

Spatial ecology of *Bufo fowleri*

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fulfilment of the requirements for the degree of Doctor of Philosophy**

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ABSTRACT

The geographic isolation of populations can result in a metapopulation effect where regional dynamics of extinction and colonization are more important to population viability than local dynamics of individuals' birth or death. When this partial isolation is maintained for many generations genetic variability can be geographically structured. Populations of temperate-zone anuran amphibians are often considered to be geographically isolated on relatively small spatial scales due to the animals presumed high site fidelity and strict dependence on moisture for respiration and breeding. As a result, temperate, pond-breeding anuran amphibian populations are considered likely candidates to test hypotheses of metapopulation theory, movement and phylogeography. Using data from the Fowler's toad, (*Bufo fowleri*) I test the applicability of metapopulation theory, the likelihood of limited movement and the strength of phylogeographic structure. Specifically, I show that the generalization of the amphibians-as-metapopulations paradigm, due to their limited dispersal capabilities, is not supported (I). *Bufo fowleri* movement is well described by an inverse power function. Whereas most individuals do not move, some move long distances. There is no sex bias to this movement and I propose the hypothesis that the animals moving the longest distances are aided by the passive action of lake currents (II). *B. fowleri* juveniles are not a dispersal stage. They move neither farther nor faster than adults. The observation of predominantly juvenile contribution to a dispersal pool is due to their abundance – there are simply many more juveniles than adults (III). My observations of amphibian population turnover do not support the predictions of two specific metapopulation models when parameterized on a local (<10km) or regional scale (~300km) (IV). *B. fowleri* populations in Canada exhibit both shallow and deep phylogeographic structure. The shallow divisions are geographically structured and the deep division is considered to be due to the historic introgression of *B. americanus* mtDNA (V). Populations within the Lake Erie watershed exhibit isolation by distance between the shallow phylogroups, but no isolation-by-distance within phylogroups across distances approaching 70km. This pattern could be due to historic small founding population(s), or be the result of high levels of contemporary gene flow (VI). Using a single-species example, this thesis demonstrates that anuran dispersal can not be as limited as is frequently thought, and the common expectation that all pond-breeding anuran amphibians will exhibit metapopulation and phylogeographic structure is not supported.

Résumé

L'isolement géographique des populations peut avoir comme conséquence un effet de métapopulation où la dynamique régionale de l'extinction et de la colonisation est plus importante que la dynamique locale du comportement et de la forme physique d'individus. Quand cet isolement est maintenu pour beaucoup de générations la variabilité génétique peut être géographiquement structurée. Des populations des amphibiens anoures tempérés sont souvent considérées être géographiquement isolées sur les échelles spatiales relativement petites dues à leur fidélité élevée d'emplacement et dépendance stricte à l'égard l'humidité pour la respiration et multiplier, et accumulent ainsi des réseaux sont considérées les candidats probables comme métapopulations. Si des populations sont isolées pour beaucoup de générations elles sont susceptibles d'exhiber phylogeographic structurent. Les espèces anoures tempérées sont ainsi favorables à l'essai des hypothèses concernant des métapopulations, la dispersion, et le phylogeography. En utilisant des données pour les hypothèses de crapaud de Fowler's, (*Bufo fowleri*) d'essai de I concernant l'applicabilité de la théorie de métapopulation, la probabilité de la dispersion limitée et la force de la structure phylogeographic. Spécifiquement, je prouve que la généralisation que les amphibiens sont susceptibles d'exhiber la structure de métapopulation en raison des possibilités limitées de dispersion n'est pas (I) soutenu. Le mouvement de fowleri de *Bufo* est bien décrit par une fonction inverse de puissance tandis que la plupart des individus ne se déplacent pas, certains déplacent de longues distances. Il n'y a aucune polarisation de sexe à ce mouvement et je propose l'hypothèse que ces animaux déplaçant les plus longues distances soient probables subissant l'accélération passive par les courants de lac (II); les juvéniles de *B. fowleri* ne sont pas une étape de dispersion? ils se déplacent pas plus loin ni plus rapidement que des adultes. Probablement l'observation de la contribution juvénile prédominante à une piscine de dispersion est une basée sur l'abondance? il y a simplement beaucoup plus de juvéniles que les adultes (III); Une fois paramétrisées avec des données amphibiens de chiffre d'affaires de population sur une échelle locale (< 10km) ou régionale (~300km), nos observations ne soutiennent pas les prévisions de deux modèles de métapopulation (IV); Les populations de fowleri de *B.* au Canada exhibent la structure phylogeographic peu profonde et profonde. Les divisions peu profondes sont géographiquement structurées et la division profonde est probablement due à l'introgession historique du mtDNA américain de *B.* (V); Populations dans l'isolement d'objet exposé de ligne de partage d'Erie de lac par la distance entre les

phylogroups peu profonds décrits dans V, mais aucun isolement par la distance dans ces phylogroups à travers des distances approchant 70km. Ce modèle a pu être dû à un petit population(s) de fondation historique, ou au résultat des niveaux élevés de l'écoulement contemporain de gène (VI). En utilisant un exemple simple spécifique d'espèce, cette thèse démontre que la dispersion amphibie anoures n'est pas aussi limitée qu'est fréquemment pensé, et l'espérance commune que tous les amphibiens anoures montreront le metapopulation et la structure phylogeographic ne sont pas soutenues comme détails.

STATEMENT ON THESIS FORMAT

This thesis has been written as a collection of papers that have been submitted to refereed journals. For the benefit of the examiners, I quote the following statement from the “Guidelines for thesis preparation” published by the Faculty of Graduate Studies and Research, McGill University: *“Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication... these texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound... the thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next...connecting texts that provide logical bridges preceding and following each manuscript are mandatory.”*

– Guidelines for Thesis preparation, Faculty of Graduate Studies and Research,
July 2002

CONTRIBUTIONS OF AUTHORS

CHAPTER 1

I surveyed the literature, and analysed the data with advice from Dr. Green.

CHAPTER 2,3 and 4

I wrote the models, analysed the results and wrote the final version with advice from Dr. Green. Dr. Green collected the original 12 years of occupancy data and the skeletochronology age estimates.

CHAPTER 5 and 6

I collected the data, performed all lab manipulations, analysed the results and wrote the final version with advice from Dr. Green. Dr. Green collected the allozyme data, but this analysis is unique.

Each chapter has benefited from the editorial comments of Dr. David Green, and he is a co-author on all manuscripts.

GENERAL INTRODUCTION

The spatial element of ecology has always been important (Andrewartha and Birch 1954), but it has been emphasized to different extents at different times. While not overtly emphasized in the mainstream ecology of the 1960's and 70's, its consideration has undergone a period of distinct growth in the past thirty years (Hanski 1999). Topics such as the spatial nature of population dynamics and the spatial partitioning of populations' genetic variability are as vibrant a part of ecology as age-structure matrices or food-web diagrams. The metapopulation approach, first outlined by Levins in (1969) and (1970), has been an especially helpful tool in the development of spatial ecology. A simple metapopulation definition is that of a collection of partially isolated breeding habitat patches, connected by occasionally dispersing individuals where each patch exists with a substantial extinction probability. Thus, long-term persistence occurs only at the regional level of the metapopulation. The most useful function of metapopulation theory has been to direct attention towards the importance of spatially structured interactions between local populations in addition to more traditionally studied processes occurring within populations.

As the metapopulation paradigm (Hanski 1999) has evolved, more realism has been added to the initial Levins approach whereby space was implicit to the model, there were an infinite number of patches and each habitat patch was of equivalent size, shape and isolation. More realistic models consider the effects of patch area, shape and isolation, and the effect of the non-habitat between patches on the likelihood of patch extinction or colonization. Accompanying this rise in theoretical realism has been an exponential increase in the number of biological systems examined for metapopulation structure (Hanski 1999). Some consider that such a rapid increase in empirical use, without clear tests of the theory's applicability, has reduced the precision with which the term metapopulation is used (Freckleton and Watkinson 2003, Pannell and Obbard 2003). For example, similar species are often considered to exhibit similar population structure and dynamics when there is no evidence to the contrary. Such sweeping generalizations are likely to obscure and confuse more frequently than to illuminate (Hanski and Simberloff 1997).

If the spatial nature of habitat breeding patch isolation is maintained for generations it can result in the geographic structuring of genetic variability. Wright (1943) first modeled and described individual sets of populations connected by migrants. He was among the first geneticists to recognize that a spatially structured population was likely to have more genetic variation than an equivalently sized panmictic population (Wright 1943, 1951). This theoretical cornerstone is the basis of phylogeography, a new paradigm spanning many disciplines. Phylogeography is a sub-discipline of biogeography and is concerned with the principles and processes governing geographic distribution of genetic lineages (Avice 2000). Phylogeographic units are often of special conservation significance because they are likely sources of independent variation that are worthy of conservation (Avice 2000). Many populations are geographically structured, however those that exhibit reduced dispersal capabilities are likely to show phylogeographic structure even at small spatial scales.

Populations of temperate anuran amphibians are often considered to be geographically isolated on relatively small spatial scales due to the animals' high site fidelity and strict dependence on moisture for respiration and breeding (Duellman and Trueb 1986, Sinsch 1990, Blaustein et al. 1994). Therefore, populations inhabiting contemporary pond networks are considered likely candidates for metapopulations (Harrison 1994, Marsh and Trenham 2000). If ponds, or populations of ponds, are isolated for many generations the populations inhabiting them are likely to exhibit phylogeographic structure. Therefore temperate anuran species are highly amenable to the testing of numerous hypotheses regarding metapopulation structure, dispersal, and phylogeographic structure. Using data from the Fowler's toad, (*Bufo fowleri*) I tested hypotheses regarding applicability of the metapopulation paradigm, the likelihood of limited dispersal, and the strength of phylogeographic structure.

In the literature, movement and dispersal are frequently used terms, but are not always equivalent (Dufty and Belthoff 2001). Turchin (1998) defined dispersal as all movements that increase the spatial spread of a population. Howard (1960) referred to dispersal as the movement an individual makes. Dingle (1996) suggested that dispersal be used to describe the process leading to a distributional outcome of movement. I agree with Dingle (1996) that ultimately dispersal is a process, subject to behavioral and

hormonal triggers, but in this thesis, I use a very general definition of dispersal that is the linear distance between an organism's starting and ending point. This definition includes movement from the natal site to the location of reproduction (natal dispersal), and movements between breeding sites (breeding dispersal (Kenward et al. 2002)). As a definition, it may combine some processes, but ultimately it leads to an efficient measure of this species' ability to move short ($0 < 10\text{km}$) and long distances. Additionally, this definition of dispersal, or movement capability, is most linked to the fulfillment of the metapopulation concept.

THE STUDY SYSTEM

Bufo fowleri (Fowler's toad) is widespread throughout the eastern United States but is restricted in Canada to sandy or rocky points and sandy beaches along the northern shore of Lake Erie, Ontario. Viable populations likely exist at only three regional localities in southern Ontario: Rondeau, Long Point, and an area stretching between the Grand to Niagara rivers. They no longer occur at any localities in western Lake Erie, where they are considered extirpated.

Adult *B. fowleri* are 50-80 mm SVL (snout-vent length) (Wright and Wright 1949, Conant and Collins 1998), and females are slightly larger than males. They are gray colored dorsally with darker patches and numerous small dark brown warts on a granular textured skin (Wright and Wright 1949, Logier 1952, Green 1984, Conant and Collins 1998). There are usually three or more warts per dark dorsal spot. The ventral surface is white or cream colored and is either without spots or with a single dark pectoral spot situated between the forelimbs. The throat is dark in males but white in females. The snout is short and blunt and the bony cranial crests on the head are weak.

Fowler's toads occur in areas with loose, well drained gravely or sandy soils, including sand dunes, sandy deciduous woodland, and rocky, poorly vegetated areas (Hubbs 1918, Brown 1974, Klemens 1993). Fowler's toads breed in the shallow water of permanent ponds, flooded low ground, temporary pools, farm ponds, roadside ditches, quiet streams, lakeshores, or along the shallows of rivers (Wright and Wright 1949, Breden 1988). Breden (1988) described breeding ponds used by the toads as shallow with sandy bottoms and gradually sloping banks, vegetated primarily with sedges and bulrushes.

At the toads' northern range limit on Lake Erie, *B. fowleri* males begin to call from the end of April to the start of May, when they reach a minimum body temperature of 14°C (Green 1997, Blaustein et al. 2001). Snakes, particularly garter snakes, *Thamnophis*, are the chief predators of toads. Hognose snakes, *Heterodon*, although now rare in Ontario, are specialized toadeaters (Edgren 1955, Smith and White 1955). Occasional predation is also due to bullfrogs (Smith and Green 2002) and gulls that have been observed to depredate juvenile toads (Appendix 1). Toads are a sit and wait predator, consuming nearly any mobile creature smaller than themselves active on the beach after dark (Judd 1957).

OUTLINE OF THE STUDY

My thesis consists of six papers, each of which focuses on a different aspect of the spatial ecology of *B. fowleri*. In the first chapter (I) I use the literature to examine the validity of the frequent characterization of amphibians as having limited dispersal abilities, strong site fidelity, and spatially disjunct breeding habitats that often result in the *a priori* assumption that amphibian populations form metapopulations. Do those studies of amphibians as metapopulations test the most elementary characteristics of a metapopulation (Hanski et al. 1995, Hanski 1999)? Is limited dispersal implicated in the assumption of breeding pond isolation? Do amphibians really exhibit limited dispersal?

Because dispersal is fundamental to the establishment and maintenance of metapopulations, in the second chapter (II) I quantify the movement characteristics of *Bufo fowleri*. Do all toads move short distances? Are toad movement frequencies easily characterized by a normal or exponential distribution? Are movement characteristics the same for male and female toads?

Whereas pond-breeding amphibians are site-loyal and polygynous as adults, juveniles should move farther and faster. In the third chapter (III) I test the prediction that juvenile toads are the principal dispersal stage. Do younger toads move farther and/or faster than older toads?

In the fourth chapter (IV) I examine the applicability of two metapopulation models to amphibian population turnover locally (<10km) and regionally (~300km). Do these populations meet the predictions of the simplest, Levins' style metapopulation model? Is the population turnover significantly different from the most frequently

invoked metapopulation model? Are these conclusions affected by low statistical power? Are many metapopulation studies in the literature affected by low statistical power?

Populations comprised of individuals which exhibit reduced dispersal capabilities are likely to show phylogeographic structure even at small spatial scales. Whereas populations of temperate anuran amphibians are often considered to be geographically isolated on relatively small spatial scales due to the animal's high site fidelity and strict dependence on moisture for respiration and breeding, in the fifth chapter (V) I examine the putative phylogeographic structure of *B. fowleri* at the northern edge of its range in the Lake Erie watershed.

In the sixth chapter (VI) I test the prediction, derived from dispersal theory, that a species at the northern edge of its range should exhibit genetic isolation-by-distance (IBD) between populations. Is there evident IBD between all populations? Is the estimate of the number of female migrants per generation (N_m) greater than, or less than, one?

RESULTS AND DISCUSSION

I. The paradigms of metapopulation structure and limited dispersal.

I reviewed the literature on amphibians as metapopulations (53 journal articles or theses) and amphibian dispersal (163 journal articles or theses for 53 anuran species and 35 salamander species) to test: 1) whether the conditions for metapopulation structure had been tested, 2) whether pond isolation was based on the assumption of limited dispersal and 3) whether amphibian dispersal is uniformly limited. I found that in the majority of cases (74%) the assumptions of the metapopulation paradigm were not tested. Although breeding patch isolation via limited dispersal and/or strong site fidelity was the most frequently implicated or tested metapopulation condition, I found strong evidence that amphibian dispersal is not as uniformly limited as is often thought. Fitting an inverse power law to the frequency distribution of maximum movements for anurans and salamanders predicts that distances beneath 11-13 km and 8-9 km respectively, are in a range that they may receive one emigrating individual. Populations isolated by distances approaching this range are more likely to exhibit a metapopulation effect than less isolated populations. Those studies which covered larger areas, also tended to report longer maximum distances – a pattern that should be noted in the research design of

mark-recapture studies in the future. Together, these results should encourage caution in the indiscriminate application of the metapopulation approach to amphibian population ecology. Some amphibian populations may be structured as metapopulations – all are not.

II. There is no sex-bias to movement and movement rates do not decay exponentially with distance, but rather obey a power law.

I used 15 years of mark-recapture data for a population of Fowler's toads (*Bufo fowleri*) to characterize dispersal distances. Although most adult *B. fowleri* showed high site fidelity, the maximum distance moved by adult toads was fifteen times greater than was once thought and there was no sex bias to dispersal. The frequency distribution of movements was better described by an inverse power function than either a normal or an exponential distribution for there was a significant 'tail' to the dispersal distribution. Using a neutral, random-walk simulation I have shown that the movement distributions I have observed in the field are not significantly different from the simulation, when recaptures are restricted to ponds, although the simulation does not replicate the tail of the distribution. These results show that 1) although predominantly of low vagility, toad movement can cover a distance much greater than previously anticipated; 2) there was no sex-bias to patterns of movement. The dispersal capabilities observed in these toads suggests that populations once thought isolated by tens of kilometers are actually connected by occasional migrants. 3) Long-distance movement was correctly estimated at a study site of approximately 10km and 4) Toad movement at Lake Erie may be stratified, with random movement occurring locally, while other forces (such as accidental transport by lake currents) are required of long distance dispersal.

III. Juvenile toads are not a dispersal stage.

Whereas pond-breeding amphibians are site-loyal and polygynous as adults, juveniles should move farther and faster. Using mark-recapture of 1000 adults and 400 juveniles over two field seasons I estimated movement rates and dispersal capabilities of juvenile *Bufo fowleri* at the northern edge of its range. Neither the distance moved, nor the frequency of movement differed between toad age classes. This is a clear departure from expectations. Juveniles appear to be the dispersing class of *B. fowleri* not due to any

quantifiable difference in dispersal strategy, but merely because there are far more of them than there are adults.

IV. The apparent metapopulation structure in *Bufo fowleri* is more likely patchy

Using a fifteen-year study of population turnover in six amphibian species collected on a local scale of ten kilometres, and occupancy data for *Bufo fowleri* sampled on a regional scale from populations across several hundred kilometres, I tested how well my observations of habitat patch occupancy fit the assumptions of a simple metapopulation model (General Metapopulation Model (GMM)) and how the observed patterns of incidence fit those predicted by the more spatially realistic, and frequently invoked, Incidence Function Model (IFM). At neither the local scale of ten kilometres nor the regional scale of hundreds of kilometres was there any evidence of metapopulation effect. My number of sample sites and years is necessarily limited – as it is in all metapopulation studies – and I found that a compromise analysis between statistical confidence and power is an appropriate test of significance for systems where unavoidably small sample size results in low power. In many metapopulation studies a reduction of statistical power is unavoidable and therefore compromise analyses are desirable. As neither the spatially implicit and simple general metapopulation model, nor the spatially explicit and popular incidence function model provided reasonable predictions or descriptions of the population dynamics for these amphibian species, it is possible that homogenising dispersal, and/or elements of population dynamics outside the pond, effectively negate any metapopulation effect. It is not my intent to dispute the general utility of the metapopulation approach for all amphibians; however, although the ‘ponds as patches’ metapopulation model of amphibian biology has merits, it is an assumption that is not always accurate. This work demonstrates that even when intuitively apparent, the assumptions of a metapopulation effect must be checked.

V. Shallow and deep phylogeographic divisions are evident within Lake Erie.

Using sequence data from the hyper-variable control region of the mitochondria I investigated phylogeographic structuring of genetic variability in the Fowler’s toad (*Bufo fowleri*) in the Lake Erie watershed at the northern edge of their distribution. A total of 540 bp of mitochondrial control region sequence data were obtained from 158 individuals from 21 populations. Inter-population sequence variation ranged from 0% to 6% and

phylogenetic analysis revealed two deeply divergent (6% sequence divergence) and three more shallow (<0.5% sequence divergence) mtDNA lineages. Shallow phylogroups represent non-overlapping geographic regions. The two deeply divergent phylogroups (1 and 2) could be due to the secondary contact of animals from two glacial refugia, incomplete lineage sorting with the most recent common ancestor with *Bufo americanus*, or the allopatric presence of introgressed mtDNA from *B. americanus* (Phylogroup 1) with the mtDNA haplotype more similar to southern populations of *B. fowleri* (Phylogroup 2). I consider the third explanation the most parsimonious – but the data does not allow the resolution of these competing hypotheses. Of all Lake Erie populations, only the populations at Long Point represent Phylogroup 2 while toads at all other locations are fixed Phylogroup 1. The mtDNA variability and divergences illustrated here indicate lineages on separate evolutionary trajectories, and whereas *B. fowleri* is a threatened species in Canada, such evolutionary significance should be reflected in future conservation protection.

VI. Extensive gene flow leads to large-scale genetic homogeneity in *Bufo fowleri*

Using mitochondrial control region sequence data from Lake Erie toads I tested the hypothesis that individuals from isolated populations at their northern range edge would exhibit low genetic diversity and isolation by distance (IBD). To determine whether disjunct breeding assemblages are likely to form metapopulations I tested the size of genetic neighborhood using genetic and demographic data. All *B. fowleri* populations in the Lake Erie watershed are characterized by low sequence diversity and the predominance of ‘private’ haplotypes. I found that populations were shown to exhibit significant isolation by distance whether geographic isolation was measured by terrestrial or aquatic-downstream distance. However, populations are likely not at equilibrium as the residuals of the IBD relationship do not increase with increasing geographic distance. Pair-wise distances among phylogroups drive the IBD trend – for within each phylogroup there is little variation across many kilometers. Genetic homogeneity across large distances may be due to the regular dispersal of individuals or the consequence of small founding populations. Breeding assemblages do not function as metapopulations, as both genetic and demographic estimates of neighborhood size are larger than disjunct breeding

populations.

CONCLUSIONS

The results of this thesis have implications for conservation. *B. fowleri* is a threatened species in Ontario and Canada (COSEWIC). I have shown here that the Long Point toads are a unique lineage within Canada and worthy of special protection. Populations within Niagara County may exchange individuals occasionally, but populations in the southwestern portion of the lake at Rondeau Provincial Park and a Long Point are effectively isolated on a management time scale. This work demonstrates that not only are Canadian populations unlikely to enjoy a rescue effect from the populations in the United States (closer to the majority of the species range), any rescue amongst Canadian populations is highly unlikely. Additionally, the populations of northern Ohio and Pennsylvania are highly divergent from the populations in the southern portions of these states. These northern populations (Ashtabula Ohio, Erie Pennsylvania) should be recognized as distinct management units within these jurisdictions.

How general are the results shown here? Amphibian dispersal is not as limited as is frequently thought, and the common expectation that all amphibians will exhibit metapopulation and phylogeographic structure is only a worthy generality if supported by specific examples. Likely many amphibian populations operate as metapopulations, however more specific attention needs to be paid to the assumptions of the term and specific tests should be carried out to test these assumptions (I, IV). As more accurate marking technologies evolve, it is becoming clear that the highly leptokurtic movement pattern demonstrated here (II, III) is not unique, and indeed is likely a general feature of the movement frequency distribution for many species. In acknowledging this fact, it is important to realize that it also will entail generally larger study areas – for some individuals will move farther than expected, and this dispersal tail will affect the ecological and genetic dynamics of the system in question. The generalization that juveniles are a dispersal stage for amphibians needs more support from specific examples where the movement capabilities of younger animals are quantitatively compared to older animals. Without such support, it is likely that the overwhelming juvenile abundance results in more juveniles partitioned into a dispersal pool – not due to any behavioral difference – just to probabilistic effect of abundance (III). The discovery of a cryptic

lineage within an area only recently deglaciated is not specific to *B. fowleri* (V). Other cryptic lineages have been documented in *Pseudacris crucifer* (Austin et al. 2002) and *Ambystoma maculatum* (Zamudio and Savage 2003) along the northern shoreline of the Lake Erie watershed. Clearly, more research is needed in this area across a wide taxonomic array to fully understand the post-glacial history of Ontario. Isolation by distance is an expected genetic signature of populations at the northern edge of their range (Green et al. 1996), however testing whether this pattern is driven by pairwise distances surrounding a barrier is a process not always completed. This work helps make it clear that apparent isolation by distance can actually hide evidence of vicariance (VI).

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CONTRIBUTIONS TO KNOWLEDGE

CHAPTER 1: ARE ALL AMPHIBIANS METAPOPOPULATIONS? : DISPERSAL AND THE METAPOPOPULATION PARADIGM IN AMPHIBIAN ECOLOGY.

- i. A literature review of the application of metapopulation theory to amphibian ecology indicates that most assumptions are not tested.
- ii. The most commonly tested, or implicated, metapopulation condition was isolation via limited dispersal/high site fidelity.
- iii. A literature review of estimates of maximum dispersal distance indicated that temperate pond-breeding amphibians are not homogenously poor dispersers but rather cover a wide range of dispersal strategies.
- iv. The generalisation that the spatial scale of investigation limits the estimation of the maximum distance moved by amphibian species is supported.

CHAPTER 2: SEX, FIDELITY AND ISOLATION: THE DISPERSAL ECOLOGY OF BUFO FOWLERI.

- i. Most adult *B. fowleri* showed high site fidelity but the maximum distance moved by adult toads was fifteen times greater than was once thought
- ii. There was no sex bias to dispersal.
- iii. The frequency distribution of movements was best described by an inverse power function for there was a significant 'tail' to the dispersal distribution.
- iv. A neutral, random-walk simulation illustrates that the movement distributions observed in the field are not significantly different from the simulation, when recaptures are restricted to ponds, although the simulation does not replicate the tail of the distribution.
- v. Comparison between the observed movement frequency distributions and two corrections demonstrated that long-distance movement was properly estimated at a study site of approximately 10km.
- vi. Toad movement at Lake Erie may be stratified, with random movement occurring locally, while other forces (such as accidental transport by lake currents) are required of long distance dispersal.

CHAPTER 3: JUVENILES NECESSARILY, NOT NECESSARILY JUVENILES: THE DISPERSAL ECOLOGY OF BUFO FOWLERI.

- i. Neither the distance moved, nor the frequency of movement differed between toad age classes.
- ii. Juveniles appear to be the dispersing class of *B. fowleri* not due to any quantifiable difference in dispersal strategy, but merely because there are far more of them than there are adults.

CHAPTER 4: "MORE APPARENT THAN REAL": AN ANALYSIS OF AMPHIBIAN POPULATION TURNOVER USING TWO METAPOPULATION MODELS.

- i. At neither the local scale of ten kilometres nor the regional scale of hundreds of kilometres was there any evidence of metapopulation effect when tested with the spatially implicit and simple general metapopulation model, and the spatially explicit and popular incidence function model.
- ii. I found that a compromise analysis between statistical confidence and power is an appropriate test of significance for systems where unavoidably small sample size results in low power.
- iii. A literature review of metapopulation research indicated low statistical power to detect a trend between sites or years using simple regression analysis.

CHAPTER 5: PHYLOGEOGRAPHY OF BUFO FOWLERI AT THEIR NORTHERN RANGE LIMIT.

- i. Two deeply divergent (6% sequence divergence) and three more shallow (<0.5% sequence divergence) mtDNA lineages were revealed through an examination of the phylogeographic structuring of genetic variability in *Bufo fowleri* in the Lake Erie watershed.
- ii. Shallow phylogroups represent concordant, non-overlapping geographic regions, while the two deeply divergent phylogroups (1 and 2) were most parsimoniously explained as the allopatric presence of introgressed mtDNA from the closely related

- B. americanus* (Phylogroup 1) with the mtDNA haplotype more similar to *B. fowleri* at the more southerly portions of their range (Phylogroup 2).
- iii. The Long Point populations represent a different phylogroup from all other populations in the Lake Erie watershed.

CHAPTER 6: ISOLATION BY DISTANCE, VICARIANCE AND GENETIC NEIGHBORHOOD IN BUFO FOWLERI AT THEIR NORTHERN RANGE LIMIT

- i. All *B. fowleri* populations in the Lake Erie watershed are characterized by low sequence diversity and the predominance of 'private' haplotypes.
- ii. Terrestrial or aquatic dispersal appear equally likely, as populations were shown to exhibit significant isolation by distance (IBD) whether geographic isolation was measured by terrestrial or aquatic-downstream distance, populations likely are not at equilibrium.
- iii. Pair-wise distances among phylogroups drive the IBD trend – for within each phylogroup there is little variation across many kilometers. Homogeneity across large distances could be due to the regular dispersal of individuals, or be the result of small founding populations.
- iv. Both genetic and demographic estimates of neighborhood size are larger than the disjunct breeding populations, suggesting that these breeding assemblages do not function as metapopulation.

**CHAPTER 1: ARE ALL AMPHIBIAN POPULATIONS METAPOPOPULATIONS? :
DISPERSAL AND THE METAPOPOPULATION PARADIGM IN AMPHIBIAN
CONSERVATION**

**Keywords: dispersal, metapopulation, amphibian,
4100 words, 220 references, 4 tables, 4 figures, 1 appendix**

PREFACE TO CHAPTER 1

A central theme to this thesis is the hypothesis that amphibian dispersal is limited, and therefore that amphibian populations are likely to be geographically structured and form metapopulations. In Chapter 1, I establish the empirical and theoretical foundations on which these assumptions are based. I test the assumptions of metapopulation structure in amphibian ecology and limited dispersal ability of amphibians by reviewing the literature on amphibians as metapopulations and amphibian dispersal. The title, “Are all amphibian populations metapopulations” was inspired by Freckleton and Watkinson’s 2003 paper “Are all plant populations metapopulations” (*Journal of Ecology* 91:321-324). Note that results from two thesis chapters (Chapter II; Chapter IV) are included as a case study, as this review was completed after the analysis of these data.

Reference style is according to submission requirements for Biological Conservation.

ABSTRACT

Amphibians are frequently characterized as having limited dispersal abilities, strong site fidelity and spatially disjunct breeding habitat. As such, pond-breeding species are often alleged to form metapopulations. Amphibian species worldwide appear to be suffering population level declines caused, at least in part, by the degradation and fragmentation of habitat and the intervening areas between habitat patches. If the simplification of amphibians occupying metapopulations is accurate, then a regional based conservation strategy, informed by metapopulation theory, is a powerful tool to estimate the isolation and extinction risk of ponds or populations. However to date, no attempt to assess the class-wide generalization of amphibian populations as metapopulations has been made. We reviewed the literature on amphibians as metapopulations (53 journal articles or theses) and amphibian dispersal (163 journal articles or theses for 53 anuran species and 35 salamander species) to evaluate whether the conditions for metapopulation structure had been tested, whether pond isolation was based only on the assumption of limited dispersal, and whether amphibian dispersal was uniformly limited. We found that in the majority of cases (74%) the assumptions of the metapopulation paradigm were not tested. Although breeding patch isolation via limited dispersal and/or strong site fidelity was the most frequently implicated or tested metapopulation condition, we found strong evidence that amphibian dispersal is not as uniformly limited as is often thought. Fitting an inverse power law to the frequency distribution of maximum movements for anurans and salamanders predicts that distances beneath 11-13 km and 8-9 km, respectively, are in a range that they may receive one emigrating individual. Populations isolated by distances approaching this range are perhaps more likely to exhibit metapopulation structure than less isolated populations. Those studies that covered larger areas also tended to report longer maximum movement distances – a pattern with implications for the design of mark-recapture studies. Caution should be exercised in the application of the metapopulation approach to amphibian population conservation. Some amphibian populations may be structured as metapopulations – but not all.

INTRODUCTION

The importance of the spatial element in ecology has long been recognized (Andrewartha and Birch, 1954), but its consideration has undergone a period of distinct growth in the past thirty years (Hanski, 1999) with increased emphasis paid to concepts such as the spatial nature of population dynamics and the spatial partitioning of populations' genetic variability. The metapopulation approach, first outlined by Levins (1969; 1970), has been especially insightful for the development of spatial ecology and its application to conservation. In simple terms, a metapopulation is a collection of partially isolated breeding habitat patches, connected by occasionally dispersing individuals whereby each patch exists with a substantial extinction probability. Thus, long-term persistence occurs only at the regional level of the metapopulation. The most useful function of metapopulation theory has been to integrate spatially structured interactions between local populations with processes occurring within populations and thereby enable better assessment of population viability.

More realistic models than the initial Levins approach (Hanski, 1999) consider the effects of patch area, shape and isolation, and the effect of the non-habitat between patches on the likelihood of patch extinction or colonization. Accompanying this rise in theoretical realism has been an exponential increase in the number of biological systems examined for metapopulation structure (Hanski, 1999). The rapid increase in empirical use of metapopulation concepts, without clear tests of the theory's applicability, may have reduced the precision with which the term metapopulation is used (Pannell and Obbard, 2003; Freckleton and Watkinson, 2003). If similar species are considered *a priori* to exhibit similar population structure and dynamics in the absence of evidence to the contrary or if departures from simple metapopulation models are not clearly detailed, conservation strategies may be misdirected (Hanski and Simberloff, 1997).

Therefore, the issue of whether or not amphibians are metapopulations is not merely semantic but relevant to their conservation and management. Indeed, where disjunct breeding patches contain individual populations that exist in a shifting balance between extinctions and recolonizations via dispersing individuals the metapopulation

approach is attractive (Hanski, 1999). Many temperate amphibians use spatially disjunct breeding habitat (Duellman and Trueb, 1986), and are often regarded as poor-dispersers with high site fidelity (Duellman and Trueb, 1986; Blaustein et al., 1994; Sinsch, 1990). Combining these two observations makes it appear highly probable that amphibian populations operate as metapopulations (Alford and Richards, 1999; Harrison, 1991). Yet the assumptions of limited dispersal, high site fidelity and evident metapopulation structure in amphibians remain to be tested.

We examined the literature for all references to amphibians and metapopulations using Current Contents, Scientific Citation Index, and other published reference lists. We tested whether the published literature for amphibians had addressed the four conditions necessary for metapopulation structure (Hanski, 1999; Hanski et al., 1995), and whether amphibians are indeed of low vagility and high site loyalty. We compiled a list of the longest distances moved by amphibians in mark-recapture and displacement studies and subsequently compared these distances to the maximum distance covered by the field site, to test whether the low-vagility hypothesis was caused by a lack of long-distance dispersal data in amphibians caused by small study areas (Marsh et al., 1999). If not recorded directly by the author, the maximum observable distance was most frequently entered as the diagonal of the figure documenting the study site – likely a liberal interpretation of maximum distance measurable. Our title was inspired by that of Freckleton and Watkinson (2003) who have asked a similar question of plant populations.

AMPHIBIANS AND HANSKI'S FOUR METAPOPOPULATION CONDITIONS:

Hanski outlines four conditions necessary to demonstrate the existence of a metapopulation effect (Hanski, 1999; Hanski et al., 1995; Hanski and Kuussaari, 1995): 1) Habitat patches support local breeding populations, 2) No single population is large enough to ensure long-term survival, 3) Patches are not too isolated to prevent recolonisation, and 4) Local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely. Someone who observes a species with high site-fidelity, limited dispersal and apparently disjunct breeding patches, may frequently and implicitly, evaluate (or accept) these assumptions *a priori*. Through

an examination of studies in the literature involving amphibians where the term metapopulation or population subdivision was used, we examined whether these studies had tested, not tested or assumed the importance of each of these conditions (Appendix 1).

We found that 74% of the possible 208 assumptions (52 articles*4 assumptions) were assumed or untested – significantly different from our null hypothesis of articles evenly distributed between categories (Likelihood chi-square value = 131.25, 3 df, $p < 0.001$) (Table 1). The most frequently tested condition was of isolation, although the dispersal ability of the amphibian species was rarely tested directly. Even though dispersal was indirectly estimated with genetics in 45% of the cases, the *a priori* assumption was that the amphibian was of low vagility. Any distance larger than one kilometre (Berven and Grudzien, 1990; Conroy and Brook, 2003; Newman and Squire, 2001; Sjogren, 1991; Vos and Chardon, 1998; Waldick, 1997), two kilometres (Hranitz and Diehl, 2000), or several hundred meters (Reading et al., 1991; Skelly et al., 1999) were referred to as critical distances beyond which amphibian dispersal would not penetrate. In fact, of the 52-amphibian/metapopulation studies, all explicitly tested or assumed that ponds were isolated due to the limited dispersal and/or high site fidelity of amphibians. Forty-two of fifty-two studies (80%) implicated this limited dispersal as the, or one of the, primary rationales behind the utility of the metapopulation process. Interestingly, studies that rejected the metapopulation paradigm (10/52) did so because there was judged to be too much dispersal among patches.

AMPHIBIAN SITE LOYALTY AND MOVEMENT

The literature contains many references that explain the poor dispersal ability of amphibians as being a consequence of their physiology and behavior (Duellman and Trueb, 1986; Blaustein et al., 1994; Sinsch, 1990). Amphibian skin is highly permeable and they therefore have a stringent dependence on moisture (Duellman and Trueb, 1986). Additionally, amphibians can show extreme site (Blaustein et al., 1994). Many individual studies have demonstrated that amphibians are found at the same location between census years and, where individuals have been followed through time, their movement is limited

(Sinsch, 1990). For instance, it has been shown that for *Bufo bufo* in England, 93% of females and 96% of males that survived between years, returned to the same breeding ponds (Reading et al., 1991). Although Seebacher and Alford (1999) reported the absence of any significant homing effect in *Bufo marinus*, previously, they had been shown return to their home site with 100% accuracy when displaced up to 70m (Brattstrom, 1962), or 165 m (Carpenter and Gillingham, 1987). Eighty-eight percent of *Rana lessonae* and *Rana ridibunda* monitored between years did not move from their capture pond (Holenweg Peter, 2001). Seventy-five percent of *Rana aurora draytonii* did not move from their site over the course of a year and 90% of these philopatric individuals were never more than 60m from the water (Bulger et al., 2003). Seventy-one percent of male *Hyla regilla* were found in the same portion of the same pond when recaptured the following year (Jameson, 1957). One hundred percent of adults and 82% of juvenile *Rana sylvatica* are faithful to their first breeding pond or natal pond respectively (Berven and Grudzien, 1990). Eighty-nine percent of *Rana muscosa* were recaptured at the same pond between breeding seasons (Pope and Matthews, 2001). *Bufo americanus* demonstrated the ability to return to within 5 m of their original site after a 235m translocation (Dole, 1972). Ninety-eight percent of *Rana pipiens* returned to their home pond after a one-kilometer displacement (Dole, 1968), while half of translocated *Rana muscosa* returned to their initial site within 20 days after a 630m translocation (Matthews, 2003). Bellis (1965) found that for 298 *Rana sylvatica* the mean distance between captures was only 11.25 m. The majority of recaptured *Pseudacris triseriata triseriata* were within 100m of their initial capture site (Kramer, 1973), while the maximum distance moved by *Pseudacris maculata* is likely only 250 m (Spencer, 1964). Eighty-nine percent of *Mixophyes iteratus* individuals were recaptured 5m or less away from where they were marked (Lemckert and Brassil, 2000).

Breeding site fidelity in salamanders' approaches 100%. Not a single one of 2500 marked *Triturus vulgaris* changed breeding ponds between seasons (Bell, 1977). Similarly, the rate of inter-pond movement in *Notophthalmus viridescens* was zero (Gill,

1978). *Plethodon cinerus* (Placyk and Graves, 2001) and *Triturus vulgaris* (Dolmen, 1981) have been found to be philopatric to the same cover object between years.

Amphibian species truly do appear to be site-loyal on average. However, if a few individuals are not described by that average – and regularly move away from their natal site – the likelihood of support for the paradigm of metapopulation structure through isolation is reduced. In this case, high rates of dispersal would effectively unite disjunct populations into a single unit – or patchy population (Harrison, 1991). We found that while the view of limited amphibian dispersal may be true for some species, it does not hold for all. Among 161 journal articles concerning 89 species recording the maximum distance moved, 46% of the amphibian species moved no farther than 400m. However, 4% were capable of movements greater than 10 km (Table 4). This literature review sampled much more of total salamander diversity (36 species of 352 worldwide, 10.23%) than anuran diversity (52 species of 3848 worldwide, 1.51%) (Duellman and Trueb, 1986), and there were evident differences between these groups. Nearly one half (41%) of the anuran species displayed maximum dispersal distances greater than one kilometre, and 6% of frogs were observed to have maximum dispersal distances greater than 10 km! This is surprising considering that one kilometre has appeared independently in the literature as a magic number beyond which amphibian populations would be isolated from dispersal events (Berven and Grudzien, 1990; Conroy and Brook, 2003; Newman and Squire, 2001; Sjogren, 1991; Vos and Chardon, 1998; Waldick, 1997); and 15 km has been defined as the maximum migratory range (Sinsch, 1990).

Although 94% of the maximum dispersal distances for salamanders are less than one kilometre, the frequency distribution of distances was also an inverse power function (Figure 3). Therefore, although most may not move very far, there is a strong likelihood that some individuals may complete surprisingly far long-distance movements. This relationship was more pronounced for terrestrial-breeding salamanders (which did not move far) than for pond-breeding species (which tended to move farther than terrestrial breeders – Results not shown). For example, *Plethodon glutinosus* is a remarkably site loyal animal across ages and sexes (Wells and Wells, 1976). Using data provided in the

paper for this species, we found that an inverse power law explained 74% of the variation in movement frequency for males (from (Wells and Wells, 1976) Figure 1). In this case, although it is clear that most individuals are unlikely to move more than 10 m, according to this relationship there is an appreciable chance (~0.4%) that an individual could move one kilometre. Indeed, recent findings document that for some pond-breeding salamanders, rates of inter-pond migration are high enough to suggest that the fit of the metapopulation paradigm to this particular species was less than was expected (Trenham, 1998).

Clearly, most amphibian species do not move very far, but surprisingly, more than 6% of anurans surveyed were capable of movements of greater than 10 km! This leptokurtic, inverse-power relationship neither supports nor rejects the model of the poorly dispersing amphibian. Concluding all amphibians are poor dispersers is as incorrect as stating that all mammals move long distances. Rather, it demonstrates that, as a group, amphibians exhibit a wide range of dispersal strategies. As has been demonstrated (Chapter II; Hayes et al., 2001; Platx et al., 1990; Stumpel and Hanekamp., 1986; Tunner, 1992; Vos et al., 2000), some amphibian species are capable of movements in distances that are surprising for presumably poorly dispersing animals. Our meta-analysis suggests that anurans have an average maximum movement recorded (2.47 km) that is two and a half times as large as the distance commonly reported as wide enough to result in population isolation. The diversity of maximum movement recorded is apparent in the wide variance (2.440 E +07). Indeed, the anuran average and variance are an order of magnitude larger than the same patterns in salamanders (Table 4). Fitting an inverse power law to the movement frequency patterns for anurans and salamanders results in the explanation of 70% and 55% of the variance. From this analysis, we have a clear prediction regarding the spatial scale at which we should expect local population differentiation if we observe where the inverse power law equals one as a prediction of the number of migrants across a distance of X. Genetically, populations tend to be locally differentiated when $N_e m \ll 1$ (Kimura and Maruyama, 1971), and a rule of thumb for the management of gene flow for isolated populations is of one-migrant-per-generation

(Wang, 2004). For salamanders, the inverse power law predicts that at least one individual is likely to move distances less than 8-9 km and for anurans at least one individual is likely to move 11-13 km. Around these distance ranges – where dispersal is possible, but not common – is likely to be a scale where the metapopulation approach is applicable for amphibians.

The perception of limited amphibian dispersal may perhaps be more a function of the scale at which amphibian-researchers operate, than a feature of the scale at which amphibians disperse (Marsh et al., 1999; Dole, 1971; Staub et al., 1995; Turner, 1960). Among those studies where there were enough data reported to estimate the longest axis of the study area, that distance was therefore the longest possible distance that could have been recorded. A regression of maximum distance dispersed on the size of the study's long axis resulted in a significant positive relationship where 72.65% of the observed variance in maximum dispersal distance is explained by having a larger study site. This relationship suggests that our understanding of the maximum distances amphibians can move is likely being underestimated by the size of sites we use to study such movement. Our review makes a simple prediction that monitoring larger areas in the future will result in the discovery of longer distance movements for both anurans and salamanders.

CASE STUDY

Evidently some amphibians function as metapopulations (Sjogren, 1991; Gill, 1978; Vos et al., 2000; Sjogren Gulve and Ray, 1996). However, examining the amphibian-as-metapopulation literature indicates that the majority of the metapopulation conditions are either not tested or are assumed to hold. Indeed, it is often taken as a foregone conclusion that amphibians operate as metapopulations. Bulger et al (2003), for example, invoke metapopulation structure without having tested any of the related (Hanski, 1999; Hanski et al., 1995; Hanski and Kuussaari, 1995) hypotheses. For species we have examined ourselves (*Bufo americanus*, *Bufo fowleri*, *Pseudacris crucifer*, *Rana sylvatica*, *Rana pipiens*, *Rana clamitans* and *Rana catesbeiana*), both locally (on a scale of ≤ 10 km) and regionally (for *B. fowleri* ≤ 300 km), we found no evidence of metapopulation effect at either scale, and concluded that while there was not enough

movement between populations separated by many tens of kilometres there was likely too much dispersal (An average of 3 *B. fowleri* individuals per year move 4 km or more) occurring between the habitat patches separated by ten kilometres or less to support a metapopulation effect (Chapter II; Chapter IV). Most of these species are common to the area we investigated, but *B. fowleri* is currently listed as THREATENED by the Committee on the Status of Endangered Species (COSEWIC) in Canada largely due to its isolated and fragmented habitat. Indeed, a metapopulation based conservation approach was intuitively appealing for this species, as, prior to our analysis, it qualitatively appeared to meet all of Hanski's four conditions.

To reconcile the acceptance of the metapopulation approach in amphibian conservation and ecology with the lack of stringent testing of hypotheses we compared those characteristics common to amphibians as metapopulations (Marsh and Trenham, 2001) (1) population dynamics determined by pond-based-processes, 2) common local extinction and colonisation, 3) local extinction occurring in suitable habitat, 4) limited dispersal causing isolation) to the studies we've reviewed and the species for which we have data.

The majority (32/52) of the studies we examined had either implicitly assumed or had tested that the 'ponds as patches' view was an accurate depiction of an amphibian population. Recently, Skelly et al (1997), Marsh and Trenham (2001) and Pope et al (2000) have critiqued the assumed primacy of the pond. For instance, with *Rana pipiens*, any apparent metapopulation structure was removed when the non-pond variable of 'summer habitat' was removed from the analysis (Pope et al., 2000). In our own work with *B. fowleri*, we know that the number of non-reproductive one year olds at year (t) explains nearly 80% of the variation in captured adults in year (t+1) (Green and Smith unpublished). Although strong, this relationship was derived using only 5 years of data and excludes one year where it was suspected that migration boosted the observed number of reproductive animals in year (t+1). Thus while population processes occurring in the pond are clearly important, there is strong evidence that events occurring outside the pond (migration, over-wintering success) also contribute to the observed population

dynamics. Therefore, instead of asking is the pond a patch (~Hanski Condition 1), we should ask is the patch only the pond? Stated another way perhaps the easily spatially delineated feature “pond” is not the disjunct habitat one should model. The application to amphibian conservation is clear, for if one adopted a metapopulation based conservation approach on the hypothesis of ponds as metapopulation patches – and summarily protected those patches – without testing whether the habitat critical to species survival was actually described by pond boundaries it is possible that the truly important habitat would not be protected.

Forty-four out of 52 articles in the literature (85%) did not examine the frequency of local extinction and colonization. A limited number of between-year occupancy transitions severely restrict the researcher’s ability to determine whether extinctions and colonizations are actually common features of the network of breeding assemblages (Thomas et al., 2002). For instance, within the local habitat patch assemblage we monitor in Ontario, there have been 38 observed colonisation and 31 extinction events for *B. fowleri* measured over 15 years at Long Point, resulting in average colonisation and extinction rates of 0.28 and 0.25 respectively. Compared to values for other amphibian species (Table 2 of Marsh and Trenham (2001)), the values for *B. fowleri* are amongst the highest and yet there is no evident metapopulation effect involved in *B. fowleri* population dynamics (Chapter IV). Due to relatively high rates of local extinction and colonisation in *B. fowleri*, a conservation strategy might be based on the faulty notion that these toads occupy local and regional metapopulations when they are actually more intimately connected by regular dispersal (i.e. a patchy population).

One finding recorded regularly in the literature was that local amphibian extinctions were deterministic, not stochastic, as habitats underwent succession (Sjogren, 1991; Skelly et al., 1999; Marsh and Trenham, 2001; Bradford et al., 2003). Indeed, at Long Point the environment is constantly undergoing succession, and ponds are likely to be exposed to deterministic degradation (as *B. fowleri* breeding habitat) as they go through succession. We suspected that at least several of the observed extinctions at Long Point were due to succession reducing habitat quality to such an extent as to cause

extinction. Perhaps the effects of this deterministic change are predominant over any stochastic changes we have measured (Skelly et al., 1999), but a quantitative analysis of this question is beyond the scope of this investigation. Clearly though, it remains important to do so (Ellner and Fussmann, 2003), as differentiating between the importance of stochastic and deterministic changes will inform conservation decisions regarding whether management should focus on landscape factors or local habitat conditions.

The supposition of limited dispersal causing isolation was the most frequently implicated reason for evident, or assumed, metapopulation structure in amphibians. In our own work with *B. fowleri*, we concluded that dispersal was likely the primary factor implicated in the lack of metapopulation structure we demonstrated – both too little and too frequent (Chapter II; Chapter IV; Chapter VI). At a local scale of ≤ 10 km, individuals dispersed over a distance and at a rate that made even isolated populations connected to the whole. Regionally (≤ 300 km), populations were too isolated for even occasional migrants to recolonise habitat following local extinction. We suspect that the generalization of limited amphibian dispersal causing population isolation, and therefore metapopulation structure, may not be warranted as frequently as the literature implies (Alford and Richards, 1999). This is especially true for pond-breeding anurans species with a high turnover of local populations and dependent upon dispersal for its persistence, for without the effect of rescue from neighboring populations, they will suffer greater cumulative local extinctions (Green, 2003). We find that movement distances for anurans are an order of magnitude greater (~ 10 km) than has previously been thought.

CONCLUSIONS

Our review demonstrates that the applicability of the metapopulation paradigm to amphibian species is largely dependant on the hypothesis of limited dispersal. As there are wide ranges of dispersal abilities within temperate pond-breeding species, we should be cautious with the indiscriminant application of the metapopulation approach to amphibians – especially where conservation decisions are to be based on the assumptions of isolation and metapopulation structure though limited dispersal. With a thorough

literature review, we have demonstrated that although temperate pond-breeding anuran amphibians are predominantly site-loyal and of low vagility, we should expect them to move distances much greater than previously anticipated. A class-wide comparison of dispersal capabilities suggests that instead of population isolation occurring at the 1 km range, occasional migrants may be expected to connect populations separated by tens of kilometers. Indeed, for both salamanders and anurans, we suggest that population differentiation is more likely to occur at scales upward of 10 km rather than 1 km. If somewhat regular movement of individuals can connect populations separated by distances smaller than this, then the effective number of populations is reduced. If reduced to one, then patch occupancy models are ineffective tools for research or conservation as they ignore local dynamics (Hanski, 1998). All amphibians are not metapopulations and not all amphibians are dispersal poor. The paradigm of pond-breeding amphibian populations as metapopulations has been adopted before there have been sufficient data available to evaluate it (Hanski and Simberloff, 1997). If the meaning of the term 'metapopulation' has lost clarity (Smedbol et al., 2002), researchers would do well to test the elementary predictions of a simple metapopulation prior to announcing that their particular study organism/population constitutes one. It is not our intent to summarily reject the positive influence the development of the metapopulation concept has had on ecology and conservation. Undoubtedly there are amphibian species for which the metapopulation assumption of pond isolation due to limited dispersal is valid. However, the generality of this assumption is not supported by data. The metapopulation approach – especially regarding habitat patch isolation due to limited dispersal – must be more stringently tested and more clearly reported.

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TABLES

Table 1:

Literature review of the metapopulation paradigm in amphibian ecology. Fifty-two studies regarding amphibians and population sub-division were examined regarding their explicit or implicit testing of the four conditions necessary for a metapopulation effect (Hanski, 1999; Hanski and Kuussaari, 1995).

	Habitat patches support local breeding populations	No single population is large enough to ensure long-term survival	Patches are not too isolated to prevent recolonisation: isolation due to limited dispersal	Local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely
Not Tested	20	37	0	44
Tested	10	9	32	3
Assumed	22	6	20	5

Table 2:

Maximum dispersal distance recorded and the longest distance able to be measured in the reported study site for 53 anuran species from 101 reports.

<i>Species</i>	Max distance recorded (m)	Longest measure of study site (m)	Ratio Study site to max distance	Reference
<i>Ascaphus truei</i>	360.00	2800.00	0.13	Daugherty and Sheldon 1982
<i>Atelopus varius</i>	20.00	20.00	1.00	Crump 1986.
<i>Bombina variegata</i>	312.80	1000.00	0.31	Beshkov and Jameson 1980
<i>Bufo americanus</i>	6437.38			Hamilton 1934
<i>Bufo americanus</i>	235.00	235.00	1.00	Dole 1972
<i>Bufo americanus</i>	1000.00			Maunder 1983
<i>Bufo americanus</i>	548.64	1254.03	0.44	Blair 1943
<i>Bufo americanus</i>	594.00			Oldham 1966
<i>Bufo americanus</i>	4023.00			Maynard 1934.
<i>Bufo baxteri</i>	99.00			Carpenter 1954
<i>Bufo baxteri</i>	423.80			Pareker and Anderson 2003
<i>Bufo boreas</i>	2400.00			Bartelet 2000
<i>Bufo boreas</i>	6000.00	15000.00	0.40	Muths et al 2003
<i>Bufo boreas</i>	2324.20			Muths 2003
<i>Bufo boreas</i>	200.00	200.00	1.00	Tracy and Dole 1969
<i>Bufo bufo</i>	3000.00			Heusser 1969
<i>Bufo bufo</i>	3621.02			Moore 1954
<i>Bufo bufo</i>	760.00	8485.28	0.09	Sinsch 1988
<i>Bufo bufo</i>	118.00			Parker and Gittins 1979
<i>Bufo bufo</i>	500.00			Haapanen 1974
<i>Bufo calamita</i>	4411.00	3605.55	1.22	Miaud et al 2000
<i>Bufo calamita</i>	400.00	2828.43	0.14	Sinsch 1997
<i>Bufo fowleri</i>	1650.00	2000.00	0.83	Breden 1987
<i>Bufo fowleri</i>	99.40	1770.00	0.06	Clarke 1974
<i>Bufo fowleri</i>	335.28	1254.03	0.27	Blair 1943
<i>Bufo fowleri</i>	1600.00			Stille 1952
<i>Bufo fowleri</i>	1280.16			Nichols 1937
<i>Bufo fowleri</i>	34000.00	34000.00	1.00	Smith and Green (unpublished)
<i>Bufo fowleri</i>	100.00			Ferguson 1960
<i>Bufo hemiophrys</i>	342.00			Breckenridge and Tester 1961
<i>Bufo japonicus formosus</i>	260.00	500.00	0.52	Kusano et al 1995
<i>Bufo marinus</i>	6400.00			Eastal and Floyd 1986
<i>Bufo marinus</i>	1300.00			Schwarzkopf and Alford 2002
<i>Bufo punctatus</i>	822.96			Tevis 1966
<i>Bufo punctatus</i>	900.00	900.00	1.00	Weintraub 1974
<i>Bufo terrestris</i>	1609.34	2449.29	0.66	Bogert 1947
<i>Bufo valliceps</i>	800.00			Blair 1953

Table 2 continued.

<i>Species</i>	Max distance recorded (m)	Longest measure of study site (m)	Ratio Study site to max distance	Reference
<i>Dendrobates pumilio</i>	20.00	20.00	1.00	McVey et al 1981
<i>Eleutherodactylus coqui</i>	4.50			Woolbright 1985
<i>Eleutherodactylus fitzingeri</i>	35.00			Hobel 1999
<i>Gastrophryne olivacea</i>	609.60			Fitch 1956
<i>Gastrophryne carolinensis</i>	914.00			Dodds 1996
<i>Geocrina alba</i>	39.00	150.00	0.26	Driscoll 1997
<i>Geocrina vitellina</i>	49.00	150.00	0.33	Driscoll 1997
<i>Heleioporus australiacus</i>	463.00			Lemckert and Brassil 2003
<i>Heleioporus eyrei</i>	2500.00			Bamford 1992
<i>Hyla arborea</i>	12600.00			Stumpel and Hanekamp 1986
<i>Hyla arborea</i>	12570.00	18000.00	0.70	Vos et al 2000
<i>Hyla arborea</i>	1500.00			Carlson and Edenhamn 2000
<i>Hyla arborea</i>	3750.00			Clausnitzer and Clausnitzer 1984.
<i>Hyla regilla</i>	1000.00	1000.00	1.00	Jameson 1957
<i>Hyla regilla</i>	1900.00			Reimchen 1991
<i>Hyla versicolor</i>	125.00	130.00	0.96	Roble 1979
<i>Leiopelma hochstetteri</i>	12.65	120.00	0.11	Tessier et al 1991
<i>Mixophyes iteratus</i>	2000.00	40000.00	0.05	Lemckert and Brassil 2000
<i>Pelobates fuscus</i>	500.00			Heis et al 2002
<i>Phyllomedusa bicolor</i>	46.60			Neckel de Oliveira 1996
<i>Physalaemus pustulosus</i>	820.00	3000.00	0.27	Marsh et al 1999
<i>Pseudacris triseriata</i>	213.00	135.00	1.58	Kramer 1973
<i>Pseudacris triseriata</i>	685.80			Spencer 1964
<i>Rana arvalis</i>	7600.00			Vos et al 2001
<i>Rana aurora</i>	300.00	787.00	0.38	Calef 1973.
<i>Rana aurora aurora</i>	24000.00			Hayes et al 2001
<i>Rana aurora aurora</i>	914.40			Dumas 1966
<i>Rana aurora draytonni</i>	3600.00	5000.00	0.72	Bulger et al 2003
<i>Rana berlandieri</i>	16000.00			Platz et al 1990
<i>Rana catesbeiana</i>	1600.20			Ingram and Raney 1943
<i>Rana catesbeiana</i>	914.40	1363.76	0.67	Raney 1940
<i>Rana catesbeiana</i>	966.00			Willis et al 1956
<i>Rana clamitans</i>	600.00			Martoff 1953
<i>Rana clamitans</i>	560.00			Lamoreux et al 2002
<i>Rana clamitans</i>	560.00			Lamoreux et al 1999
<i>Rana clamitans</i>	4800.00			Schroeder 1976
<i>Rana dabmatina</i>	300.00			Ponsero and Joly 1998

Table 2 continued.

<i>Species</i>	Max distance recorded (m)	Longest measure of study site (m)	Ratio Study site to max distance	Reference
<i>Rana lessonae</i>	900.00	1000.00	0.90	Sjogren Gulve 1998
<i>Rana lessonae</i>	1760.00	2830.00	0.62	Holenweg Peter 2001
<i>Rana lessonae</i>	15000.00			Tunner 1992
<i>Rana lutieventris</i>	2066.00	3905.00	0.53	Pillioid et al 2002
<i>Rana lutieventris</i>	560.00			Bull and Hayes 2001
<i>Rana mucosa</i>	1020.00	1414.21	0.72	Pope and Mathews 2001
<i>Rana pipiens</i>	1609.34			Merrell 1970
<i>Rana pipiens</i>	85.00	720.00	0.12	Dole 1965
<i>Rana pipiens</i>	2100.00	720.00	2.92	Dole 1965
<i>Rana pipiens</i>	8000.00	8000.00	1.00	Seburn et al 1997
<i>Rana pipiens</i>	5000.00	720.00	6.94	Dole 1971
<i>Rana pretiosa</i>	1280.16	2133.60	0.60	Turner 1960
<i>Rana pretiosa</i>	45.00			Carpenter 1954
<i>Rana ridibunda</i>	1760.00	2830.00	0.62	Holenweg Peter 2001
<i>Rana sevosia</i>	299.00	1202.00	0.25	Richter et al 2001
<i>Rana sevosia</i>	2000.00			Franz et al 1988
<i>Rana sevosia</i>	1600.00			Carr 1940
<i>Rana sylvatica</i>	60.00	186.00	0.32	Regosin et al 2003
<i>Rana sylvatica</i>	500.00			Howard and Kluge 1985
<i>Rana sylvatica</i>	2530.00	2287.00	1.11	Berven and Grudzien 1990
<i>Rana sylvatica</i>	89.61	65.84	1.36	Bellis 1960
<i>Rana temporaria</i>	460.00			Seitz et al 1992
<i>Syrhophus marnocki</i>	299.92			Jameson 1955
<i>Scaphiopus holbrooki</i>	825.00			Pearson 1955

Table 3:

Maximum dispersal distance recorded and the longest distance able to be measured in the reported study site for 35 salamander species from 61 reports.

<i>Species</i>	Max distance recorded	Longest measure of study site	Ratio Study site to max distance	Reference
<i>Ambystoma californiense</i>	670.00			Trenham et al 2001
<i>Ambystoma californiense</i>	129.00			Loredo et al 1996
<i>Ambystoma jeffersonianum</i>	250.00	567.00	0.44	Douglas and Monroe 1981
<i>Ambystoma jeffersonianum</i>	1600.00			Bishops 1941
<i>Ambystoma jeffersonianum</i>	625.00			Williams 1973
<i>Ambystoma jeffersonianum</i>	231.00			Wacasey 1961
<i>Ambystoma laterale</i>	405.00	692.00	0.59	Faccio 2003
<i>Ambystoma macrodactylum</i>	1170.00	39000.00	0.03	Funk and Dunlap 1999
<i>Ambystoma maculatum</i>	220.00	567.00	0.39	Douglas and Monroe 1981
<i>Ambystoma maculatum</i>	125.00			Williams 1973
<i>Ambystoma maculatum</i>	500.00	500.00	1.00	Shoop 1968
<i>Ambystoma maculatum</i>	402.00			Gordon 1968
<i>Ambystoma maculatum</i>	750.00	711.00	1.05	Madison 1997
<i>Ambystoma maculatum</i>	200.00			Wacasey 1961
<i>Ambystoma maculatum</i>	249.00			Kleeberger and Werner 1983
<i>Ambystoma opacum</i>	30.00	567.00	0.05	Douglas and Monroe 1981
<i>Ambystoma opacum</i>	450.00			Williams 1973
<i>Ambystoma opacum</i>	1000.00	600.00	1.67	Pechmann et al 2001
<i>Ambystoma talpoideum</i>	287.00	400.00	0.72	Semlitsch 1981
<i>Ambystoma talpoideum</i>	1000.00	600.00	1.67	Pechmann et al 2001
<i>Ambystoma texanum</i>	125.00			Williams 1973
<i>Ambystoma tigrinum</i>	600.00	600.00	1.00	Pechmann et al 2001
<i>Ambystoma tigrinum</i>	5.10			Semlitsch 1983
<i>Ambystoma tigrinum</i>	286.00			Madison and Farrand 1986
<i>Amphitama tridactylum</i>	297.00			Cagle 1948
<i>Aneides aeneus</i>	106.00			Gordon 1961
<i>Aneides vagrans</i>	15.24			Williams and Gordon 1961
<i>Aneides vagrans</i>	20.30	1300.00	0.02	Davis 2002

Table 3 continued.

<i>Species</i>	Max distance recorded	Longest measure of study site	Ratio Study site to max distance	Reference
<i>Cryptobranchus alleganiensis</i>	900.00			Nickerson and Mays 1973
<i>Cryptobranchus alleganiensis</i>	85.00	100.00	0.85	Peterson 1987
<i>Cryptobranchus alleganiensis</i>	900.00			Wiggs 1977
<i>Desmognathus fuscus</i>	19.80	50.00	0.40	Ashton 1975
<i>Desmognathus fuscus</i>	17.20			Barbour et al 1969
<i>Desmognathus fuscus</i>	40.00	40.00	1.00	Barthalmus and Bellis 1969
<i>Desmognathus quadromaculatus</i>	25.00	180.00	0.14	Freeman 2003
<i>Dicamptodon tenebrosus</i>	66.25			Johnston and Frid 2002
<i>Ensatina eschscholtzii platensis</i>	150.00			Staub et al 1995
<i>Eurycea bistineata</i>	420.00			Johnson and Goldberg
<i>Eurycea quadridigitata</i>	600.00	600.00	1.00	Pechmann et al 2001
<i>Gyrinophilus porphyriticus</i>	490.00	1000.00	0.49	Lowe 2003
<i>Hynobius nebulosus tokyoensis</i>	90.00	332.00	0.27	Kusano and Miyashita 1984
<i>Necturus beyeri</i>	65.00			Shoop and Gunning 1967
<i>Necturus maculosus</i>	256.00			Shoop and Gunning 1967
<i>Notophthalmus perstriatus</i>	709.00			Dodd 1996
<i>Notophthalmus viridescens</i>	1000.00	6522.00	0.15	Gill 1978
<i>Plethodon cinereus</i>	90.00	90.00	1.00	Kleeberger and Werner 1982
<i>Plethodon glutinosus</i>	91.50	32.00	2.86	Wells and Wells 1976
<i>Plethodon jordani</i>	60.00			Madison and Shoop 1970
<i>Plethodon jordani</i>	150.40	180.00	0.84	Madison 1969.
<i>Plethodon kentucki</i>	13.95	15.00	0.93	Marvin 1998
<i>Plethodon vehiculum</i>	8.50	14.00	0.61	Ovaska 1988
<i>Rhyacotriton cascade</i>	6.09	11.66	0.52	Nijhuis and Kaplan 1998
<i>Salamandra atra aurorae</i>	30.00	933.00	0.03	Bonato and Fracasso 2003
<i>Salamandra salamandra</i>	30.00			Rebello and Leclair 2003
<i>Taricha rivularis</i>	12874.75	12874.00	1.00	Twitty et al 1967
<i>Taricha rivularis</i>	4023.36	4023.36	1.00	Twitty et al 1964
<i>Triturus alpestris</i>	500.00			Perret et al 2003
<i>Triturus alpestris</i>	200.00			Joly and Grolet 1996
<i>Triturus vulgaris</i>	80.00	30.90	2.59	Griffiths 1984
<i>Triturus vulgaris</i>	182.00	400.00	0.46	Warwick 1949
<i>Triturus vulgaris</i>	50.00			Bell 1977
<i>Triturus vulgaris</i>	123.00			Dolmen 1981

Table 4:

Summary table for the analysis of maximum reported distance moved for 164 studies of 90 species of amphibians.

	AMPHIBIANS	FROGS	SALAMANDERS
Number of species in dispersal studies	90	53	37
Number of dispersal studies	164	101	63
% max dispersal \leq 1 km	72	58	94
% max dispersal \leq 400 m	46	32	65
% max dispersal \geq 10 km	4	6	2
AVERAGE (m)	1746.35	2486.31	577.67
STANDARD DEVIATION (m)	4092.09	4909.83	1677.49
VARIANCE (m ²)	1.67E+07	2.41E+07	2.81E+06

FIGURE HEADINGS:

Figure 1:

Frequency histogram of the maximum distance moved by amphibians from 161 journal articles (89 species). 200m size bins. 28% of the reviewed studies had maximum movement distances greater than 1km. $y = 22.286x^{-0.7666}$, $R^2 = 0.72$, $SE = 0.21$.

Figure 2:

Frequency histogram of the maximum distance moved by anurans from 101 journal articles (53 species). 200m size bins. 42% of the reviewed studies had maximum movement distances greater than 1 km. $y = 13.908x^{-0.6453}$, $R^2 = 0.69$, $SE = 0.22$.

Figure 3:

Frequency histogram of the maximum distance moved by salamander species from 62 journal articles (36 species). 200m size bins. Six percent of the reviewed studies had maximum movement distances greater than 1km. $y = 6.08x^{-0.4759}$, $R^2 = 0.58$, $SE = 0.25$

Figure 4:

Relating the maximum movement recorded with the maximum size of the study area. $R^2 = 0.72$, $SE = 1280.4$.

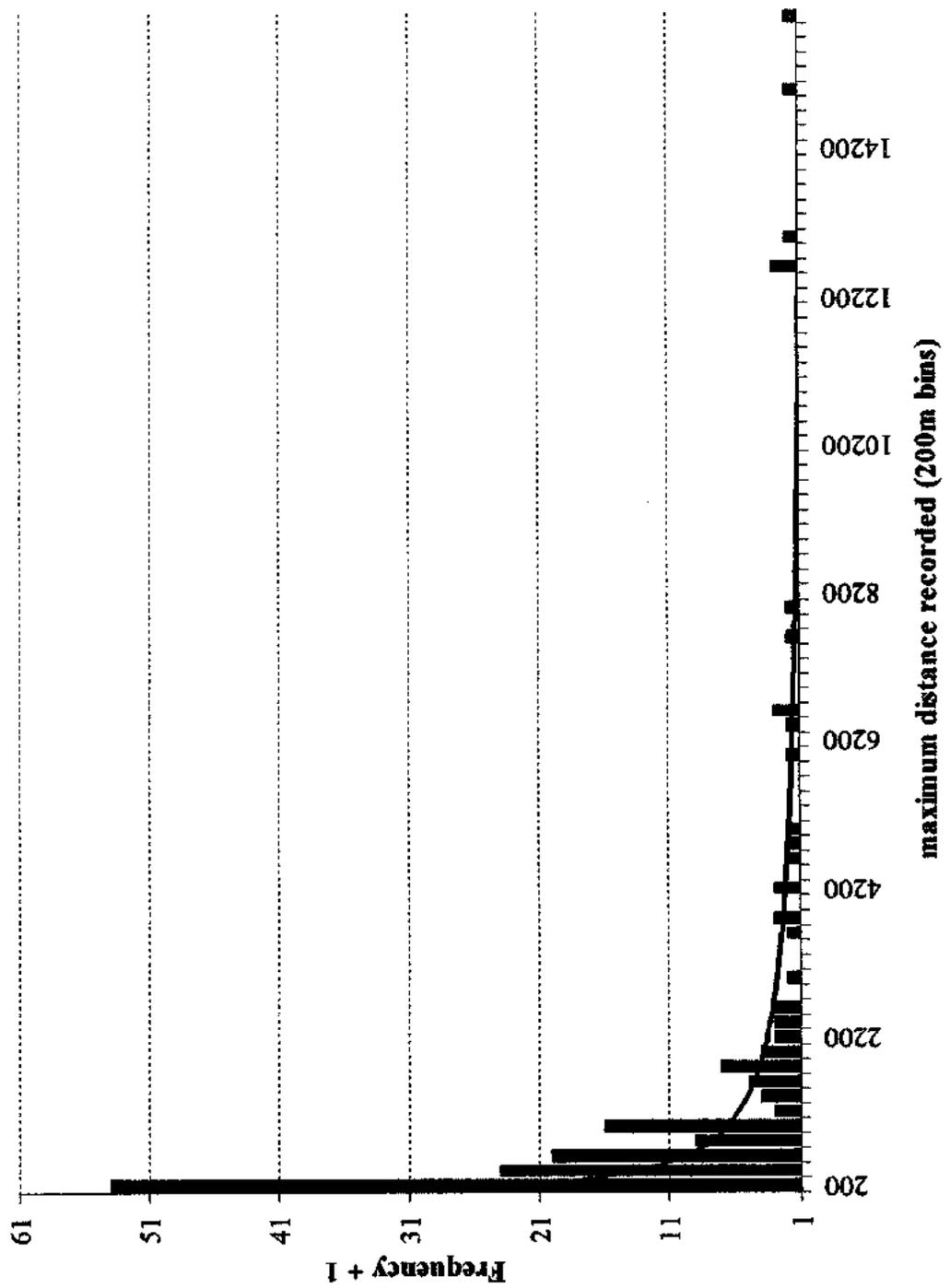
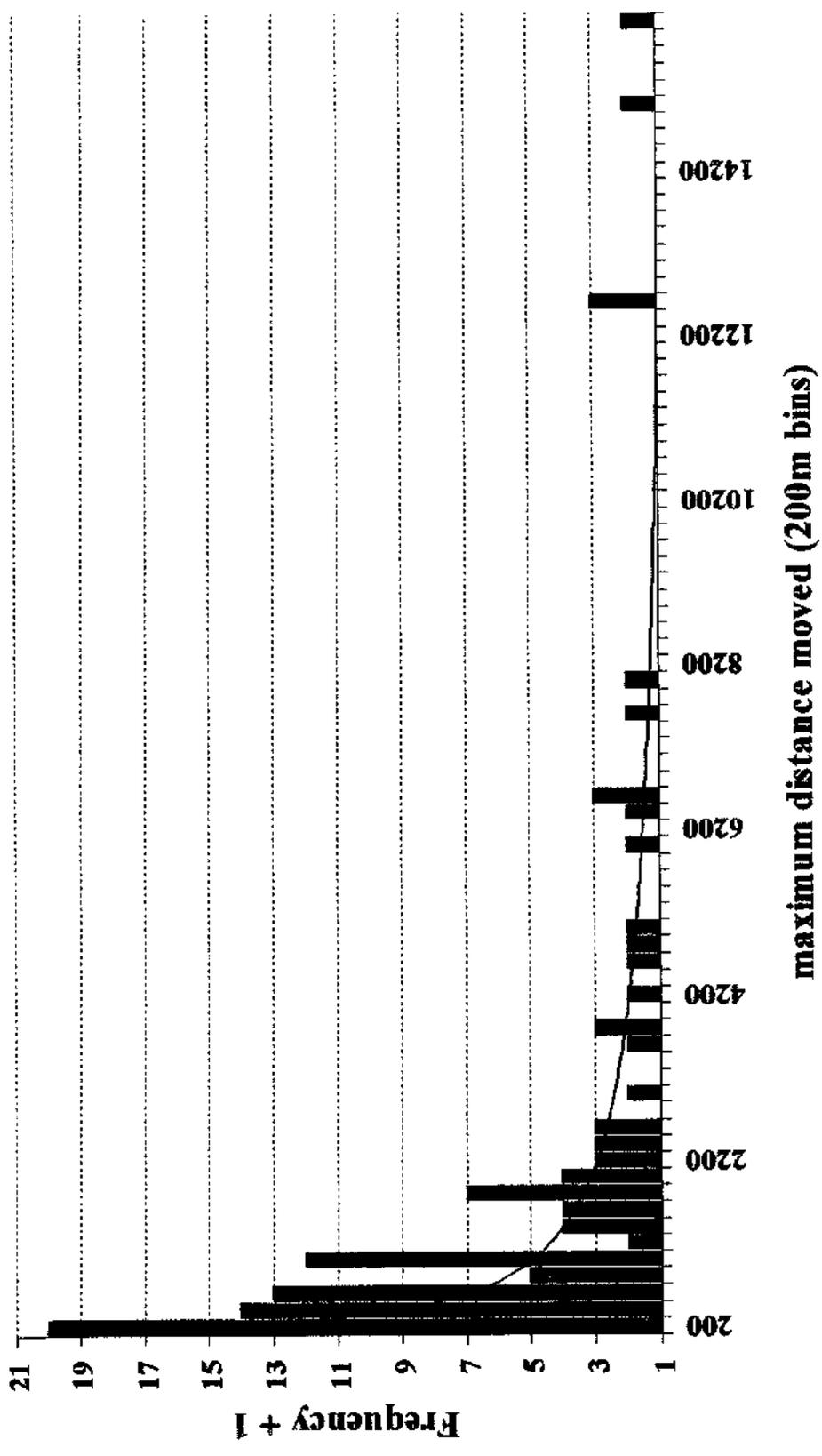


FIGURE 1

FIGURE 2



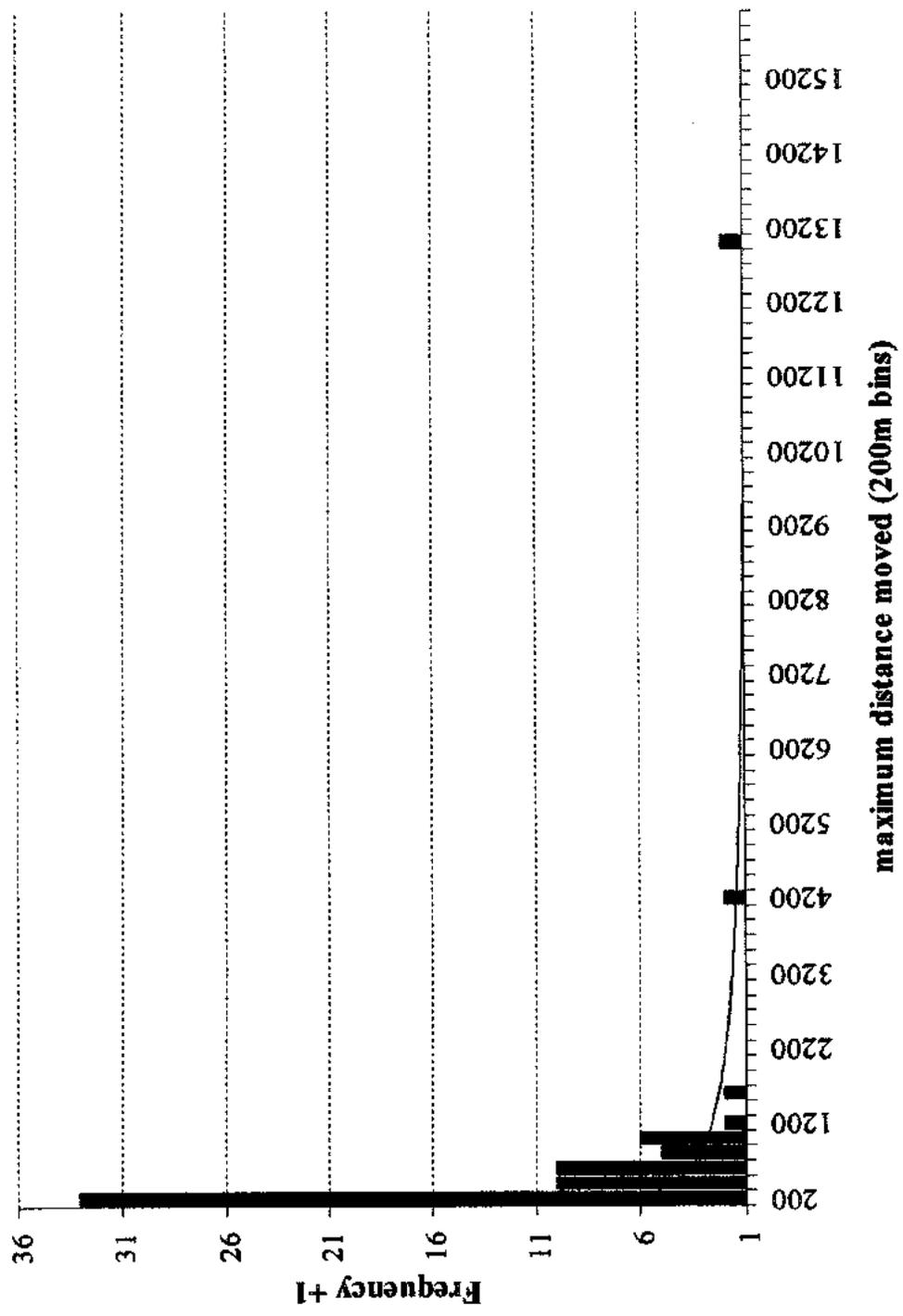
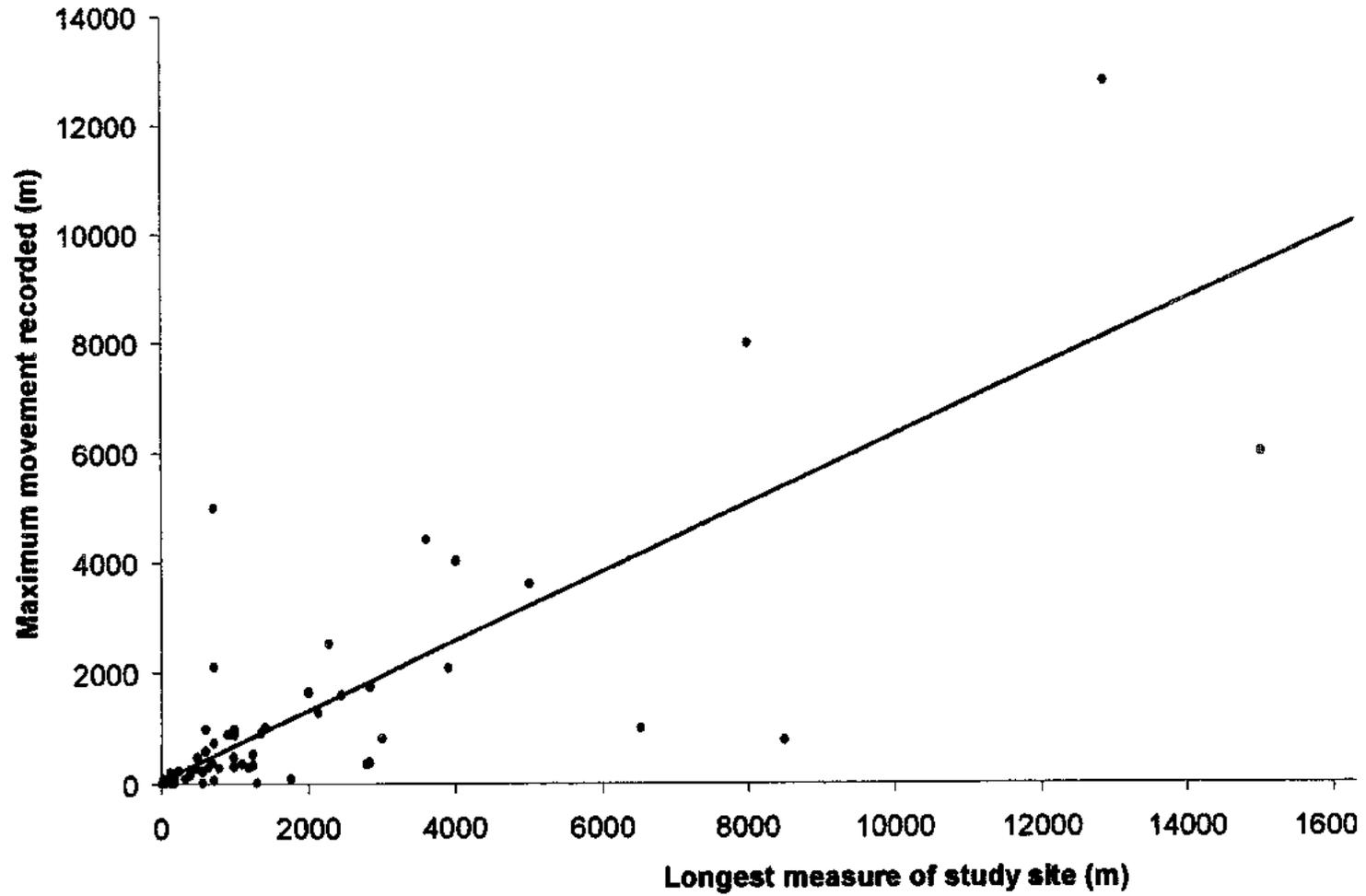


FIGURE 3

FIGURE 4



Appendix 1:

Testing of metapopulation conditions. 0 = not tested, 1 = tested, and 2 = assumed

Condition 1: Habitat patches support local breeding populations	Condition 2: No single population is large enough to ensure long- term survival	Condition 3 Patches are not too isolated to prevent recolonisation (ie. Limited dispersal allows isolation with potential recolonisation)	Condition 4 Local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely	Source
2	2	2	2	Alford and Richards 1999
1	1	1	0	Berven and Grudzein 19990
2	1	2	0	Blaustein et al 1994
2	1	1	0	Bradford et al 2003
2	0	1	0	Call 1997
2	0	2	0	Carlson and Edenhamn 2000
2	2	2	0	Corser 2001
0	0	1	0	Driscoll 1997
1	0	1	0	Gill 1978
2	0	2	0	Goldber 2002
2	0	2	0	Halley et al 1996
1	1	2	0	Hartwell 1990
2	0	2	0	Hecnar and M'Closkey 1996
0	0	1	0	Hels 2002
2	0	2	0	Hranitz and Diehl 2000
0	0	1	0	Johnson and Semlitsch 2003
2	0	1	0	Knapp et al 2003
2	2	2	2	Laan and Verboom 1990
0	2	1	0	Marsh et al 1999
0	0	2	0	Marsh and Trenham 2001
2	0	1	0	Monsen 2002
0	0	1	0	Muths et al 2003
2	2	1	2	Newman and Squire 2001
0	0	1	0	Osborne and Norman 1991
1	0	2	0	Perret et al 2003

Appendix 1 continued

Condition 1: Habitat patches support local breeding populations	Condition 2: No single population is large enough to ensure long- term survival	Condition 3 Patches are not too isolated to prevent recolonisation (ie. Limited dispersal allows isolation with potential recolonisation)	Condition 4 Local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely	Source
0	0	2	0	Pope et al 2000
0	0	1	0	Reading et al 2001
2	2	1	2	Reh and Seitz 1990
2	0	1	0	Ritland et al 2000
0	0	2	0	Routman 1993
2	0	1	2	Rowe et al 2000
0	0	1	0	Scribner et al 1994
0	0	1	0	Scribner et al 2001
0	0	2	0	Semlitsch and Bodie 1998
0	0	2	0	Semlitsch et al 1996
2	0	1	0	Seppa and Laurila 1999
0	0	1	0	Shaffer et al 2000
0	0	1	0	Sinsch 1992
1	1	1	0	Sjogren 1991
1	1	1	0	Sjogren-Gulve 1994
0	0	1	1	Skelly and Meir 1997
2	0	1	0	Skelly et al 1999
0	0	1	0	Tallmon et al 2000
1	1	1	1	Ter Braak and Etienne 2003
1	1	1	0	Trenham 1998
2	0	1	0	Vos et al 2001
2	0	1	0	Vos and Chardon 1998
1	1	1	1	Vos et al 2000
0	0	2	0	Woodford and Meyer 2003
2	0	2	0	Conroy and Brook
0	0	2	0	Ebisuno and Gentilli 2002
1	0	2	0	Joly et al 2001

**CHAPTER 2: SEX, FIDELITY AND ISOLATION: THE DISPERSAL ECOLOGY
OF *BUFO FOWLERI***

**Keywords: amphibian, dispersal, power law, *Bufo fowleri*, long distance dispersal,
drift,**

4600 words, 91 references, 1 table, 6 figures

PREFACE TO CHAPTER 2

In demonstrating the lack of support for “amphibian as metapopulation” paradigm I demonstrated that the most frequently invoked condition to support metapopulation structure was limited dispersal. I now investigate the hypothesis of limited dispersal with a single species example and quantify the movement capabilities for *Bufo fowleri* using mark-recapture data collected on a local scale (<10km). If toads can move between the maximally separated ponds on a local scale, this could be considered to be a reason for the lack of support for either simple metapopulation model. Additionally, if toads move large distances locally, this suggests a reduced likelihood of phylogeographic structuring – examined in Chapter 5.

Reference style is according to submission requirements for the Journal of Animal Ecology.

SUMMARY

- 1) Amphibians are generally considered especially restricted by their biology and behavior to short distance movements. If true, it is logical to view disjunct breeding assemblages of pond-breeding frogs for example, as isolated populations. We used 15 years of mark-recapture data for a population of Fowler's toads (*Bufo fowleri*) to characterize dispersal distances and test this assumption.
- 2) Although most adult *B. fowleri* showed high site fidelity, the maximum distance moved by adult toads was fifteen times greater than was once thought and there was no sex bias to dispersal. The frequency distribution of movements was better described by an inverse power function than either a normal or an exponential distribution for there was a significant 'tail' to the dispersal distribution. Although sex-based resource theory predicted that males should move farther than females there was no sex-bias to patterns of movement. An examination of the literature indicates that the simple predictions of Greenwood do not apply to amphibians as readily as to birds and mammals.
- 3) Using a random-walk simulation we have shown that the movement distributions observed in the field are not significantly different from the simulation, when recaptures are restricted to ponds, although the simulation does not replicate the tail of the distribution.
- 4) The dispersal capabilities observed in these toads suggests that populations once thought isolated by tens of kilometers are actually connected by occasional migrants. Toad movement at Lake Erie may be stratified, with random movement occurring locally, while other forces (such as accidental transport by lake currents) are required of long distance dispersal. Among amphibians, this type of passive movement is likely not unique to *B. fowleri*.

INTRODUCTION

Habitat destruction, fragmentation and deterioration have resulted in populations of many species becoming patchily distributed across the landscape (Saunders et al., 1991) When a set of populations is patchily distributed over a landscape where there is significant environmental stochasticity, we expect variance in population persistence in the local habitat patches (Green, 2003; Thomas et al., 1996). Yet, species in such landscapes do not simply become extinct. This is because immigrants may rescue populations that have gone locally extinct, or are on the verge of local extinction. Thus the rate and extent of movement over the landscape determines the persistence of the species overall and consequently extinctions cannot be predicted without understanding the movement characteristics and resultant population level genetic relationships for the animal in question (Petit et al., 2001). For many species with low variance in demographic characteristics, this effect may not be noticeable. However, when there is high variance in reproductive success and resources, as in many species of amphibians, the importance of dispersal for population persistence may be high. Indeed, at the population level, dispersal is the source of novelty and resilience that mutation is to the individual, and thus studies of dispersal are as crucial to ecology, as estimates of mutation to genetics. Temperate anuran amphibians often utilize patchily distributed breeding habitat and tend to breed in the same ponds year after year and thus dispersal ability is a critical population parameter. Although the literature for amphibians in general (Blaustein et al., 1994; Sinsch, 1990), suggests that any movement is limited and occasional, the dispersal ability of specific amphibians remains poorly characterized.

Bufo fowleri is a small bufonid with a widespread distribution in eastern North America, but in Canada, populations are limited to the sandy beaches on the northern shoreline of Lake Erie. Here they are isolated from one another by uninhabitable stretches of shoreline development and by the lake itself. As with many pond-breeding amphibians their abundance fluctuates wildly (Green, 1997), and we would expect them to be incapable of the long distance movements necessary to recolonise isolated populations. Thus, estimating extinction risk is intrinsically tied to estimating dispersal

potential. Movement estimates for *B. fowleri* in the literature range from maximums of 100m (Clarke, 1974), and 335m (Blair, 1943), to 1280m (Nichols, 1937), 1600m (Stille, 1952), and 2000m (Breden, 1982, 1987, 1988). Urban populations of *B. fowleri* in Missouri rarely moved between adjacent streetlights (Ferguson, 1960). Despite these estimates, movement patterns have not been quantified for this species and we here ask whether previous estimates were accurate, whether toad movement is normally distributed or random, whether there is a sex-bias to movement tendencies, and whether our estimates of movement are biased by the finite nature of our study site.

Differential competition for resources between the sexes has been implicated in the evolution of dispersal and the maintenance of a sex bias in dispersal. Johnson and Gaines (1990) have stated that if it is solely inbreeding avoidance that selects for dispersal, then there should be no sex-bias in dispersal capabilities. However, Waser et al (1986) argues that the costs of inbreeding should result in female-biased dispersal in polygynous systems – a prediction rarely supported by data. Perrin and Goudet (2001) demonstrate that inbreeding depression alone will not produce sex biases in dispersal but can enhance any existing biases. Greenwood (1980) reviewed the literature and found that amongst monogamously mating birds the dispersing sex was female, whereas it was the male in polygynous mammals. Female biased dispersal is to be expected when, prior to female selection of males, there is some kind of resource partitioning by males. Male biased dispersal is to be expected when the distribution of males is determined by the distribution of females, not by any *a priori* resource partitioning. In *Bufo*, the mating system is polygynous (Wells, 1977), and in *B. fowleri* males do not appear to compete for females (Green, 1992; Laurin & Green, 1990). Sexual selection is likely by female choice (Sullivan, 1983, 1992) where females judge males and likely do not breed more than once a year. Therefore, our null hypothesis was that with no *a priori* partitioning of resources, *B. fowleri* would exhibit male-biased dispersal. Our alternative hypothesis was that in an unstable environment with typically high mortality at all life history stages some proportion of both sexes will move. To test the hypothesis of sex biased dispersal

we have compared site-specific and known-point mark-recapture distance records for adult males and females.

Even when movement is limited, animals emigrating outside the study site plague mark-release-recapture studies. Such individuals cannot be recaptured, and thus the animal's capability of long distance movement is not properly considered. To reduce the chance that 1) we were not biased in our estimation of the kurtosis of the movement frequency distribution and 2) that we had not underestimated the long distance movements of *B. fowleri*, we considered three methods to correct for these biases – two analytical and one simulation.

Many population models estimate isolation through an assumption that an individual's movements are normally distributed, or random (Halley et al., 1996; Okubo, 1980). We test whether this assumption holds true for *B. fowleri* by testing whether the observed distribution of movement is significantly different from a normal distribution (Dobzhansky & Wright, 1943); and through a simulation approach where the random, rooks-style movements of individuals in a simplified landscape were modeled, and then compared to the observed movements using non-parametric statistics.

MATERIALS AND METHODS

We studied *B. fowleri* at Long Point, Ontario, a 35 km long sand spit and dune formation which lies approximately east-west on the north shore of Lake Erie (Green, 1992, 1997). The habitat patches at Long Point where we have monitored movement are outlined in Figure 1. Each year, beginning in late April, every adult toad in the study area was found, hand-captured, marked and recorded. All study sites were inspected nightly throughout the entire breeding season until late June. The position of each individual was recorded by site (1991-2000), with GPS (2001), and with differentially corrected GPS (2002 and 2003). Toads were measured (snout-vent length), sexed, and a unique combination of toes was clipped to allow for the identification of individual animals (Green, 1992).

For each recaptured animal the distance, time and direction between captures was calculated and the statistical properties of the movement frequency distributions were investigated using a Kruskal-Wallis test (Sokal & Rohlf, 1995). We tested the hypothesis that the movement distribution was normal using Lilliefors' test for normality (Conover, 1980), and also estimated the kurtosis and symmetry of the distribution. The movement frequency data were compared to two alternative models: I (Exponential) and II (Power). The significance of each model was calculated by regression analysis of $\ln(\text{frequency} + 1)$ and distance and $\ln(\text{frequency} + 1)$ and $\ln(\text{distance})$ respectively. Statistical comparisons were made using Systat (v.9), and Matlab (v 6.12).

Habitat exists to the east and west of the study site, and it is possible that animals captured near the eastern and western perimeters were not recaptured because they had moved off the site. We have used three methods to estimate the effect that these missed individuals would have upon our measurement of the movement frequency distribution. The first was simply to restrict analysis to those animals first captured in the center of the study site thereby increasing the likelihood of recapture. The second method adjusted the observed frequency distribution according to the probability that the dispersal was contained within the study site (Barrowclough, 1978), defined for R (radius of study site), r (distance from site center to capture point), and x (distance from capture to recapture).

When $0 < r < R-x$, the probability is 1, and when $R > r > R-x$ the probability (p) is described by:

$$p = \{ \pi - \cos^{-1} [R^2 - x^2 - r^2] / 2xr \} / \pi$$

Each x has an associated correction. For each 100m-distance category, the total number of animals observed was divided by the average correction factor associated for movements in that category to give the corrected distribution. The third method we developed was "Idealized Site" (IS) and is loosely based on Baker et al (1995). We considered the network of habitat patches described here (Figure 1) which is roughly linear with no habitat to the north or south, but with amphibian habitat to the east and west. Occasional surveys were made into these areas, but the study site is large enough to logistically preclude regular visits to all areas. We mirrored the observed landscape of toad breeding habitats to the east and west, creating a hypothetical transect of 25 km with 56 habitat patches. Within this new transect, we considered dispersal events of distance i , originating from site j , and a circle with radius i . In reality, i from j goes in one of two equally probable directions (observed/alternate). The observed sites covered by distance $j=x$. For that same distance event, the sites in the alternate direction = y . Thus, the probability of observing an equivalently sized dispersal event, in the direction opposite to what actually occurred, is $y/(x+y)$. The IS correction factor was the average of these values grouped by equivalently sized dispersal events. Dividing the observed frequency of the dispersal event by the IS correction factor provides an estimate of the animals missed by the structure of the study site. The methodology assumes that all endpoints are equi-probable, and this allows the use of averages. The IS methodology was written in Matlab v6.1.

We wrote a simple simulation model using Visual Basic (v.6), and Microsoft Excel (v.9) that tested whether the observed movement frequency distributions could have been reproduced by randomly moving individuals in a simplified landscape based on patch network at Long Point. In the model, square grid cells were coded as pond, non-pond or lake. Individuals began at the center of the simulated landscape and were allowed 50 rook's-move movements with no mortality and the Euclidean distance

between the release-point and the end-point was then calculated. The simulation was repeated for 1000 individuals. Using the non-parametric Kolmogorov-Smirnov test in Systat (v.9), we compared the distribution of a randomly selected sub-sample (255) of the simulation to the frequency distribution of movements we observed when captures were restricted to the center of the study site (i.e. Figure 3).

RESULTS

While 70% of recaptured toads were within 100 m of their initial capture site, approximately 2% of the animals marked moved the maximum distance of the study site (~8.5 km) (Figure 2). The movement frequency distributions were highly skewed leptokurtic (kurtosis= 79.74, skewness=8.81), and were neither normal (Lilliefors's test = 1) nor exponential, but were well described by an inverse power relationship that explained 63% of the observed variation in the movement frequency when recaptures were measured for adults with DGPS (Table 1). There was no significant difference between the movement distances or frequencies of adult males and females (Mann Whitney U P = 0.4). Only eleven percent of either 1280 captured males or 400 captured females had moved to another habitat patch upon recapture between 1991 and 2000. The average between-site distance moved by male toads was 390 m and by females was 385 m. The movement frequency distributions for both sexes were highly skewed leptokurtic (adult males kurtosis= 80.04, skewness=8.84, adult females kurtosis= 74.25, skewness= 8.38). For both males and females, a power relationship was the best description of the movement frequency data (Figures 1 and 3, Table 1). An average of 2.7 toads / year moved a distance larger than 4 km when 736 recaptures between 1991 and 2001 were analysed.

On May 24, 2000 a juvenile Fowler's toad was hand captured and given a site-specific toe-clip in a breeding pond at the base of Long Point, Ontario, Canada. On August 28, 2001, this toad (now distinguishable as a female) was recaptured near the tip of the Long Point peninsula. The distance between the two points was 34 km. This is the longest recorded between-captures movement of *B. fowleri* and may be the longest for any anuran amphibian (Sinsch, 1990).

The frequency distribution created by only using those individuals initially captured in the center of the study site was not different from the pattern demonstrated by the entire data set (Figure 4). Both the Barrowclough and Ideal Site Correction (Figure 5) increased the estimated frequency of animals moving the maximum distance of the study site. Yet neither correction was significantly different from the uncorrected data.

(Observed compared to Ideal Site Correction Mann-Whitney U test statistic = 4073.000, $p=0.937$. Observed compared to Barrow Correction Mann-Whitney U test statistic = 3909.000, $p=0.744$).

The simulation of random toad movement at Long Point did not describe what we observed in the field. In the simulation, in which recaptures occurred anywhere across the landscape, the shoulder of the dispersal curve (those animals moving moderate distances) was significantly larger than we observed in the field (Kolmogorov-Smirnov test, ($p=0.001$)). When the simulation recaptures were limited to cells coded as ponds, the resultant dispersal frequency was leptokurtic and although not significantly different from what we observed in the field (Kolmogorov-Smirnov test, ($p=0.774$)) it did not produce individuals moving long distances, or the tail of the distribution (Figure 6).

DISCUSSION

Even though it is true that most toads show limited dispersal ability, there is a significant tail to the *B. fowleri* movement distribution where some 2% of recaptured animals have consistently moved surprisingly far away. Amphibians are not ordinarily conceded to be capable of long distance movements, although this may be due to a lack of data (Marsh et al., 1999) and an expression of the scale of investigation rather than the abilities of the animals (Chapter I; Dole, 1971; Turner, 1960). The previous mark-recapture estimate of the longest distance moved by an amphibian was 15 kilometers (Tunner, 1992), and here *B. fowleri* at Long Point moving more than twice this distance. In fact, according to the power relationship detailed here, we should expect at least 0.15% of toads at Long Point to move 15km. Thus, despite high site fidelity, the wide dispersal range of toads make the utility of the simple metapopulation approach appear highly unlikely. Here, very high dispersal frequencies would effectively create one large population (Levin, 1974; Mouquet & Loreau, 2002; Taylor, 1988).

Movement frequency and distance fundamentally describe population isolation and the eventual likelihood of extinction in a metapopulation. The frequency of movement between isolated habitat patches determines whether an individual population functions as a patchy population or as a non-equilibrium metapopulation (Harrison, 1994). In a patchy population, dispersal between disjunct habitats is frequent enough to preclude the development of independent histories for each patch (Harrison, 1994). In a non-equilibrium metapopulation, movement between patches is so infrequent that the population dynamics are completely uncoupled – a condition with far higher extinction risk (Harrison, 1994). Clearly, for a species whose populations are patchily distributed across a landscape, dispersal allows re-colonization of habitat patches and is vital for species persistence at the regional level. When the preconception of limited dispersal is coupled with the observation of apparently disjunct habitat, it is not surprising that amphibians are considered likely candidates for simple metapopulations. However, the long distance dispersal tail, and relatively high rate of long distance movements we have documented here might indicate that we should not expect the protective metapopulation

effect to prevent synchronous dynamics in these seemingly disjunct toad populations on the local scale of less than 10 km (Quinn & Hastings, 1987). However, dispersal among local populations can have a wide variety of complicated effects on population dynamics (Kneitel & Miller, 2003). Our data regarding the apparent lack of metapopulation structure suggests that high rates of long distance movement may effectively connect disjunct populations into a single population – but the data do not allow us to reject the metapopulation approach in general.

In truth, when an environment is both spatially and temporally variable, we should expect selection for dispersal (Gillespie, 1981), and even a “poorly dispersing” animal inhabiting such an environment will be capable of, at least occasional, long distance movement. At Lake Erie, toad habitat is highly variable – dominated by sand and waves (Davidson-Arrott & Stewart, 1987; Hamblin, 1971; Wood, 1960). The sand dunes and adjacent ponds provide necessary habitat for toad breeding and over-wintering. Dune structure is built up by the wind and then broken down by large, unpredictable storms. If left undisturbed, these habitats undergo deterministic succession and become unusable for toad breeding, and storms that return ponds to early succession can also result in drastic over-winter die-off. Toad habitat is thus both spatially and temporally variable. Any pond exists in a perpetual early succession environment where deterministic successional change will eventually be stochastically altered and returned to early stages by storms. If toads were incapable of longer distance movements, they would certainly have been driven locally extinct either by stochastic weather events (which destroy individuals and habitat) or deterministic succession (which destroy habitat).

The study area was not closed. Marked animals moved off and unmarked animals moved in and so we applied various methods to account for such movements. If the leptokurtic distribution of dispersal events seen over the entire data set were a function of recaptures missed at the edges (Baker et al., 1995), we would expect to see a more normal distribution of movement events within the sub-sample of animals initially captured in the center of the study site. However, the pattern in the subset was the same: a power relationship of the proportion of recaptures that moved a certain distance. Additionally,

although the Barrowclough and Ideal Site corrections increased the probability of an animal moving the maximum length of the study site, both corrections were still best described by an inverse power law and were not significantly different from the uncorrected distribution pattern. We therefore conclude that our study site was large enough to reliably measure the dispersal potential of this toad without underestimating the long-distance dispersal potential. The leptokurtic pattern demonstrated was not due to recaptures missed because of a spatially finite study site.

Because of the polygynous mating system, with no resource partitioning by males, we expected dispersal to be male biased. However we found movement in *B. fowleri* had no sex bias. Indeed, the elegant generalities documented by Greenwood (1980) regarding dispersal and the mating systems of birds and mammals do not appear to apply as readily to amphibians. Greenwood (1980) recognized that where there is any partitioning of resources by males, prior to the selection of males by females, there should be female biased dispersal. Males are highly territorial in *Rana catesbeiana* (Howard, 1978), *R. clamitans* (Wells, 1978), *R. lessonae* (Radwan & Schneider, 1988; Sjogren Gulve, 1994), *Dendrobates pumilio* (Duellman, 1966; Wells, 1977), *Hyla arborea* (Brzoska et al., 1982) and *Eleutherodactylus coqui* (Drewry, 1970; Woolbright, 1985), and Greenwood's hypothesis predicts that dispersal in these species should be female-biased. However, there is no sex bias reported to movements in *R. clamitans* (Lamoureux et al., 2002), *R. lessonae* (Holenweg Peter, 2001), *E. coqui* (Woolbright, 1985), *D. pumilio* (McVey et al., 1981), and *H. arborea* (Stumpel & Hanekamp, 1986) and *H. arborea* was further found to exhibit male-biased dispersal by Vos et al (2000). Although indirect genetic evidence has recently indicated that dispersal in *R. catesbeiana* is female biased (Austin et al., 2003) – supporting Greenwood's hypothesis – mark-recapture data from the 1940's indicates that male *R. catesbeiana* actually move larger distances than females (Raney, 1940). *Bufo boreas* is known to be territorial during the breeding season (Nussbaum et al., 1983) and – as predicted by Greenwood's hypothesis – they do exhibit female biased dispersal (Muths, 2003).

When females appear to invest heavily in offspring, and can only breed once per year while males can breed more than once per breeding season – with a comparatively low metabolic investment – we expect selection for male biased dispersal. However, in the case of *Atelopus oxyrhychus* (Dole & Durant, 1974), *Rana aurora draytonii* (Bulger et al., 2003; Calef, 1973), *Rana pretiosa* (Licht, 1969; Watson et al., 2003) and *Rana sylvatica* (Berven & Grudzien, 1990; Howard, 1988) – which appear to meet these conditions – there was no sex bias to dispersal. *Bufo punctatus* (Turner, 1959) and *Bombina variegata* do not have male territories but have quick, opportunistic breedings – and as predicted – dispersal is male biased (Beshkov & Jameson., 1980; Weintraub, 1974).

In the specific case of *B. fowleri*, the sexual equivalence in movement frequency is reflective perhaps of the apparently random nature of local movement, combined with the passive influence of lake currents on long distance dispersers – such a process would not result in any sex bias to movement. In any case, one aspect is clear: anuran amphibians do not fit Greenwood's hypothesis as well as do birds and mammals. Either the hypothesis is wrong, or there are elements of anuran life history that invalidate the theories use in the same manner as with birds and mammals.

In a mark-recapture study of amphibians, individuals are primarily observed at breeding sites. To emulate this, the random movement simulation recaptures were restricted to 'ponds', and the dispersal frequency diagram was then very leptokurtic, as we observed in the field. However, the simulation did not reproduce the fat dispersal tail. Therefore, other factors may be involved in the production of large movements. We hypothesize that *B. fowleri* movements at Lake Erie would be more similar to the multi-stage wind/bird mediated dispersal of some seeds (Gomez, 2003) than to sex-based vertebrate dispersal theory. In this model – the stratified movement hypothesis – the long distance movements we have observed are part of a multi-stage, mixed process, where much local movement is random diffusion, but long distance movement is due, at least in part to the passive dispersal of toads via the currents of the lake. Qualitative observations of toad biology support the possibility of such a model. *B. fowleri* forage immediately

beside the lakeshore, and bufonids can survive up to a week in fresh water (Czopek, 1962; Schmid, 1965). The circulation patterns in Lake Erie are such that passive displacement of particles in the central basin can be as large as 5 kilometers per day in midsummer (Schwab & Bennett, 1987), those weeks of the year when young-of-the-year toads become active. Beletsky et al (1999) estimated that the mean summertime flow rate in Lake Erie is approximately 1.4 cm/s, or just over a kilometer per day. For post-metamorphic toads, it is more likely that the lake currents would provide periods of high-speed transmission along the lakeshore, and long distance movements would likely have both passive aquatic and active terrestrial components. At Long Point, toads tend to lay their eggs in adjacent dune ponds; cut off from the lake, however in other areas of Lake Erie toads can lay their eggs directly in the lake. Here, the passive dispersal via the lake would then extend to include all life history stages (eggs, tadpoles and adults), and could play a greater role in long-distance movement. If the long distance movements recorded by toads were indeed facilitated by the passive transmission of individuals via lake currents, then we would expect to see more long distance movement events finishing in the downstream direction of the predominant current. Although this hypothesis is difficult to test, as the currents can change throughout the year and there can be different currents acting at different distances from the shore and at different depths, a simple comparison of mark recapture data between east (mean summer current downstream) and west (mean summer current upstream) revealed no significant difference. It is clear that lake currents exist that could displace adult toads. The lotic or lentic transmission of adult and/or juvenile amphibians may be more common than is currently appreciated and clearly has an analogously important role to play in the transmission of long-distance dispersers – and therefore the geographic scale of genetic homogeneity – as does the dispersal of pollen and seeds by wind. Although not well known, the passive aquatic transmission of adult amphibians has been invoked in the movements of *Atelopus varius* (Crump, 1986), *Rana clamitans* (Martof, 1953; Schroeder, 1976), *Rana luteiventris* (Engle, 2001), *Rana muscosa* (Pope & Matthews, 2001), *Hyla regilla* (Reimchen, 1990), *Rana pretiosa* and *Bufo boreas* ((Carpenter, 1954)) and *Bufo americanus* (Dole, 1972),

while field observations of passively moved adults have been reported for *Rana pipiens*, and some unnamed "salamanders" (Dubois & Stoll., 1995), *Bufo fowleri* (Blair, 1943), *Bufo punctatus* (Tevis, 1966), and *B. americanus* (Blair, 1943; Dubois & Stoll., 1995; Maynard, 1934). The passive movements of larvae have been reported for *Salamandra salamandra* (Klewen, 1986) and *Eurycea bislineata* (Johnson & Goldberg., 1975; Stoneburner, 1978). In the case of individual toads being carried long distances by lake currents we might expect that lakeshore populations would tend to become genetically homogenized. This prediction is testable using Mantel tests of genetic distance and downstream aquatic distance (Chapter VI). Interestingly, although populations of *B. fowleri* around the lake exhibit geographic structuring of genetic variation which roughly corresponds with the basin structure of Lake Erie, (which does not support our theory of passive dispersal), there is apparent genetic homogeneity within basins across distances of greater than 50 kilometers – an observation which does support our theory of relatively high rates of movement across large areas.

We have shown that 1) although predominantly site loyal, *B. fowleri* can move surprising distances with unanticipated frequency. This leads us to reconsider the isolation of apparently disjunct and separate breeding populations. With such regular and long distance dispersal connecting populations, they are likely not functioning as a metapopulation, but are rather a patchily distributed single population. Consequently, their susceptibility for regional extinction is likely higher. 2) There was no apparent sex bias to *B. fowleri* dispersal. Polygynous mating systems have been observed to foster a male biased sex dispersal in other animals, and perhaps the absence of such a pattern here is reflective of the apparently random nature of movement locally, and the passive influence of lake currents on long distance dispersers which would not differentiate between sexes. 3) Although finite, our study site is large enough to reliably estimate the long distance dispersal characteristics of this toad and to conclude that the movement frequency distribution is highly leptokurtic. 4) We propose that for these toads, and perhaps for many amphibians, passive aquatic drift facilitates long distance movement

with currents in adjacent water bodies. Ignoring the importance of this vector for amphibians would be as negligent as ignoring the importance of wind to trees.

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TABLES

Table 1:

Comparing movement estimates using movements measured among-sites with differentially corrected global positioning system (DGPS) to an exponential (Model I), or power (Model II) model. In MODEL 1 (the exponential model), DISTANCE is the untransformed distance (m), in MODEL 2 (the power model); DISTANCE is the natural logarithm of the distance from the previous capture. The comparisons between male and female frequency distributions were made with distances measured among sites.

	Model I R ²	Significance (p)	Model II R ²	Significance (p)
All individuals, DGPS	0.081	0.005	0.633	< 0.0001
Males among sites	0.245	< 0.0001	0.577	< 0.0001
Females among sites	0.247	< 0.0001	0.703	< 0.0001

FIGURE HEADINGS

Figure 1:

Nested diagrammatic maps of the Fowler's toad breeding habitat patches (C) monitored nightly at Long Point, Ontario Canada (B). Box C contains the filled outlines of *B. fowleri* breeding ponds along the Long Point shoreline and has a width of approximately 10km.

Figure 2:

Movement frequency distribution of male *B. fowleri*. Between capture distances were made for 609 males between 1991 and 2001. Movement bins along the x-axis are for 100m. All captures were made the same year. Inset figure is of the same data log/log transformed.

Figure 3:

Movement frequency distribution of female *B. fowleri*. Between capture distances were made for 129 females between 1991 and 2001. Movement bins along the x-axis are for 100m. All captures were made the same year. Inset figure is of the same data log/log transformed.

Figure 4:

Movement frequency distribution of adult recaptures when first capture is restricted to sites in the center of the study area. Movement west is negative, movement east is positive. N=255.

Figure 5:

Movement frequency distribution of adult recaptures when corrected by the Barrowclough, and Ideal Site methodology.

Figure 6:

Frequency distribution of simulated Fowler's toad movements in an idealized Long Point, when recaptures were only recorded when an individual completed a movement at a pond. White bars are from the simulation, black bars are the observed values for *B. fowleri* recaptures when initial captures were restricted to the center of the study site.

FIGURE 1

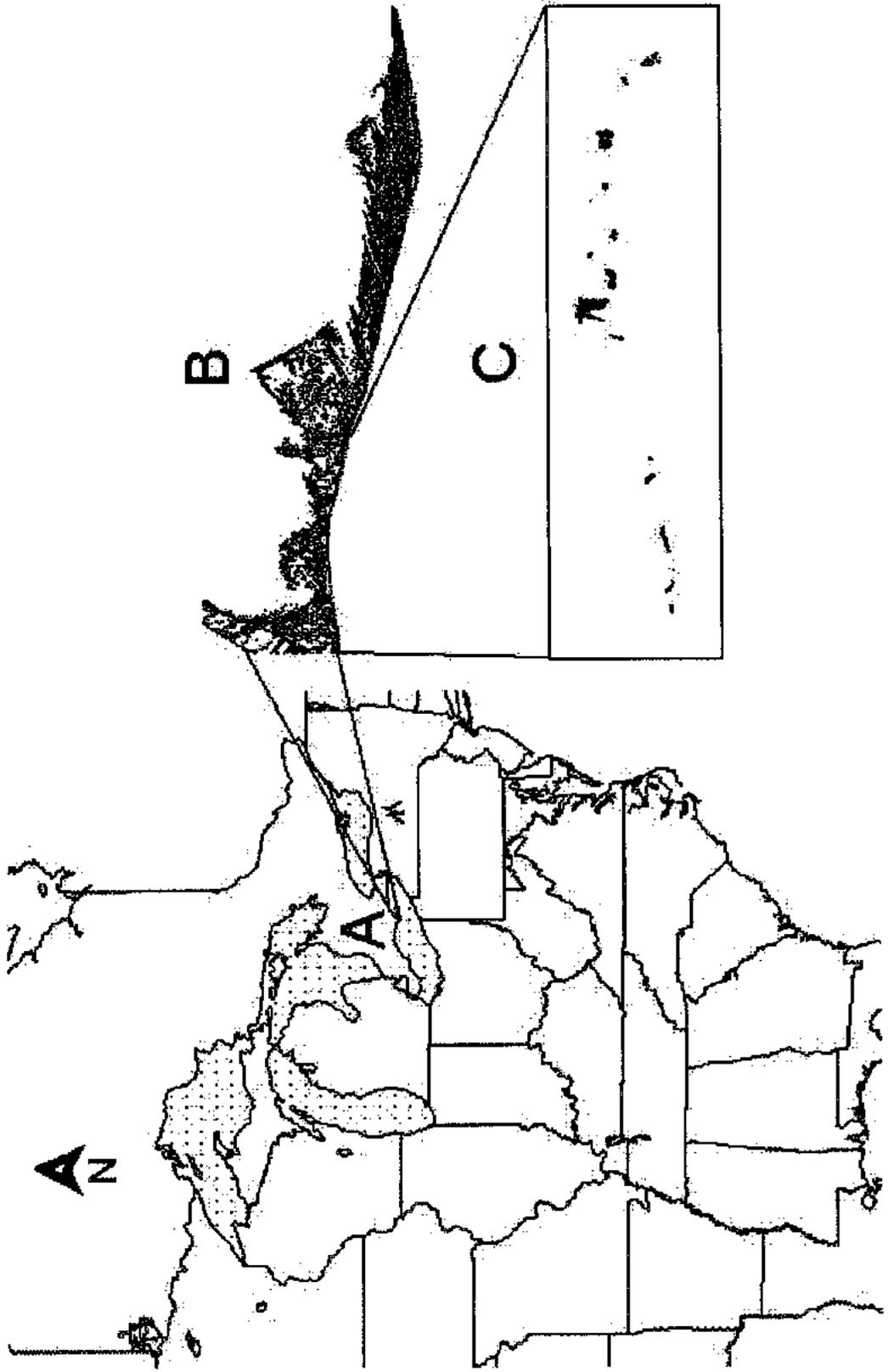
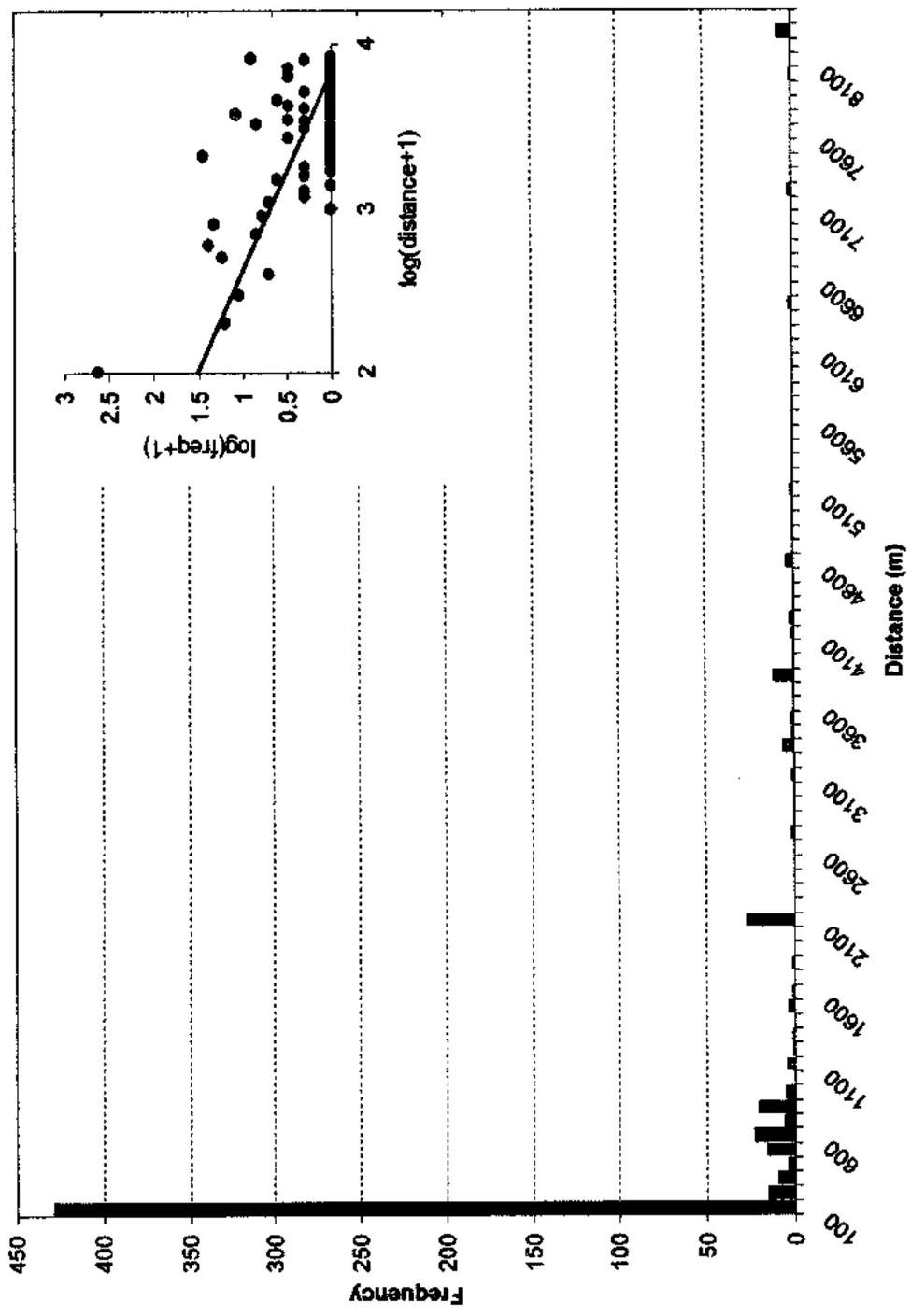


FIGURE 2



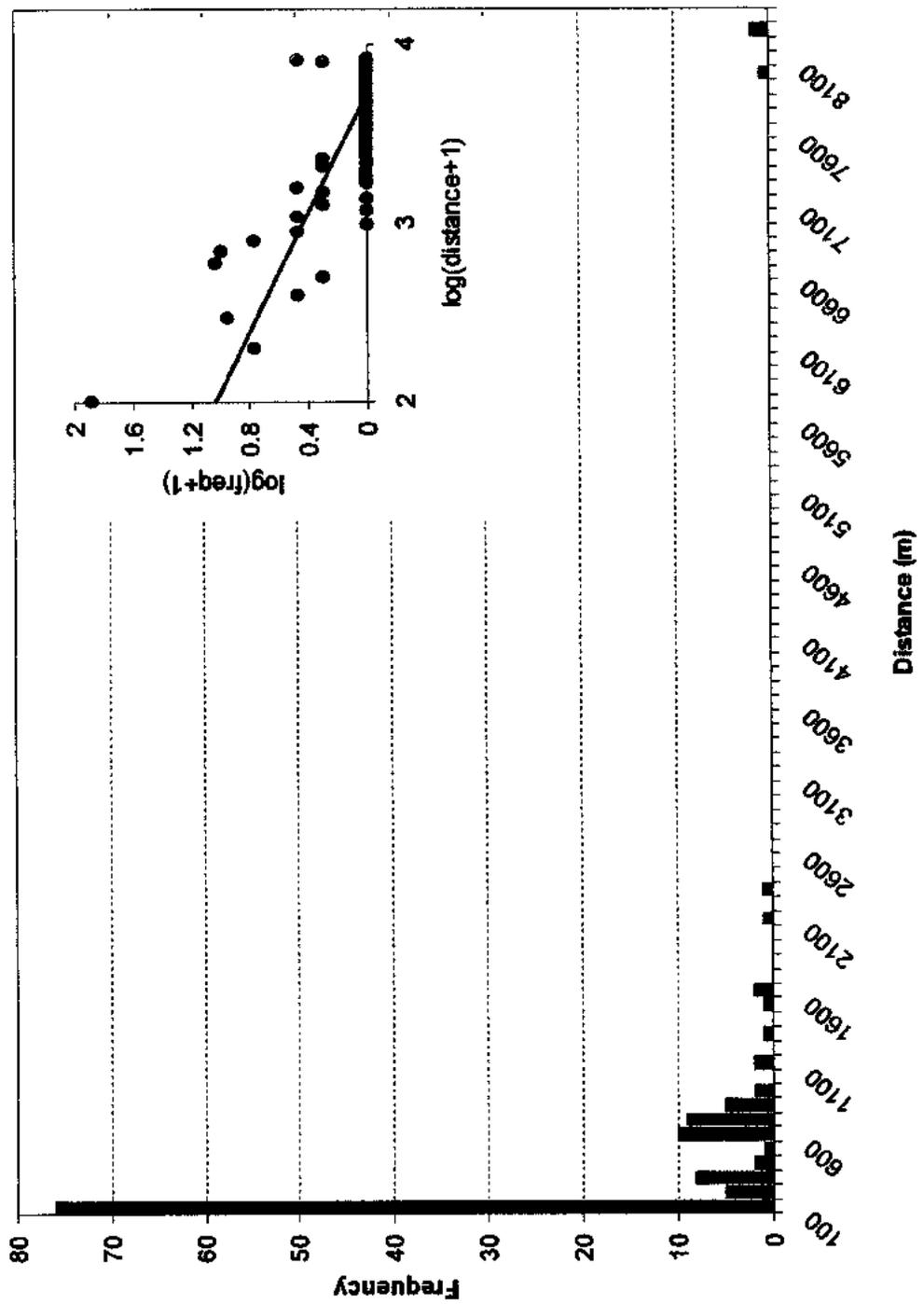


FIGURE 3

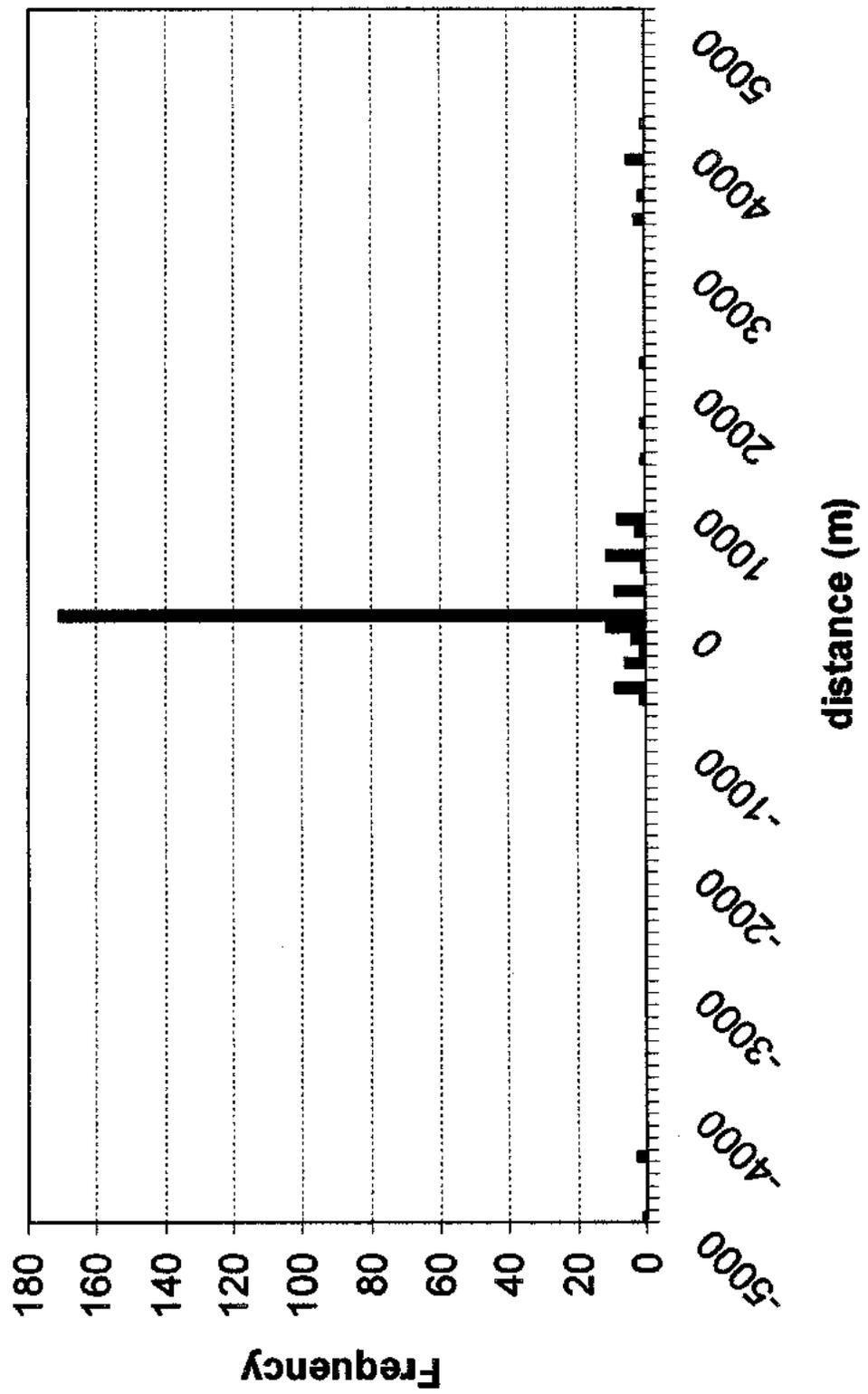


FIGURE 4

FIGURE 5

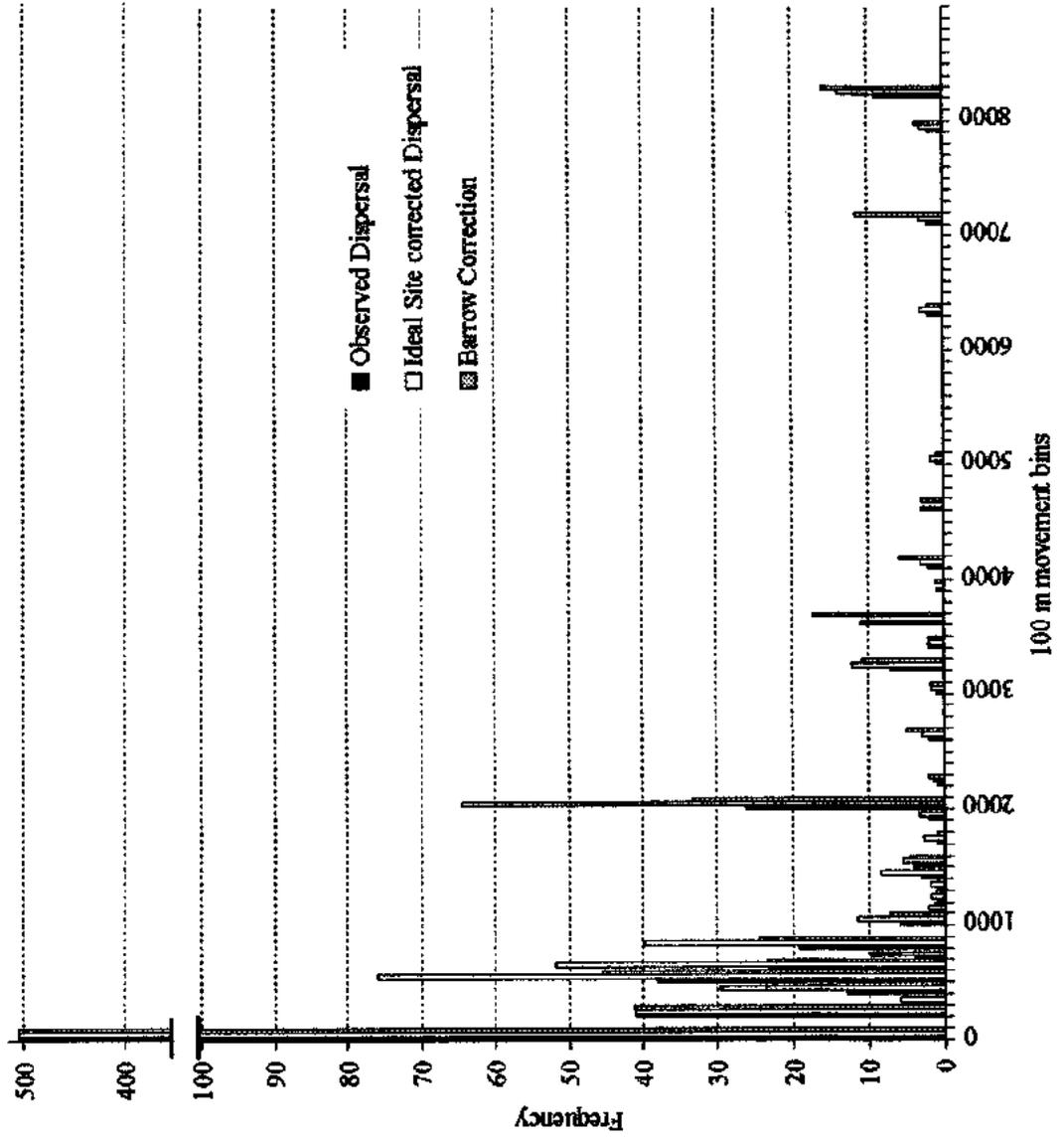
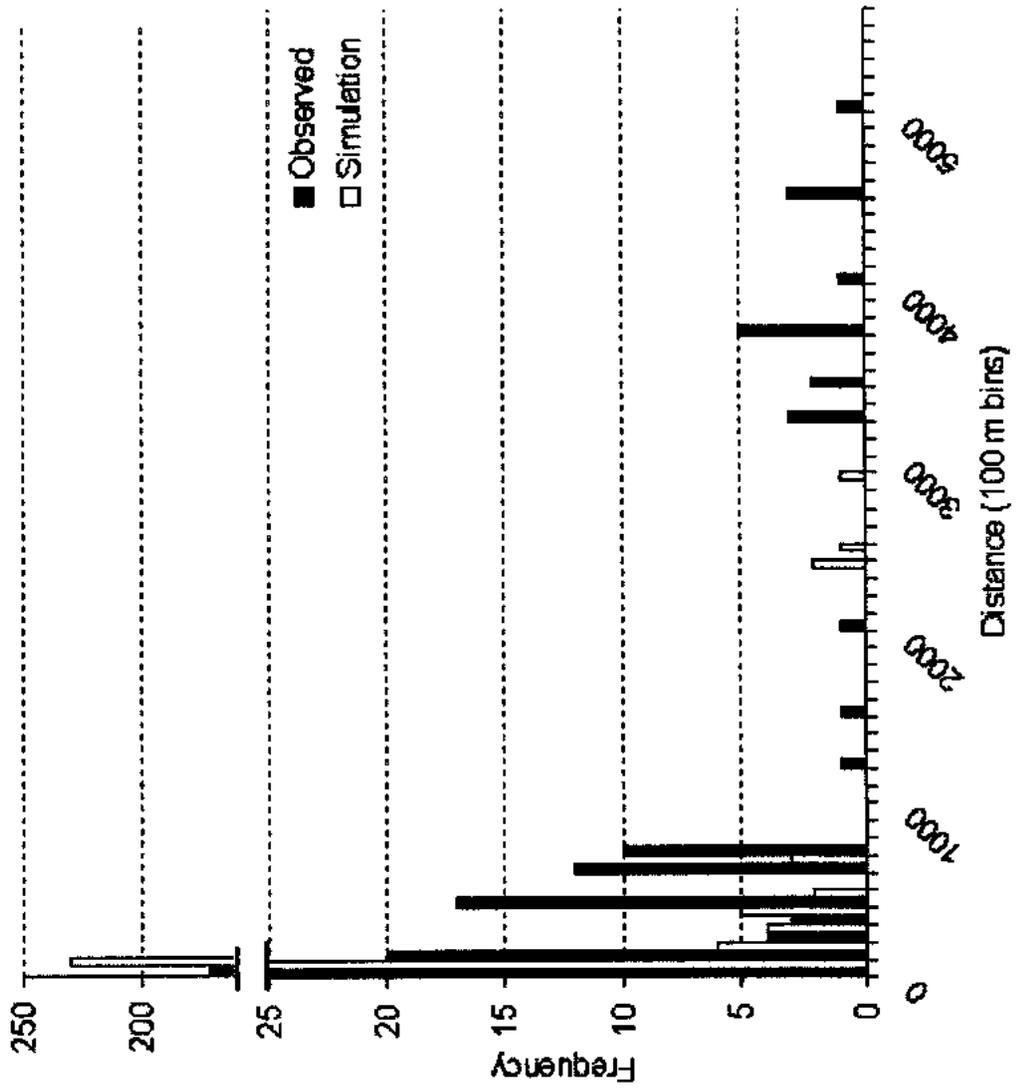


FIGURE 6



**CHAPTER 3: JUVENILES NECESSARILY BUT NOT NECESSARILY
JUVENILES: THE DISPERSAL ECOLOGY OF *BUFO FOWLERI***

Keywords: amphibian, dispersal, age, juvenile, *Bufo fowleri*

2220 words, 52 references, 1 figure

PREFACE TO CHAPTER 3

Having demonstrated that *Bufo fowleri* movement is well approximated by an inverse power function and that there is no sex bias to movement, I now consider the movement capabilities for juveniles. As adults are largely site-loyal, the mating system is polygynous and the survivorship has been described as Type III juveniles should move farther and faster more frequently. Those adults who survive the initial cull will continue to occupy whatever resource is limiting, and thus juveniles must move to find their own. I test the resulting prediction that younger animals should move farther than older animals.

Reference style is according to submission requirements for Oikos.

ABSTRACT

Amphibians are often portrayed as having limited dispersal ability, and those individuals that do disperse are expected to do so as juveniles. Indeed, because pond-breeding amphibians are site-loyal and polygynous as adults, juveniles should move farther and faster. We used mark-recapture of 1000 adults and 400 juveniles over two field seasons to estimate movement rates and dispersal capabilities of *Bufo fowleri* at the northern edge of its range. Neither the distance moved, nor the frequency of movement differed between toad age classes. This is a clear departure from expectations. Juveniles appear to be the dispersing life history stage of *B. fowleri* not due to any quantifiable difference in dispersal strategy, but merely because there are far more of them than there are adults.

INTRODUCTION

In populations of any organism, there will be individuals who disperse (Hamilton and May 1977, Van Valen 1971) but which individuals and why will vary. Dispersal can be caused by increased density (Grant 1977, Sutherland, Gill and Norris 2002), inbreeding avoidance, (Buechner 1987, Dobson 1979, Dobson 1982, Dobson and Jones 1986), colonization of new habitats, abandonment of degrading habitat (Denno, Roderick, Peterson, Huberty, Dobel, Eubanks, Losey and Langellotto 1996), and kin competition (Ronce, Clobert and Massot 1998). Complicating any study of dispersal is the fact that, in most cases, no one of these reasons alone will explain any observed pattern. Rather, the root causes will interact (positively and negatively) and as a result the question of who disperses and why is very complex (Dobson and Jones 1986).

One of the most commonly ascribed, but controversial, selective pressures favoring the evolution of dispersal is inbreeding avoidance (Perrin and Goudet. 2001), and in this explanation, it is often the younger animals that are expected to disperse (Dole 1971, Halley, Oldham and Arntzen. 1996, Hranitz and Diehl 2000). Lomnicki (1988) demonstrated that in a variable environment, where there is a high probability of empty habitat for each generation, lower ranking (especially younger) individuals should disperse. In most species of pond-breeding frogs there seems to be observational support for this expectation: adults are more or less philopatric whereas juveniles are observed to disperse and thereby connect otherwise disjunct populations (Berven and Grudzien 1990, Bulger, Norman and Seymour 2003, Daugherty and Sheldon 1982, Dole 1971, Roble 1979, Schroeder 1976). Indeed, in general amphibians are described as poor dispersers; constricted by biology and behavior to being highly site loyal and likely to exhibit population subdivision on small spatial scales (Blaustein, Wake and Sousa 1994, Sinsch 1990).

Bufo fowleri is a small terrestrial bufonid, common in many areas in the eastern United States, but restricted in Canada to early succession beaches along the Lake Erie shoreline. Recruitment and immigration of juveniles is likely a major factor in the population maintenance of *B. fowleri* (Green 2003), yet the actual dispersal ability or

propensity of juveniles is poorly understood (although see Breden (1987)). *B. fowleri* has been characterized as having a Type III survivorship curve (Breden 1982, Breden 1988, Deevey 1947) where most individuals die during early life history stages and then mortality rates decline for older individuals resulting in a J-shaped mortality curve. Pre-sexual animals (juveniles) of this type of survivorship should move farther and faster more frequently because those adults who survive the initial cull will continue to occupy whatever resource is limiting, and thus juveniles must move to find their own (Hamilton and May 1977, Lomnicki 1988, Ronce, Clobert and Massot 1998).

Our null hypothesis was that juveniles would move faster and/or farther than adults, while our alternative hypothesis was that some proportion all ages will move in an unstable environment with typically high mortality at all life history stages. To test the hypothesis of juvenile dispersal we compared the dispersal rate frequency for juvenile and recently metamorphosed toads to adults, and compared recapture distances for toads of known ages.

MATERIALS AND METHODS

Field techniques have been described elsewhere (Chapter II, Green 1992, Green 1997) and we therefore restrict our description to those techniques not already explained. Adult and juvenile toads were marked at 2 study sites; the Canadian Wildlife Services Thoroughfare Beach at Long Point, Ontario, Canada (N42.575 W080.369), and the James N. Allan Provincial Park Beach west of Dunnville, Ontario Canada (N42.848 W079.664). All captured toads were measured (snout-vent length), adults were sexed, and a unique combination of toes was clipped to allow for the identification of individual animals (Green 1992). Location for adult recaptures was recorded by site from 1988 to 2000, by global positioning system (GPS – Garmin II+) in 2001 and using differentially corrected GPS in 2002-03 (DGPS - Garmin II+ with GBR23 differential correction beacon). Phalanges were stored in 10% buffered formalin and were used in skeletochronological estimates of age on individuals captured in 1994, 1995, and 1997- 1999 (Kellner and Green 1995). The movements of 227 known-age-animals were grouped according to age and sex (23 females, 204 males).

The dynamic nature of the beach has proven prohibitive to establishing and maintaining pitfall traps and therefore to monitor movement we conducted repeated visual encounter surveys along a two-kilometer long section of beach. Each night, all active juveniles were captured and their position was recorded using differentially corrected GPS. All animals were released after measurements and position readings were taken at the capture point. Due to the lack of vegetation along the beach, there is strong likelihood that we were capturing most or all, active animals on any particular night. The young age of these animals prevented the external determination of their sex.

In 2002, we individually marked 421 juveniles using only non-additive toe-clips (ie. no more than one toe per appendage was clipped). All juveniles were captured and released on the Thoroughfare Unit at Long Point, Ontario. In August of 2003, we individually marked 70 recently transformed metamorphs using the same clipping criteria. These animals were captured and released at James N. Allan Provincial Park. In each case, we calculated the distance and the movement rate between all captures, and between initial and final capture for each individual. The frequency distribution of these observations was transformed to a percentage and then compared to the equivalent data for adult males and females collected in 2001 and 2002.

Using a Kruskal-Wallis test (Sokal and Rohlf 1995) we compared the movement frequency distributions of adults to juveniles and metamorphs. We tested the hypothesis that each movement distribution was normal using Lilliefors's test for normality. The kurtosis of each distribution was also estimated. Statistical comparisons were made using Systat (v.9), and Matlab (v 6.12).

RESULTS

In 2000 and 2001, we did not toe-clip juveniles because results in the literature suggested that toe clips negatively affected recapture probability for small animals (Clarke 1972). In order to determine whether juveniles move faster and farther than adults we used a special case of the marking scheme (only non-additive clips) that we suspected would minimize any potential negative effects of clipping, while still allowing us to identify individuals and track their movement. Measured by recapture rate- within

and between years- this methodology appears to have been successful for there were no apparent negative effects of the non-additive toe-clipping scheme on juvenile recapture rate. Approximately 80% of the juveniles were recaptured at least once within the same season and 14% of the individuals marked in 2002 as juveniles were recaptured the following spring as adults – a rate not different from the between year recapture rates for adults (results not shown).

There was no significant effect of age on the distance moved between captures for either adult males (Age classes 1-5, Total N= 204, Kruskal-Wallis $p=0.834$) or females (Age classes 1-4, Total N=23, Kruskal Wallis $p=0.863$).

The movement rate between captures (m/s) was recorded for all movements. The movement frequency distributions for all ages of toads were highly skewed leptokurtic (adult kurtosis= 29.69 $p<0.001$, skewness= 5.15, juveniles kurtosis= 29.79, $p<0.001$, skewness= 5.17, metamorph kurtosis= 29.85, $p<0.001$, skewness= 5.18). There was no significant difference between the resulting distributions of adult, juvenile and metamorph rates (Kruskal Wallis-test, $p=0.117$) (Figure 1).

DISCUSSION

Younger toads moved neither faster, nor farther, than adult toads. Were our results to align with standard dispersal theory we would have observed younger toads moving longer distances more frequently and at a faster rate. Indeed, dispersal in anurans, and in many vertebrates, is usually attributed to the young. Dole (1968, 1971) noted that the longest distances recorded by *Rana pipiens* were by juvenile frogs and that connections between populations were likely made by juveniles rather than adult frogs. Schroeder (1976) documented that most between population movement is accomplished by immature *Rana clamitans*. Sjogren-Gulve (1998) observed that while only 1% of adult *Rana lessonae* dispersed between localities, 35% of juveniles dispersed to neighboring ponds. Bulger et al (2003) invoked the importance of juvenile dispersal, yet never observed any. Even a limited literature review of amphibian dispersal estimates reveals that the trend of juvenile dispersal is not ubiquitous. Older *Bufo japonicus* individuals moved farther than younger ones (Kusano, Maruyama and Kaneko 1995).

Eighty-three to one hundred percent of juvenile *Bufo bufo* were recaptured as adults at their natal pond (Reading, Loman and Madsen 1991). In *Rana lutiventris* younger individuals do not move farther (Turner 1960). Dispersal is not restricted to a specific life-history stage in *Rana lessonae* (Holenweg Peter 2001). Joley and Grolet (1996) noted that both mature and immature newts colonized newly available sites, although colonization was predominantly by pre-sexual individuals. Juvenile *Bombina variegata*, *Plethodon glutinosus* and *Ambystoma talpoideum* are more sedentary than adults are (Beshkov and Jameson. 1980, Semlitsch 1981, Wells and Wells 1976).

That younger toads do not move faster, or farther, than adult toads does not support the dispersing juvenile hypothesis. However, there may be an order of magnitude more juveniles than adults and thus an equally large differential in the likelihood that any recaptured disperser will be a juvenile. The judgment that juvenile toads contribute differentially more than adults to a "dispersal pool" may be one biased by abundance. Since all life history stages seem to have the same dispersal characteristics, juvenile toads comprise a large proportion of the dispersal pool simply because of their abundance.

Because the greatest mortality rates in *B. fowleri* are amongst the young (Green 2001), juveniles of any particular generation should move away from their natal site. The relatively constant rate of adult mortality makes it unlikely that any resource held by adults would be available to juveniles. Indeed, we would expect any animal approaching such a Type III survivorship (Breden 1988, Deevey 1947) curve to have more motile juveniles than adults. However, in actuality, adult *B. fowleri* mortality is not constant as in the hypothetical Type III curve. It is high for all age classes because of the highly variable early succession habitat that these toads inhabit (Green 2003). Numerous theoretical models (Jarvinen 1976, Levin, Cohen and Hastings 1984, McPeck and Holt 1992, Oliveri, Michalakis and Gouyon 1995, Roff 1974, Roff 1974, Van Valen 1971, Vepsalainen 1974) and some field studies (Peroni 1994) have shown that environmental variability can result in higher dispersal frequencies. Even if there is a 99% chance of mortality during dispersal, at least half of all offspring should disperse (Hamilton and May 1977, Motro 1982). In this case, where there is high mortality probability

throughout the life of the individual, we should expect both dispersing and philopatric juveniles. Some juveniles should be philopatric since many adults may actually die and subsequently expose resources. Yet, some adults will not die and will continue to occupy resources; so some juveniles should leave. In this hypothesis, the actual *B. fowleri* survivorship curve is more like a two stage Type II; and here both dispersing and philopatric individuals have a chance at inheriting resources (Hamilton and May 1977).

The similarity between all age classes may be taken as more evidence (Chapter II, Chapter V) that currents in Lake Erie could act as a passive dispersal vector. The combination of essentially random, terrestrial movement and facilitated longer distance aquatic-dispersal results in the stratified movement patterns where the vast majority of movement is diffusive while some reliable small portion is much longer than random diffusion would predict. Certainly, occasional forays into Lake Erie result could result in the accidental, passive acceleration of movements over longer distances than mark-recapture terrestrial measurements would suggest (Chapter II). As there is equivalent exposure to the lake for all ages and sexes, the stratified movement hypothesis (Chapter II) would predict that the movement, and rate, frequency distributions should be the same for all age classes.

Although sex-based resource theory led us to expect that juveniles would move farther than adults would, this is not what we have found; there was no significant difference between ages for displacement or movement rate. We conclude that differentially large juvenile abundance leads to differentially large probability of contributing to a dispersal pool, and likely an incorrectly large expectation that juveniles are a dispersal stage for this species. This finding fits within theoretical expectations for a variable environment where likely all components of a population should disperse. In *B. fowleri*, the perception that juveniles disperse more frequently than adults may be driven more by the abundance of the life history stage, than by any significantly different behavioral strategy. In the end, toads achieve the long distance dispersal expected in their variable environment, but not by the predicted behavioral means of juvenile dispersal.

Rather all post-larval life history stages appear equally likely to move long distances or quickly.

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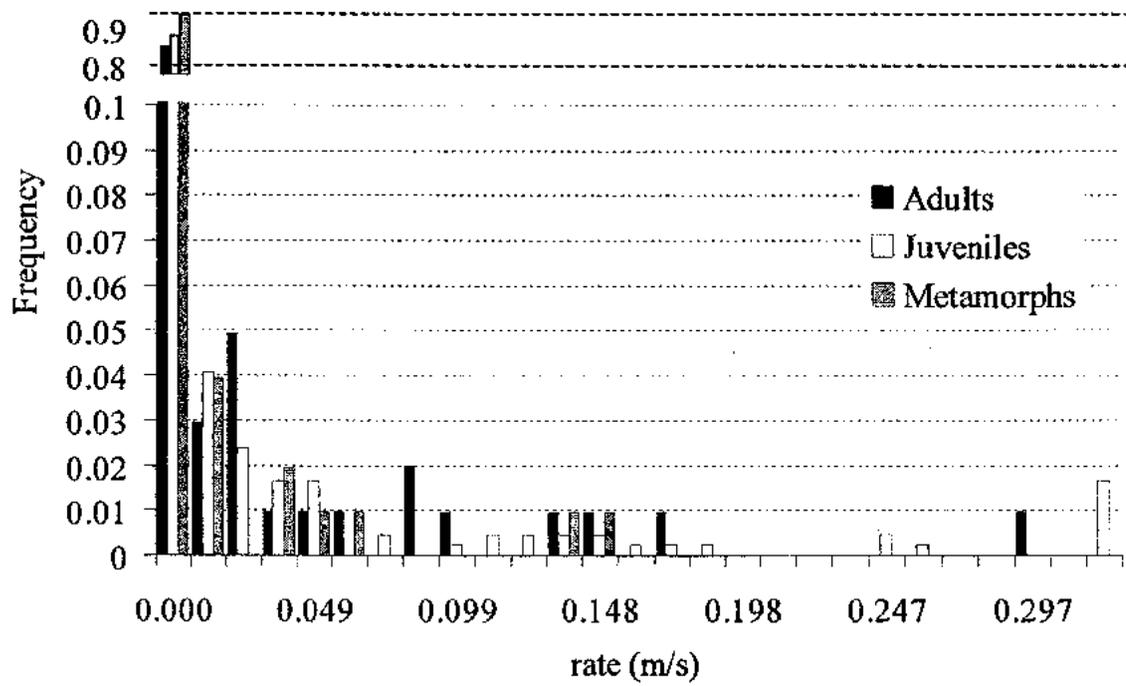
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FIGURE HEADING

Figure 1:

Frequency distribution of rates for all observed movements for adults (101), pre-sexual juveniles (410), and post-metamorphic juveniles (103). The three distributions are not significantly different (Kruskal-Wallis 4.286, $df=2$, $p=0.117$).

FIGURE 1



**CHAPTER 4: "MORE APPARENT THAN REAL": AN ANALYSIS OF
AMPHIBIAN POPULATION TURNOVER USING TWO METAPOPOPULATION
MODELS.**

**Keywords: metapopulation, general metapopulation model, incidence function
model, power, amphibian, *Bufo fowleri*
6200 words, 67 references, 4 tables, 8 figures**

PREFACE TO CHAPTER 4

The utility of two metapopulations models – a version of the most simple, Levin's style (General metapopulation model of Gotelli and Kelly (1993)), and perhaps the most frequently cited (Incidence Function Model – Hanski 1994 (Seven percent of articles published between 2000 and 2003 with metapopulation and model in the keywords also contained incidence when searched using Current Contents in December 2003)) are considered with population turnover data from 6 amphibian species over 18 breeding habitat patches through 15 years of observation at Long Point, Ontario. The large-scale utility of the models is tested with one species, *Bufo fowleri*, for populations along the north shore of Lake Erie over nearly a century. The quote in the title refers to a sentence from Marsh and Trenham (2000), "*For many species, however, the ponds-as-patches view may be an oversimplification and metapopulation structure may be more apparent than real.*" Page 40.

Reference style is according to submission requirements for Oikos.

ABSTRACT

Temperate anuran amphibians are often portrayed as poorly dispersing, site loyal animals where breeding habitat is naturally fragmented into ponds. Thus pond networks are considered to be metapopulations. Using a fifteen-year study of population turnover in six amphibian species collected on a local scale of ten kilometres, and occupancy data for *Bufo fowleri* sampled on a regional scale from populations across several hundred kilometres, we tested how well our observations of habitat patch occupancy fit the assumptions of a simple metapopulation model (General Metapopulation Model (GMM)) and how the observed patterns of incidence fit those predicted by the more spatially realistic, and popular, Incidence Function Model (IFM). At neither the local scale of ten kilometres nor the regional scale of hundreds of kilometres was there any evidence of metapopulation effect. Our number of sample sites and years is necessarily limited – as it is in all metapopulation studies – and we found that a compromise analysis between statistical confidence and power is an appropriate test of significance for systems where unavoidably small sample size results in low power. In many metapopulation studies a reduction of statistical power is unavoidable and therefore compromise analyses are desirable. As neither the spatially implicit and simple general metapopulation model, nor the spatially explicit and popular incidence function model provided reasonable predictions or descriptions of the population dynamics for these amphibian species, it is possible that homogenising dispersal, and/or elements of population dynamics outside the pond, effectively negate any metapopulation effect. It is not our intent to dispute the general utility of the metapopulation approach for all amphibians. However, although the, ‘ponds as patches’ metapopulation model of amphibian biology has merits; it is an assumption that is not always accurate. Our work demonstrates that even when intuitively apparent, the assumptions of metapopulation structure must be checked.

INTRODUCTION

Metapopulation theory (Hanski 1998, Levins 1969) describes the extinction and colonisation of populations inhabiting distinct habitat patches linked by dispersal. As applied to real populations, this theory relies on the existence of several features: separate breeding populations in discrete habitat patches, the absence of any population large enough to sustain all others, sufficient isolation to make dispersal between habitat patches rare but not impossible, and sufficiently asynchronous local dynamics so that populations at all habitat patches do not go extinct at once. It differs from other, individually based, population-modeling approaches in its focus on the presence or absence of a species at a habitat patch, and not on the dynamics within the patch. This in turn assumes that any local extinction leaves empty habitat that another individual could move quickly into - that is, a stochastic extinction that has not altered the patch quality. The metapopulation approach further differs from individually based population modeling in that the occupancy of any patch at time $(t+1)$ is only dependent on time (t) , not $(t-1)$ (Moilanen 1999). As a consequence of the metapopulation structure there is a resilience of multiple populations that single populations lack since the assumed asynchrony between habitat patches makes simultaneous extinction of habitat patches unlikely. Thus, regional persistence occurs only at the level of the metapopulation (metapopulation effect) since all local populations are subject to extinction, and persistence is expected to increase with asynchrony.

Amphibian populations have long been thought to constitute metapopulations (Bradford, Neale, Hash, Sada and Jaeger 2003, Carlson and Edenhamn 2000, Gibbs 1993, Gill 1978, Hecnar and M'Closkey 1996, Pope, Fahrig and Merriam 2000, Ritland, Dupuis, Bunnell, Hung and Carlson 2000, Seppa and Laurila 1999, Sinsch 1997, Sjogren Gulve 1994, Sjogren Gulve and Ray 1996). In many, but not all, temperate anuran species the breeding aggregation is restricted to spatially delineated habitat patches, such as ponds. Individuals tend to be loyal to these patches, and, are often thought of as poor dispersers (Blaustein, Wake and Sousa 1994, Sinsch 1990). In this simplified 'ponds as patches' view, the space between the pond is envisioned as non-habitat, and therefore many

amphibian populations may be modelled as metapopulations: connected by occasional dispersers forestalling local extinction. Indeed, “frogs in ponds” have specifically been acknowledged as likely candidates for simple metapopulations (Hanski 1997, Harrison 1991) with the potential to conform to the General Metapopulation Model (GMM) of Gotelli and Kelley (1993), and the Incidence Function Model (IFM) of Hanski (1994).

The GMM is based on a simple equation with two colonisation parameters (a and b) and two extinction parameters (c and d), which describe the fraction of sites occupied (f):

$$df/dt = (1 - f)(a + bf) - (f)(c - df)$$

The contributions of migration (a), the effect of adding another occupied site on colonisation (b), the extinction probability of a single site (c), and the effect of adding another occupied site on extinction (d) are estimated by simple linear regression of the probabilities of colonisation and extinction with site occupancy (Gotelli and Taylor 1999). The GMM predicts that the extinction probability will be negatively correlated, and colonisation probability will be positively correlated, with increasing site occupancy.

The Incidence Function Model (IFM) (Hanski 1994, Hanski 1999, Hanski and Gilpin 1997, Moilanen 1999) is likely the most popular metapopulation model. The IFM is a first order Markov chain relating the incidence (probability of occurrence) of a population (J_i) to the probability of extinction, (E_i) and colonisation, (C_i).

$$J_i = C_i / (C_i + E_i) = [1 + e'/S_i^2 A_i^x]^{-1}$$

The probability of colonization is related to the connectivity of the habitat patch in question S_i , (the inverse of isolation), whereas the area of the patch (A_i) largely drives the probability of extinction. The other parameters, e' and x scale the extinction probability for the minimum patch area and the severity of environmental stochasticity respectively.

Metapopulation models such as these are an attractive explanation for population changes over the landscape for they implicitly consider a landscape fragmented into habitat and non-habitat with local extinction – an increasingly common feature of many anthropogenically altered habitats. However, the literature holds few examples where a model's elementary assumptions or predictions were tested (Hanski 1999). Indeed, the

long-term mark-recapture studies needed to test the assumptions of the models are few in number as they are difficult to fund and maintain. We possess occurrence data for six amphibian species (*Rana catesbeiana*, *Rana clamitans*, *Rana pipiens*, *Pseudacris crucifer*, *Bufo americanus* and *Bufo fowleri*) at eighteen habitat patches over 15 years, and are therefore in a unique position to test the predictions of this simple and this popular metapopulation model.

Beginning this study, we hypothesized that the amphibian populations we monitored were likely to exhibit metapopulation dynamics. This was largely due to our preliminary assumption that these populations appeared to meet the four required conditions for the demonstration of metapopulation dynamics (Hanski 1999, Hanski, Pakkala, Kuussaari and Lei 1995):

- 1) Habitat patches appeared to support local breeding populations
- 2) No single population appeared large enough to ensure long-term survival
- 3) Patches seemed not too isolated to prevent recolonisation
- 4) Local dynamics appeared sufficiently asynchronous to make simultaneous extinction of all local populations unlikely.

Given that the populations qualitatively appeared to meet the criteria for simple metapopulation dynamics, we considered it reasonable to test whether these populations quantitatively matched the predictions of the models. Thus, using fifteen years of local amphibian population data, and 90 years of regional data for *B. fowleri*, we investigated whether the local and regional population dynamics for six amphibian species met the assumptions and predictions of these metapopulation models. Specifically with the GMM, we tested our expectations of the relationship between the fraction of occupied sites and the probability of colonisation or extinction. For each species we used least squares regression to estimate GMM coefficients for colonisation and extinction, then used a null simulation to evaluate the significance of the observed relationships, and further completed a compromise analysis which helps mitigate against the Type II error that plagues field studies, of limited size and duration. With the IFM we tested with

simulation whether the observed patterns of incidence were significantly different from those predicted by the model when parameterized with a snapshot of occupancy data.

MATERIALS AND METHODS

The study sites were at Long Point, Ontario, Canada; a sand-spit that extends 35 km southeast from the north shore of Lake Erie (Figure 1). Data were derived from nightly surveys of 19 breeding sites conducted along an eight km long study transect between the end of April and the middle of June each year from 1988 through (Green 1989, Green 1992, Green 1997, Laurin and Green 1990). *Bufo fowleri* abundance at each site was estimated through hand-capture of uniquely marked individuals. The other species were recorded as present or absent based on the observation (aural and visual) of calling males. The chances of incorrectly marking a site as empty were reduced through the use of a repeated, intensive survey conducted every night throughout the breeding season of the species. Intensive call count surveys have been shown to be reliable indicators of anuran species presence at breeding ponds (Bishop, Pettit, Gartshore and Macleod 1997, Shirose, Bishop, Green, MacDonald, Brooks and Helferty 1997).

GENERAL METAPOPULATION MODEL

Field data were reduced to a 15-year and 19-site occurrence matrix, except for those of *P. crucifer* for which we fashioned an occurrence matrix of 10 years and 19 sites. From the occurrence matrix we calculated year-to-year probabilities of extinction and colonisation for each species according to Gotelli and Taylor (Gotelli and Taylor 1999). The colonisation probability between years ($P_{i,t}$) was calculated as the number of sites unoccupied in year (t) that are occupied in year ($t+1$) divided by the number of sites surveyed in year t . Conversely, the between-year extinction probability ($P_{e,t}$) was calculated as the number of sites occupied in year t that are unoccupied in year $t+1$ divided by the number of sites occupied in year t . The fraction of sites occupied in year t , f_t was the number of sites occupied in year t divided number of sites surveyed in year t . The relationships between f_t and $P_{i,t}$ and $P_{e,t}$ were fit with a least squares regression model as the GMM predicts a linear relationship.

For each species our null simulation consisted of reshuffling the occurrence matrix 1000 times and calculating the between year probabilities of extinction, colonisation and site occupancy. Site occupancy (f) was fit to both $P_{i,t}$ and $P_{e,t}$ using least squares regression. The frequency distribution of the slope and intercept from these null regressions was calculated, and the observed least squares regression value was compared to the 1000 null values. The observed values were deemed significantly different from random if 95% of the random values were either greater than or less than the observed value in the direction predicted by the GMM (Gotelli and Taylor 1999). This constitutes a one-tailed test of the null hypothesis. This was used as a starting point for our analysis but we then adjusted for individual effect sizes (ie. the strength of the relationship between site occupancy and either colonisation or extinction probability) using the program G*Power as will be described below. If the observed relationship between site occupancy and colonisation supported the GMM, then a (the intercept of the regression of colonisation probability and site occupancy is the expected likelihood of colonisation for a site in an empty landscape) would be less than the majority of the randomly generated (i.e. null model) values. The observed b (slope of the same relationship) would be greater than the null values. Otherwise we accepted the null hypothesis that the intercept did not vary significantly from 0.5 and the slope was not different from zero. Likewise if the relationship between site occupancy and extinction supported the GMM, then the observed coefficient c (the intercept of the linear regression of site occupancy and extinction) should have been greater than the majority of the randomly generated values and coefficient d (the slope of the same relationship) should have been less than the null slopes. Otherwise we accepted the null hypothesis that the intercept did not vary significantly from 0.5 and the slope was not different from zero. For each least squares regression we used the program G*Power to estimate statistical power using methods outlined in Erdfelder et al (1996). The null simulation was written using Matlab 6.1 (The Mathworks).

To determine whether the metapopulation approach was appropriate at a larger spatial scale we tested the GMM and IFM predictions using historic occurrence data for

B. fowleri along the northern shoreline of Lake Erie - the extent of its Canadian range. *B. fowleri* is a habitat specialist (Green 1989) whose range in Canada is severely restricted and it is therefore reasonable to predict and plot from maps and historic data. Occupancy data from 1920 to present day were obtained from field surveys and the Ontario Natural Heritage Information Centre (NHIC). Throughout the 90 years covered by this data set, no site was surveyed continuously, and the exact locations of individuals from older records were sometimes questionable. We therefore had to resolve potential errors of time and space for these occurrence records. For instance, the patch occupancy transition between years of: 1-no data-no data-0, may contain two extinctions and one colonization or only one extinction and no colonizations. Incorrectly determining this would artificially boost our colonization expectations. The same transition measured through space could be either a result of a more patchy distribution, or of missing the incidence of two populations. For the GMM, we solved this problem by restricting our analysis to five Canadian sites and 9 time periods. We considered the five populations (Peelee, Rondeau, Long Point, Grand River, Niagara) based in part on genetic evidence for isolation (Chapter V). To infer the absence of a species from a site, we reduced the rows of the presence/absence matrix to decades. If a population had existed at that site, its presence would more likely be observed within a ten-year span than on a year-to-year basis. Thus only if a population was absent from a site for a decade did we conclude that a local extinction had occurred. For the IFM we tested both the original data set (90 years and 17 sites) and the reduced data set described above.

Male longevity would affect our measure of between-year metapopulation effect if males skip breeding seasons. Both *R. catesbeiana* and *R. clamitans* are long-lived compared to other species we examined, and males may not breed every year (Howard 1976, Shiroye and Brooks 1995). Therefore, we additionally tested a reduced occurrence matrix for all species on 2 and 3-yr cycles.

The number of replicates in any long-term study, even of 15 years duration, is necessarily limited both in terms of the number of sample years and the number of sites sampled. Therefore statistical power of any test will be small, making it extremely

difficult to test any metapopulation model against real data at a traditional critical value of 0.05 (Thomas and Krebs 1997). To mitigate against Type II error we completed a compromise between α (the probability of making a Type I error) and β (the probability of making a Type II error where the null hypothesis of “no change in probability of extinction or colonization” is not rejected when it should have been). Effectively, power is increased for a given effect size by increasing the critical value for significance (Cohen 1988, Erdfelder, Faul and Buchner 1996). In most cases the effect size of the regression was small, and we used the Compromise Analysis function of G*Power to obtain a compromise between the demands for a low alpha-risk and a large power level, given a fixed sample size. For each coefficient the critical value examined was increased from the 0.05 value used in Gotelli and Taylor (Gotelli and Taylor 1999) to a level more appropriate for each effect size (Table 1). In all comparisons we set q (ratio of β/α) to 2 indicating that we considered the risk of incorrectly failing to reject a false null hypothesis (Type II Error – not rejecting the null when it is in fact false) to be two times as serious as incorrectly accepting it (Type I Error – the rejection of null when it is true) (Cohen 1988). Henceforth, this procedure will be called the Null Compromise Analysis (NCA).

THE INCIDENCE FUNCTION MODEL

Since more data than simply occupancy are required for the IFM (dispersal abilities and habitat patch area), we have only considered the utility of this model for *B. fowleri*. We have a greater understanding of the biology of this species, and can make more meaningful parameter estimates.

The IFM was parameterized locally using the occupancy data for *B. fowleri* from Long Point, and regionally with data from the northern Lake Erie shoreline. On a local scale, patch area was determined in 2001 and 2002 using differentially corrected global positioning system (DGPS) unit (Garmin II+ with GBR23) readings taken of toads, and of perimeter walks of apparent toad habitat. Patch areas and among patch distances were all calculated using Matlab 6.1 (The Mathworks). Parameters relating incidence to habitat patch size and isolation were initially estimated from field data and then these

estimates were optimized by iteration using the Stochastic Patch Occupancy Simulator (SPOM v 1.0b) (as described in Moilanen (Moilanen 1999, Moilanen 2000)). From Equation 2, parameter α represents the constant that determines the survival rate of migrants over distances between patches (d_{ij}). As $1/\alpha$ is the average dispersal distance, this parameter value was calculated locally from mark-release-recapture data for males and females, and regionally from mtDNA variability – which is effective dispersal (Chapter V, Chapter VI). Parameter b scales population size to patch area. Initial estimates of b were estimated by linear regression of $\log(\text{abundance})$ against $\log(\text{patch area})$. Parameter x represents the susceptibility of populations to environmental fluctuations. When x is less than 1, even large populations in abundant habitat can go extinct (Hanski, Pakkala, Kuussaari and Lei 1995), and as we have seen the largest, most abundant toad populations go extinct; our initial estimate of x was less than one. Parameter y measures how fast the probability of colonisation approaches 1 with an increasing number of migrants. These initial parameter values were tested by 4800 iterations using the SPOM program and the resultant ideal values were used in the population simulation (as described in Moilanen (Moilanen 1999, Moilanen 2000)). To test the efficacy of the IFM's predictions, occupancy values resulting from one thousand 15-year simulations – initialised with occupancy data from 1988 (the first year of study) – were compared to the observed patterns of occupancy throughout the 15-year study.

On a regional scale, patch 'area' was calculated as the shoreline distance of available habitat surrounding historical records using ArcView (v3.2). The precedent of replacing habitat area with habitat edge distance has been utilised successfully in the past (Moilanen, Smith and Hanski 1998). The edges of this distance were determined by biological knowledge through field surveys between 1999 and 2003, or through the occurrence of a habitat change which would likely result in the absence of the species (i.e. the presence of shoreline agriculture, cottage development or bluffs). Alpha (α) values were tested that included the average dispersal distance measured in mark-recapture studies (~600m), and mtDNA variability within Lake Erie (~21km) (Smith and Green unpublished). Values of the other parameters were initially loaded as for the local

populations at Long Point, and all values were then optimized as above (Table 3). One thousand, seventeen-year simulations were initialised with both the entire 90-year data set, and with a single year of patch occupancy (1985). This year was selected as we have high confidence regarding the quality of occupancy data since one of us (DMG) completed regular toad surveys along the Lake Erie shoreline that year.

On both scales (regional (Lake Erie) and local (Long Point)) the observed values of the proportion of occupied patches were considered different from the simulation if they fell outside the 80% percentiles of the observed trend. Eighty percent was chosen with regard to the power of determining a trend through either 15 or 17 years with a regression. Using the 80% critical values results in a much smaller chance (33%) of committing Type II error for a medium effect size. Using the traditional 95% critical value results in power of 34% (ie. 66% chance of making a Type II error for a medium effect size).

To investigate whether or not our suspicions regarding the low statistical power of many metapopulations studies was valid, we surveyed 27 published metapopulation studies and calculated their ability, using a correlation or regression analysis, to correctly reject an incorrect null hypothesis at a traditional confidence level of 0.05 for both small (0.1) and large (0.5) effect sizes when trends were measured between years and between sites.

RESULTS

GENERAL METAPOPOPULATION MODEL

As the fraction of occupied sites increased, there was a minimal effect on colonisation probability. Four species did increase, as theory would predict, but that rate of increase was only significant for *R. clamitans*. For both toad species, colonisation probability decreased as site occupancy increased (Figure 2, Table 1). When measured between sites, colonisation probability increased as site occupancy increased only for *B. fowleri* (Figure 3, Table 2). Only in *R. clamitans* was the observed intercept between years for colonisation probability and site occupancy (α_i) significant at $P < 0.05$, though it was significant for all species using the compromise analysis. For *R. clamitans*, P .

crucifer and *B. fowleri*, the observed a , was significant at $P < 0.05$ and significant also for *R. catesbeiana* when the compromise analysis increased the significance threshold. For *R. clamitans*, and *P. crucifer*, this is likely reflective of low values of $P_{i,s}$ as the slope of the regression between site occupancy and colonisation probability measured between years (b_t) did not approach significance.

The slope of the regression between site occupancy and colonisation probability measured between years (b_t) or between sites (b_s) was not significant for any species at $P < 0.05$. When the significance threshold was increased using compromise analysis, b_t for *R. catesbeiana* and b_s for *B. fowleri* were significantly different from the null expectation.

The extinction coefficients were never significant in the direction predicted by the GMM. Long Point populations exhibit low values of c (the intercept between site occupancy and extinction probability – the GMM predicts high values), which indicates a low probability of extinction for an occupied site in an empty landscape (Gotelli and Kelley 1993). The GMM predicts low values of d (the slope of the regression of site occupancy and extinction probability), while at Long Point values of d tended to be higher than expected. The extinction probability decreased with increasing site occupancy measured between years, as theory would predict, in only *R. pipiens* and *R. catesbeiana*. For all other comparisons, extinction probability measured between years (Figure 4, Table 1), and sites (Figure 5, Table 2), actually increased as site occupancy increased. For all species, the observed extinction coefficients c (intercept) and d (slope) were not significantly different from the null model at $P < 0.05$. When the significance threshold was increased using compromise analysis only the slope for the linear regression of site occupancy and extinction probability measured between years for *R. pipiens* was significantly different from the null expectations.

When the GMM was applied to the reduced historic data of *B. fowleri* occurrence across the north shore of Lake Erie, neither extinction nor colonisation probabilities between years changed with site occupancy as predicted by the GMM ($P(a) = 1$, $P(b) = 0.738$, $P(c) = 1$, $P(d) = 0.386$).

When we examined all species $P_{i,t}$, $P_{a,t}$ and f_t on a two and three year cycle none of the coefficients tested was significant except for the 2 year cycle slope between site occupancy and colonisation probability (b_t) for *R. catesbeiana* (P(0.043)).

THE INCIDENCE FUNCTION MODEL

When 1000, 15-year simulations initialised with *Bufo fowleri* occupancy data for 1988 predicted the incidence for 2002; the predicted values only explained 2% of the observed variation (Regression results not shown). Additionally, we compared the efficacy of the IFM's predictions by contrasting the observed mean incidence for each study year to each year of the simulation (Figure 6). The observed incidence generally fell outside the 80% confidence intervals of the simulation mean incidence.

When the IFM was loaded with a single year of occupancy (for either the entire, or the reduced Lake Erie data set for *B. fowleri*) it did not accurately predict the patterns of incidence we observed in 2002. A 17-year simulation was parameterized using *B. fowleri* occupancy data from the northern Lake Erie shoreline in 1985. When the resultant incidences were compared to the actual incidence in 2002 they explained less than 1% of the observed variance. When the mean occupancies for the period from 1985 to 2002 were compared to the occupancies of 1000 simulations parameterized with data from 1985, the observed data were lower than the 80% percentiles (Figure 7).

DISCUSSION

There is no discernable metapopulation effect apparent in the local dynamics of amphibians at Long Point. When parameterized with data from mark-recapture studies, observations of calling males at a local level, or occupancy data from historical records across a wider geographical range, both the GMM (Gotelli 1991, Gotelli and Kelley 1993), and the IFM (Hanski 1994) failed to describe the observed trends for any of the six species we examined. This does not mean that some metapopulation approach might not ultimately describe the population dynamics of some of these species; however it should caution against the *a priori* acceptance that a simple metapopulation approach is appropriate with a certain species because of a qualitative assessment of their spatial ecology.

One important consideration, which our study is amongst the first to explicitly acknowledge and test, is the low power that affects metapopulation ecology. In the majority of metapopulation studies, any regression or correlative conclusion made on data from both among-sites and among-years at a 0.05 confidence level would result in less than 50% chance of correctly rejecting a false null hypothesis (Table 4). All field studies are necessarily constrained in time and space, and ours is no different. On those occasions when a metapopulation model has been presented with occupancy data, often the data set is very small and the resultant statistical power is quite low. We compiled a short list ($n=28$) of metapopulation studies where the number of sites and years of analysis was reported. We then investigated the power of that sample size to correctly identify a small and large effect size using linear regression (Table 6). In all but one instance, there was a $> 85\%$ chance of making a Type II error of identifying a small effect size through time at a traditional critical value of 0.05. Verboom et al (1991), for instance, is a common reference to a natural system operating as a classic metapopulation. The nuthatch populations used were from three different sites: two were surveyed for three years, and the other for two. Each site contained 18, 43 and 64 patches. Thus, in this case the power to correctly reject the null hypothesis is vanishingly low. Clearly, when the number of years and sites are beyond the control of the researcher, a compromise analysis, as advocated here, is useful. Indeed, as we have shown, regressions based on 19 sites and 15 years lead to low statistical power at a 0.05 confidence level. However, we have also demonstrated that the null compromise analysis has considerable value in strengthening our conclusions by reducing both the chance of Type I and Type II error. We could not completely reject the potential of a slight metapopulation effect influencing colonisation in the GMM. However, increasing the critical values when examining extinction only strengthened our conclusion that there was no metapopulation effect on extinction.

In the introduction of this analysis, we listed the factors that made it appear likely that these populations met the conditions of the metapopulation paradigm. We considered that although some of these conditions would be hard to test for some species

where data were lacking; all in all it was likely that metapopulation theory would describe the population dynamics of this system. Therefore, our analysis was designed to test several assumptions of a Levins-style model (the GMM) and the predictive capabilities of the incidence function model. As we have detailed, in fact, there is likely no discernable metapopulation effect for amphibian populations at Long Point and a broader geographic scale for *B. fowleri*. Since both these models require a minimum of data it would be rash to make ostentatious claims regarding their definitive performance. However, in the contexts of these specific systems, and with metapopulation approach in general, it is useful to re-consider the assumptions and conditions necessary for metapopulation persistence listed earlier and make a supplementary judgement regarding whether in fact the condition is not met, or the assumption is broken.

An examination of the trends of extinction and colonisation probabilities between years and sites suggests that patches are not equally connected, and that populations for *R. clamitans* may not be in equilibrium (data not shown) – two primary assumptions of the GMM. Amphibian populations at Long Point inhabit early succession environments and as such they may be experiencing changes in extinction and colonization as habitat quality changes and thus would not be at equilibrium. Furthermore, due to succession, patches may not be homogenous in quality nor equally connected (the mean-field assumption is rarely met in the real world – especially if sites are separated along a peninsula as a Long Point, or around the shoreline of Lake Erie). Indeed, it is a common occurrence in the amphibian-as-metapopulation literature that local extinctions are deterministic, not stochastic, as habitats undergo succession (Bradford, Neale, Hash, Sada and Jaeger 2003, Marsh and Trenham 2000, Sjogren 1991, Skelly, Werner and Cortwright 1999). Differentiating between stochastic and deterministic extinction is clearly important, but beyond the scope of the current investigation.

One IFM assumption is that habitat patches support local breeding populations and our data for *B. fowleri* support this assumption. However, we do speculate that measuring patch dynamics simply only on the level of the pond may not be enough for *B. fowleri*, and more work is required regarding the importance of the terrestrial

environment (Pope, Fahrig and Merriam 2000). Specifically, the area of importance may not exclusively be the breeding pond. The area we may need to consider may be the amount of terrestrial habitat needed to allow daily burial during the summer, or deeper burial to survive the winter.

Another IFM condition is that patches are not too isolated to prevent recolonisation. However dispersal events that regularly link habitat patches also invalidate the use of metapopulation theory to explain population dynamism, for frequent dispersal could cause population dynamics to become synchronous (Ranta, Kaitala, Lindstrom and Linden 1995). Here, the individuals inhabiting habitat 'islands' then operate as a patchily distributed, single population, and not as a true metapopulation (Harrison 1991, Harrison 1994). Amongst the species investigated here, *B. fowleri* is known to be capable of movement much farther in distance and frequency than is usually thought possible for an amphibian – including up to 34 kilometres in a single year (Chapter I, Chapter II). Even if such an event is rare, merely its possibility indicates that there may be enough movement between patches to prevent the development of asynchronous local dynamics. In fact, animals do move larger than expected distances at a relatively high rate (~ 3 animals / year move more than 4 km).

Modeled on the regional level, the average dispersal distance necessary to prevent metapopulation extinction are many times larger than the average dispersal distance measured using mark-release-recapture or mtDNA variability data (Table 3). Therefore, we are either underestimating the long distance dispersal frequency, or the populations are too isolated to be a metapopulation. We consider the latter more likely than the former as our long distance estimates are already amongst the highest reported for amphibians. Thus we are presented with the case where locally there may be too much movement, and regionally there may not be enough.

No one of the populations surveyed here is large enough to ensure long-term survival of the patch network – as assumed by the IFM. However as there are currently populations to the east and the west of the study site there is a possibility that 1) a population in one of these areas is large enough to act as a source for the system we have

studied here, or 2) the populations to the east and west together with those documented here to form a metapopulation. Nevertheless, it is unlikely that populations outside those studied here are acting as a mainland. In the course of this study populations to the east have been locally extinct, and there is no reason to suspect that populations to the west would not be susceptible to similar extinction events. The data to refute the second hypothesis do not exist, but the extremely long distances moved by toads that we have observed in the course of three years (34km) suggests that even when patchy populations are scaled up to the entire size of Long Point, there is too much dispersal (in distance and rate) for a metapopulation process to prevail.

The IFM assumes that local dynamics are sufficiently asynchronous to make simultaneous extinction of all local populations unlikely. In analysing the GMM, we observed that extinction probabilities between sites and years were not statistically independent (data not shown). As the 19 local populations studied here are within 10 km of each other, it is conceivable that stochastic weather events could effectively reduce the number of independent populations (the Moran Effect (Moran 1953a,b)), making the classic metapopulation effect less likely (Hanski 1998). However, we suspect that the synchronization of population dynamics expected if all these spatially segregated populations experience the same weather perturbations are not evident at Long Point (Figure 8).

Thus, our analysis of the assumptions and conditions of the IFM suggests that at a local level, high rates of dispersal and the effects of large weather events, together or separately, may reduce the number of populations until what remains is a single population whose resources (breeding ponds) are patchily distributed. At a regional level, the high levels of dispersal documented locally are, however, not large enough to cause the formation of a functioning simple metapopulation. Such patchily distributed populations, out of the recolonisation range of dispersing individuals has been termed a non-equilibrium metapopulation (Harrison 1991, Harrison 1994). In a non-equilibrium metapopulation each population is an independent entity and, as such, the trajectory for each population is decided by factors operating much faster than the metapopulation

dynamics of a balance between extinction and colonization (Harrison 1991, Harrison 1994). Whether a functioning metapopulation exists between these two scales is really a moot point, for no populations in the Lake Erie watershed exist at such an intermediate scale. Thus, the simple metapopulation approach, unsuccessful at the local scale, also fails on the regional scale for a species with such apparently localized distribution and dispersal. If this is an example of a non-equilibrium metapopulation its persistence is extremely sensitive to the internal dynamics of each patch, and likely this is an area of research where more fruitful conservation science should take place. In the end however, to discard the hypothesis of a metapopulation effect operating at the scale of 100's of kilometers would require more precise historic data regarding site occupancy. Such data are unobtainable. Genetic evidence of population isolation would be the better method to test the potential for a large-scale metapopulation effect. Indeed, several predictions regarding amphibian populations (rescue effect between these populations, size of genetic neighborhood coincident with breeding assemblage habitat size) are amenable to testing with genetic analysis of mtDNA variability (Chapter VI).

Clearly, the metapopulation paradigm has been well received by the ecological and conservation literature (Hanski and Simberloff 1997). Many variants have been proposed (Hanski and Simberloff 1997), and demonstrated to be useful when presented with real data (Hanski, Pakkala, Kuussaari and Lei 1995, Moilanen, Smith and Hanski 1998, Vos, Braak and Nieuwenhuizen 2000). However, as the paradigm ages, it is applied less stringently (Pannell and Obbard 2003). Population extinction, for instance, once a hallmark of the metapopulation approach (Andrewartha and Birch 1954) is often no longer viewed as necessary (Pannell and Obbard 2003). With this reduction in stringency comes a reduction in precision and accuracy. If a patchy population and a metapopulation are two different things, subject to different stimulus and trajectories, then the associated assumptions should be tested prior to the acceptance of one or another paradigm. Metapopulation models have been made to appear attractive for conservation biology as they offer explanations for regional processes of extinction and colonisation. Unfortunately real population data sets are invariably too small to allow quantitative

assessments of the assumptions upon which these models rest. In the case of temperate anuran amphibians, long-term mark-release-recapture studies, although labour intensive, produce exactly the sort of data necessary for tests invaluable to the development of conservation strategies. It is not our intent to challenge the utility of the metapopulation paradigm for conservation biology in general, or amphibian biology in particular.

However, we have demonstrated here that neither the GMM nor the IFM could accurately describe the dynamics of this specific system nor did this amphibian system meet the expectations of a classic metapopulation model. It is risky to assume that temperate pond-breeding anuran amphibians exist as classic metapopulations without a close examination of, and attention to, the assumptions upon which such simple models rest.

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TABLE HEADINGS

Table 1:

The critical value (α) and power ($1-\beta$) values determined through compromise analysis on the given effect size calculated between years. For all species $q = 2$, total sample size = 14, except *P. crucifer* where $N=10$.

	Species	Effect size d (observed r)	Alpha	Power
<i>Colonisation effect</i>	<i>R. catesbeiana</i>	0.384	0.1539	0.6921
	<i>R. clamitans</i>	0.511	0.0983	0.8033
	<i>B. americanus</i>	0.005	0.3311	0.3379
	<i>P. crucifer</i>	0.2	0.2401	0.5198
	<i>R. pipiens</i>	0.187	0.2463	0.5075
	<i>B. fowleri</i>	0.145	0.2661	0.4678
<i>Extinction effect</i>	<i>R. catesbeiana</i>	0.092	0.291	0.418
	<i>R. clamitans</i>	0.618	0.0572	0.8857
	<i>B. americanus</i>	0.084	0.2947	0.4105
	<i>P. crucifer</i>	0.079	0.2971	0.4059
	<i>R. pipiens</i>	0.207	0.2368	0.5264
	<i>B. fowleri</i>	0.245	0.2188	0.5624

Table 2:

The critical value (α) and power ($1-\beta$) values determined through compromise analysis on the given effect size calculated between sites. For all species $q = 2$, total sample size = 17

	Species	Effect size d (observed r)	Alpha	Power
<i>Colonisation effect</i>	<i>R. catesbeiana</i>	0.071	0.2974	0.4052
	<i>R. clamitans</i>	0.089	0.2881	0.4237
	<i>B. americanus</i>	0.663	0.0302	0.9396
	<i>P. crucifer</i>	0.235	0.2126	0.5748
	<i>R. pipiens</i>	0.415	0.1238	0.7523
	<i>B. fowleri</i>	0.388	0.1365	0.7271
<i>Extinction effect</i>	<i>R. catesbeiana</i>	0.062	0.302	0.396
	<i>R. clamitans</i>	0.2	0.2306	0.5387
	<i>B. americanus</i>	0.014	0.3263	0.3473
	<i>P. crucifer</i>	0.523	0.0772	0.8456
	<i>R. pipiens</i>	0.001	0.3328	0.3343
	<i>B. fowleri</i>	0.201	0.2301	0.5397

Table 3:

Parameter values for the incidence function model (IFM) determined following 1000 simulations after initial parameterization using biological information. α represents the constant that determines the survival rate of migrants over distances between patches. Parameter b scales population size to patch area. Parameter x represents the susceptibility of populations to environmental fluctuations. Parameter y measures how fast the probability of colonisation approaches 1 with an increasing number of migrants. See text for details on how values for each parameter were initially parameterized.

Test	α	b	y	u	x	average dispersal distance for α in meters	
Long Point local habitat patches	0.002315		0	0.59112	1.53163	0	432.0
Lake Erie historical data (90 year/17 sites)	0.000007	0.51025		0.003198	36.7147	0.4643	142857.1
Lake Erie historical data reduced (9 decades/5 sites)	0.000013	0.174		0.005943	238.773	0.94907	76923.1

Table 4:

A meta-analysis of the statistical power of metapopulation studies. The power of a correlation or regression on population turnover between sites and years was investigated assuming a small (0.1) or large (0.5) effect size (Cohen 1988) at a critical value of 0.05.

Source	Comparison between sites	N	Small Effect			Large Effect		
			Size	Power	β	Size	Power	β
Hanski and Singer. 2001.	Sites	1700	0.1	0.9937	0.6%	0.5	1	0.0%
Carlson and Edenhamn. 2000.	Sites	378	0.1	0.6201	38.0%	0.5	1	0.0%
Telfer et al 2001	Sites	218	0.1	0.4329	56.7%	0.5	1	0.0%
Vos et al 2000.	Sites	198	0.1	0.4055	59.5%	0.5	1	0.0%
Eber and Brandl 1996.	Sites	197	0.1	0.4041	59.6%	0.5	1	0.0%
Thomas and Harrison 1992	Sites	157	0.1	0.3464	65.4%	0.5	1	0.0%
Kindvall 1996	Sites	115	0.1	0.2815	71.9%	0.5	1	0.0%
Berendonk and Bonsall 2002	Sites	79	0.1	0.221	77.9%	0.5	0.9997	0.0%
Moilanen et al 1998	Sites	76	0.1	0.2187	78.1%	0.5	0.9996	0.0%
Crone et al 2001	Sites	71	0.1	0.2083	79.2%	0.5	0.9991	0.1%
Verboom et al 1991	Sites	64	0.1	0.196	80.4%	0.5	0.998	0.2%
Hanski et al 1995.	Sites	50	0.1	0.1725	82.8%	0.5	0.9913	0.9%
Lei and Hanski. 1998.	Sites	50	0.1	0.1725	82.8%	0.5	0.9913	0.9%
Verboom et al 1991	Sites	41	0.1	0.1538	84.6%	0.5	0.9738	2.6%
Stelter et al 1997	Sites	30	0.1	0.134	86.6%	0.5	0.925	7.5%
Pfister 1998.	Sites	29	0.1	0.1299	87.0%	0.5	0.9081	9.2%
Verboom et al 1991	Sites	18	0.1	0.1059	89.4%	0.5	0.7344	26.6%
Petit et al 2001	Sites	12	0.1	0.0908	90.9%	0.5	0.5493	45.1%
Corser 2001	Sites	12	0.1	0.0908	90.9%	0.5	0.5493	45.1%
Nieminen and Hanski. 1998.	Sites	10	0.1	0.088	91.2%	0.5	0.5097	49.0%
Gotelli and Taylor. 2000	Sites	10	0.1	0.0851	91.5%	0.5	0.4673	53.3%
Neve et al 1996	Sites	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Hels 2002	Sites	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Sinsch and Seidel 1995	Sites	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Driscoll 1997	Sites	3	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Saether et al. 1999	Sites	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Spendelov et al 1995	Sites	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%

Table 4 continued.

Source	Comparison between years	N	Small Effect			Large Effect		
			Size	Power	β	Size	Power	β
Moilanen et al 1998	Years	20	0.1	0.1127	88.7%	0.5	0.799	20.1%
Gotelli and Taylor 2000	Years	11	0.1	0.088	91.2%	0.5	0.5097	49.0%
Corser 2001	Years	8	0.1	0.0821	91.8%	0.5	0.4219	57.8%
Kindvall 1996	Years	6	0.1	0.0715	92.9%	0.5	0.2637	73.6%
Crone et al 2001	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Petit et al 2001	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Hanski et al. 1995	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Eber and Brandl 1996	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Hanski and Singer	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Spendelov et al 1995	Years	5	0.1	0.067	93.3%	0.5	0.2014	79.9%
Hels 2002	Years	3	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Vos et al 2000	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Carlson and Edenharn 2000	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Telfer et al 2001	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Verboom et al 1991	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Verboom et al 1991	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Verboom et al 1991	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Lei and Hanski. 1998	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Nieminen and Hanski. 1998	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Pfister 1998	Years	3	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Thomas and Harrison 1992	Years	2	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Driscoll 1997	Years	3	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Sinsch and Seidel 1995	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Neve et al 1996	Years	2	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Saether et al. 1999	Years	4	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Berendonk and Bonsall 2002	Years	3	0.1	0.0616	93.8%	0.5	0.1348	86.5%
Stelter et al 1997	Years	unknown	0.1			0.5		

FIGURE HEADINGS

Figure 1:

Nested diagrammatic maps of the amphibian breeding assemblages utilized here. Regional populations are considered across Lake Erie (A), local populations at Long Point (B) were measured as breeding ponds (C). Box C contains the filled outlines of *B. fowleri* breeding ponds along the Long Point shoreline and has a width of 10km.

Figure 2:

The relationship between colonisation probability (P_i) and site occupancy measured by patch state transition between years. * = Significantly different from 1000 null simulations at $p=0.05$. ** = Significantly different from 1000 null simulations for the appropriate confidence level determined by null compromise analysis (NCA). Intercept = a_i , slope = b_i .

Figure 3:

The relationship between colonisation probability (P_i) and site occupancy measured by patch state transition between sites. * = Significantly different from 1000 null simulations at $p=0.05$. ** = Significantly different from 1000 null simulations for the appropriate confidence level determined by NCA. Intercept = a_s , slope = b_s .

Figure 4:

The relationship between extinction probability (P_e) and site occupancy measured by patch state transition between years. * = Significantly different from 1000 null simulations at $p=0.05$. ** = Significantly different from 1000 null simulations for the appropriate confidence level determined by NCA. Intercept = c_i , slope = d_i .

Figure 5:

The relationship between extinction probability (P_e) and site occupancy measured by patch state transition between sites. * = Significantly different from 1000 null simulations at $p=0.05$. ** = Significantly different from 1000 null simulations for the appropriate confidence level determined by NCA. Intercept = c_s , slope = d_s .

Figure 6:

Comparing the proportion of occupied patches from 1000 15-year simulations to the observed pattern for 15 years of field study. Dashed lines are the average proportion of

occupied patches, and the 80 and 20% percentiles for each year. Solid line is, the best fit equation of the observed proportion of occupied patches: $y = 0.0776 \cdot \log(\text{time}) + 0.3372$. When the observed dynamics fall outside the 80% percentiles of predicted dynamics there may be a trend in the data.

Figure 7:

Comparing the proportion of occupied patches from 1000 17-year simulations to the observed pattern for 17 years of occupancy data from across Lake Erie. Dashed lines are the average proportion of occupied patches, and the 80 and 20% percentiles for each year. Solid line is, the best-fit equation of the observed proportion of occupied patches: $y = -0.0267 \log(\text{time}) + 0.2272$. When the observed dynamics fall outside the 80% percentile of predicted dynamics there may be a trend in the data.

Figure 8:

Abundance of male *B. fowleri* at 18 habitat patches over 15 years. There is little evidence for spatial synchrony.

FIGURE 1

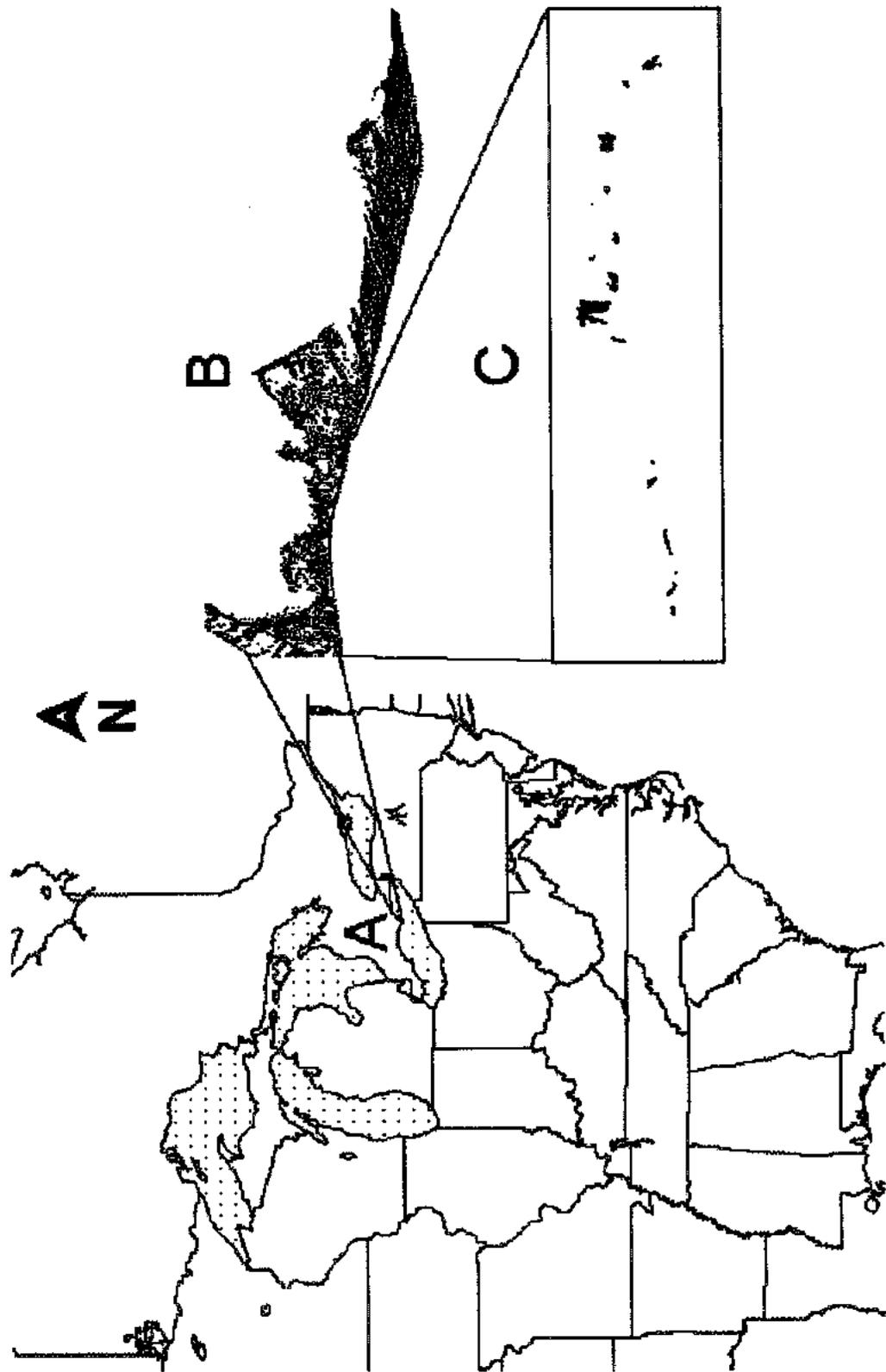


FIGURE 2

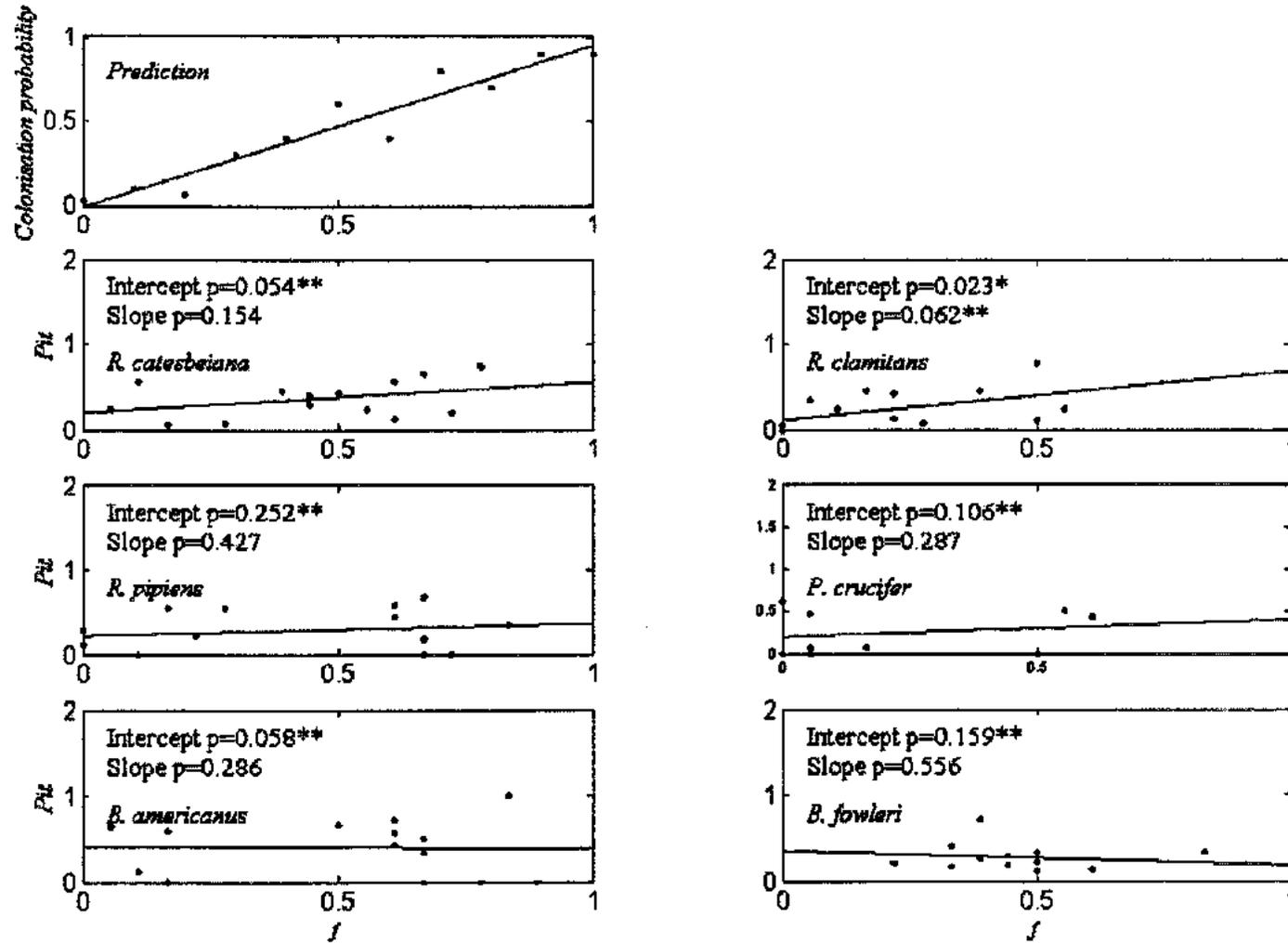


FIGURE 3

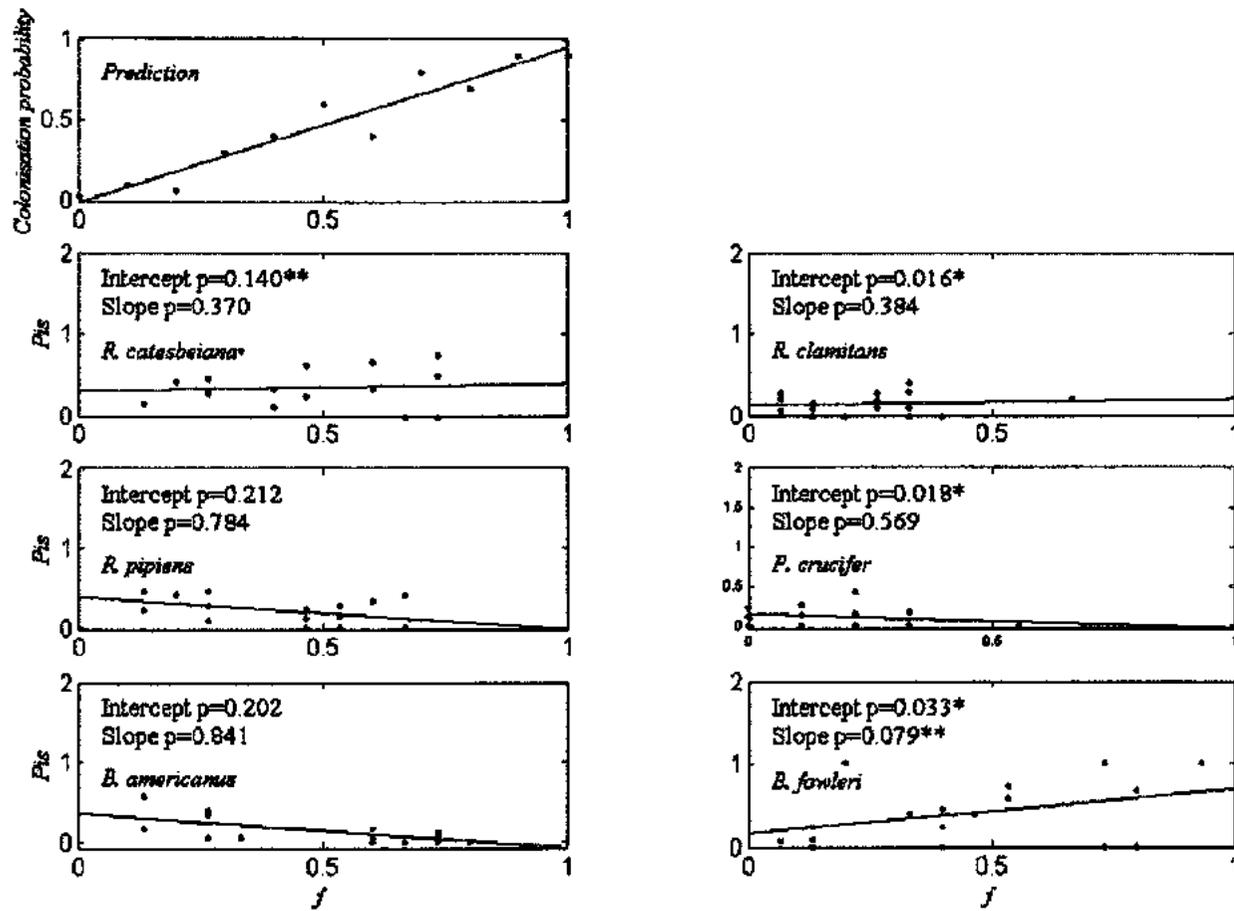


FIGURE 4

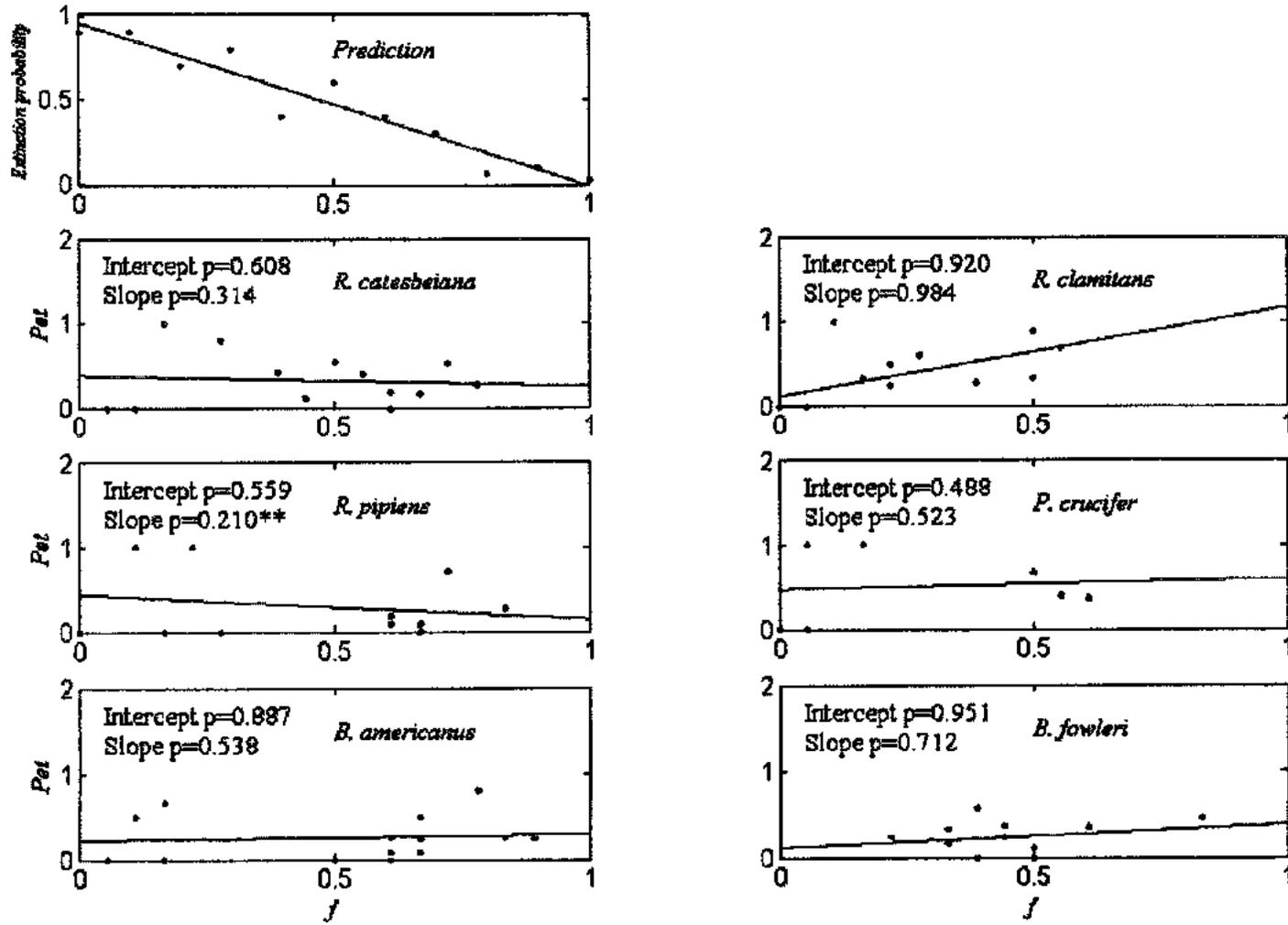
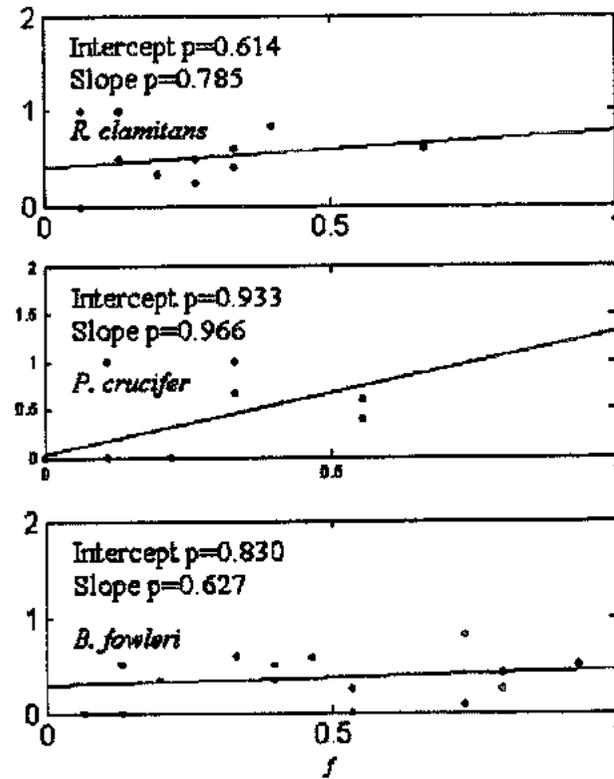
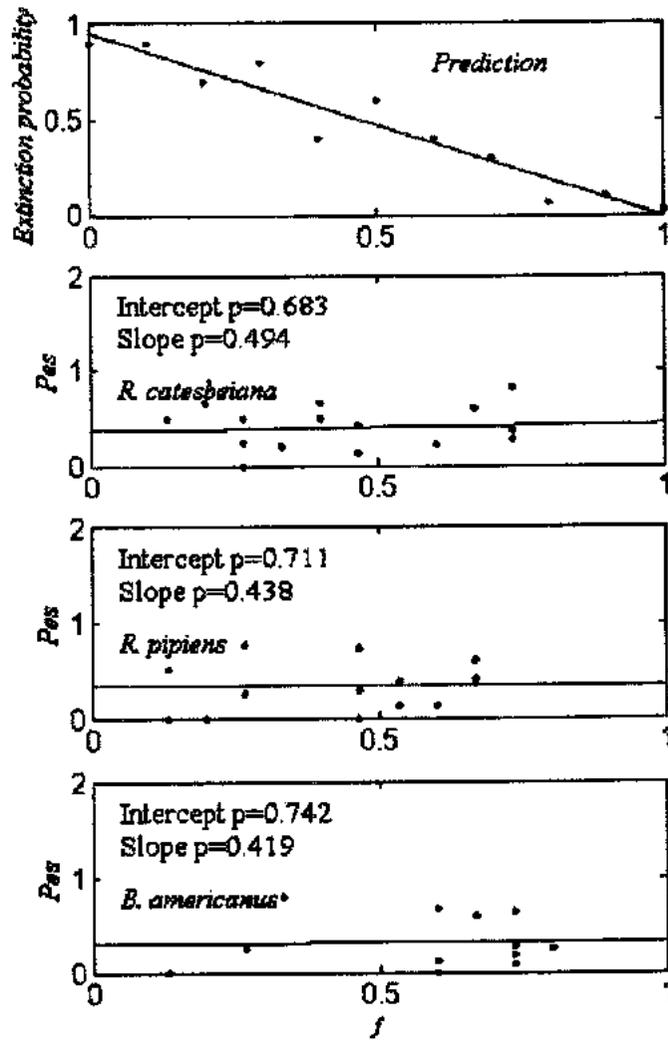


FIGURE 5



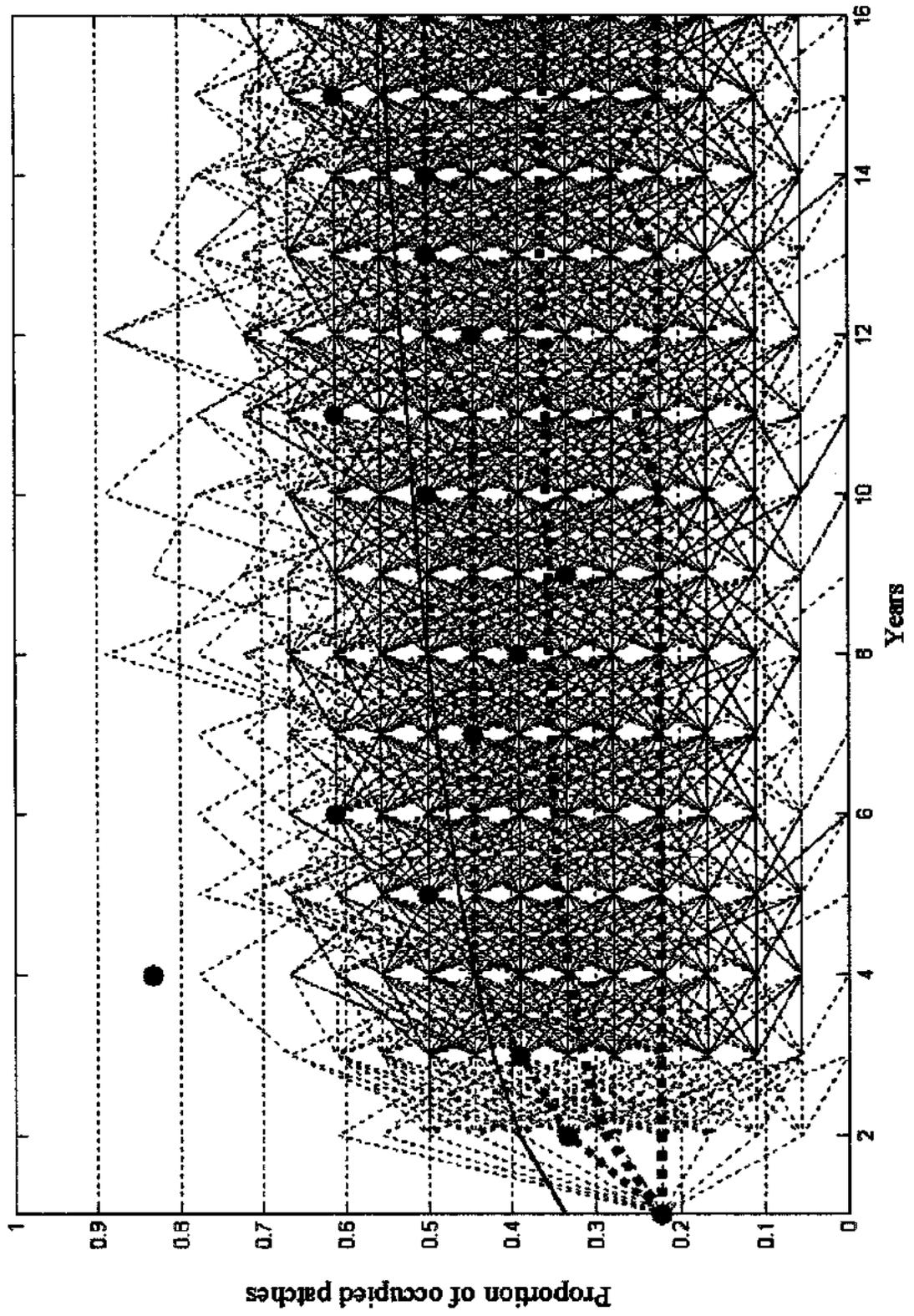


FIGURE 6

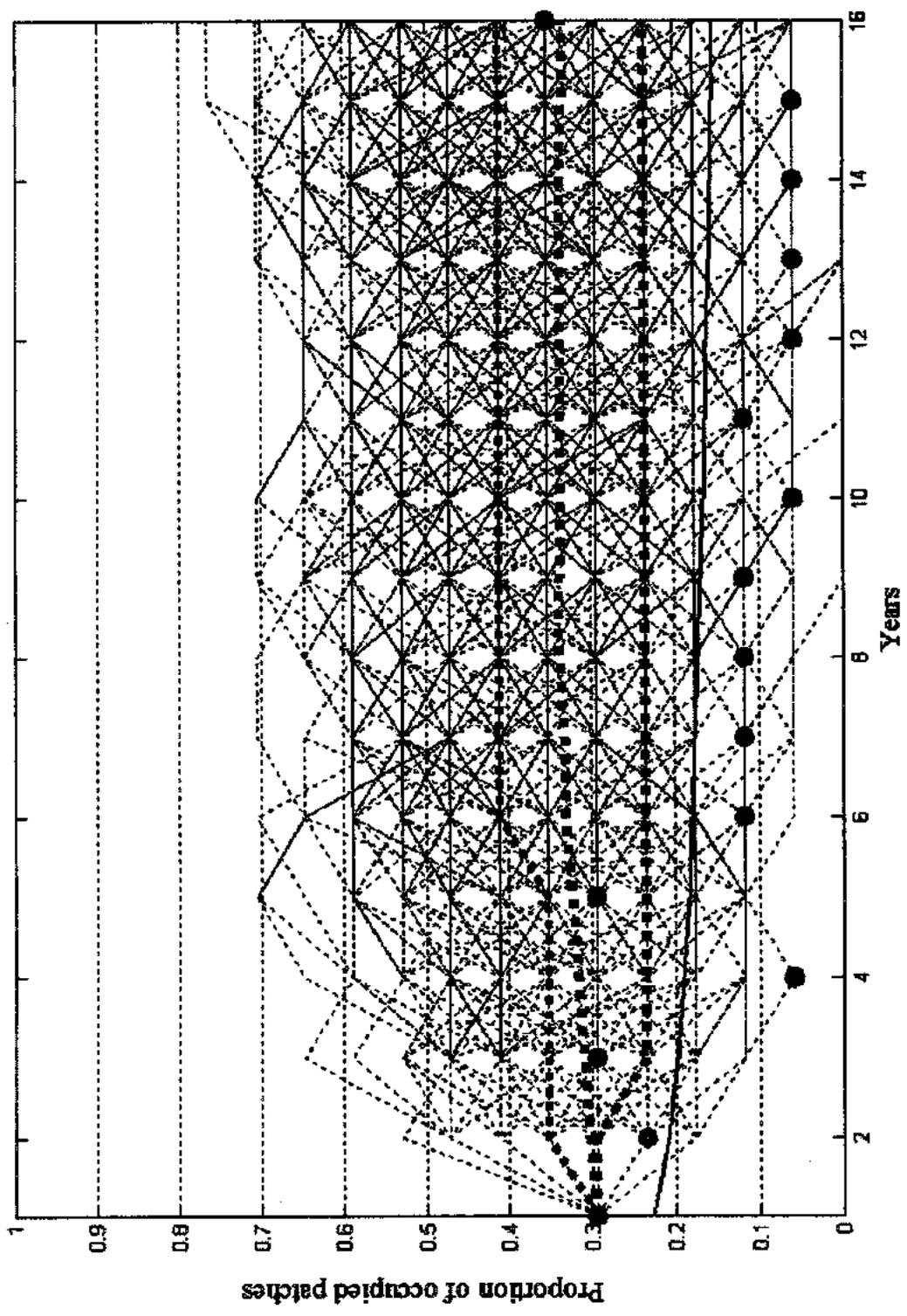
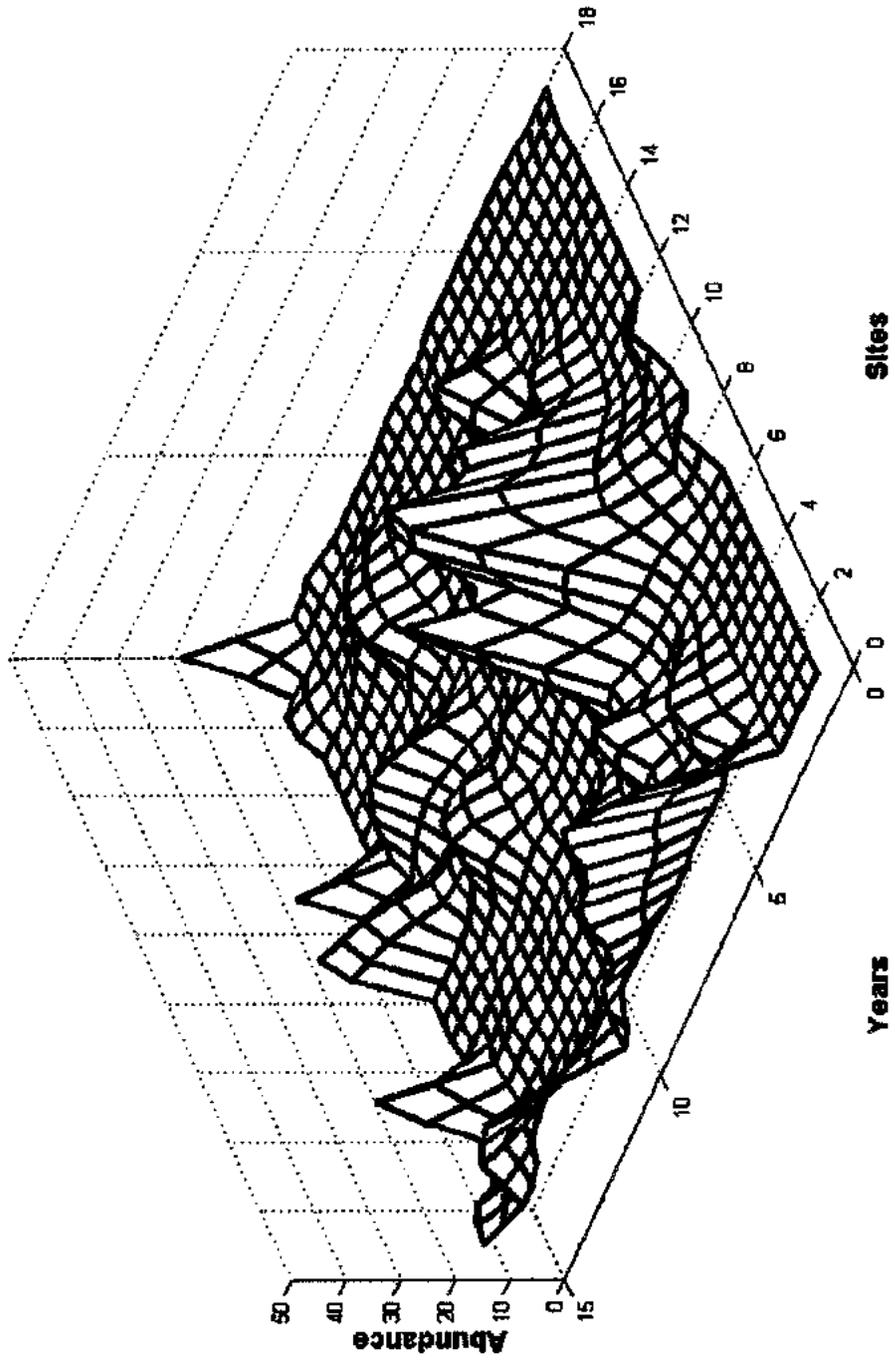


FIGURE 7

FIGURE 8



**CHAPTER 5: PHYLOGEOGRAPHY OF *BUFO FOWLERI* AT ITS NORTHERN
RANGE LIMIT**

**Keywords: phylogeography, amphibian, mtDNA, *Bufo fowleri*
3700 words, 51 references, 2 tables, 3 figures, 1 appendix**

PREFACE TO CHAPTER 5

Having demonstrated the lack of support for two ecological metapopulation models in regional *Bufo fowleri* population turnover – and the surprising local movement capabilities for male, female and juvenile toads – I now examine the phylogeographic structure for *Bufo fowleri* across the Lake Erie watershed. If those populations considered in the metapopulation analysis are isolated, then it is likely that they will have experienced enough genetic drift to exhibit shallow phylogeographic structuring of genetic variability. If the large capabilities for movement demonstrated locally scale up, then it is likely that occasional migrants connect otherwise isolated populations. These migrants will swamp any local genetic drift and there will be no geographic structuring to genetic variability.

Reference style is according to submission requirements for Molecular Ecology.

ABSTRACT

Many of the species that recolonised previously glaciated areas in the Great Lakes basin of North America over the past 10-12 000 years exhibit genetic evidence of multiple invasion routes and present-day secondary contact between deeply divergent lineages. With this in mind, we investigated the phylogeographic structure of genetic variability in Fowler's toads (*Bufo fowleri*) at the northern edge of its distribution where its range encircles the Lake Erie basin. Because *B. fowleri* is so closely tied to habitats along the Lake Erie shoreline, we would expect to find clear evidence of the number of invasions leading to the species' colonization of the northern shore. A 540 bp sequence from the mitochondrial control region was amplified and analyzed for 158 individuals from 21 populations. Inter-population sequence variation ranged from 0% to 6%. Phylogenetic analysis of p-distance using the neighbor-joining method revealed two deeply divergent (6% sequence divergence) mtDNA lineages (Phylogroup 1 and 2), possibly arising due to secondary contact of populations that entered the region from two separate glacial refugia. However, the phylogeographic pattern was not simple. The populations at Long Point, on the north shore of Lake Erie, clustered with the population from Indiana Dunes on Lake Michigan to form Phylogroup 2 whereas all other *B. fowleri* populations examined from both sides of Lake Erie constituted Phylogroup 1. Furthermore, mtDNA sequences from the related species, *Bufo americanus*, obtained from populations outside the range of *B. fowleri*, clustered with mtDNA haplotypes of *B. fowleri* Phylogroup 1, indicating the possibility of partial introgression of mitochondria from one species to the other.

INTRODUCTION

Holman (1995) proposed that species of amphibians and reptiles initially re-entered what is now Ontario post glacially from present day Indiana and Ohio across the area between present day Lakes Huron and Erie. Additional recolonisations from the north and east would have been delayed (<10 000 ypb) until the receding Champlain Sea to the east revealed the St. Lawrence lowlands (Holman, 1995). The genetic signature of two post-glacial routes of recolonisation is evident in the Spring Peeper (*Pseudacris crucifer*) (Austin *et al.*, 2002) and the Spotted Salamander (*Ambystoma maculatum*) (Zamudio, Savage, 2003). Both species display deep inter-population genetic divergences not predicted by the geographic separation of contemporary populations. Other species, such as, *Bufo fowleri* with very different temperature requirements (McKenney *et al.*, 1998), would likely have arrived after as a secondary (sensu (Holman, 1992)) invader. There is fossil evidence to support the hypothesis of primary (*Pseudacris*, *Ambystoma*), and secondary (*B. fowleri*) invasion of post-glacial North America (Holman, 1992; Holman, 1995). As southern Ontario has been shown to be a zone of secondary contact for two primary invaders, it is therefore reasonable to ask whether such a zone exists for a secondary invader such as *B. fowleri*.

Contemporary populations of *B. fowleri* have a precarious hold on the northern Lake Erie shoreline. They are isolated from each other by shoreline development, agriculture and other non-viable habitat. They are further isolated from populations on the southern shoreline by many kilometers of the open waters of Lake Erie. Where there is population isolation with occasional dispersing individuals connecting, or founding, geographically disjunct populations there is likely to be phylogeographic structure (Avice, 2000). Thus the geographic subdivision of genetic variability is related to the scale of the dispersal ability of the species (Avice, 2000). If the individuals of a species can disperse relatively far, then many populations are likely within an area of panmixia, or genetic neighborhood (Wright, 1951), and we would not expect to see the accumulation of small mutations that delineate a lineage on a unique trajectory. However, if the individuals do not move long distances then it becomes more likely that we would find geographic

structuring of genetic variability. Amphibians, for example, are expected to be consigned by their biology and behavior to have relatively isolated populations associated with discrete habitats such as breeding ponds (Blaustein *et al.*, 1994; Rowe *et al.*, 2000; Sinsch, 1990). Amphibians are therefore likely to exhibit phylogeographic structure on relatively small spatial scales on the order of kilometers. Geographic structuring of genetic variability has been shown in the true toads of the genus *Bufo*. *Bufo calamita* exhibits small but significant geographic sub-structuring of microsatellite DNA with pairwise between pond distances ranging from 0.5-16 km (Rowe *et al.*, 2000). *Bufo woodhousei* populations spaced between 150 and 250 km apart exhibits phylogeographic structure at the ND1 region of mtDNA (Masta *et al.*, 2003). Although island populations of *Bufo bufo* showed no significant isolation by distance among islands separated by less than 10 kilometers (Seppa, Laurila, 1999), urban and rural populations of *B. bufo* did exhibit significant isolation at a scale of approximately 30km, when the additional isolating effects of roads were taken into account (Hitchings, Beebee, 1998). Populations of *B. bufo* separated by 5-15 kilometers of pasture and small urban developments were estimated to have more than 2 migrants per generation on average (Scribner *et al.*, 1994). Populations of *Bufo fowleri* did not demonstrate isolation-by-distance when populations were separated by maximums of approximately 35 kilometers in Mississippi (Hranitz, Diehl, 2000) or 120 kilometers in Ontario (Green, 1984) when genetic variation was assayed using allozymes.

The variability, or resolution, of the genetic region used determines the spatial scale within which genetic structure is expected (Parker *et al.*, 1998; Scribner *et al.*, 1994). For instance in toads, rapidly changing microsatellites display geographic structure on a scale of a kilometer or less (Rowe *et al.*, 2000), while more slowly mutating regions of the mitochondria (ND1 – (Masta *et al.*, 2003), tRNA^{Ile}, tRNA^{Met}, tRNA^{Gln}, and ND2 – (Macey *et al.*, 1998), 16S and cytochrome-b – (Mulcahy, Mendelson, 2000), and 12S – (Liu *et al.*, 2000)) show geographic structure on a scale of hundreds of kilometers. Allozymes – under the control of nuclear genes with generally very conservative rates of mutation – do not show any geographic structuring to genetic variation at scale of

hundreds of kilometers (Green, 1984). Amongst mitochondrial markers, the D-loop or control region is not constrained by function and is thus a rapidly evolving locus useful for delineating moderately divergent lineages (Goebel *et al.*, 1999). We selected this locus with the expectation that it would expose phylogeographic structure within the Lake Erie watershed where tens and hundreds of kilometers separates contemporary populations.

Using sequence data from the highly variable control region of the mitochondria of *Bufo fowleri* we tested the hypothesis that as southern Ontario is a likely zone of secondary contact between two post-glacial refugia, there would be a large genetic divergence evident between some *B. fowleri* populations along the northern shoreline of Lake Erie. Such deep divisions would not be predicted by contemporary geographic distance. This area has only been free of glacial ice for approximately 10 000 years and therefore the majority of phylogeographic structuring among contemporary populations would likely be shallow.

MATERIALS AND METHODS

Toads were sampled from sites across the northern edge of the Fowler's toad distribution (Appendix 1). Additional samples were obtained from Ross and Union Counties in Ohio and Leigh County in Pennsylvania courtesy of S. Masta. For adult toads, toe clips were preserved in the field in 70% ethanol and total DNA extractions were completed following Fetzner (1999). For juveniles, tissue samples from the heart, liver and skeletal muscle were frozen and maintained at -80°C and total DNA extractions were completed following Fetzner (1999) and Chase *et al.* (1998). Control region mtDNA was amplified using published primers (Goebel *et al.*, 1999). Thermocycling conditions (Robocycler) for double-stranded DNA (dsDNA) amplification began with 39 cycles each of which each had a 30-s denaturation at 94°C , a 45-s annealing at 52°C , and a 1.5-min extension at 72°C . A 10-min extension at 72°C followed the final cycle. Reaction mixtures for PCR contained 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 0.4 mM of each oligonucleotide, 2.5 mM MgCl₂, 0.5 U/100 μl Taq DNA polymerase (Sigma), and 0.1 μM each primer in a reaction volume of 25 μl . A negative

control was included for all PCR reactions. Amplified DNA was examined on 2.5% agarose gel and then cleaned with the Qiaquick PCR purification kit (Qiagen). Cleaned DNA was sequenced using Amersham Biosciences DYEnamic ET Terminators Chemistry Cycle Sequencing System with Thermo Sequenase II DNA Polymerase.

Sequences were aligned and manipulated using ClustalW v1.4 (Higgins *et al.*, 1994) DNAsp v3.5 (Rozas, Rozas, 1999), and Bioedit v5.0 (Hall, 1999). Phylogroups were examined through haplotype clustering using neighbor-joining (NJ – (Saitou, Nei, 1987)) Minimum Evolution (ME –(Rzhetsky, Nei, 1993)) and Maximum Parsimony (MP – (Fitch, 1971)) in Mega2 v.2.1 (Kumar *et al.*, 2001). Genetic distance was calculated using p-distance and Kimura 2-parameter (Kimura, 1980) across all populations using 1000 bootstrap replications. The genetic diversity at each population was calculated using Arlequin v2.0 (Schneider *et al.*, 2000) for nucleotide diversity (Nei, 1987; Tajima, 1983), and haplotype diversity (Nei, 1987).

Geographic structuring was further evaluated using the analysis of molecular variance (AMOVA) options in Arlequin v.2.0. The AMOVA tests whether genetic variation between groups, between populations within groups and within populations is significant. Using the AMOVA approach, we tested various alternative hypotheses suggested by geography, history and NJ-tree visualizations. Φ statistics (analogous to F-statistics) were calculated using Arlequin.

Combining the tree-making approach with a frequency distribution of pairwise genetic distances methods can illustrate where hybridization has occurred (Liebers *et al.*, 2001), and allow other inferences regarding the population history of these NJ revealed sites. A uni-modal frequency distribution suggests that the population has recently gone through a bottleneck or a founding event. A distinct, bi-modal distribution of distances (spanning 0.2 p-dist or more), this would indicate the presence of a deeply divergent mtDNA haplotype in the population (Liebers *et al.*, 2001).

Previously published allozyme frequencies (Green, 1981; Green, 1984) for seven variable loci (6PGD, CK1, EST1, GP2, IDH1, SOD, LDH1) were reanalyzed using the AMOVA approach to determine whether there was concordance between mtDNA and

mtDNA phylogeographic structure. Three populations at Long Point, and four populations in the eastern basin of Lake Erie were included in the allozyme analysis.

RESULTS

A 540 bp PCR product was amplified for 158 individuals from 21 populations. The product corresponds to region 880 – 1460 of the control region in *Xenopus laevis* (Roe *et al.*, 1985). Reference sequences have been deposited in Genbank (Accession numbers AY529737 – AY529865).

PCR products were obtained from mitochondrial rich tissue (muscle and toe clip containing muscle), displayed no sequence ambiguity and ghost bands were never observed in post-PCR agarose gel electrophoresis – all suggesting that these were not nuclear pseudogenes (Bensasson *et al.*, 2001). Indeed, no Numts have been found in Amphibia (Bensasson *et al.*, 2001).

Analyses using different distance methods were equivalent, and p-distance is presented here, as it is the more simple measure (Nei, Kumar, 2000). Toads at all sites were characterized by low genetic diversity. In only two cases was the same haplotype recorded from more than one population. With this exception, different haplotypes were found in each location or population sampled (Table 1).

All tree-building algorithms (NJ, ME, MP) produced identical phylogroups demonstrating both shallow (sequence divergence of <1%) and a deep (sequence divergence of 6%) division (Figure 1). Shallow divisions correspond to significant geographic subdivision of mtDNA variability for *B. fowleri* populations in the Lake Erie watershed (Table 2). Toads from Rondeau Ontario and Ashtabula Ohio were identical (mean pairwise $F_{st} = 0$) and comprised a southwest Phylogroup 1b. Toads collected in Ontario from populations between the mouth of the Grand River and the Niagara River formed Phylogroup 1a. Toads from Presque Isle, Pennsylvania, on the southern shore of the eastern basin of Lake Erie were next most similar to Phylogroup 1a and formed Phylogroup 1c. Long Point, Ontario, animals clustered together to form Phylogroup 2a, and were more similar to animals from Indiana, southern Ohio, and, southern Pennsylvania than any animals on Lake Erie. All *B. americanus* individuals clustered

with Phylogroup 1. This phylogroup structure explained 55% of the variation with a nearly equivalent proportion found within populations (40%). Populations within phylogroups were significantly (but minimally, ~4.5%) differentiated (Table 1). Pairwise frequency distributions of genetic distance were different from the expected Poisson distribution in all but Phylogroup 1c (Figure 2).

There was no concordance between the mtDNA and nDNA phylogeographic structure. An AMOVA using allozymes frequencies revealed that the variance between Phylogroups 1 and 2 ranged from 0-10% ($P < 0.001$), whereas variance among populations within phylogroups was higher (5.4-855.71%, $P < 0.001$). Variance within populations was very high (35.57-96.77%, $P < 0.001$).

DISCUSSION

A deep phylogeographic division, such as we have found within *B. fowleri* in the Lake Erie basin, has been explained in other species as the genetic signature of secondary contact between two previously isolated post-glacial lineages (Austin *et al.*, 2002; Vallianatos *et al.*, 2001; Zamudio, Savage, 2003). But the pattern is not so clear in these toads and other explanations may be entertained.

The deep sequence divergence in Lake Erie watershed populations of *B. fowleri* may be due to incomplete lineage sorting leading to the paraphyletic retention of ancestral haplotypes. This would indicate two colonizations of southwestern Ontario (Austin *et al.*, 2002; Holman, 1995), one from the Midwest evident in Phylogroup 2 and another from the northeast, following the receding Champlain Sea, evident in Phylogroup 1. However, this scenario is complicated by the contemporary spatial arrangement of populations. There are currently Phylogroup 1 populations of *B. fowleri* both to the east and to the west of Long Point, which is Phylogroup 2. This makes the hypothesis of parapatry complicated, for it invokes either the missed (or unsuccessful) colonization of Long Point by Phylogroup 1 mtDNA, or the initial colonization of Lake Erie from individuals to the east and the subsequent founding of the western basin populations via the Central basin south shore (approximately Pennsylvania).

Alternatively, the pattern of mtDNA distribution may reflect past hybridization with closely related *B. americanus*. The two species are known to hybridize (Green, 1984) and introgression coincident with a small population bottleneck could produce this genetic signature. Hybridization is frequent, and populations are known to fluctuate in size (Green, 1997). Fixation of introgressed mtDNA of one species within individuals from populations of another species is not new and has been demonstrated in salmonid fishes (Wilson, Bernatchez, 1998), and hylid frogs (Lamb, Avise, 1986).

Consider a population much like contemporary Long Point – intermixing *B. americanus*, *B. fowleri* and occasional F₁ hybrids. In this scenario, inter-specific mating of female *B. americanus* and male *B. fowleri* produced hybrids. After this mating, and the metamorphosis of the F₁ generation, there was an extreme population bottleneck. The bottleneck reduced the numbers of all individuals and there was no selective advantage to either species or the hybrids. Afterwards, the *B. americanus* female/*B. fowleri* male F₁ female hybrids (intermediate between the species in breeding temperature preference and timing) would be most likely to find and breed with *B. fowleri* males (Green, Parent, 2003). If founding populations at Lake Erie sites were small, and/or remained small for several generations, fixation could occur quite rapidly (Avise, Saunders, 1984). This simple chronology could lead to the fixation of the *B. americanus* mtDNA in an otherwise *B. fowleri* population. To test this hypothesis, groups in the hierarchical AMOVA were reduced to Phylogroup 1 (putative *B. americanus* mtDNA) and Phylogroup 2 (putative *B. fowleri* mtDNA). Group structure here was strongly significant and explained a large portion (56%) of the observed variation (Table 2).

Judging by the phylogeographic structure within Lake Erie, any bottleneck/hybridisation/founding event would likely have occurred prior to the recolonisation of the north shore of Lake Erie. Otherwise there would not have been enough time for the populations to drift towards the shallow geographic structuring we observed today. Indeed, the widespread nature of the hybridization suggests that it is older (Neigel *et al.*, 1991), as it is the more pervasive genotype.

Interestingly, for the small number of toads from Indiana Dunes National Lakeshore, there was also a second mode evident in the frequency histogram, but here, the Phylogroup 2 individual did not cluster with Phylogroup 1. Instead, this animal clustered with *Bufo terrestris* (data not shown). Masta et al (2002) describe the Northern, Southern and Eastern clades of *B. fowleri*. *B. fowleri* at Long Point cluster with individuals from Masta's Northern clade. The clustering of *B. fowleri* mtDNA from the southern shore of Lake Michigan with mtDNA from *B. terrestris* is likely indicative of the overlap of Masta *et al*'s Southern and Northern clades at this locality (Masta *et al.*, 2002).

The deep phylogeographic divisions between Long Point (2b) and the northeastern basin of Lake Erie (1a) revealed by mtDNA are not concordant with allozyme data. Whereas greater than 50% of the observed mtDNA variation was partitioned among the phylogroups less than 10% of the observed allozyme variation was explained by between-group differences. Here, the majority of variation was distributed among- and within-populations. If toad dispersal was sex-biased towards males (as in humpback whales – (Palumbi, Baker, 1994), this is the discordant genetic pattern we would expect – more philopatric female behavior resulting in greater geographic structuring of a matrilineally inherited genetic marker (Avise, 1995). However, as we have shown elsewhere, there is no sex bias to dispersal in *B. fowleri* (Smith and Green unpublished) and it is therefore an unlikely explanation for the discordance. Differing rates of evolution might also cause this nuclear/organelle discordance since the effective population size of nuclear genes is four times larger than for mitochondrial (Avise, 2000). The so called, three times rule (Avise, 2000) predicts that if a matrilineal tree has required time x to achieve reciprocal monophyly, then on average $3x$ time would be required for a nuclear tree to achieve the same shape. Our acceptance of this hypothesis is limited by the fact that populations sampled from Phylogroup 1 for allozymes are only from 1a, and we cannot therefore test the shallow phylogroup structure amongst 1a, 1b and 1c. The deep division between Phylogroup 1 and 2 is likely due to historic introgressive hybridization, or the “ghost of hybrids past” (Wilson, Bernatchez, 1998), and we feel that this ghost explains both the

nuclear/organelle discordance and the deep phylogeographic division within the Lake Erie watershed.

In the end, our data do not allow for the unequivocal resolution of the competing hypotheses explains the deep phylogeographic division apparent within the Lake Erie watershed. We consider the hypothesis of secondary contact between two formerly isolated post-glacial lineages to be improbable due to the geographic complexity of these hypotheses. In other cases where this hypothesis was accepted there was no evidence of one phylogroup nestled between representatives of the other – as we see with *B. fowleri*. Within Lake Erie, we consider the hypothesis of stochastic post-founder hybridization and fixation to be the more parsimonious explanation for the deep phylogeographic structure we have documented here.

The only nuclear loci for which we have information are the allozyme data from Green (1984) where there was no significant difference between our mtDNA Phylogroups 2b and 1a. Indeed, according to Avise's 3x rule (2000), we should not expect the alignment of nuclear and mitochondrial markers until there has been three times the amount of time necessary for reciprocal monophyly of mtDNA. This condition is hard to satisfy in a temperate area which has only been deglaciated for approximately 10 000 years. Phylogroup 1 and 2 represent two distinct mtDNA lineages and show a high magnitude of sequence divergence between geographically isolated populations. We consider the likelihood of demographic connection between phylogroups on ecological timescales to be highly unlikely. Therefore, Phylogroups 1 and 2 are phylogenetically distinct and deserve high conservation priority (Vane-Wright *et al.*, 1991). In a Canadian context, this is particularly true for Phylogroup 2 as it only occurs at one Canadian locality. The shallow phylogroup structure demonstrated within Phylogroup 1 should likely be used to describe management units for the conservation of this species within the Lake Erie watershed. Within Phylogroup 1a there may be enough inter-population migration to allow site recolonisation in the event of a catastrophic decline in abundance. However, Phylogroups 1b and 1c appear much more isolated. A local extinction here would likely be permanent, as we have seen at Point Pelee/Pelee Island (Green, 1989).

The similarity of the northern Ohio and Rondeau populations is likely due to a founder effect, and not the regular transport of many individuals via over 100km of open lake water.

B. fowleri populations on the north shoreline of Lake Erie have a precarious existence. Our work indicates that their long viability is even less certain. The Long Point population is significantly different from the remainder of the Lake Erie populations close enough to provide immigrants that would forestall any local extinction. Toads at this peninsula are a unique lineage and protection reflecting the unique and distinct nature of this population is warranted.

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FIGURE HEADINGS

Figure 1:

Neighbor-Joining tree of sequence divergence (p-distance) from control region mtDNA of *B. fowleri* of populations from the northern edge of their distribution. 1000 bootstrap values >80% are shown.

Figure 2:

Comparative illustration of mtDNA phylogeography for *B. fowleri* on Lake Erie. On the right is the paired NJ-tree and frequency histogram for all Lake Erie populations. On the left are the paired images of the neighbor-joining tree and the frequency histogram of pair-wise distances for that branch. X-axis on frequency distributions is made of 0.5% bins of sequence divergence (p-distance). Phylogram scale bars show p-distance.

Figure 3:

Comparative map of mtDNA phylogeography for *B. fowleri*. Map shows the divisions of groups into Phylogroups 1a, 1b, 1c, and 2a and 2b. The geographic location of each population is the tip of the appropriate branch of the neighbor-joining tree. Branch length is not proportional to distance.

TABLE

Table 1:

Measures of genetic diversity for Lake Erie Fowler's toad populations.

<i>Site</i>	<i>Phylogroup</i>	<i>Sample size</i>	<i>No. haplotypes</i>	<i>Polymorphic loci (S)</i>	<i>Mean number of pairwise differences between haplotypes (d)</i>	<i>Tajima's D</i>	<i>Haplotype Diversity</i>	<i>Sequence Diversity $\pi = n_d / n$</i>
Ashtabula	1c	4	4	29	17.16666	-0.40253	1	0
Crystal Beach	1a	6	6	36	15.81	1.25057	0.9091	0.00652
James N Allan	1a	11	11	50	14.038055	-0.20353	0.9302	0
Kraft Road	1a	10	9	48	15.102041	-0.41158	0.9184	0
LP Anderson	2b	10	11	67	20.867796	0.31265	0.9153	0.00054
LP Big Creek	2b	11	11	71	19.141148	0.3094	0.9211	0.01184
LP Crown	2b	10	10	90	28.67848	1.75498	0.9114	0.00121
LP Hastings	2b	10	10	59	15.006742	0.07275	0.9101	0.00121
LP Thoroughfare	2b	11	11	40	13.944954	0.4357	0.9174	0.00099
Point Abino	1a	13	13	57	19.91464	0.54202	0.929	0.00148
PresQueIsle	1b	10	10	63	14.691824	0.11908	0.9057	0.01196
Rondeau	1c	11	11	45	14.706745	1.31581	0.914	0
Windmill Point	1a	10	10	63	23.537689	0.53559	0.9045	0.00163

Table 2:

Analysis of molecular variance of Fowler's toad populations. Ten models representing different groupings of populations were tested, and the two models explaining the most variation are presented. Model I are the groups revealed by the NJ tree in Figure 1. Model II is a test of the deeper Phylogroups 1 and 2.

Model	Populations in Groups		Variance Component	% variance explained
I	Phylogroup 2a, Phylogroup 1a, Phylogroup 1b, Phylogroup 1c	Among Groups	Φ_{CT} 0.5536	55.36 P < 0.00001
		Populations within Groups	Φ_{SC} 0.10201	4.55 P < 0.00001
		Within Populations	Φ_{ST} 0.59914	40.09 P < 0.00001
II	Phylogroup 1 and 2	Among Groups	Φ_{CT} 0.56095	56.09 P < 0.00001
		Populations within Groups	Φ_{SC} 0.26229	11.52 P < 0.00001
		Within Populations	Φ_{ST} 0.67611	32.39 P < 0.00001

FIGURE 1

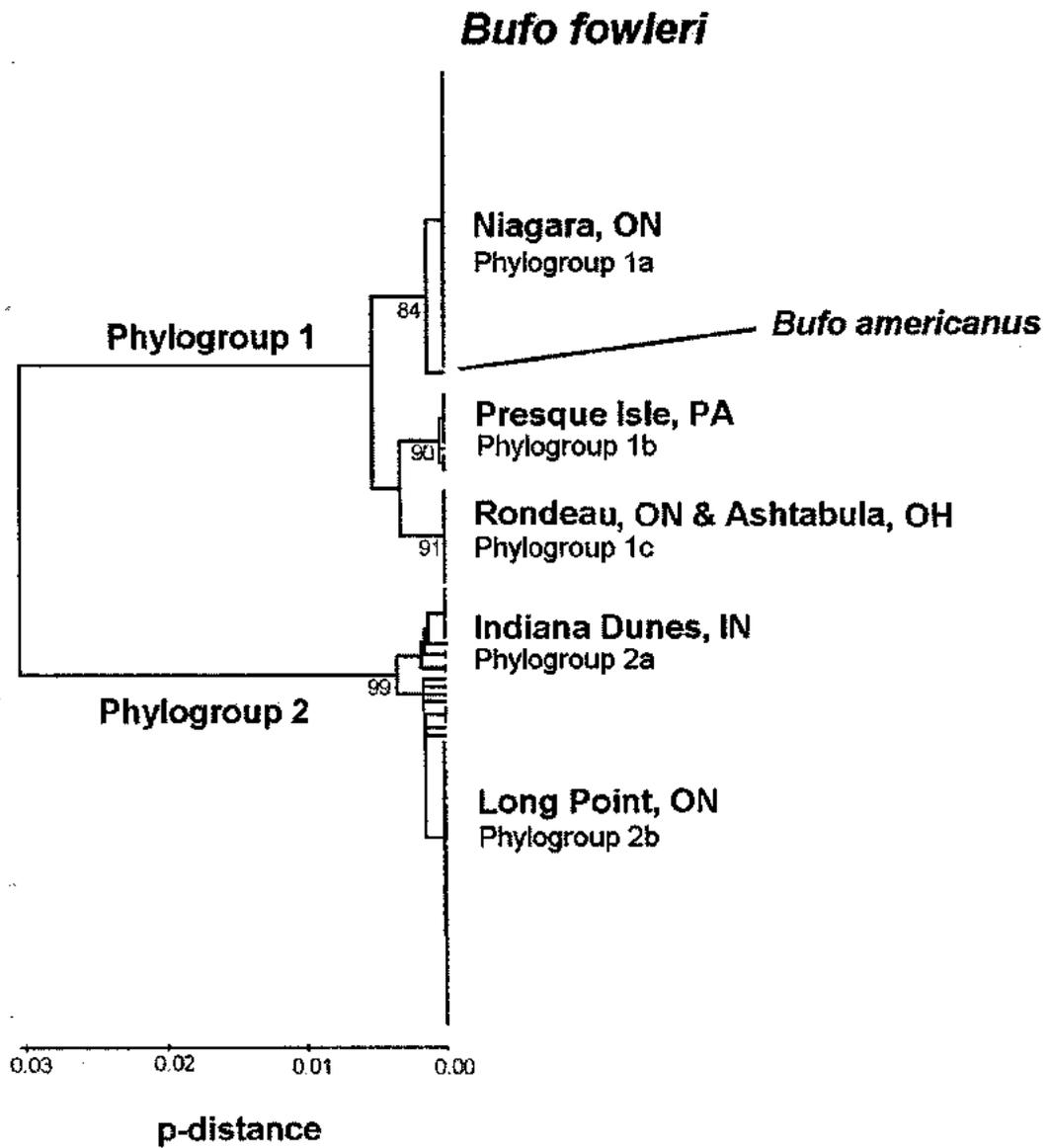


FIGURE 2

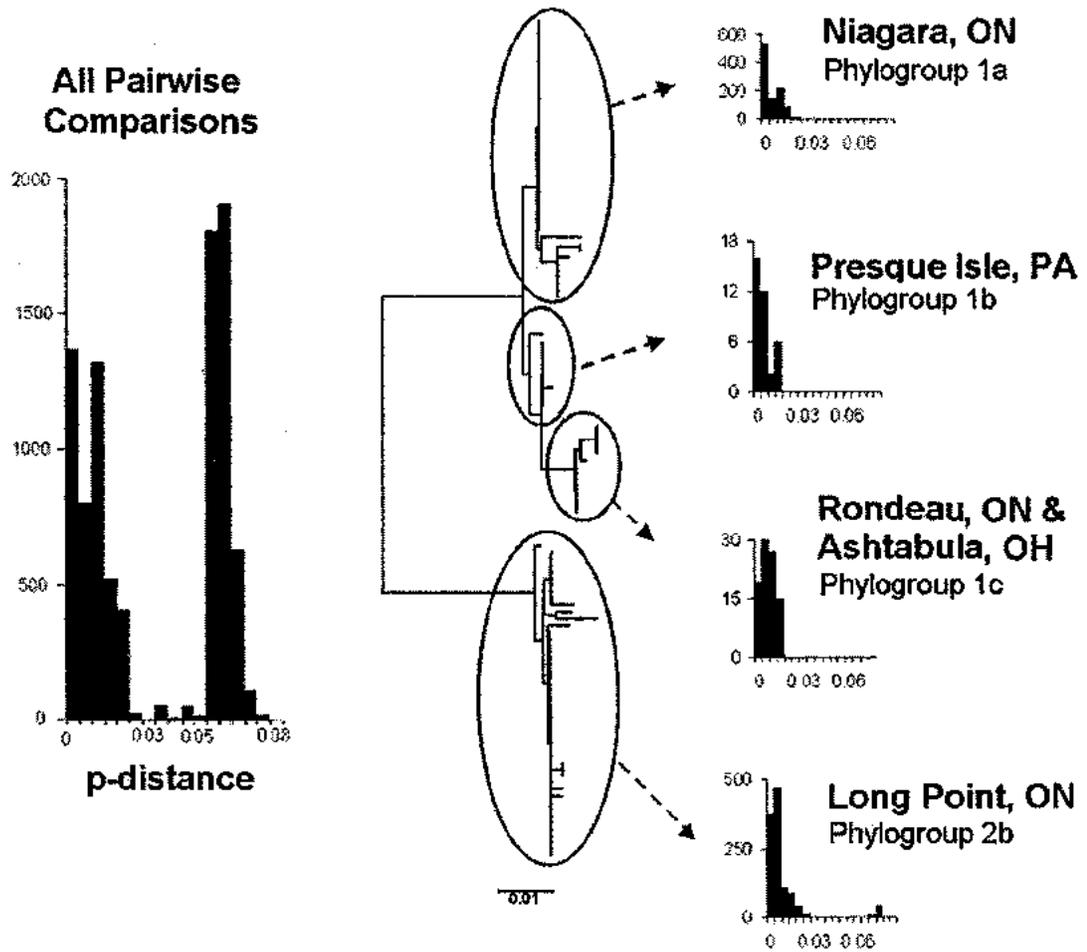
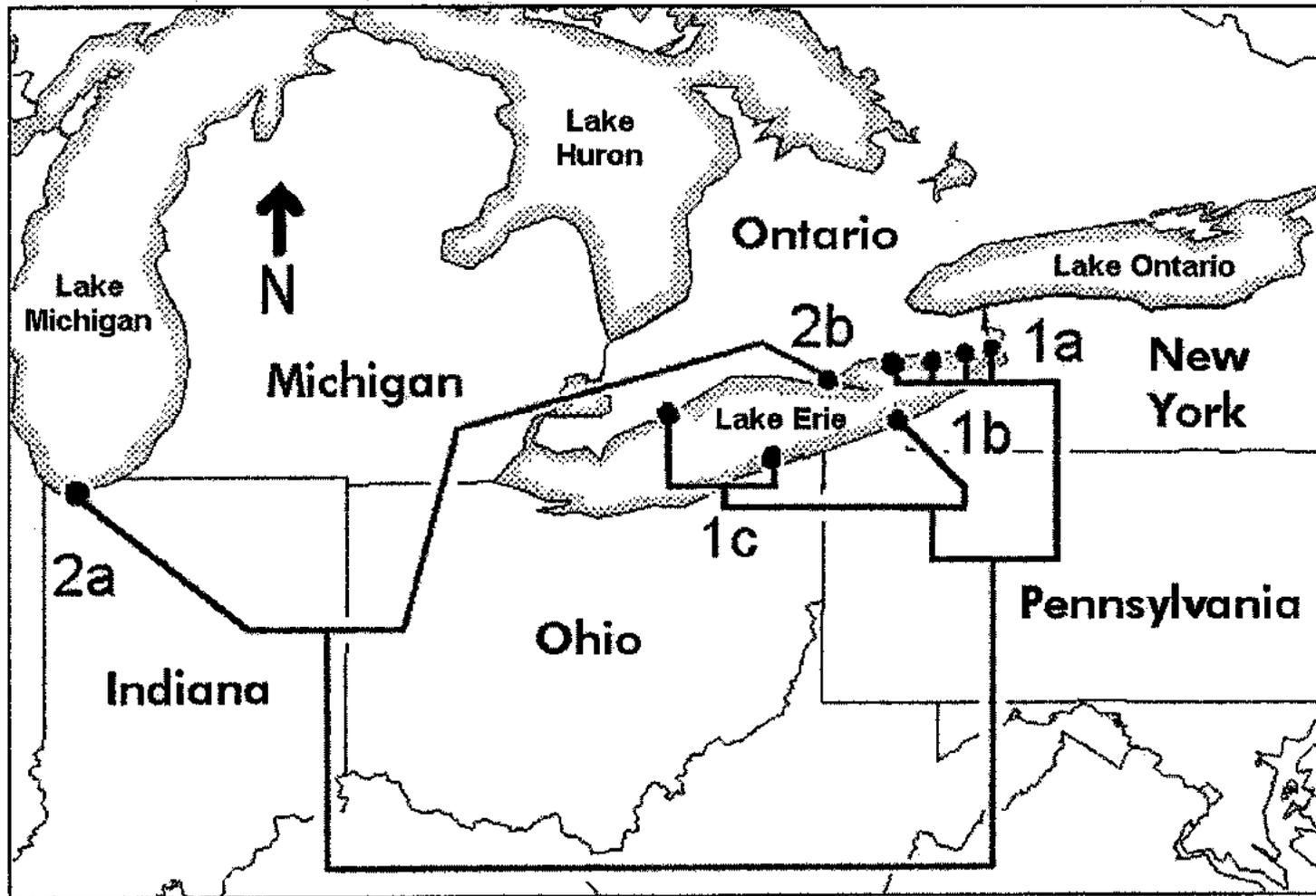


FIGURE 3



APPENDIX 1:

Tissue sample collection information.

SITE DESCRIPTION	Latitude	Longitude	N	Accession Number	GenBank accession numbers
Thoroughfare Beach at Long Point Ontario	N42.576184	W080.374029	10		AY529737, AY529738, AY529743, AY529742, AY529762, AY529763, AY529764, AY529778, AY529780, AY529808, AY529760, AY529761, AY529767, AY529766,
Hastings Beach at Long Point Ontario	N42.577043	W080.447500	9		AY529768, AY529779, AY529806, AY529809, AY529810 AY529745, AY529746, AY529748, AY529747,
Big Creek at Long Point Ontario	N42.573738	W080.536957	10	RM 4473-4478	AY529749, AY529797, AY529798, AY529799, AY529846, AY529864 AY529739, AY529740, AY529750, AY529744,
Crown Marsh at Long Point Ontario			9		AY529752, AY529758, AY529759, AY529777, AY529796 AY529753, AY529754, AY529756, AY529755,
Anderson Property, south beach, Long Point tip, Ontario	N42.540725	W080.105915	10	RM 4479-4493	AY529757, AY529770, AY529801, AY529802, AY529803, AY529804

SITE DESCRIPTION	Latitude	Longitude	N	Accession Number	GenBank accession numbers
Rondeau Provincial Park Ontario	N42.260483	W081.905983	10	RM 4496-4505	AY529775, AY529776, AY529795, AY529794, AY529807, AY529814, AY529815, AY529830, AY529831, AY529845 AY529773, AY529800, AY529818, AY529811, AY529825, AY529861, AY529862, AY529863, AY529771
Windmill Point Ontario	N42.875900	W078.999397	9		
Port Burwell Ontario	N42.587873	W080.403630	3	NMC15771, NMC15777, NMC16914	
Rock Point Ontario	N42.841106	W079.547845	1	NMC21953	
Point Pelee Ontario	N41.75726	W082.63228	6	NMC543-4, NMC4981-2	AY529865, AY529865
Turkey Point Ontario	N42.67744	W080.32874	1	ROM 5586-8	
Point Abino Ontario	N42.51415	W079.5545	10		AY529751, AY529836, AY529838, AY529837, AY529839, AY529841, AY529842, AY529844, AY529848, AY529858 AY529781, AY529783, AY529788, AY529787, AY529821, AY529849, AY529850, AY529851, AY529852, AY529853
James N Allan Provincial Park Ontario	N42.841106	W079.547845	14		

SITE DESCRIPTION	Latitude	Longitude	N	Accession Number	GenBank accession numbers
Crystal Beach Ontario	N42.862167	W079.053353	6		AY529772, AY529774, AY529816, AY529805, AY529820, AY529854 AY529812, AY529813, AY529819, AY529817, AY529822, AY529823, AY529843, AY529847, AY529855 AY529790, AY529792, AY529826, AY529793, AY529828, AY529829, AY529834, AY529835, AY529856, AY529857
Kraft Road Ontario	N42.879746	W078.951236	10		AF462519
Indiana Dunes National Lakeshore Indiana	N41.618496	W087.208496	11	RM 4468-72	AF462520
Rosa County Ohio	N39.3286	W083.0598	1	SEM 2306	AF462513
Union County Ohio	N40.3050	W083.3745	1	SEM 2307	AY529789, AY529791, AY529833, AY529832 AY529741, AY529769, AY529784, AY529782, AY529785, AY529786, AY529824, AY529827, AY529840, AY529859
Leigh County Pennsylvania	N40.6144	W075.5899	1	SEM 2283	
Ashtabula Ohio	N41.901303	W080.809760	4	RM 4494-5	
Presquisle State Park Pennsylvania	N42.10171	W080.6402	10		
<i>Bufo terrestris</i> Oklahoma	-	-	1	DMG 2305	
<i>Bufo americanus</i> Ontario	-	-	10	ROM 21664	AF190229
<i>Bufo americanus</i> Quebec	-	-	1	RM 2681	

RM = Redpath Museum
NMC = Canadian Museum of Nature
ROM = Royal Ontario Museum
SEM = Susan E. Masta collection
DMG = David M. Green collection

**CHAPTER 6: ISOLATION BY DISTANCE AND GENETIC NEIGHBORHOOD
IN THE FOWLERS' TOAD (*BUFO FOWLERI*) AT ITS NORTHERN RANGE
LIMIT**

**Keywords: isolation by distance, amphibian, mtDNA, genetic neighbourhood, *Bufo
fowleri***

2500 words, 2 tables, 2 figures, 33 references and 1 appendix

PREFACE TO CHAPTER 6

Having demonstrated the phylogeographic structure of *Bufo fowleri* genetic variability in the Lake Erie watershed, I test whether there is evident isolation-by-distance between all populations. Additionally, I test whether estimations of genetic neighbourhood size are larger or smaller than the observed distances between ponds at Long Point. If neighbourhood size estimates are larger than the inter-pond distances it suggests that the metapopulation effect is not significant and seemingly isolated ponds are in fact a single population. The existence of some type of metapopulation would be supported by neighbourhood size estimates that are smaller than inter-pond distances.

Reference style is according to submission requirements for Molecular Ecology.

ABSTRACT

Fowler's toads (*Bufo fowleri*) are common to eastern North America and reach the northern periphery of their range on Lake Erie in southern Ontario, Canada. Using mitochondrial control region sequence data from Lake Erie toads we tested the hypothesis that individuals from isolated populations at their northern range edge would exhibit increasing genetic difference in correlation with geographic separation, i.e. isolation-by-distance (IBD). To determine whether disjunct breeding assemblages are likely to form metapopulations we tested the size of genetic neighborhood directly and indirectly using genetic and demographic data. We found that populations exhibited significant IBD whether geographic isolation was measured by terrestrial or aquatic-downstream distance. Pair-wise distances among regions (roughly coincident with the basin structure of Lake Erie) drive the IBD trend – for within each region there is little variation across many kilometers. Genetic homogeneity across distances up to 50 km may be due to the regular dispersal of individuals or the consequence of small founding populations. Breeding assemblages are not functioning as metapopulations, as both genetic and demographic estimates of neighborhood size are larger than the disjunct breeding populations.

INTRODUCTION

Despite rigorous attempts in the field to quantify long distance movement with mark-recapture methods, such direct measures of dispersal have an inherent constriction – all field sites end (Baker *et al.*, 1995; Slatkin, 1985; Slatkin, 1993). Therefore, indirect measures of historic dispersal, or gene flow, are used to estimate the rate and distance of rare long-distance dispersal (Slatkin, 1985). On a regional scale, population connectivity or isolation can be inferred from this information. For instance, if high rates of movement for a particular species, on a local scale, “scale- up” to the regional level, frequent dispersers should swamp any population on a local trajectory and there should be no significant relationship between genetic and geographic distance (isolation by distance –IBD). Alternatively, increasing geographic distance will cause increasing genetic distance at neutral loci if movement between populations is infrequent (Wright, 1943). Genetic estimates of dispersal are effective measures (movement plus reproduction); not just the ability to disperse (as in direct mark-recapture methods). Thus, genetic estimates of dispersal are more sensitive to long-distance dispersal than are direct methods (Slatkin, 1985). Amphibians particularly (Blaustein *et al.*, 1994; Sinsch, 1990), and especially at their northern range edge (Green *et al.*, 1996), are expected to show IBD on relatively small spatial scales. Their populations are often disjunct demes between which movement (through both direct and indirect estimates) appears rare and predominantly short distance. However, although characterized in general as being of low vagility and high site fidelity, direct measures of dispersal with one species (Fowler’s toad (*Bufo fowleri*)) led us to suspect that this species moves farther than the poorly-dispersing-amphibian generalization leads one to expect (Chapter II). Specifically, the movement frequency distribution we documented was an inverse power function, where although most individuals did not move very far, some moved quite far, causing a tail at the end of the distribution (Chapter II). These occasional long distance dispersers are the individuals who would connect populations separated by tens of kilometers within the Lake Erie watershed. We investigated the null hypothesis that individual populations in the Lake Erie watershed are isolated enough for the effects of genetic drift to have created

a situation where there is evident isolation-by-distance with an equilibrium between gene flow and genetic drift. Alternatively, the long distance dispersal we have characterized elsewhere with mark-recapture data will cause genetic homogeneity over relatively large distances.

Long distance dispersal is also related to functional difference between a patchy population (where resources are not homogenously distributed across a landscape and dispersal does not limit between patch movement) and a metapopulation (where resources are not homogenously distributed and dispersal between these resources is limited). Elsewhere, we presented evidence that what initially appeared to be a metapopulation of *B. fowleri* at Long Point was more likely a single population where breeding aggregations were patchily distributed (Chapter IV). Wright's consideration of a genetic neighborhood, or area of panmixis (Wright, 1969), has been used as a tool to differentiate between patchy populations and metapopulations (Driscoll, 1999). If the genetic neighborhood spans several apparently disjunct breeding aggregations, then such patchy aggregations can likely be considered one population and the use of metapopulation theory would be inappropriate. If, however, the genetic neighborhood is roughly coincident with the isolation of patchy breeding aggregations, then each population is experiencing a degree of isolation that suggests that the population network is functioning as a genetic metapopulation (Driscoll, 1999). We tested this hypothesis with two measurements of the genetic neighborhood - one genetic and one demographic.

MATERIALS AND METHODS

Samples were collected from extant Lake Erie populations (Figure 1, Appendix 1). Tissues were predominantly toe clips from adults, but also included muscle, heart and liver tissue from juveniles. Tissues were preserved in 70% ethanol and total DNA extractions were performed according to (Fetzner, 1999). The highly variable control region was amplified using published primers (Goebel *et al.*, 1999). Thermocycling conditions (Robocycler) for double-stranded DNA (dsDNA) amplification began with 39 cycles each of which had a 30-s denaturation at 94°C, a 45-s annealing at 52°C, and a 1.5-min extension at 72°C. A 10-min extension at 72°C followed the final cycle. Reaction

mixtures for PCR contained 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 0.4 mM of each oligonucleotide, 2.5 mM MgCl₂, 0.5 U/100 µl Taq DNA polymerase (Sigma), and 0.1 µM each primer in a reaction volume of 25 µl. Amplified DNA was sequenced using Amersham Biosciences DYEnamic ET Terminators Chemistry Cycle Sequencing System with Thermo Sequenase II DNA Polymerase.

Sequences were aligned and manipulated using ClustalW v1.4 (Higgins *et al.*, 1994) DNAsp v3.5 (Rozas, Rozas, 1999), and Bioedit v5.0 (Hall, 1999). Genetic distance was calculated using p-distance and Kimura 2-parameter (Kimura, 1980) for all populations using 1000 bootstrap replications. Arlequin v2.0 (Schneider *et al.*, 2000) was used to calculate pairwise values of F_{st} and N_m .

Isolation by distance was tested by comparing the pairwise matrix of genetic distance ($\log(F_{st})$ or p-dist) against a pairwise measure of geographic separation (Euclidean distance, $\log(\text{km})$) with a Mantel test (Mantel, 1967) in Arlequin. Additionally, we tested the strength of the association when geographic separation was measured as contemporary downstream aquatic or contemporary upstream aquatic distance via the predominant large-scale currents (Hamblin, 1971; Saylor, Miller, 1987). In this analysis, we omitted pairwise values of $F_{st} = 0$. Such a strategy thus treats these localities as a single population - justified by the lack of detectable subdivisions (Barber, 1999).

Gene flow rates were estimated using the regression method of Slatkin (1993) where the log of gene flow (Slatkin's N_m statistic ($N_m = (1/F_{st}-1)/2$), which approximates the number of female migrants per generation (Slatkin, 1993)) is regressed on the log of distance. Values of N_m equal to, or greater than, 1 are generally considered sufficient to overcome the effects of genetic drift, and to therefore prevent population differentiation (Slatkin, Barton, 1989). We compared pairwise values of N_m to Euclidean distance for all populations, and for among- and between- major regions of Lake Erie.

Two methods were used to estimate the size of the genetic neighborhood. Method 1 followed Slatkin (1993) where the intercept of the regression of $\log(N_m)$ on $\log(\text{distance})$ is a rough estimate of the neighborhood size. Method II follows Wright

(1969) by directly calculating the radius of an area of panmixis (i.e. genetic neighborhood) using the equation:

$$2R = 2 \sqrt{(4/3S^2T)},$$

Where S^2 is the variance of dispersal (calculated for between site and known exact locations between captures) and T is the generation time of the individual (estimated as two years). For Method 1, the estimate of genetic neighborhood is given in number of individuals, and we compared this number to the estimated number of individuals per kilometer of beach at Long Point for different years from 1988 to 2000 (Table 2, Data from Green unpublished). For Method II the estimate is given in meters and was also compared to the observed number of adults at Long Point over one kilometer.

RESULTS

A 540 bp PCR product corresponding to base pairs 880 – 1460 of the control region in *Xenopus laevis* (Roe *et al.*, 1985) was amplified for 158 individuals from 21 populations. Sequences obtained were deposited in Genbank (Ascension numbers AY529737 – AY529865) (Appendix 1).

Isolation by distance was demonstrated within Lake Erie for all populations excluding Long Point. Mantel tests between genetic distance ($\log(F_{st})$ and p-dist) and geographic distance (Euclidean, $\log(\text{km})$) demonstrated a significant positive relationship ($r = 0.817$, $p = 0.005$). Additionally, the relationship between $\log(F_{st}/(1-F_{st}))$ and $\log(\text{distance})$ (Rousset, 1997) was significant ($r = 0.68$, $p = 0.001$). Contemporary average currents are circular and run counter-clockwise within the Lake Erie basins (Hamblin, 1971; Saylor, Miller, 1987), and when geographic separation was modeled relative to currents, Mantel tests between p-dist and either upstream or downstream distances were both significant (downstream $r = 0.80$, $p = 0.002$; upstream $r = 0.69$, $p = 0.01$).

There was a strongly negative relationship between distance and N_m ($r = -0.660545$, $p = 0.0039$) (Figure 2a), and this relationship was driven by the difference between the among-region and the within-region comparisons (Figure 2b).

For all Lake Erie (Long Point included), the neighborhood size calculated using Slatkin's (1993) method (the intercept of a regression of N_m and km) was approximately 445 individuals. When only extant sites on Lake Erie were included, and Long Point excluded, the approximate neighborhood size was 1374 individuals. When only individuals from the North East basin were included, the approximate neighborhood size was 456 individuals. The diameter of Wright's (1969) genetic neighborhood estimated using differentially corrected GPS measured mark-recapture data was 12.42km. Using recapture distances measured between-sites the diameter was 4.01km. Converting both Method I and Method II estimates to meters (Table 2) the maximum estimate of neighborhood size was 37km, the minimum was 0.8km, and the mean value was 7km.

DISCUSSION

Using sequence data from the highly variable control region of the mitochondrion we have shown that the patchily distributed *B. fowleri* populations of the Lake Erie watershed: 1) have apparent IBD between three primary regions (Niagara, Northern Pennsylvania, Southwestern Basin) when geographic distance is measured by aquatic or terrestrial separation, 2) have genetic homogeneity over greater than 50km of shoreline distance, and 3) have genetic neighborhood sizes which are generally larger than the size of the disjunct breeding assemblage.

When the geographic structuring of nuclear variability for *B. fowleri* across Lake Erie was estimated using allozymes (Green, 1984), no significant correlation between genetic and geographic distance was detected. However, using the increased resolution of the mtDNA control region, we have found significant IBD amongst Lake Erie *B. fowleri* populations. Yet, the pattern of IBD is neither simple, nor consistent across the entire geographic area we surveyed. The significant relationship between genetic distance and geographic separation contains two trends. There is significant IBD between the larger geographic areas of 1) Presque Isle, Pennsylvania, 2) Ashtabula, Ohio and Rondeau, Ontario, and 3) Niagara County, Ontario (Figure 2). However, no IBD is apparent within each area. Thus the among-group distances drive the apparent IBD trend (Figure 2b), for the within-group genetic variation is homogenous over relatively large spatial scales.

Thus either demographic (small-founding population) or genetic (population bottleneck) events could have reduced the genetic variability within each population group.

Alternatively, appreciable numbers of individuals are dispersing among populations each generation (Figure 2).

Species with limited dispersal should exhibit IBD if there has been enough time to reach equilibrium - no IBD, with low values of N_m , can indicate the lack of ongoing gene flow (Slatkin, 1993). No IBD with high values of N_m can suggest that a species has recently colonized an area and has subsequently had low abundance (Slatkin, 1993). Within the Niagara region values of N_m are generally greater than 1 across distances of greater than 50km suggesting recent colonization and fluctuating abundance - not the absence of gene flow. Indeed, direct estimates of movement using mark-recapture (Chapter II) suggested that this species of toad can move much farther than is commonly thought possible for amphibians. However we cannot reject the alternative hypothesis, that pairwise distances within each region are a function of a small founding population (Leblois *et al.*, 2000; Rafinski, Babik, 2000; Raybould *et al.*, 1996).

We were not able to exclude the possibilities of terrestrial, aquatic-upstream or aquatic-downstream connections between populations. Comparing Mantel tests for the strength of association between terrestrial and aquatic distance between populations has not allowed us to discount either measure of geographic separation. However, we hypothesize that movement among populations occurs in the Eastern Basin population it is at least partially aquatic. In the Eastern Basin of Lake Erie, due to anthropogenic development, dune ponds are not as available a habitat for breeding toads. Indeed, we frequently observe breeding activity and fertilized eggs in the lake (Smith and Green unpublished). Between toad breeding populations the landscape is highly developed, and the shoreline has been reinforced and altered. In this case, we envisage contemporary dispersal between populations along the shoreline to be highly unlikely. Rather, we speculate that the individuals dispersing between Eastern Basin populations are eggs, tadpoles or adult toads carried via the predominantly west to east currents along the Canadian shoreline (Hamblin, 1971; Saylor, Miller, 1987). Toads have been observed to

withstand 5-7 days floating (Schmid, 1965), or submersion (Czopek, 1962). Aquatic connections appear more likely among these populations since all life history stages of the animal are open to passive transport via lake currents. Conversely, at Long Point the aquatic transport of individuals would likely be restricted to post-metamorphosis individuals as eggs are laid, and tadpoles develop, in dune ponds generally disjunct from the lake (Smith and Green, unpublished).

Estimates of the size of the genetic neighborhood vary between years using Method I, and vary between Methods I and II, as we would expect with a species whose abundance fluctuates so widely (Green, 1997; Green, 2003). However, all estimates are consistently large enough to encompass all, or nearly all, of the 19-habitat patches studied at Long Point. Thus the populations observed at Long Point are likely patchy, as the estimates of neighborhood size include many apparently disjunct populations. This tends to support conclusions made elsewhere (Chapter IV) that the asynchronous dynamics reflective of an ecological metapopulation are prevented by the apparently large capability for animals to move frequently between the disjunct populations at Long Point. Patchily distributed breeding assemblages are essentially homogenous on a genetic scale at the resolution of the mitochondrion.

Using highly variable mtDNA control region sequence data we have shown here that there is apparent IBD between three primary Lake Erie regions (Niagara, Northern Pennsylvania, Southwestern Basin) when geographic distance is measured by aquatic or terrestrial. The size of genetic neighborhood is generally larger than the size of the disjunct breeding assemblage, which does not support the existence of metapopulation structure at Long Point. Together these results reflect the underlying complexity behind the relatively simple questions of population isolation and gene flow within temperate watersheds.

ACKNOWLEDGEMENTS

Many students and volunteers provided the field workforce crucial to and responsible for the collection of this data between 1988 and 2002. MAS gives special thanks to Elaine Bazinet Smith, Clark Smith and Alison Judd for support and encouragement. The Ontario Ministry of Natural Resources, Canadian Wildlife Services and Ontario Parks all granted permission to conduct research at Long Point. We thank in particular John Marchington and the staff at Long Point Provincial Park, Paul Ashley, Jeff Robinson and the Big Creek Unit of the Canadian Wildlife Service, David and Ann Judd, and Ed Dirse for assistance in the field throughout the duration of this field study. S. Masta generously shared tissue; A. Goebel was helpful in answering questions early on. We gratefully acknowledge the support of the Natural Sciences and Engineering Council (NSERC) and the World Wildlife Fund Canada Endangered Species Recovery Fund for grants to DMG and the Mountain Equipment Co-op Environment Fund, McGill University, NSERC and Fonds Nature et Technologies for grants and scholarships to MAS.

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FIGURES

Figure 1:

Extant *Bufo fowleri* populations within Lake Erie.

Figure 2:

a) Genetic estimates of migration between population pairs of *B. fowleri* within the Lake Erie watershed ($\text{Log}(N_m)$ vs $\text{Log}(\text{km})$). Pairwise comparisons with values of N_m less than 1 are generally considered sufficient for the accumulation of genetic drift while values greater than 1 there is often a homogenizing effect of gene flow.

b) Within-region values of N_m are filled circles, among-region comparisons are open circles.

TABLES

Table 1:

Pairwise distance matrix for extant Lake Erie *Bufo fowleri* populations. Pairwise Euclidean geographic distance in kilometers are above diagonal. Pairwise values for the F_{st} estimator, Φ_{st} are the below diagonal.

	Ashtabula OH	Niagara 1	Niagara 2	Niagara 3	LP 1	LP 2	LP 3	LP 4	LP 5	Niagara 4	Presqueisle PA	Rondeau ON	Niagara 5
Ashtabula OH		179.57	141.34	187.84	92.084	78.301	82.463	80.952	83.209	176.64	65.919	98.669	184.26
Niagara 1	0.5512		49.282	8.8533	93.008	124.52	115.41	118.09	112.47	3.237	115.11	242.97	4.8775
Niagara 2	0.805	0.0583		57.955	49.595	77.122	68.341	70.955	65.713	46.084	83.126	195.27	53.947
Niagara 3	0.8595	0.1004	0.1377		101.8	133.38	124.26	126.94	121.32	12.085	123.08	251.82	4.0204
LP 1	1.6552	1.6407	1.885	2.0045		34.515	25.521	28.055	22.186	89.836	41.398	151.05	97.882
LP 2	1.5697	1.4597	1.6133	1.7982	0.1562		9.2424	2.9179	12.462	121.29	56.636	118.46	129.38
LP 3	0.773	0.8027	1.0082	1.0961	0.1109	0.1506		2.6924	3.3387	112.18	52.334	127.56	120.27
LP 4	1.9565	1.888	2.0432	2.1267	0.128	0.277	0.0857		5.91	114.86	53.232	124.88	122.95
LP 5	2.4795	2.3521	2.4685	2.5849	0.0878	0.1817	0.1271	0.1039		109.24	50.197	130.53	117.33
Niagara 4	0.5348	0.0372	0.0617	0.0942	1.709	1.4552	1.0398	1.678	1.9817		112.34	239.74	8.0895
Presqueisle PA	0.4139	0.295	0.4681	0.6658	1.7015	1.45	0.9139	1.5537	1.9144	0.4292		148.88	119.66
Rondeau ON	0	0.5802	0.7715	0.8654	2.181	1.9467	1.256	1.9767	2.4185	0.6369	0.4286		247.83
Niagara 5	0.4975	0.0628	0.0828	0.1137	1.5201	1.3324	0.9905	1.4966	1.7675	0.0404	0.4426	0.6422	

Table 2:

a) Estimating the size of the genetic neighborhood using genetic and demographic information in Method 1.

Method I	Intercept of $\log(N_m)$ and $\log(km)$ for all populations on Lake Erie	Estimated Number of females per km of beach	Estimated Genetic Neighbourhood (m)
Year	445		
1988		12	37087
1989		54	8241
1990		248	1795
1991		487	914
1992		367	1213
1993		515	864
1994		115	3870
1995		49	9082
1996		47	9469
1997		33	13486
1998		223	1996
1999		125	3560
2000		89	5000

Table 2 continued:

b) Estimating the size of the genetic neighborhood using genetic and demographic information in Method II.

Method II	Recapture distance measured by DGPS (N=250)
Mean Dispersal Distance (m)	981.6593
Variance in Dispersal Distance (m)	1.44E+07
Generation Time (years)	2
Diameter of Panmictic Circle (m) (Estimated genetic neighbourhood)	12412

Figure 1:

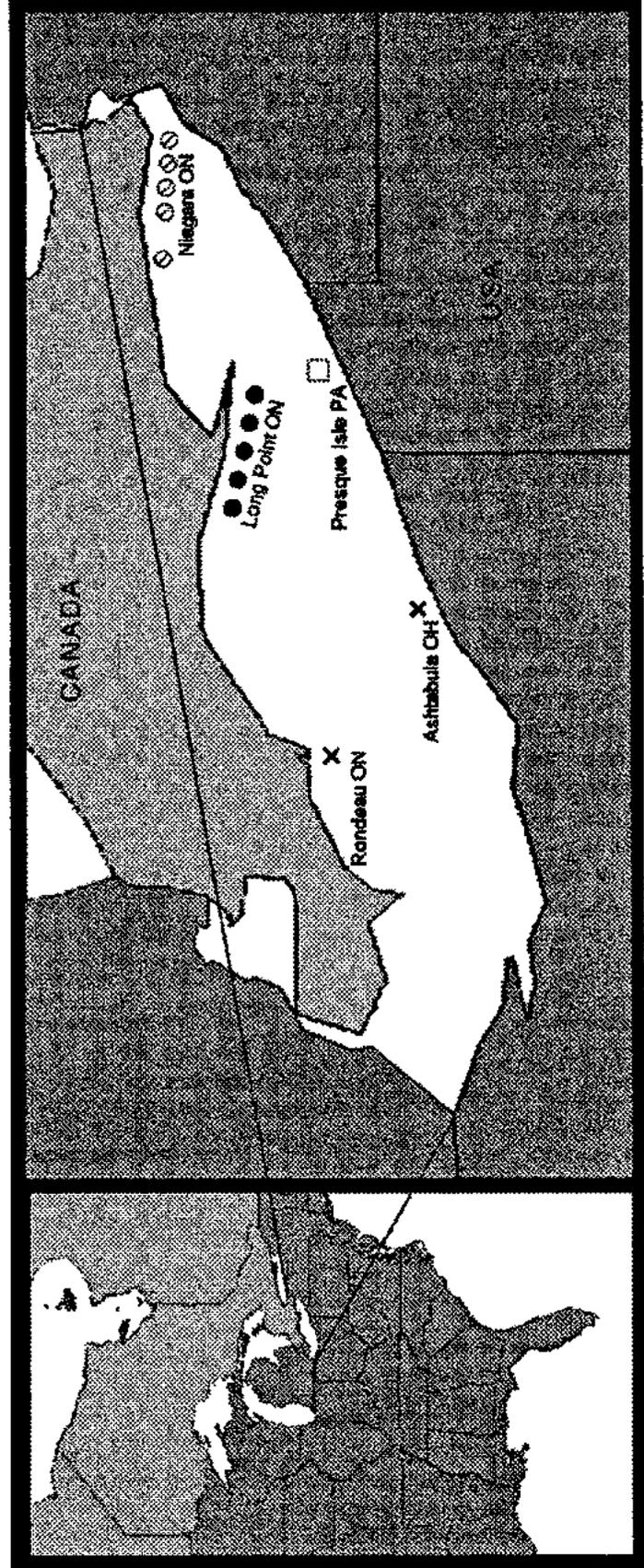
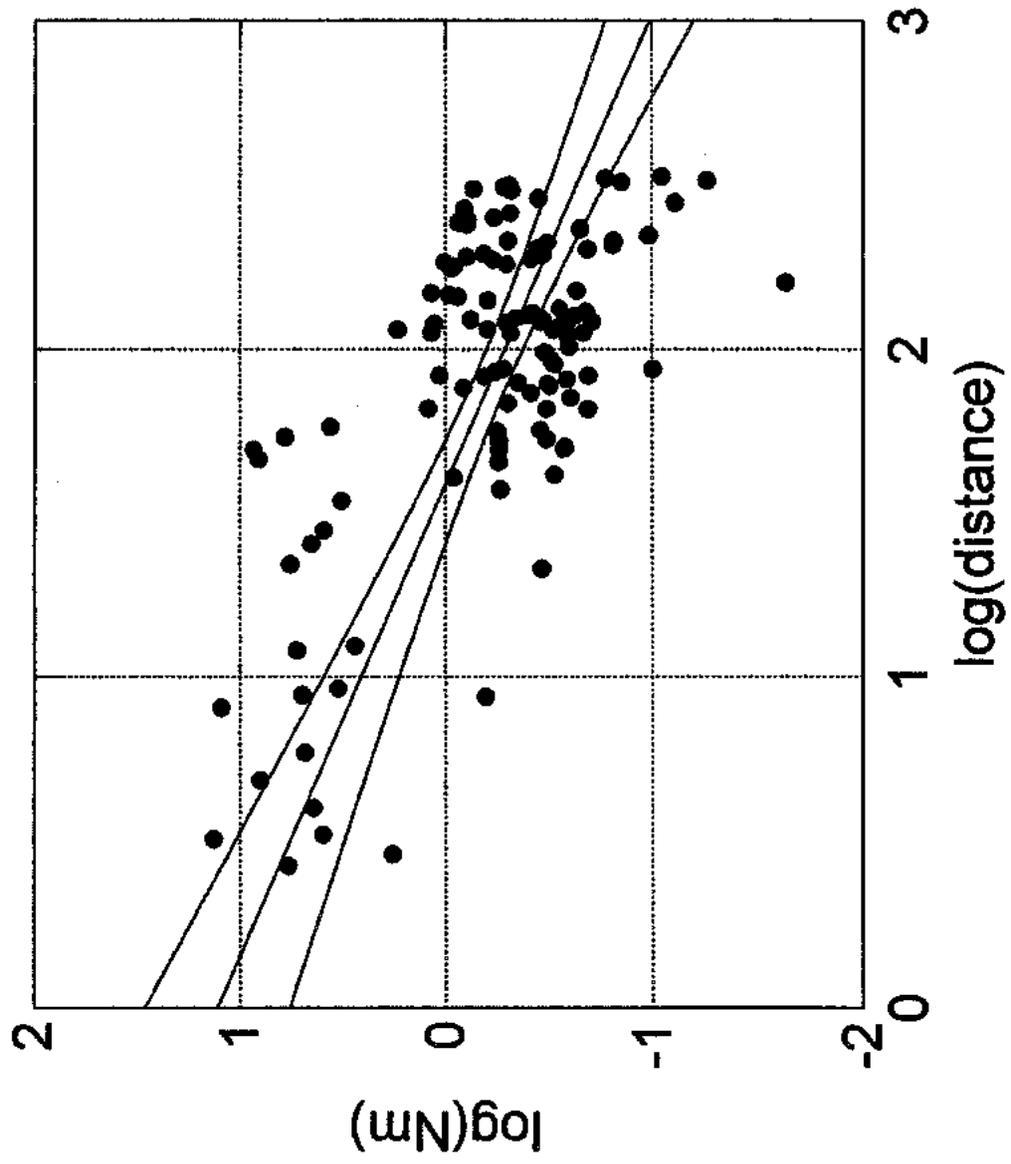


Figure 2:
2a



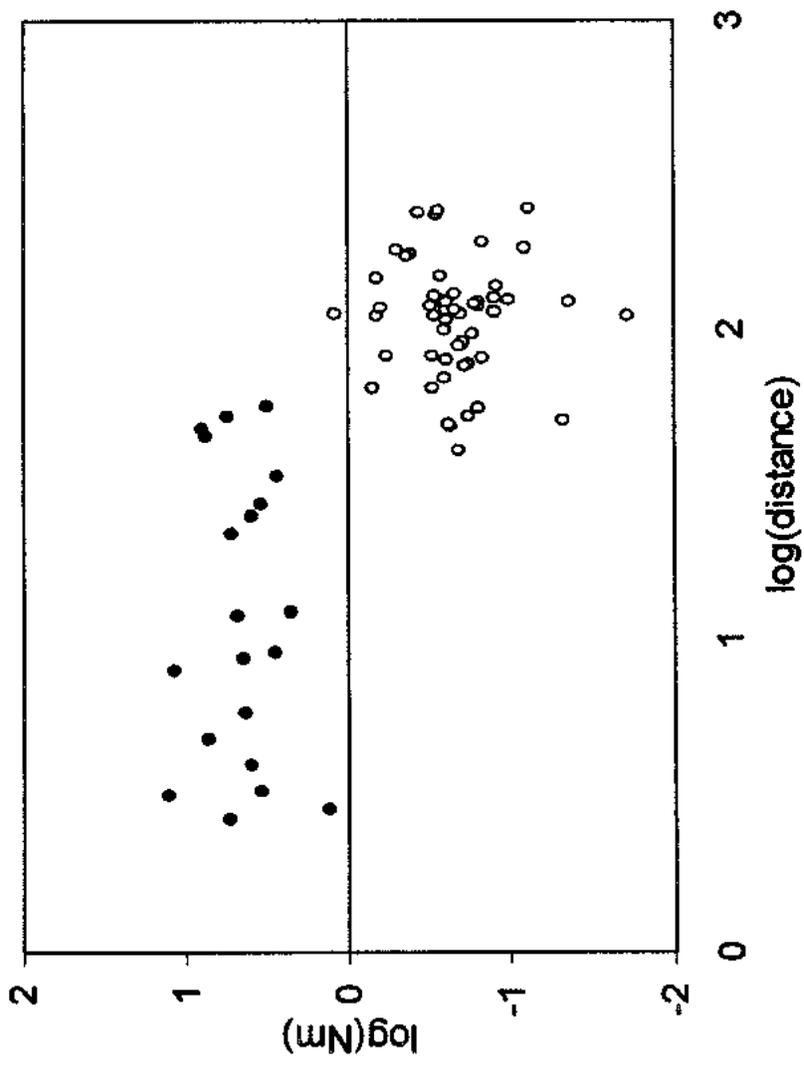


FIGURE 2b

Appendix 1:

Tissue sample collection information.

SITE DESCRIPTION	Latitude	Longitude	N	Accession Number	GenBank accession numbers
LP5 - Thoroughfare Beach at Long Point Ontario	N42.576184	W080.374029	10		AY529737, AY529738, AY529743, AY529742, AY529762, AY529763, AY529764, AY529778, AY529780, AY529808, AY529760, AY529761, AY529767, AY529766, AY529768, AY529779, AY529806, AY529809, AY529810
LP4 - Hastings Beach at Long Point Ontario	N42.577043	W080.447500	10		AY529745, AY529746, AY529748, AY529747, AY529749, AY529797, AY529798, AY529799, AY529846, AY529864, AY529739, AY529740, AY529750, AY529744, AY529752, AY529758, AY529759, AY529777, AY529796
LP2 - Big Creek at Long Point Ontario	N42.573738	W080.536957	10	RM 4473-4478	AY529753, AY529754, AY529756, AY529755, AY529757, AY529770, AY529801, AY529802, AY529803, AY529804, AY529775, AY529776, AY529795, AY529794
LP3 - Crown Marsh at Long Point Ontario			9		AY529807, AY529814, AY529815, AY529830, AY529831, AY529845
LP1 - Anderson Property, south beach, Long Point tip, Ontario	N42.540725	W080.105915	10	RM 4479-4493	
Rondeau Provincial Park Ontario	N42.260483	W081.905983	11	RM 4496-4505	

Appendix I continued

SITE DESCRIPTION	Latitude	Longitude	N	Accession Number	GenBank accession numbers
Niagara5 - Windmill Point Ontario	N42.875900	W078.999397	10		AY529773, AY529800, AY529818, AY529811, AY529825, AY529861, AY529862, AY529863, AY529771
Niagara4 - Point Abino Ontario	N42.51415	W079.5545	10		AY529751, AY529836, AY529838, AY529837, AY529839, AY529841, AY529842, AY529844, AY529848, AY529858, AY529781, AY529783, AY529788, AY529787, AY529821, AY529849, AY529850, AY529851, AY529852, AY529853
Niagara2 - James N Allan Provincial Park Ontario	N42.841106	W079.547845	14		AY529772, AY529774, AY529816, AY529805, AY529820, AY529854, AY529812, AY529813, AY529819, AY529817, AY529822, AY529823, AY529843, AY529847, AY529855
Niagara1 - Crystal Beach Ontario	N42.862167	W079.053353	6		AY529789, AY529791, AY529833, AY529832, AY529741, AY529769, AY529784, AY529782, AY529785, AY529786, AY529824, AY529827, AY529840, AY529859
Ashtabula Ohio	N41.901303	W080.809760	4	RM 4494-5	
Presque Isle State Park Pennsylvania	N42.10171	W080.6402	10		

SUMMARY AND CONCLUSIONS

In the thesis introduction, I outlined several reasons why a temperate anuran amphibian would likely be a useful organism to test hypotheses of metapopulation, dispersal and phylogeography. While this thesis has helped demonstrate that utility, it has also challenged several of the assumptions upon which the generality was based. The population dynamics of *Bufo fowleri* do not meet the predictions of two specific metapopulation models and does not have uniformly limited movement capabilities. Although the utility of the metapopulation approach has in no way been conclusively ruled out – these results caution against the immediate acceptance of the metapopulation paradigm in amphibian ecology and conservation. If a metapopulation approach proves useful in the future it will likely be one that accommodates within patch processes of local population dynamics – and not the simple Levin’s style approach. I have provided evidence that not all amphibians are metapopulations, nor are all amphibians uniformly limited dispersers. Agreeing to discuss them as such does a disservice to the organisms, and will hinder truthful conclusions in the future. The intuitively appealing parallels between temperate pond-breeding amphibians and the Levin’s model could easily be misconstrued into inadequate and inaccurate conservation strategies if the, “amphibian as metapopulation” paradigm was accepted and understood to mean a Levin’s style model where only regional dynamics matter. Species-specific studies of spatial ecology, such as this one, provide the quantifiable data necessary for the utility of such generalizations. Testing these assumptions with other species, using a deep and varied toolbox, should be the foremost challenge for spatial ecology.

Thesis Appendix 1

I include one note here that describes background details relevant to my thesis. The known predators of *Bufo fowleri* did not previously include *Larus delawarensis* and during the collection of data for my thesis I observed this species to consume *B. fowleri*. The note addresses the consumption of newly emergent juvenile *B. fowleri* by *L. delawarensis*. (Smith and Green submitted).

Smith, M. A., and D. M. Green. submitted. *Bufo fowleri* (Fowler's toad):
Predation. Herpetological Review.

Bufo fowleri (Fowler's toad): **Predation.**

In Canada, *Bufo fowleri* occurs in three disjunct populations (Rondeau, Long Point and the Eastern Basin (largely encompassed by Niagara County)). This disjunct distribution and fluctuations in abundance have resulted in the Committee on the Status of Endangered Species (COSEWIC) listing *B. fowleri* as a Threatened species. As a threatened species, knowing potential sources of predation is especially important. Here we report the predation of a juvenile *B. fowleri* by two Ring-Billed gulls (*Larus delawarensis*) in James N. Allan Provincial Park, Ontario, Canada within the Niagara region (N42.848 W079.664).

On the late afternoon of August 16, 2003 while conducting a survey of toadlet abundance along the park beach, I observed a pair of Ring-billed gulls on the beach in front of me, pulling at a juvenile toad. When the gulls were disturbed, they flew out approximately 20m into Lake Erie where the toad was dropped. The gulls landed beside the toad and swallowed it.

To my knowledge, this is the first reported predation of *B. fowleri* by gulls. Estimating the occurrence and sources of predation for such a threatened species has important conservation and management ramifications.

Acknowledgements: Ontario Ministry of Natural Resources, and Ontario Parks for permission to conduct research. David Judd for hospitality in the field.

Thesis Appendix 2:

Research compliance certificates



Centre for Biodiversity
and Conservation Biology:
Herpetology
phone: (416) 586-5899
FAX: (416) 586-5553
email: rossm@rom.on.ca

Royal
Ontario
Museum

100 Queen's Park
Toronto Ontario
Canada M5S 2C6

TO: Dr. D.M. Green
Redpath Museum
McGill University
859 Sherbrooke O.
Montreal
Quebec H3A 2K6

Category: Loan 02-03 H17

Authorized By: Dr. R.W. Murphy

Method of Shipment: Air Parcel Post

Date of shipment: 12 December 2002

PLEASE STORE R.O.M. SPECIMENS IN 70% ETHANOL UNLESS OTHERWISE NOTED.
PLEASE RETURN R.O.M. MATERIAL IN ALCOHOL PROOF CONTAINERS.
DISSECTING, OR ANY OTHER WORK WHICH INFLUENCES THE CONDITION OF THE MATERIAL, REQUIRES WRITTEN PERMISSION.
IF LOANED MATERIAL IS MENTIONED IN A PUBLICATION, THE AUTHOR IS ASKED TO SEND A REPRINT.

SPECIES

ROM NUMBER

Bufo fowleri, tissues in ethanol

Turkey Point	2805, 2819*
Turkey Point	3559
Turkey Point	4877, 4879-4904 incl.
Turkey Point	5580-5593 incl.
Port Dover	7514
Wainfleet	7441-2
Ont., Norfolk Co., Lake Erie beach, W.W. Judd, 1955.	13192-4 incl.

Note that there is no mention of Dunnville (or any other specific location) in the records for these three specimens

* 2813 is a *Eumeces fasciatus*. We substituted 2819, from the same collection as 2805.

Received in good order (Date)

Signed

Green copy is to be retained by the recipient; the pink copy is to be signed and returned on receipt of the material



ONTARIO PARKS

659 Exeter Road
London, Ontario
N6E 1L3
www.OntarioParks.com

May 14, 2003

Mr. Alex Smith
Redpath Museum
McGill University
859 Sherbrooke Street West
Montreal, PQ
H3A 2K6

Dear Mr. Smith:

Subject: Application to Conduct Research – Phylogeography and metapopulation ecology of the Fowler's toad in Canada

We have reviewed and approved your request to renew your application to conduct research on Fowler's toads in Long Point, Rondeau, James N. Allen, Rock Point, Port Burwell, and Turkey Point, provided the conditions listed below are met. Permission is extended to you and Dr. David Green to conduct research between May and October 2003. This letter and the approved research application should be carried with you during your fieldwork. They will serve as your authorization for accessing the provincial parks and identification while you are conducting your study.

The following conditions apply to your research application approval:

- The following Ontario Parks staff are to be notified prior to commencing fieldwork and when it is complete:
 - John Marchington, Park Superintendent, Long Point (519) 586-2133
 - Sandy Dobbyn, Natural Heritage Education Leader, and Rick Hornsby, Park Superintendent, Rondeau (519) 674-1750
 - Mark Custers, Park Superintendent, Rock Point, James N. Allen (905) 774-3163
 - Henry Valks, Park Superintendent, Port Burwell (519) 874-4601
 - Mike Postma, Park Superintendent, Turkey Point (519) 426-7138
- Your Scientific Collector's Permits from both the Chatham Area Office and the Niagara Area Office must be valid for the dates during which you conduct your research.
- You will provide the **Zone Ecologist** at the address on this letterhead with GPS coordinates of locations where you located Fowler's toads as well as sites that you visited but were unable to find Fowler's toads. These coordinates will then be submitted by the Zone Ecologist to the Natural Heritage Information Centre.



ONTARIO PARKS

659 Exeter Road
London, Ontario
N6E 1L3

Telephone: 519-873-4502
Facsimile: 519-873-4645
www.OntarioParks.com

May 14, 2001

Mr. Alex Smith
Redpath Museum
McGill University
859 Sherbrooke St. West
Montreal, QC
H9X 1Y3

Dear Mr. Smith:

Subject: Research Application
Fowler's Toad Population Ecology in Canada

We have reviewed and approved your request to conduct research on the Fowler's Toad (*Bufo woodhousii fowleri*) in Long Point, Rock Point, Port Burwell, Rondeau and James N. Allen, given that the conditions below are met. Permission is extended to Dr. David Green, yourself and students Ernest Lo, Esther Duffy, Dan Brouillette, Faith Au Yeung and Suzanna Atkinson to conduct the research between May and October 2001. Please carry a copy of this letter, the original research application and a copy of your research proposal during all fieldwork in Ontario Parks.

Conditions of Approval

1. You are required to contact the below noted Ontario Parks staff well in advance of your fieldwork. Upon arrival at the operating/staffed parks, please contact the following:

John Marchington, Park Superintendent, Long Point (519) 586-2133

Henry Valks, Park Superintendent, Port Burwell (519) 874-4691

Sandy Dobbyn, Natural Heritage Education Leader and Rick Hornsby, Park Superintendent, Rondeau (519) 519-674-1750

Mark Custers, Park Superintendent, Rock Point, James N. Allen (905) 774-6642
2. A Scientific Collector's Permits from both the Chatham Area Office (Aylmer District) and Niagara Area Office (Guelph District), including your permit to euthanise the number of *Bufo woodhousii fowleri* specified in your proposal (20 per park), is necessary. Please send a copy of these to the address noted in the letterhead, and please carry a copy of these while conducting research.
3. In addition to the stipulations that may be made on the Scientific Collector's Permit, Ontario Parks stipulates that only juvenile toads may be euthanised and that no more than 20 toads or 10% of the juvenile population, whichever is the lesser, may be euthanised.



DIVISION OF WILDLIFE

Division of Wildlife Headquarters
1840 Belcher Drive
Columbus, Ohio 43224-1329
(614) 265-6300

Ohio Department of Natural Resources

WILD ANIMAL PERMIT

PERMIT NUMBER: 864
SOCIAL SECURITY NUMBER: N/A

SCIENTIFIC COLLECTION
Others authorized on permit

NO

M. ALEX SMITH
859 SHERBROOKE ST., W.-REDPATH MUSEUM
MONTREAL, QC H3A 2K6

MICHAEL J. BUDZIK
Chief, Division of Wildlife

8/22/00
DATE ISSUED

is hereby granted permission to take, possess, and transport at any time and in any manner specimens of wild animals, subject to the conditions and restrictions listed below or any documents accompanying this permit.

This permit, unless revoked earlier by the Chief, Division of Wildlife, is effective
from: 3/16/00 to: 3/15/01

This permit must be carried while collecting wild animals and be exhibited to any person on demand.

THIS PERMIT IS RESTRICTED TO THE FOLLOWING

Permittee must notify the local wildlife officer or the Wildlife District office at least 24 hours prior to collecting. When collecting in Lake Erie, permittee must notify the Lake Erie Enforcement office at (419) 625-8062.

Locations of Collecting:
OTTAWA, ERIE COUNTY

Equipment and method used in collection:
HAND

Name and number of each species to be collected:
FOWLERS TOADS FOR RESEARCH.

RESTRICTIVE DOCUMENTS ACCOMPANYING THIS PERMIT? NO

This permit is not valid for collecting migratory birds, their nests, or eggs unless a current permit from the U.S. Fish and Wildlife Service has been obtained.

NO ENDANGERED SPECIES MAY BE TAKEN

