal behaviour to frogs but juvenile newts demonstrated a significant preference for taller grass possibly reflecting a greater risk of desiccation. If future climate changes lead to a significant drying of terrestrial habitats, then native urodeles might be impacted upon more severely than anurans. The microhabitat selection of efts also appears to be in direct contrast to that of adults (Marnell, 1998). These results reinforce the need to

identify significant habitat features for all life-stages. Juvenile dispersal may play an important part in explaining the presence and abundance of frogs in habitats where reproductive success is known to be poor.

6.2 Future research directions

Although this study demonstrated a wide and abundant frog distribution, differences in habitat requirements mean care should be taken when inferring habitat suitability for other species. Ideally, similar surveys for occupancy and relative abundance should be conducted simultaneously and repeated in several years to prevent misleading conclusions being drawn from 'snapshot' data. More detailed survey of terrestrial microhabitat is needed in all future studies, for instance, to record the type, location and amount of rank vegetation, scrub, log piles, rubble, etc. Although habitat type could not be related to frog distribution in this study, use of a geographical information system (GIS) and detailed land cover data may prove more useful in examining distribution patterns in areas with more inimical habitat, i.e. arable or urban/industrial areas, at scales of ≥ 1 km.

The findings of this thesis suggest that acidification could potentially affect patterns of frog distribution and relative abundance via reduced reproductive success. Therefore, effects of acidity on recruitment of *B. bufo* and *Triturus* spp. also need to be assessed. Immigration by dispersing juveniles may currently sustain the presence of *R. temporaria* (and other species) in acidic habitats. Future research needs to evaluate the relative contributions made by both recruitment and immigration to distribution patterns. Specifically, long term records of juvenile recruitment would show whether poor recruitment in one or two years is likely to be off-set by recruitment in preceding and subsequent years. Monitoring output at acidic ponds in particular would highlight ponds with consistent, chronically poor recruitment. Predicting reproductive success from water chemistry is likely to be unfeasible given the complex interactions of chemical variables (Freda, 1991). Visual assessments of *Saprolegnia* infection rates could, however, be a relatively easy way of currently measuring reproductive success in acidic ponds. Better evaluation of capture rates with the pitfall traps used in this study would also permit more accurate estimates of recruitment to be made. Studies

may have to be conducted continuously for many years for true sink habitats to be identified (Meyer *et al.*, 1998).

Levels of movement between ponds by both adults and juveniles also need to be gauged, although this is difficult to quantify. Adults are considered to be relatively sedentary but limits of local movements can be monitored using passive integrated transponders (PIT tags) with subsequent monitoring of potential breeding ponds each year. Radiotracking would provide useful information on location and distances of summer habitats and hibernation sites and the use of interconnecting terrestrial habitat.

Currently, it is only possible to batch mark juveniles at emigration (with toe-clipping or subcutaneous wire tags) and observe where they reappear as sexually mature adults (Berven and Grudzien, 1990; Sinsch, 1997). However, this could be used to show the proportion of adults breeding in acidic ponds that have migrated from adjacent non-acidic areas. It might be possible to relate numbers of immigrants to the proximity and number of surrounding circumneutral ponds which could help predict which acidic ponds may be at risk of isolation. Until a tracking technique is devised that allows more continuous monitoring, it will be impossible to assess average and maximum dispersal distances across different terrains. Assessment of habitat use and survival and growth rates within different habitats will have to be assessed experimentally (e.g. de Maynadier and Hunter, 1999; Rothermel and Semlitsch, 2002). Similarly, the effectiveness of different barriers such as rivers, roads and other open areas, to dispersal will have to be evaluated experimentally. Such experiments may involve artificially constructed ponds placed close to natural or artificial barriers with subsequent monitoring of dispersal behaviour, perhaps under different climatic conditions.

Molecular techniques could also be used to monitor levels of gene flow, although these only show historical movements and may only represent occasional migration of one or two individuals (Storfer, 2003). However, genetics studies could help to delimit populations and indicate where barriers to migration may occur, such as rivers, roads or possibly large areas of coniferous forest.

To date, there has been relatively little research to show how size, or condition, of juveniles relates to population dynamics (e.g. Berven, 1990; Smith, 1987) but experimental studies may provide some valuable insights. Hypotheses could be tested to determine how size at metamorphosis affects growth, survival and dispersal of native species in different habitats and under different environmental conditions. For example, do 'heavier' metamorphs have increased survival rates or dispersal ability? Does foraging quality differ in unimproved grassland, improved grassland, deciduous woodland or coniferous woodland and how might this limit population density? To what extent do different foraging conditions actually affect growth and survival? Additionally, experimental studies can show how different habitats and climatic conditions may influence rate of dispersal (Stevens *et al.*, 2004) or direction of dispersal (de Maynadier and Hunter, 1999) for each species.

A GIS was used in this study to collate and extract data from different surveys to facilitate analysis of distribution patterns in relation to particular habitat features. Additional studies may benefit from spatial analysis of occupancy and abundance in relation to variation in habitat quality to identify geographic areas where distribution may be limited. In the future, it may be possible to map or model likely dispersal routes and distances through different habitats in response to known sources of juvenile recruitment using a GIS. This might permit better understanding of potential threats to populations resulting from habitat modifications and allow more specific targeting of local and regional conservation plans.

6.3 Wider implications for amphibian ecology

Using frog spawn as an indicator of presence-absence or relative abundance is a common and recommended survey technique followed by observation or netting to detect larval stages, i.e. to identify a 'recruiting' pond (Griffiths and Raper, 1994; Griffiths *et al.*, 1996). However, the mere presence of spawn coupled with variability in tadpole capture rates may give misleading impressions regarding habitat quality. At worst, potential sink habitats may not be identified and the importance of terrestrial habitat connecting poor quality ponds with more productive habitats may be underrated leading to possible isolation. Monitoring and assessment of potential impacts on all life-stages will, therefore, be important in identifying potential sites

where populations could decline in the future. Specifically, evaluation of breeding pond quality needs to take into account metamorphic output and variation in output between years and between adjacent ponds. This will also apply to other species when ponds subject to inputs from environmental contaminants or acidity are used for breeding. In addition, care must be taken in acidified areas not to assume incorrectly that signs of egg deposition, coupled with signs of chemical reversal, indicate biological recovery. This study also provides evidence to show that different behaviours, i.e. dispersing vs. non-dispersing, may result in differences in microhabitat use. Furthermore, dispersing life-stages may utilise different habitats to more sedentary stages. This will need to be considered for habitat management of both anurans and urodeles across all regions and stresses the importance of maintaining a suitable habitat matrix wherever possible. Future climate change predictions mean that for areas with a predicted reduction in summer rainfall coupled with an increase in temperature, mediation against the possible loss of suitable breeding sites/terrestrial habitat cover would also have to be considered at some point.

6.4 Conclusions

This study contributes to the understanding of possible factors controlling amphibian populations in rural upland habitats and proposes that all life-stages could potentially be impacted upon by differences in habitat quality. It has been demonstrated that rural upland habitats can be valuable habitats for amphibians and the common frog in particular. Inferring the impacts of habitat quality from presence and abundance of one life-stage could, however, be misleading and prevent identification of sub-optimal habitats. In central Wales, acidity could potentially exert controls on distribution which are probably negated by the current pond density and terrestrial habitat matrix. Further research is required to better understand rates of recruitment and immigration and how terrestrial habitats mediate juvenile survival and dispersal. It is also necessary to investigate how similar factors influence current distribution of other amphibian species in upland habitats.

6.5 References

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Appendix

The classification of terrestrial habitat surrounding study ponds in Chapter 2 was based on the Phase 1 habitat classification numerical codes obtained from the Habitat Survey of Wales dataset. A definitive list of the habitat types described in the text is given here. Habitat definitions for each code are taken from *Handbook for Phase 1 Habitat Survey – A Technique for Environmental Audit. England Field Unit. Nature Conservancy Council. Revised reprint, 2003.*

Code	Phase 1 category	Definition
A1.1.1	Semi-natural broadleaved woodland	Vegetation dominated by trees more than 5m high when mature, forming a distinct, although sometimes open, canopy. The stands do not obviously originate from planting and comprise 10% or less conifer in the canopy.
A1.2.2	Planted coniferous woodland	A woodland of any age comprising coniferous trees that obviously has been planted. 10% or less broadleaves in the canopy.
A4	Felled coniferous woodland	Felled areas of trees where the future land use is uncertain, for instance when it is not clear whether the area is to be replanted or used for crops.
B1.1	Unimproved acid grassland	Often unenclosed grassland, as on hill-grazing land, and occurs on a range of acid-soils (pH less than 5.5). Generally, species poor with less than 25% wet or dry dwarf shrub cover. Species that are indicative of acidic conditions when frequent or abundant include <i>Deschampsia flexuosa</i> , <i>Nardus stricta</i> , <i>Juncus squarrosus</i> , <i>Galium saxatile</i> , <i>Rumex acetosella</i> .
B4	Improved grassland	Meadows and pastures that have lost species typically associated with unimproved sward after being subjected to heavy grazing, drainage or applications of inorganic fertilisers, slurry or high doses of manure. Substantial improvement is indicated by a bright green, lush and even sward dominated by grasses, a low diversity of forb species and more than 50% <i>Lolium perenne</i> , <i>Trifolium repens</i> and other agricultural species. Species that are indicative of improved grasslands include <i>Lolium perenne</i> , <i>Cynosurus cristatus</i> , <i>Trifolium repens</i> , <i>Rumex acetosa</i> , <i>Taraxacum officinale</i> , <i>Bellis perennis</i> , <i>Ranunculus acris</i> , <i>Ranunculus bulbosus</i> . <i>Rumex</i> spp., <i>Urtica dioica</i> and <i>Cirsium</i> spp. indicate localised soil enrichment by grazing animals.
C1.1	Bracken	Areas dominated by <i>Pteridium aquilinum</i> , or with scattered patches of this species.

Code	Phase 1 category	Definition
B5	Marshy grassland	Occurs on more or less level areas rather than on the banks of watercourses. The following communities are included: vegetation with more than 25% cover of <i>Molinia caerulea</i> on peat less than 0.5m deep (cf. mire), vegetation with less than 25% dwarf shrub cover on peat less than 0.5m deep (cf. heathland), vegetation with more than 25% cover of <i>Juncus acutiflorus</i> , <i>J. effusus</i> , <i>J. inflexus</i> , <i>Carex</i> spp. or <i>Filipenda ulmaria</i> . (Distinguished from <i>J. effusus</i> – <i>Holcus lanatus/Deschampsia cespitosa</i> grassland which is classified under neutral grassland. Swamp has a water table distinctly above the substratum for much of the year and dominated by reed grasses or large sedges. Flushes have a flow/seepage of water through them and bryophytes for a conspicuous component of the vegetation.)
D1.1	Dry acid heath	Dry dwarf shrub heath usually occurring on deep podzols developed on base-deficient sands, gravels and clays. Vegetation with greater than 25% cover of ericoids or small gorse species in relatively dry situations. Typical lowland dry dwarf shrub heath species include <i>Calluna vulgaris</i> , <i>Vaccinium myrtillus</i> , <i>Erica cinerea</i> , <i>Ulex minor</i> and <i>Ulex galli</i> . Typical upland heath species include <i>Empetrum nigrum</i> , <i>E. hermaphroditum</i> , <i>Arctostaphylos uva-ursi</i> and <i>Vaccinium vitis-idaea</i> .
D5	Dry heath/acid grassland mosaic	A common mixture of dry heath (D1) and acid grassland (B1.1 unimproved or B1.2 semi-improved) found on hill and moorland.
E3.2	Basin mire	A type of fen that develops in a waterlogged basin and contains very little open water. The water table within the basin is level, but small flushes may occur around the edges and there is a limited through-flow of water. Vegetation may be dominated by <i>Sphagnum</i> spp., together with <i>Carex rostrata</i> and ericoids, or by tall swamp plants such as <i>Phragmites australis</i> , <i>Schoenoplectus (Scirpus) lacustris</i> , <i>Typha</i> spp. and in base-rich situations, <i>Cladium mariscus</i> .
I1.2.1	Acid/neutral scree	An accumulation of weathered acidic or neutral rock fragments of all sizes. Fragments usually are angular in shape and found at the foot of a cliff.
J1.1	Arable	Areas that include arable cropland, horticultural land (for example, nurseries, vegetable plots, flower beds), freshly-ploughed land and recently reseeded grassland, such as rye grass and rye-clover leys, often managed for silage.
NA	Not accessed	-

