The Relationship Between Roads and Amphibians: Effects of Traffic Noise and Mitigation of Road Mortality

by

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Preface

Co-authorship Statement

The following thesis is formatted using the integrated thesis format; each data chapter was produced as an independent manuscript. Chapters 2 and 3 have been published, and Chapter 4 is currently in press, all in peer-reviewed journals. The text of each published chapter has been reproduced in the following document in whole, but with minor changes to formatting.

I was responsible for completing the majority of work described in this thesis; however, I did receive the following assistance. I proposed and developed the research questions in co-operation with Lenore Fahrig. I was primarily responsible for the design of the studies used to address these questions. I carried out all fieldwork for Chapters 2 and 3, and collected one year of data used in Chapter 4. I was responsible for all data analysis and wrote all first drafts of each of the chapters.

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Abstract

Anuran abundance is negatively correlated with road traffic; this may be due in part to the interruption of mate attraction by traffic noise. The impact could be small if anurans can alter their vocalization characteristics to avoid masking of their calls by traffic noise. I compared vocalizations of four anuran species in locations with low traffic noise vs. sites with high traffic noise. I then broadcast traffic noise at low traffic sites, and compared the anuran vocalizations before vs. during the broadcast traffic noise. Finally, I compared vocalizations at high traffic sites to those produced at low traffic sites while broadcasting traffic noise. Some species produced different vocalization characteristics in the presence and absence of traffic noise. Broadcast traffic noise immediately altered vocalizations such that they became similar to individuals of the same species found in locations with high traffic noise.

To test whether alterations compensate for an effect of traffic noise on mate attraction, I: (i) recorded a male calling at a quiet site; (ii) played traffic noise at the same male and recorded its altered call; and (iii) used these recordings to attract females to a trap at sites either with or without broadcast traffic noise. Results indicate that when necessary, males change their calls to compensate for potential effects of traffic noise on mate attraction. If my results apply to anurans in general, the previously documented negative effects of roads on populations are likely not caused by traffic noise; I hypothesize that road mortality is the main cause.

Culvert-type ecopassages, along with fencing have been used to mitigate road mortality on herpetiles. Effectiveness of the ecopassage itself, independent of fencing, is largely untested. I tested the hypothesis that pre-existing drainage culverts actually

mitigate anuran road mortality. I found no evidence that culverts alone reduce road kill. In contrast, fencing with culverts was effective at reducing road mortality. Overall, my results suggest that mitigation of road effects on anurans should focus on ways of excluding the animals from the road surface.

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1 Chapter: General Introduction

In Southern Ontario, no point on the landscape is greater than 1.5 km from a road (Crowley 2006). Roads affect both abiotic and biotic components of ecosystems, often to the detriment of the species inhabiting them (Coffin 2007). The construction of roads has been shown to impact both the quality and quantity of available wildlife habitat (Theobald et al. 1997); this impact typically occurs through fragmentation (Forman and Alexander 1998, Spellerberg 1998, Andrews 1990, Lesbarrères and Fahrig 2012). Roads dissect continuous habitat patches, fragmenting them into smaller patches with higher edge-to-interior ratios. The use of roads leads to direct wildlife mortality in many instances (Ashley and Robinson 1996, Smith and Dodd 2003, Taylor and Goldingay 2004) and has been linked to declines in wildlife populations (Langton 1989). The physical presence of roads coupled with the impacts of direct mortality, restricts animal movements (Forman and Alexander 1998) and can fragment populations (Hels and Buchwald 2001, Gibbs 1998, Trombulak and Frissell 2000, Gibbs and Shriver 2005); this is often the most documented effect of roads. Previous literature reviews (Spellerberg 1998, Andrews 1990, Trombulak and Frissell 2000, Coffin 2007, Fahrig and Rytwinski 2009) of roads and their ecological effects note the barrier effect of roads and the role this effect has on the long-term viability of wildlife populations near roads. The extent to which roads impact wildlife populations has been linked to species-specific behavioural traits (i.e., road avoidance, movement speed, etc., Jaeger et al. 2005, Fahrig and Rytwinski 2009). Additionally, the physical characteristics of the road, traffic volume, and the spatial configuration of the road relative to the surrounding landscape (Vos and Chardon 1998, Forman and Alexander 1998, Trombulak and Frissell 2000, Mazerolle et

al. 2005, Glista et al. 2007) are all thought to play a role in the impacts of roads on wildlife populations.

Roads have been shown to fragment habitats and isolate local wildlife populations (Andrews 1990, Vos and Chardon, 1998). These isolated populations can have reduced genetic diversity (Sjörgren-Gulve 1994, Sacccheri et al. 1998, Lesbarrères et al. 2003) increasing the likelihood of extirpation through stochastic events such as disease (Bennett 1991). Fragmentation of wildlife populations by roads has been documented in small (Oxley et al. 1974) and large mammals (Nellemann et al. 2001), avian communities (Develey and Stouffer 2001), and herpetofauna (Patrick et al. 2010). When roads function as a barrier to movement, wildlife populations become subdivided into isolated groups with no exchange of genetic diversity. The loss of genetic exchange is thought to result in increased likelihood of local population loss (Johnson and Collinge 2004). Therefore, mitigation of the barrier effects of roads is essential to the maintenance of wildlife movement.

Road-kill numbers appear to be on the rise and correlate to increases in infrastructure networks on a global scale. It is thought that road mortality is the leading source of human-caused wildlife mortality (Forman and Alexander 1998). The direct mortality of animals due to vehicle collisions is a primary and obvious effect that reduces animal populations (Romin and Bissonette 1996, Trombulak and Frissell 2000, Gibbs and Shriver 2002, Glista et al. 2008). When traffic volume is high, even small roads can represent a significant source of mortality affecting wildlife populations (Langton 1989, Rosen and Lowe 1994, Fowle 1996). Anthropogenic factors such as traffic volume (Fahrig et al. 1995, Mazerolle 2004) and environmental factors such as temperature and

precipitation (Forman et al. 2003, Shepard et al. 2008) affect wildlife road mortality. Populations of slow moving animals, and those that regularly cross roads, suffer in particular from the negative effects of increased mortality due to vehicle collisions (Jaeger et al. 2005). Traffic mortality accounts for more than half of all known deaths for the endangered Florida panther (*Felis concolor*, Harris and Gallagher 1989, Harris and Scheck 1991). Approximately 10% of mortality in Iberian lynx (*Felis pardina*) populations has been linked to road traffic; this is considered to be its second most important mortality factor (Ferrerias et al. 1992). Ashley and Robinson (1996) documented over 30,000 road-killed amphibians over a four-year period along a 3.6 km stretch of road. Traffic density has been shown to have a significant negative effect on leopard frog (*Rana pipiens*) abundance (Carr and Fahrig 2001). Mazerolle (2004) found vehicles had killed over half of amphibians encountered on roads, and Hels and Buchwald (2001) estimated that annually, roads could kill as much as 25% of an adult amphibian population. Identifying and understanding the factors influencing wildlife road mortality can help in the development of effective management strategies (Shepard et al. 2008).

It has been proposed that by increasing the permeability of roads through the use of wildlife passages or culverts, some of the detrimental impacts of roads can be alleviated (Yanes et al. 1995, Guyot and Clobert 1997, Aresco 2005a). The most commonly proposed solution to road impacts is the use of eco-passages (Romin and Bissonette, 1996). These structures have been designed with the intent of providing safe passage for individuals under the road surface, thereby increasing habitat connectivity and permeability of the road (Carr et al. 2002) while reducing direct mortality (Forman et

al. 2003). In some cases, exclusion fencing is used to both limit access to the road surface and increase the likelihood of individuals locating the culvert (Clevenger et al. 2001a, Dodd et al. 2004, Aresco 2005a), particularly for animals that typically avoid crossing structures (Glista et al. 2009). Installation of barrier fencing to assist individuals in locating crossing structures is often considered vital to the success of crossing structures (Jackson and Griffin 1996). The design and installation of crossing structures and fencing is costly, as is the maintenance of these structures; their inclusion in the design of new roads is only recently becoming more common.

In North America mitigation of road effects on wildlife is primarily focused on the facilitation of movements across roads by large mammals. This focus on large mammals is partially motivated by an interest in reducing potential for human injury resulting from collisions between large mammals and vehicles. While the majority of North American overpass-style ecopassages have been designed with a focus on ungulates (Ward et al. 1976, Singer and Doherty 1985, Romin and Bissonette 1996), these structures have been found to be used by large carnivores (Florida panthers, Foster and Humphrey 1995; black bears *Ursus americanus*, and grizzly bears *Ursus arctos*, Sawaya et al. 2014), and small mammals (McDonald and Cassady-St. Clair 2004). These large crossing structures for wildlife appear to be the most effective design for accommodating the needs of a broad range of wildlife species; however, they have not been shown to be effective for smaller bodied species such as reptiles and amphibians. In addition to the use of overpasses, underpasses and tunnels have been used to help facilitate the movement of wildlife across roads and highways in Europe, Australia, Canada, and the U.S. Tunnels are typically installed to facilitate crossings by reptiles and

amphibians (Jackson and Tying 1989, Jackson 1996, Evink 2002). Effectiveness of tunnels is dependent on a number of variables, including: size, placement, noise levels, substrate, vegetative cover, moisture, temperature, and light (Jackson and Griffin, 1996).

In addition to increasing animal mortality and decreasing their movements, roads may harm wildlife through the disturbance caused by traffic noise. Species that establish breeding territories or attract mates using acoustic signals may be particularly impacted by traffic noise. Kumar et al. (1998) identified vehicular traffic as the major source of noise in urban areas, where flat surfaces reflect sound, thus increasing noise level (Singal 2005). While natural environments can also be noisy (waterfalls, canyons, etc.) it is the intensity of the urban acoustic environment that causes it to differ significantly from its natural counterparts (Singal 2005, Warren et al. 2006). Vehicle noise is produced by the engine and tires, and varies directly with size of vehicle and speed of travel (Singal 2005). Traffic noise is therefore a function of the number of each type of vehicle as well as the speed of each vehicle. Anthropogenic sounds produced by automobiles and other vehicles may have detrimental effects on species that rely on acoustical advertisement to attract mates (Sun and Narins 2005).

Morton (1975) observed that environmental factors appeared to influence the long range acoustic signals by imposing selection pressures that in turn benefit individuals that maximize their broadcast range and the number of potential receivers. The assumption was that these selection pressures would result in a matching between successful signals and the environment in which they were broadcast (Morton 1975). However, Couldridge and van Staaden (2004) suggest that selection pressure on acoustic signals cannot be described by environmental variables alone. Discrepancies observed between an optimal

acoustic signal and its environment may be due to predatory or competitive pressures (Couldridge and van Staaden 2004). Endler (1992) proposed that signals should evolve in such a manner as to maximize the signal-to-noise ratio for a specific receiver given the surrounding environmental conditions. The simplest way to increase the signal to noise ratio is for the sender to increase the intensity or amplitude of the signal (Endler 1992, Forrest 1994).

Recent studies have focused on the effects of anthropogenic noise on the population size and behaviour of species that use acoustic signals for communication. Slabbekoorn and Peet (2003) found that great tits (*Parus major*) that inhabit noisy urban locations sing with a higher minimum frequency, resulting in a song that is not masked by the predominantly low-frequency urban noise. Brumm (2004) documented noise-dependent vocal amplitude regulation by free-ranging nightingales (*Luscinia megarhynchos*) in response to daily fluctuations in traffic noise. Traffic noise is also negatively correlated with the call rate in amphibians (Sun and Narins 2005). For species that lack plasticity in communication ability, anthropogenic noise could affect breeding opportunities and contribute to a decline in species density and diversity (Slabbekoorn and Peet 2003, Sun and Narins 2005). Research on the role of habitat acoustics on animal behaviour can provide a basis for predictions concerning the effects acoustic environmental variables may have on species signal design, detection, signal timing, and the species expected to be the most greatly influenced by noise disturbance (Warren et al. 2006).

It has been estimated that globally one-third of amphibian species are experiencing population declines (Stuart et al. 2004). Typically, these declines are

thought to be the result of changes in the habitat, environmental conditions (e.g., solar radiation, contamination, climate change), disease, or exploitation. While roads are often discussed as a source of amphibian decline in terms of physical habitat loss (i.e., due to the road footprint) and fragmentation, roads and their associated traffic noise may reduce amphibian abundance by reducing the effectiveness of vocalization-based mate attraction (Barrass 1985, Forrest 1994, Warren et al. 2006, Lengagne 2008). Traffic noise may mask vocalizations thereby reducing their efficacy in attracting mates (Narins 1982, Bee and Swanson 2007). If amphibians that rely on acoustic communication for mate attraction are unable to successfully obtain a mate in the presence of traffic noise, the amount of habitat lost to roads, and therefore the potential for roads to cause amphibian declines, may be greater than previously understood.

In addition to potential impacts of roads on amphibian populations due to traffic noise, roads have been shown to be a source of mortality in adjacent populations. Vehicular traffic is negatively correlated with amphibian abundance (Fahrig et al. 1995, Carr and Fahrig 2001), and the density of paved roads is negatively correlated with amphibian species richness (Findlay and Houlihan 1997, Houlihan and Findlay 2003). Nystrom et al. (2007) found that over a five-year period, ponds adjacent to roads with high levels of traffic were associated with the disappearance of calling male amphibians, while ponds adjacent to roads with limited traffic did not show any noticeable declines in chorusing males. Since both road density and traffic levels have increased over the past few decades (Statistics Canada 2000), their effects may be partially responsible for amphibian declines over the same period. It has been proposed that by increasing the permeability of roads through the use of wildlife passages or culverts, some of the

detrimental impacts of roads on amphibian populations can be alleviated (Aresco 2005a, Woltz et al. 2008, Patrick and Gibbs 2010). To this end, numerous types of road crossing structures have been developed (Jochimsen et al. 2004); however, the efficacy of these structures at mitigating road mortality has yet to be tested.

My work begins by studying the impacts of traffic noise on amphibian breeding behaviour to determine if noise plays a significant role in the negative association between roads and anuran populations (Chapters 2 and 3). By analyzing vocalization characteristics (i.e., frequency, volume, rate) of male anurans before and during the broadcast of high volume traffic noise, in locations with limited or no traffic volume and those of male anurans found in locations with high traffic I sought to determine how anurans respond vocally to the presence of traffic noise (Chapter 2). I found that anuran vocalization characteristics in some of the anurans I studied are plastic, and can change quickly to respond to changes in traffic noise. I then focused on determining whether traffic noise affects the ability of individual anuran males to attract mates (Chapter 3). Box traps ‘baited’ with an anuran vocalizations in the presence or absence of traffic noise were used to attract female amphibians. I then compared the number of females attracted in different treatments to determine how anuran responses to traffic noise affect reproductive success. My conclusion from this work was that individual male anurans are capable of changing their calls to compensate for a potential effect of traffic noise on mate attraction. Based on the results of this work it appears that traffic noise is not a component of the negative effects of roads on the amphibian populations that I studied. If these results apply to anurans in general, then the previously documented negative effects of roads on anuran populations are likely not caused by noise disturbance. The final

portion of my work was to focus on evaluating methods for mitigating road mortality, which I suggest is the most likely mechanism driving the negative effects of roads on amphibian populations (Chapter 4). By using a Before-After-Control-Impact (BACI) design, I artificially manipulated access to a series of existing drainage culverts to determine if there was a response in road-kill numbers. I found no evidence that culverts alone mitigate road-kill effects on anuran populations. Instead, my results suggest that culverts alone do not reduce anuran mortality, but that reduction of mortality requires that individuals be excluded from the road surface. I conclude that the previously documented negative effects of roads on anuran populations are not likely the result of traffic noise and suggest that efforts to reduce road effects on anurans should focus on excluding individuals from the road surface.

2 Chapter: Plasticity in the vocalizations of anurans in response to traffic noise

(Adapted from: Cunningham, G.M. and L. Fahrig. 2010. Plasticity in the vocalizations of anurans in response to traffic noise. *Acta Oecologica* 36: 463-470.)

2.1 Chapter Summary

Many species use acoustic signals to attract mates, and such signals can be degraded by anthropogenic noise. Anuran abundance is negatively correlated with road traffic, which could be due in part to the interruption of mate attraction by traffic noise. However, this impact could be small if anurans can alter their vocalization characteristics to avoid masking of their calls by traffic noise. I predicted that: (i) anuran vocalization characteristics (dominant frequency, mean amplitude and call rate) should be different in areas with different traffic noise levels; (ii) increases in traffic noise can cause immediate changes in amphibian vocalization characteristics; (iii) these altered vocalizations are similar to those at high traffic sites. To test the first prediction I compared vocalizations of four species of anuran at breeding sites in locations with low traffic noise vs. sites with high traffic noise. For the second prediction I broadcast traffic noise at low traffic (quiet) sites, and compared the anuran vocalizations before vs. during the broadcast traffic noise. For the third prediction I compared vocalizations at high traffic sites to those produced at low traffic sites while broadcasting traffic noise. Three species of anurans found at locations with low traffic noise produced vocalizations with different characteristics than individuals of the same species found in locations with high traffic noise. Broadcast traffic noise immediately altered amphibian vocalization characteristics such that they became similar to those of the same species found in locations with high traffic noise. I

conclude that plasticity in the vocalizations of anurans allows for the maintenance of acoustic communication in the presence of traffic noise.

2.2 Introduction

The spread of urban centers can have large effects on the natural environment. One such effect is anthropogenic noise ('noise pollution'), which can be harmful to animals, interfering with their acoustic signaling for communication (Slabbekoorn and Peet 2003, Sun and Narins 2005). The effectiveness of an acoustic signal depends on the distance a receiver is from the signal's origin (Forrest 1994), the acoustic properties of the signal, and the degree to which the environment disrupts signal transmission through, for example, background interference (Ryan and Rand 1993, Castellano et al. 2003). If animals in a particular area are unable to communicate due to background interference, the habitat quality in that area is effectively reduced; this may result in declines in species density and distribution (Sun and Narins 2005).

It has been estimated that globally one-third of amphibian species are experiencing population declines (Stuart et al. 2004). Beebee and Griffiths (2005) listed habitat alteration, environmental contaminants, UV-B irradiation, disease, introduced species, exploitation and climate change as the primary factors explaining these declines. Roads are an additional possible cause. Amphibian abundance is known to be negatively correlated with road traffic (Fahrig et al. 1995, Carr and Fahrig 2001), and amphibian species richness is negatively correlated with the density of paved roads (Findlay and Houlihan 1997, Houlihan and Findlay 2003). Several authors have suggested that traffic mortality (road kill) is the main reason for these negative relationships (Fahrig et al. 1995, Hels and Buchwald. 2001, Mazerolle 2004). However, it is also possible that roads

reduce amphibian abundance through disruption of vocalization-based mate attraction by traffic noise (Barrass 1985, Forrest 1994, Warren et al. 2006, Lengagne 2008). Bee and Swanson (2007) suggest that traffic noise acts to reduce the effective transmission distance (ETD) of a vocalization, resulting in vocalizations becoming lost in the noise such that females cannot orient themselves towards a breeding chorus (Narins 1982). On the other hand, if males can alter their vocalizations to avoid masking by traffic noise, there may be no net effect on reproductive success. While differences in vocalization characteristics in areas with and without traffic noise have been observed in several species of birds (Slabbekoorn and Peet 2003, Brumm 2004, Parris et al. 2009) and anurans (Bee 2000, Lardner and bin Lakim, 2002, Penna and Hamilton-West 2007, Parris et al. 2009) it is not yet known whether this is a plastic or fixed response.

The purpose of this study was to test the predictions that: (i) anuran vocalization characteristics (dominant frequency, mean amplitude and call rate) are different in areas with different traffic noise levels; (ii) increases in traffic noise cause immediate changes in amphibian vocalization characteristics; (iii) these altered vocalizations are similar to those observed at high traffic (noisy) sites. To test the first prediction I compared vocalizations of anurans at breeding sites with low traffic noise vs. at sites with high traffic noise. For the second prediction I broadcast traffic noise (mean 76 dBA) at low traffic (quiet) sites, and compared the anuran vocalizations before vs. during the broadcast noise. For the third prediction I compared vocalizations of anurans at breeding sites with high traffic noise to the vocalizations produced at low traffic sites while broadcasting traffic noise.

2.3 Methods

I tested these predictions using four species of anurans in eastern Ontario: green frogs (*Lithobates clamitans melanota*), northern leopard frogs (*Lithobates pipiens*), gray treefrogs (*Hyla versicolor*), and American toads (*Anaxyrus americanus*). I also included spring peepers (*Pseudacris crucifer*) but these data had to be omitted (see below for explanation). Sample sites in Eastern Ontario, located south of Ottawa and north of highway 401 between Brockville and Long Sault, were selected from National Topographic Data Base (NTDB) maps using Geographic Information System software. Sites consisted of wetlands, ponds and ditches (Figure 1). Sample sites were selected to provide the greatest distribution of locations across the region given the limitations imposed by the locations of high and low traffic roadways. Sampling locations were a minimum of 3 km apart to avoid pseudoreplication (Eigenbrod et al. 2008a). If no anurans of a particular species were found calling at a sampling location, the site was removed from the study for that species. Sites were divided into two categories (HIGH and LOW noise) and were selected such that they represented the greatest possible difference in traffic noise levels between categories. LOW sites were those with traffic noise not exceeding 50 dBA while HIGH sites were those with traffic noise exceeding 60 dBA (Figure 2). I obtained traffic noise levels from audio recordings (see below). Forty-four LOW and 39 HIGH sites were sampled between May 1 and August 1, 2007, between half an hour after sunset and midnight.

At each sampling location I made audio recordings of the vocalizations produced by a single male anuran with a digital sound recorder (Sampson Zoom H4 with stereo unidirectional electret condenser microphone) using a sampling rate of 96 kHz, at 1.5m

above the ground placed at the water's edge. At HIGH locations audio recordings were made for 3 min. Vocalizations at LOW sites were recorded for a total of 6 min. Three minutes into the recording at LOW sites, I broadcast traffic noise (mean 76 dBA at 5 m) from stereo speakers (Altec Lansing, Model - VS4121BLK), located directly below the recording equipment, for 3 minutes. This resulted in a 3-minute recording of vocalizations before the broadcast of traffic noise (BEFORE) and a 3-minute recording of vocalizations during the broadcast (TREATMENT) at the low traffic sites. The sampling unit was the vocalization of a single male at a given site, found within 5 m of the recording equipment. Vocalizations attenuate with increasing distance from their source (Marten et al. 1977); however, the attenuation rate over a 'hard' boundary such as water is very low (Forrest 1994). Therefore, I assumed that anuran vocalizations produced at the water surface did not attenuate appreciably over this short distance. During the initial 3 min recording, a sound meter (Galaxy Audio, Model - CM-130) was used to record the mean amplitude of the site background noise. If there was more than one anuran species calling at a given site, I returned to that site for successive nights, such that each night an individual of a different species was recorded. This was done to limit the exposure of individuals to the broadcasted traffic noise at LOW sites while collecting the BEFORE recording. The number of calling bouts recorded per species varied due to the innate characteristics of each species call. For species with long calls (e.g. American toads), I recorded between six and 10 bouts per sample while species with shorter calling bouts (e.g. green frogs and leopard frogs) allowed for the recording of a greater number of bouts (4 – 33 bouts) within the same 3 minute sampling period.

2.3.1 Vocalization Characteristics

All recordings were amplified using Raven Pro v1.3 (Cornell Lab of Ornithology, 2007), until the mean site noise level within the recording was equal to that of the reading from the sound meter during recording. The use of a digital recorder with a stereo microphone results in the production of audio files with two channels (Left and Right). I used the channel with the lowest traffic noise levels to extract the vocalizations. The remaining channel was used to determine the site-specific traffic noise level (see below). Raven Pro v1.3 was used to isolate calls produced by individual males from each recording. For TREATMENT recordings, the interactive signal detector tool was used to remove the traffic noise from the recording. This tool allows the software to identify the known traffic noise and remove it, leaving behind the male vocalizations for analysis. Recordings that were found to have traffic noise levels higher than the amplitude of anuran vocalizations were subjected to multiple runs through the signal detection software, and any remaining traffic noise was filtered out of the recording manually. I also listened to each recording during processing to ensure no vocalizations were removed by the detector tool.

Extraction of vocalization characteristics of the calls produced by individual males was completed in Raven Pro (v1.3). Individual calls were subjected to a Fourier transformation to define signal frequency and magnitude. Each Fourier coefficient was then squared and the resulting values were used to produce columns of pixels within a spectrogram (Charif et al. 2007). The values of the power spectrum between the lower and upper frequency bounds of the call were summed, and then divided by the number of frequency bins in the call to provide a mean amplitude value in dB for a given call (Charif et al. 2007). Mean amplitude values for all calls taken from a three-minute

recording were then averaged to produce the mean amplitude for an individual male. This process was repeated to produce a single dominant frequency value for an individual male. The spectrogram and playbacks of a recording were used to determine the number of calls per minute, i.e., the number of calls divided by three (minutes of recording).

2.3.2 Site Specific Traffic Noise

To obtain a measure of the site-specific traffic noise I selected the channel from each recording that contained the highest traffic noise level. As traffic noise is known to be composed of primarily low frequencies, the mean amplitude of all elements below 400 Hz contained within each recording was used as a measure of the traffic noise at each location. Sounds below 400 Hz are below that of vocalizations produced by the species in this study (Figure 3).

2.3.3 Broadcast Traffic Noise

Traffic noise for broadcasting was obtained by recording traffic from Provincial Highway 401, 5 m from the edge of the paved road surface at a height of 1 m. Highway 401 connects the two largest cities in Canada—Toronto and Montreal - and forms the southern boundary of the study area. At the point of recording, Highway 401 is a four-lane divided highway with an average traffic volume of 18,300 vehicles/day, including a high proportion of transport trucks (Eigenbrod et al. 2008b); it offers the highest traffic noise level in the region. A sound level meter (Galaxy Audio, Model - CM-130) was used to measure the mean amplitude (dBA) of the traffic noise during recording (76 dBA). During broadcast, the sound volume of the traffic recording was set such that the mean amplitude at 5 m was 76 dBA.

2.3.4 Analysis

Temperature is known to affect the vocalization characteristics of anurans (Parris et al. 2009). I compared the median ambient temperature at HIGH and LOW sites using a Mann-Whitney U test, to ensure this variable was not contributing to any observed differences in vocalization characteristics. This test was conducted separately for each species, using the subset of sites at which each species was present. Results were considered significant at $\alpha=0.05$.

To test the prediction that anuran vocalization characteristics (dominant frequency, mean amplitude and call rate) are different in sites with different traffic noise levels, I compared vocalization characteristics at LOW sites before noise was broadcast (BEFORE) to vocalization characteristics at HIGH sites using Mann-Whitney U tests. To test the prediction that traffic noise causes immediate responses in amphibian vocalization characteristics, I compared vocalization characteristics at LOW sites before traffic noise was broadcast (BEFORE) to vocalization characteristics at the same sites during the 3-minute broadcast of traffic noise (TREATMENT), using Wilcoxon's signed ranks tests. To determine if the experimentally altered vocalizations are similar to those at high traffic sites, I compared vocalization characteristics from TREATMENT at LOW sites to vocalization characteristics at HIGH sites using the Mann-Whitney U test. Significance for the comparison of vocalization characteristics was then corrected by the Benjamini-Hochberg procedure for controlling false positives in multiple comparisons (Thissen et al. 2002). All statistical analyses were conducted with SPSS (v13.0)

2.4 Results

Mean traffic noise was 43.8 dBA (SD = 2.66) at LOW sites and 73.2 dBA (SD = 4.91) at HIGH sites (Figure 2). Temperature was not significantly different between the

LOW and HIGH sites where green frogs, leopard frogs, gray treefrogs or American toads were sampled (Table 1). However, ambient temperature was significantly higher at the LOW sites than at the HIGH sites where spring peepers were sampled (Table 1). Due to the confounding nature of temperature on the vocalization characteristics of anurans and the difference in ambient temperatures between the LOW and HIGH spring peeper sites, the spring peeper data were removed from further analyses. The spectral signatures of vocalizations produced at quiet sites by green frogs and leopard frogs overlapped the traffic noise spectrum, whereas the vocalizations produced by American toads and gray treefrogs did not (Figure 3).

Prediction 1: Vocalizations at low traffic vs. high traffic sites

The call rates of green frog, leopard frog and gray treefrog vocalizations were significantly higher at LOW sites than at HIGH sites (Figure 4, Table 2). Green frogs produced vocalizations with significantly lower mean amplitude and significantly higher dominant frequency at HIGH sites compared to LOW sites (Table 2, Figure 4). Leopard frogs did not show significant differences in the mean amplitude of their vocalizations (Table 2) but did demonstrate a significantly higher dominant frequency of vocalizations at HIGH sites than LOW sites (Table 2, Figure 4). Gray treefrogs did not produce vocalizations with significantly different mean amplitude or dominant frequency at LOW vs. HIGH sites (Table 2, Figure 4). No significant differences were observed in any of the American toad vocalization characteristics at LOW vs. HIGH sites (Table 2, Figure 4).

Prediction 2: Vocalizations at low traffic sites before vs. during broadcast of traffic noise

Green frog and leopard frog vocalizations during the TREATMENT sampling period had significantly lower call rates and mean amplitudes than during the BEFORE

sampling period (Table 2, Figure 4). Both green and leopard frogs produced vocalizations with significantly higher frequencies during the TREATMENT sampling period when compared to the BEFORE period (Table 2, Figure 4). Gray treefrog call rates during the TREATMENT sampling period were significantly reduced compared to the BEFORE sampling period; however, there was no significant difference in the mean amplitude or dominant frequency of their vocalizations between these two sampling periods (Table 2, Figure 4). When exposed to broadcast traffic noise, American toads did not significantly change their vocalizations (Table 2, Figure 4).

Prediction 3: Vocalizations at high traffic sites vs. at low traffic sites during the broadcast of traffic noise

There were no significant differences in calling characteristics between HIGH sites and LOW sites during TREATMENT periods for any of the four species (Table 2, Figure 4).

2.5 Discussion

The results of my experimental field-based study of four anuran species indicate that (a) three species of anurans found at locations with low traffic noise produce vocalizations with different characteristics than individuals of the same species found in locations with high traffic noise; (b) experimentally broadcasting traffic noise elicits immediate changes in amphibian vocalization characteristics in the same three species; and (c) these experimentally altered vocalizations are similar to those of the same species found in locations with high traffic noise.

To the best of my knowledge, this study is the first demonstration that anuran vocalization characteristics respond immediately to increased traffic noise, and that these

altered vocalizations are similar to the vocalizations anurans make at sites with chronically high traffic noise. My results suggest that these species employ a plastic vocalization response to contend with communication difficulties associated with anthropogenic noise. It has long been recognized that such plasticity allows individuals to have a broader tolerance to environmental conditions and therefore higher fitness in multiple or highly variable environments (Padilla and Adolph 2005, Ghalambor et al. 2007, Wells and Schwartz 2007). Natural noise levels in the anuran environments in this area are variable in space and time due to noise sources such as rapids/waterfalls, wind, and noise produced by conspecifics, other chorusing anuran species, and other vocal taxa. Therefore, it seems likely that the plasticity I observed in anuran vocalizations is an adaptive response to this spatio-temporal variability in ambient noise.

This interpretation is in contrast to previous studies, which suggested that spatial differences in calling characteristics are “dialects”, or fixed adaptive responses to local noise conditions (Ryan and Wilczynski 1988). In my study area it is unlikely that populations (within species) are genetically distinct. The total area covered by my study sites has a diameter of about 65 km, and anuran habitats – wetlands, streams, and forests – are well distributed and common in the area. Dispersal ranges of my study species are on the order of 1-6.5 km (Smith and Green 2005). Therefore, frequent genetic mixing across the study region is likely, such that genetically determined dialects are unlikely to develop. It seems possible that previously identified “dialects” in other regions are simply the expression of a plastic response to local environmental noise.

One possible confounding variable that I was unable to measure is the effect of body size on the dominant frequency of calls. It is well documented that larger anurans

produce calls with lower dominant frequencies (Forester and Czarnowsky 1985, Wells 2007). If HIGH traffic sites contained smaller males than LOW traffic sites, this could explain my first result (above). I do not have information on body sizes of individuals at my sites so I cannot test this possibility directly. However, given that individuals from LOW traffic sites increased the dominant frequency of their vocalizations and produced calls more similar to those found at HIGH traffic sites when exposed to artificial traffic noise (TREATMENT), differences in body size are likely not responsible for my results.

It is also possible that my results are confounded by population sizes of anurans near vs. far from high-traffic roads. Anuran populations are known to be reduced near high-traffic roads (Eigenbrod et al. 2009), so at these sites the number of individuals and species of chorusing anurans should be lower. This means that the differences in calling characteristics between LOW and HIGH could be due to a response to differences in chorus sizes rather than a response to the traffic noise. However, again, given that individuals from LOW traffic sites produced calls more similar to those found at HIGH traffic sites when exposed to artificial traffic noise (TREATMENT), differences in chorus sizes are likely not responsible for my results.

If elevated levels of (low-frequency) traffic noise cause individuals to produce calls with a higher dominant frequency, it is possible that the largest males in the population (those with the lowest call frequencies) could be at a disadvantage in attracting females. While previous research indicates that female anurans prefer male vocalizations with lower dominant frequencies (Gerhardt 1994, Howard and Palmer 1995), recent studies suggest that females become less choosy in the presence of traffic noise. Wollerman and Wiley (2002) suggested that in noisy environments, female

anurans switch from discriminating among available male vocalizations to simply locating a male. In environments dominated by low frequency noise, males with higher dominant frequencies may be more easily located by females and be chosen instead of the males that females would normally choose, i.e., the larger males with lower call frequencies. This shift in mating towards smaller males could result in lower genetic quality of offspring, potentially leading to population declines. This hypothesis remains to be tested.

It is of interest to note that the two species in my study with the shortest breeding seasons, the gray treefrog and American toad, did not alter the dominant frequency of their vocalizations, and demonstrated limited or no alteration of their calling characteristics in response to traffic noise (Figure 4). If vocalizations are only required to attract mates over a short time during which there is only limited spatio-temporal variability in ambient noise, these species may not need the same plasticity in vocalization characteristics as species with longer breeding seasons. On the other hand, the lack of observed plasticity in vocalization characteristics in these species may simply be because their innate vocalizations are not masked by ambient noise (Figure 3). It would therefore be interesting to see whether these species alter their vocalizations in the presence of higher frequency noises.

Previous work on calling energetics in frogs has revealed that calling is probably the most energetically expensive activity a male frog undertakes during his lifetime (Pough et al. 1992). Wells and Schwartz (2007) suggest that due to the energetic costs of calling, individuals should use vocalizations that conserve energy while maximizing the effective transmission distance. It is possible that the observed acoustical responses to

traffic noise by green frogs and leopard frogs in my study are the result of a trade-off. The reductions in call rate and mean amplitude are coupled with significant increases in the call frequency (Figure 4). An increase in the dominant frequency of a vocalization comes at an increased energetic cost; however, this cost is considerably less than that of increasing the mean amplitude or calling rate of the vocalization (Parris 2002). The associated reduction in calling rate and power could result in a vocalization with no net increase in energetic expenditure for the increased dominant frequency.

Two primary hypotheses have been developed to explain how species attempt to overcome the masking effects of their environment: the Lombard effect hypothesis and the acoustic adaptation hypothesis (AAH). The Lombard effect hypothesis states that in noisy environments individuals should increase the power of their vocalizations (Pick et al. 1989). Results of my study indicate that when faced with the task of calling in a noisy environment, anurans do not increase the power of their vocalizations; therefore, my results do not support the Lombard effect hypothesis. The AAH states that the environment in which acoustic communication takes place should favour vocalization characteristics that minimize attenuation and distortion (signal degradation) with distance (Brown and Handford 2000). The AAH predicts that individuals should increase the frequency of their vocalizations such that they are not masked by lower frequency noise (e.g. Slabbekoorn and Peet 2003, Rabin et al. 2003) or that individuals should alter the temporal characteristics of their calls (Lengagne et al. 1999) such that they are not masked by other noise in the environment. Both green frogs and leopard frogs significantly altered multiple calling characteristics in response to increased levels of

traffic noise (Figure 4), suggesting that they are attempting to produce vocalizations that minimize attenuation of the acoustic signal, in support of the AAH.

It remains unclear whether the alterations in calling characteristics I observed compensate for possible effects of traffic noise on breeding. Without data on reproductive success in the presence of traffic noise, it is not possible to determine whether traffic noise contributes to amphibian population declines (Warren et al. 2006). Although previous studies have examined the impact of traffic noise on species that use vocalizations to attract mates (Narins 1982, Slabbekoorn and Peet 2003, Brumm 2004, Sun and Narins 2005, Parris et al. 2009), two key questions remain unanswered: (1) how effective are altered vocalizations at attracting potential mates, and (2) are there costs associated with altered vocalizations that affect the survival of individual males? Future efforts should focus on determining whether a reduction in breeding success occurs in the presence of traffic noise or alterations in vocalization characteristics by amphibians in the presence of noise are truly compensatory.

Table 1. Median temperatures (T) and number of males recorded (n) at LOW (<50dBA) and HIGH (>60dBA) traffic noise locations for five species of anuran. Sample sizes differ among species because each species was present at a subset of sites. Only one male of each species was present at a subset of sites. Only one male of each species was recorded at a given site.

					Mann-Whitney U	
		LOW sites		HIGH sites		(LOW vs. HIGH T)
Species		Median T	n	Median T	n	
Green Frog		20.0	25	20.0	23	-1.103 0.270
Leopard Frog		12.5	12	10.0	5	-1.377 0.195
Gray Treefrog		20.0	11	21.0	8	-0.840 0.968
American Toad		14.5	14	10.5	6	-1.255 0.239
Spring Peeper		17.0	17	11.0	11	-2.650 0.007

Table 2. Comparisons of vocalization characteristics for four species of anuran at LOW (<50dBA traffic noise) vs. HIGH (>60dBA traffic noise) sites, at LOW sites before the broadcast of traffic noise vs. during the broadcast of traffic noise (TREATMENT) and at LOW sites during the broadcast of traffic noise (TREATMENT) vs. at HIGH sites. Test statistic is from the Mann-Whitney U Test; sample sizes for each treatment are indicated in Table 1. Note that sample sizes for LOW and TREATMENT are equal. P-values have been corrected by the Benjamini-Hochberg procedure for controlling false positives in multiple comparisons.

	LOW vs. HIGH		LOW vs. TREATMENT		TREATMENT vs. HIGH	
	Z	p	Z	p	Z	p
Green Frog						
Call Rate	-2.728	0.009	-2.837	0.009	-0.486	0.634
Mean amplitude	-4.419	<0.001	-3.374	<0.001	-0.566	0.671
Dominant frequency	-5.351	<0.001	-4.341	<0.001	-2.915	0.233
Leopard Frog						
Call Rate	-2.294	0.032	-2.563	0.023	-1.010	0.464
Mean amplitude	-1.868	0.110	-2.646	0.024	-1.470	0.244
Dominant frequency	-2.842	0.023	-2.646	0.036	-0.490	0.730
Gray Treefrog						
Call Rate	-3.125	0.006	-2.521	0.048	-0.755	0.596
Mean amplitude	-1.620	0.362	-0.700	0.544	-0.811	0.695
Dominant frequency	-0.463	0.694	-1.260	0.374	-1.390	0.426
American Toad						
Call Rate	-1.245	0.408	-1.309	0.429	-0.856	0.586
Mean amplitude	-1.179	0.490	-1.423	0.464	-0.236	0.864
Dominant frequency	-1.887	0.597	-0.474	0.715	-1.887	0.299

Figure 1. Sampling locations with traffic noise >60dBA (HIGH) and <50dBA (LOW) in Eastern Ontario. Inset shows location of study within Eastern North America.

Figure 2. Traffic noise levels (dBA) at HIGH sites and LOW sites. Lines within boxes represent the median values, upper and lower limits of boxes represent the 75th and 25th percentiles, and upper and lower whiskers represent the 90th and 10th percentiles.

Figure 3. Spectral overlap of the broadcast highway noise and vocalizations of four species of anurans from sites with low traffic noise. Rectangles represent the range of acoustic characteristics of species vocalizations.

Figure 4. Vocalization characteristics of four species of anuran at low-traffic sample sites (noise levels ≤ 50 dBA), before (LOW) and during (TREATMENT) the broadcast of traffic noise (mean 76 dBA at 5 m), and at high-traffic sample sites (noise levels > 60 dBA) (HIGH). * indicates a significant difference between LOW and HIGH (* $p < 0.05$, ** $p < 0.001$), ▲ indicates a significant difference between LOW and TREATMENT (▲ $p < 0.05$, ▲▲ $p < 0.001$), and ● indicates a significant difference between TREATMENT and HIGH (● $p < 0.05$, ●● $p < 0.001$). Upper and lower limits of boxes represent the 75th and 25th percentiles. Lines within the boxes represent the median values and upper and lower whiskers represent the 90th and 10th percentiles.

3 Chapter: Mate attraction by male anurans in the presence of traffic noise

(Adapted from: Cunningham, G.M. and L. Fahrig. 2013. Mate attraction by male anurans in the presence of traffic noise. *Animal Conservation* 16: 275-285.)

3.1 Chapter Summary

I previously found that males of two anuran species - *Hyla versicolor* and *Lithobates clamitans melanota* - alter their mating calls in response to traffic noise. To test whether these alterations compensate for an effect of traffic noise on mate attraction I: (i) recorded a male calling at a quiet site; (ii) played traffic noise at the same male and recorded its altered call; and (iii) used these recordings to attract females to a trap at sites either with or without broadcast traffic noise. The calls produced without traffic noise attracted fewer females when they were played at sites with traffic noise than when they were played at sites without noise. However, the calls of the same individuals produced with traffic noise attracted as many females at sites with noise as at sites without noise, and they attracted as many females as did the call of the same male made without noise and played at sites without noise (the "natural" situation). Therefore, for these species, traffic noise does not affect mate attraction; males change their calls to compensate for a potential effect of traffic noise on mate attraction. A third species - *Anaxyrus americanus americanus* - does not alter its call in response to traffic noise, and its call made in the absence or presence of traffic noise was equally able to attract females in the absence or presence of traffic noise, indicating that traffic noise does not negatively affect mate attraction. Therefore it appears that traffic noise does not negatively affect mate attraction in these three species of anurans. I suggest that, if my results apply to anurans in general, the previously documented negative effects of roads on anuran populations are likely

caused mainly by road mortality. If this is true, road mitigation for anurans should focus mainly on reducing this mortality.

3.2 Introduction

Many studies have shown negative effect of roads on anuran (frog and toad) abundance and distribution (Fahrig et al. 1995, Vos and Chardon 1998, Carr and Fahrig 2001, Houlahan and Findlay 2003, Pellet et al. 2004, Nyström et al. 2007, Skidde et al. 2007, Eigenbrod et al. 2008a, 2009; reviewed in Fahrig and Rytwinski 2009). Most authors assume that the negative effects of roads on anurans are due to road mortality. This is a reasonable assumption, since very high anuran mortality rates have been recorded on roads. For example, Ashley and Robinson (1996) documented over 30,000 road-killed amphibians over a four year period on a 3.6 km stretch of road; Hels and Buchwald (2001) estimated that roads can kill as much as 25% of an adult amphibian population annually; and Bouchard et al. (2009) found that 28% of frogs attempting to cross a low-traffic road were killed by vehicles.

In addition to direct mortality, some authors have suggested that roads could affect anuran populations indirectly if traffic noise interferes with the ability of calling males to attract females (Warren et al. 2006, Bee and Swanson 2007, Lengagne 2008). On the other hand, several studies have shown that anuran males of some species alter their mating calls in the presence of traffic noise (Penna et al. 2005, Penna and Hamilton-West 2007, Parris et al. 2009, Cunningham and Fahrig 2010). It is possible that such alterations compensate for a potential negative effect of traffic noise on the ability of males to attract mates, but to date there are no studies of the effect of traffic noise on mate attraction in anurans.

In Chapter 2, I recorded the calls of individual males of three anuran species, first in the absence of traffic noise and then while I played traffic noise at the same individuals. Two species, the gray treefrog (*Hyla versicolor*) and the green frog (*Lithobates clamitans melanota*), altered their calls in the presence of traffic noise while the third species, eastern American toad (*Anaxyrus americanus americanus*), did not. Here I hypothesize that gray treefrogs and green frogs change their calls in the presence of traffic noise to reduce negative effects of traffic noise on the ability of males to attract mates. I further hypothesize that American toads do not change their calls in the presence of traffic noise because traffic noise does not interfere with their ability to attract mates. The purpose of the current study was to test these hypotheses.

3.3 Methods

To test for the effects of traffic noise on mate attraction in the gray treefrog, green frog and American toad, I used the recorded mating calls of a typical individual male (of each species) from Chapter 2. These were 24-bit recordings in WAV format made using a Sampson Zoom H4 recorder with stereo unidirectional electret condenser microphone at a sampling rate of 96 kHz. The individual's call was recorded (in the field) first in the absence of noise and then while I played traffic noise at it. For the current study, I selected a single representative male of each species, using its recorded call without traffic noise and then with traffic noise, to attract females in the absence or presence of traffic noise. Using a single male of each species allowed me to control for other factors that affect recorded calls such as distance from the recorder, vegetation height, and wind speed and direction. Power spectra of the recorded calls (Figure 5) were

created through fast Fourier transformation (FFT) using Sigview (2.4); call characteristics in comparison with the means are shown in Table 3.

Here I refer to calls produced by a male in the absence of traffic noise as “CAN” and calls produced by the same male in the presence of traffic noise as “CPN”. My overall design was to broadcast the CAN and CPN, to attract females to box traps, either in the absence or presence of traffic noise (NT and T, respectively; Table 4), during the breeding seasons of each of the three species (Table 5). Based on my hypotheses (above) I made the following predictions. First, for the gray treefrog and the green frog, the number of females captured should be lowest in the CAN-T treatment since the call is not adjusted to compensate for traffic noise. Furthermore, if the adjusted calls *completely* compensate for the traffic noise, I predicted that there would be about the same number of females attracted in the CAN-NT and CPN-T treatments. In addition, if the altered calls made by these two species do not carry a cost in terms of mate attraction, I predicted that there should be about the same number of females attracted to the CAN-NT and CPN-NT treatments. For the American toad, I predicted no differences in the number of females attracted in any of the treatment combinations, since I hypothesized that the reason this species does not adjust its call in the presence of traffic noise is that traffic noise does not interfere with mate attraction.

I selected four study sites in eastern Ontario, approximately 40 km southwest of Ottawa. All sites were vegetated wetlands (i.e., cattail marsh) previously known to contain all three focal species during their respective breeding seasons, and had traffic noise levels not exceeding 50 dB, a level of traffic noise corresponding to traffic volumes shown to have no detectable effect on abundance of these anuran species (Eigenbrod et

al. 2008b). The recorded male calls were then broadcast at the four trapping locations, where the treatments (Table 4) were cycled through the trapping locations through time (Table 5). Note that calls of only one species were used each night (Table 5). Each night two trapping locations were exposed to the CPN broadcast and two locations to the CAN broadcast. Within each pair of locations, traffic noise was broadcast at one location, while no traffic noise was broadcast at the other. Calls were broadcast from a portable Ultra Hydra 1GB portable media player through a waterproof marine speaker (Pyramid MDC7) mounted to the lid of a 2.5 litre plastic container (Figure 6D). The broadcasts consisted of a 3-minute recording of a calling male (Figure 5, Table 3), which was broadcast in a continuous loop for the whole four-hour trapping period (below). In treatments involving broadcast traffic noise (CPN-T and CAN-T), a second portable media player and speaker were used to broadcast traffic noise on the edge of the wetland. The amplitude of the broadcast traffic noise and the broadcast calls were adjusted, until values measured using a sound meter (Galaxy Audio, Model e CM-130) matched the amplitude of the recorded traffic noise and calls (Cunnington and Fahrig 2010). The traffic noise broadcast was set at a mean of 76 dB at 5 m and the amplitudes of the broadcast male calls at 5m are shown in Table 3.

Note that, since use of MP3 as a file type would have resulted in the loss of some sound elements, I made the original recordings in WAV format. However, the portable media players I used did not recognize WAV format. They did recognize WMA format, which retains more information than does MP3 format. Therefore, the WAV format field recordings of traffic noise and of individual male calls were converted to WMA format for the broadcasts. I suggest that this change in file format did not have a substantive

effect on my results, as the spectral characteristics of the two audio files were similar, apart from a small reduction in amplitude in the conversion from WAV to WMA (Figure 7), which was corrected in the field using a sound meter (see above). In Chapter 2, I found that the vocal responses of these anurans to this broadcast traffic noise were the same as their vocal responses to actual traffic noise. Therefore, despite the alterations to the signal, the anurans appear to "hear" them in the same way that they hear the actual traffic noise, at least for the portion of sound that is relevant to their responses to traffic noise. There was still the possibility that this conclusion cannot be extrapolated to the female responses to broadcast male calls; it is possible that females hear a real calling male differently from the broadcast of the same male call. I could not test this directly but, in retrospect, given the support for my predictions (see Results); the assumption seems to have been reasonable. If the male broadcasts were "noise" and not representative of actual male calls, I should not have seen the predicted differences in female attraction for the different treatments and species.

A single box trap (Figure 6A) was placed in the water 5 m from the edge of the wetland (and the broadcast traffic noise) at each of the four sample sites. The speaker broadcasting the male call was placed inside the screened portion of the trap and was therefore floating in the water. Individuals approached the trap (in the open position) by first sitting on the large flat area; to get closer to the recorded call they would cross over the raised lip (which was sloped towards the centre of the trap) and enter the central screened area. The screened area was open only at the top, and the lip around the top edge retained trapped individuals. Traps were placed such that the water line was level

with the large flat area of the trap and therefore with the base of the screened lip (Figure 6C).

In the spring of 2008, traps were placed in the trapping sites one week before the onset of sampling to allow individuals to become acclimatized to their presence. The treatment at a given site on a given night was systematically selected such that no site was exposed to successive nights of the same treatment and each of the four treatments was conducted for the same number of nights for a given species at a given site (Table 5). As broadcasts could not be started simultaneously at all four sites, trap sites were visited consecutively to begin trapping, with the same order being repeated at the end of the trap night. The consecutive visits to trap sites resulted in a 20 min difference between the start of trapping at the first site and the start of trapping at the fourth site; however, by repeating the same order of visits to sites at the end of each trap night, I standardized the trapping time (effort) across locations. To ensure that no bias in effort was created by the lag time, I systematically selected the starting site (i.e. of sites A-D) for each trap night such that each site began the trap night an equal number of times (Table 5).

Trap nights began 30 minutes after sunset and lasted for approximately 4 hours during the peak of the focal species breeding season. Traps were emptied at the end of each night. Individuals were considered captured if they were found on or in any portion of the trap. Traps were closed at the end of each night to ensure no individuals were captured outside of the trapping period (Figure 6B). The air temperature of each trap site for each night of sampling was obtained by averaging the temperature recorded at the beginning and end of each night.

As no site received the same treatment two nights in a row (Table 5), I used each night as an independent sample in my analysis. Due to relatively high proportions of nights with zero captures (54-59%; see Results), logistic regression was the appropriate modeling framework. The results represent the probability of the treatment attracting at least one female. I modeled the occurrence of captures (presence/absence of at least one female) in a logistic regression analysis, separately for each species, with predictor variables treatment (CPN-T, CAN-T, CPN-NT, CAN-NT; Table 4) and site (A, B, C, D) as factors, and temperature as a covariate; I included the interaction effect between treatment and site. I modeled site and site*treatment as fixed effects rather than random effects because there were only four levels of the site variable, i.e., four sites (Bolker et al. 2009). I also conducted post-hoc pairwise comparisons of treatment effects using the least significant difference (LSD) method for logistic regression. Note that, since I was comparing responses within species (for different treatments), not between species, it was not necessary to assume that the traps were equally effective at capturing the three species. In addition, by rotating the treatments through the same four sites (Table 5) and including site and site*treatment as in the analyses, I controlled for site variables (e.g., local abundance) that could affect the numbers of females attracted to the traps.

3.4 Results

I captured gray treefrogs on 22 of 48 trap-nights, green frogs on 20 of 48 trap-nights, and American toads on 13 of 32 trap-nights. Only species whose vocalization was being broadcast were trapped on a given night (i.e., no non-target species were captured). The raw data, showing numbers of captures per night, are given in Appendix A. The probability of capture of gray treefrog females and of green frog females was lower when

broadcasting the mating call made in the absence of traffic noise at sites when traffic noise was broadcast (CAN-T) than in the other three treatments (Figure 8). For the gray treefrog, the overall p -value for the treatment effect was 0.037 (Table 6); in the post hoc LSD pairwise comparisons, the probability of capture for CAN-T was significantly lower than for all the other treatments ($p < 0.05$ for all comparisons), and the other treatments were not significantly different from each other ($p \geq 0.2$ for all comparisons). For the green frog, the overall p -value for the treatment effect was 0.006 (Table 6); in the post hoc LSD pairwise comparisons, the probability of capture for CAN-T was significantly lower than for all the other treatments ($p \leq 0.01$ for all comparisons), and the other treatments were not significantly different from each other ($p \geq 0.5$ for all). There was no effect of treatment on the probability of capture of American toads (overall p for treatment = 0.588 (Table 6); $p > 0.2$ in all post-hoc LSD pairwise comparisons). The significance level of the treatment effect did not change substantially for any species when simpler models were considered, although some simpler models were more parsimonious than the full models (Table 7).

3.5 Discussion

To the best of my knowledge, this study is the first test of the hypothesis that alteration of mating calls by anurans compensates for effects of traffic noise on mate attraction. My results support the predictions that, for the species whose males alter their calls in the presence of traffic noise (gray treefrog and green frog: Chapter 1), (i) calls made in the absence of traffic noise should attract fewer females in the presence of traffic noise than do calls made in the presence of traffic noise, and (ii) calls made in the presence of traffic noise should attract as many females in the absence of traffic noise as

do calls made in the absence of traffic noise. These results support the hypotheses that (i) the alteration of male mating calls in the presence of traffic noise completely compensates for the effect of traffic noise on mate attraction, and (ii) the altered calls do not carry a cost for mate attraction. In addition, my results support the prediction that, for a species whose males do not alter their calls in the presence of traffic noise (American toad: Chapter 2), there should be no difference between the mate-attraction ability of male calls made in the presence or absence of traffic noise, played at sites with or without traffic noise. This supports the hypothesis that the species that does not alter its call does not need to alter it, because its unaltered call is effective in attracting mates in the presence of traffic noise. Therefore, it seems that, when necessary to do so, male anurans are able to change their calls to attract females in the presence of traffic noise, such that traffic noise has no effect on mate attraction, at least for the species I have studied.

I hypothesize that my results may apply to anurans in general and not just to the species I studied. Studies of anuran mating calls in the presence of background noise have found that males alter their calls in response to noise in a variety of ways. Species shown to alter their calls include *Microhyla butleri*, *Rana nigrovittata*, *Kaloula pulchra*, *Rana taipehensis* (Sun and Narins 2005), *Litoria ewingii* (Parris et al. 2009), *Eupsophus calcaratus*, *E. emiliopungini* (Penna et al. 2005, Penna and Hamilton-West 2007), *Hyla versicolor*, and *Lithobates clamitans melanota* (Cunnington and Fahrig 2010). At least for *H. versicolor* and *L. clamitans melanota*, this is a flexible behaviour, such that the male changes its call when the noise begins. I hypothesize that this flexibility in mating calls has evolved as a response to natural variability in space and time in background noise levels due to waterfalls and rapids, wind, and noise made by conspecifics and other

species. For example, *Hyla ebraccata* (Schwartz and Wells (1983a, b) and *Hyla chrysoscelis* (Love and Bee 2010) decrease their calling rate in response to chorus noise, a response similar to the response to traffic noise of *Hyla versicolor* (Cunnington and Fahrig 2010). A flexible call allows males to attract females in various noise environments.

I suggest that the reason traffic noise does not reduce mate attraction by the non-altered American toad call (CAN) is that its "natural" call characteristics already avoid potential masking by traffic noise. American toad calls naturally occur at frequencies well above that of traffic noise (American Toad ~ 1430 Hz, Traffic ~ 400 Hz), which should facilitate their detection by females. On the other hand, gray treefrogs, which do adjust their calls in the presence of traffic noise (see Chapter 2), also call at frequencies well above that of traffic noise, suggesting that call frequency is not the entire explanation for the American toad results. A second aspect of the natural American toad call that could make it immune to traffic noise is its long duration. Toads have much longer calls than other anurans in my study area, including gray treefrogs and green frogs. Since shorter calls are more likely to be masked by time-varying noise, the long calls of American toads may make them relatively immune to effects of traffic noise on female attraction. It has been shown in some anuran species, including gray treefrogs, that call duration is an important component of mate selection (Schwartz et al. 2001, Gerhardt and Brooks 2009), and that males increase call duration while simultaneously reducing call rate, in response to chorus noise (Wells and Taigen 1986, Martinez-Rivera and Gerhardt 2008). In Chapter 2, I found reduced call rates for gray treefrogs and green frogs in the presence of traffic noise, which could indicate an associated increase in call duration (not

measured) in these species, if they respond to traffic noise in a similar way as to chorus noise. In contrast, American toads did not show a change in call rate (see Chapter 2), so probably did not change their call duration, again supporting the idea that their natural call is already sufficiently long to avoid masking by traffic noise.

It is particularly surprising that, in the absence of traffic noise, the altered calls (CPN) of gray treefrogs and green frogs attracted as many females as did their unaltered calls (CAN). This is surprising because many laboratory studies have demonstrated female choice for particular male call characteristics in anurans (Gerhardt 2005, Castellano and Rosso 2006, Höbel and Gerhardt 2007, Bee 2008, Marquez et al. 2008, Richardson et al. 2010), including in *Hyla versicolor* (Schwartz et al. 2001, Gerhardt and Brooks 2009, Schwartz et al. 2010). It would therefore seem reasonable to assume that calls that are altered due to traffic noise should be less attractive to females in the absence of traffic noise than are "normal" calls, as the alterations should move them away from the optimum for female choice. One possible explanation for the lack of reduced attractiveness is that the alterations to the calls due to traffic noise may not compromise the particular call components that females cue in on for mate selection. Species vary widely in the call components that females select on but, at least in the case of *Hyla versicolor*, as mentioned previously, call duration appears to be an important element of mate choice by females (Schartz et al. 2001, Gerhardt and Brooks 2009). If call duration is unaffected or even increases in response to traffic noise, as seen in response to chorus noise for some species (e.g., Wells and Taigen 1986, Martinez-Rivera and Gerhardt 2008), this could explain why the altered calls remain successful in attracting females even in the absence of traffic noise. An alternative possible explanation is that the

assumption of strong female choice based on call characteristics may not apply in the field; studies conducted in the field often indicate rather weak or even random mate choice by females (Schwartz et al. 2001, Friedl and Klump 2005, Bee 2008; but see Pröhl 2003), possibly because of interference from background noise (Wollerman and Wiley 2002). Whatever the explanation, my results indicate that the alteration to calls in response to traffic noise does not affect female attraction to them, at least in the species I studied.

There is the possibility that the individuals attracted to the calls in my experiment included both males and females. Males are known to use other males' calls to locate breeding aggregations and to gain access to the females there (Bee 2007), including in *Rana clamitans* (Bee and Perrill 1996). I suggest that even if males were attracted to my male calls, it is very unlikely that this could produce artefactual results, i.e. that altered male calls (CPN) actually do not compensate for the effect of traffic noise on mate attraction. Such a bias would require that males are *more* attracted to the CPN calls than to the CAN calls, even in the absence of traffic noise, which could mask a reduced attraction by females to the CPN calls. Since this scenario seems unlikely, I suggest that the possible occurrence of males within my samples did not bias my results.

The sample size in my study was constrained by the limited calling seasons of the species and the number of ponds that I could study in a single night (four). Therefore the sample size was 8-12 trials per treatment per species. In this context, I decided to create the treatment recordings using a single male for each species. I used the results of Chapter 1 to select a single male whose calling characteristics, recorded in the field, were typical of the average for that species in the absence of traffic noise, and whose call

changed in response to traffic noise (also recorded in the field) in a manner typical for that species. I acknowledge that the use of a single male for each species limits the inferential strength of my study, but I suggest that it was appropriate to use my previous knowledge on the calling characteristics of these species to select a single typical individual, thus eliminating variation in the response that would be caused by variation in distance of individuals from the recorder, wind direction, vegetation height and density, and other factors.

The timing of trap nights for each of the focal species was selected in an attempt to sample individuals during the peak of each species breeding season (Cunnington unpublished data). Timing of arrival at breeding grounds is positively correlated with matting success (Morbey and Ydenberg 2001). It is thought that individuals arriving at breeding sites early are able to select the highest quality territories, thereby increasing the likelihood of successful mate attraction (Morbey and Ydenberg 2001). As traps were placed in the sampling ponds prior to the onset of the breeding season, it is possible that there was an “advantage” of the broadcast male in that it was present at the breeding site prior to the onset of the season. It is unlikely that the traps being present prior to the breeding season had an effect on my results for a number of reasons. The broadcast of vocalizations did not commence until after the individual species breeding seasons had already commenced; therefore, the broadcast did not gain any competitive advantage over local males in being on the site first. Traps were not located in optimal territories but were instead placed to facilitate access for starting the broadcast recordings and releasing captured individuals. Finally, by targeting the peak of the breeding season for each

species, the broadcasts were required to “compete” for mates in a full chorus setting thereby removing any advantage to being on the breeding grounds first.

As discussed in Chapter 2, previous studies have shown that calls produced by larger anurans produce have lower dominant frequencies than those produced by smaller bodied individuals (Forester and Czarnecky 1985, Wells 2007). Body size is considered representative of genetic fitness in male anurans, with larger individuals being considered ‘more fit’ (Howard 1988, Cherry 1992). In a recent study by Hoskin and Goosem (2010) the dominant frequencies of male vocalizations of two species of anurans (*Litoria rheocola* and *Austrochaperina pluvialis*) were found to be significantly higher closer to roads when compared to vocalizations produced by individuals of the same species at locations away from roads. Individual male body sizes of both *L. rheocola* and *A. pluvialis* were also smaller near roads when compared to individuals captured farther from roads (Hoskin and Goosem 2010). If traffic noise results in selection for individuals with higher frequency vocalizations, this selection pressure may result in a loss of genetic fitness within local populations as well as an overall reduction in body size of anurans living near roads. Future study could be directed to determining the long-term effects of traffic noise on body size and fitness of anurans.

Although my study indicates, in three species of anurans, that mate attraction is not affected by traffic noise, and that the alteration of male calls in response to traffic noise does not carry a cost to mate attraction, it is still possible that traffic noise lowers fitness in these species. Males using altered calls may have lower survival, if production of these calls requires more energy. Taigen and Wells (1985) suggested that calling represents the most energetically expensive activity a male frog undertakes during his

lifetime. Male gray treefrogs have been shown to experience significant weight loss after only a few nights of calling (Fellers 1976). Parris (2002) indicated that an increase of 3 dB in the mean amplitude of a call resulted in a doubling of the energy required to produce the call. Ideal calls would have an optimal balance between the conservation of energy and maximization of the call transmission distance (Pough et al. 1992); in altered calls, conservation of energy may be reduced in favour of maintaining a longer transmission distance. Again, further research would be required to determine whether there is a net energetic cost associated with altered calls and whether this cost translates into a negative effect at the population level.

In conclusion, the results of my study indicate that alteration of calling characteristics by anurans is compensatory, eliminating potential negative effects of traffic noise on mate attraction, at least for the three species I studied. Given this result, I suggest that the previously documented negative effects of roads on anuran populations (Fahrig et al. 1995, Ashley and Robinson 1996, Findlay and Houlahan 1997, Carr and Fahrig 2001, Hels and Buchwald 2001, Houlahan and Findlay 2003, Mazerolle 2004, Nystrom et al. 2007, Eigenbrod et al. 2008a) are likely due mainly to road mortality. If this inference is correct, it provides clear direction for mitigation of road effects on anurans. It implies that the main objective of such mitigation should be to keep anurans off roads thereby eliminating road mortality. At least for the species I studied, measures to remove or reduce traffic noise (e.g. noise barriers) are apparently unnecessary, or at most of secondary importance relative to measures, such as fencing and ecopassages, aimed at keeping anurans off roads.

Table 3. Vocalization characteristics of the individual males used in the experiment, in the presence and absence of broadcast traffic noise (see also Figure 5). Values in parentheses are the mean values across individuals, from Chapter 2.

	Mean Frequency (Hz)		Mean Power (dB)		Mean Call Rate (calls/min)	
	No traffic noise	Traffic noise	No traffic noise	Traffic noise	No traffic noise	Traffic noise
		broadcast		broadcast		broadcast
Gray Treefrog	1976 (1994)	1957 (1945)	78.3 (81.5)	85.3 (84.4)	11.3 (18.5)	6.7 (10.2)
Green Frog	497 (489)	738 (724)	72.6 (74.8)	62.0 (65.9)	4.7 (5.2)	2.7 (2.0)
American Toad	1426 (1448)	1451 (1454)	79.2 (79.8)	81.6 (82.2)	2.0 (2.3)	1.3 (2.0)

Table 4. Treatments applied to determine if alteration of calls in the presence of traffic noise compensates for potential negative effects of traffic on mate attraction in anurans. Male calls, produced in either the absence or presence of traffic noise, were broadcast to attract females at four different sites, with treatments rotating through sites across sampling dates (see Table 3). Traffic noise was broadcast at mean 76 dB at 5 m.

Treatment name	Male call	Traffic noise broadcast at the trapping site?
CAN - T	Male recorded in the absence of traffic noise.	Yes
CPN - T	Same male as above, recorded in the presence of traffic noise.	Yes
CAN - NT	Male recorded in the absence of traffic noise.	No
CPN - NT	Same male as above, recorded in the presence of traffic noise.	No

Table 5. Dates in 2008 on which trapping of three species of anurans was conducted at the four sites (A, B, C, D). AT = American toad, GTF = gray tree frog, GF = green frog. Treatments are defined in Table 4. The sequence of site-treatments (left to right) within each date indicates the order in which the treatments began (and ended, four hours later).

Species	Date	Site / Treatment			
GTF	May 30	B / CAN-NT	A / CPN-T	D / CPN-NT	C / CAN-T
GTF	May 31	B / CPN-T	D / CAN-T	C / CAN-NT	A / CPN-NT
GTF	Jun 1	A / CPN-T	B / CAN-NT	D / CPN-NT	C / CAN-T
GTF	Jun 2	C / CPN-NT	A / CAN-NT	B / CAN-T	D / CPN-T
GTF	Jun 4	C / CAN-NT	B / CPN-T	A / CPN-NT	D / CAN-T
GTF	Jun 9	D / CAN-T	C / CAN-NT	B / CPN-T	A / CPN-NT
GTF	Jun 11	A / CAN-T	D / CAN-NT	C / CPN-T	B / CPN-NT
GTF	Jun 12	C / CPN-NT	B / CAN-T	D / CPN-T	A / CAN-NT
GTF	Jun 16	D / CAN-T	A / CPN-NT	C / CAN-NT	B / CPN-T
GTF	Jun 23	A / CAN-T	C / CPN-T	B / CPN-NT	D / CAN-NT
GTF	Jun 24	B / CAN-T	D / CPN-T	A / CAN-NT	C / CPN-NT
GTF	Jun 25	D / CAN-T	C / CAN-NT	A / CPN-NT	B / CPN-T
GF	July 1	B / CPN-NT	A / CAN-T	D / CAN-NT	C / CPN-T
GF	July 2	B / CAN-T	D / CPN-T	C / CPN-NT	A / CAN-NT
GF	July 4	A / CPN-T	B / CPN-T	D / CAN-T	C / CAN-NT
GF	July 7	C / CAN-T	A / CPN-T	B / CAN-NT	D / CPN-NT
GF	July 8	C / CAN-NT	B / CPN-T	A / CPN-NT	D / CAN-T
GF	July 9	D / CPN-T	C / CPN-NT	B / CAN-T	A / CAN-NT
GF	July 14	A / CPN-T	D / CPN-NT	C / CAN-T	B / CAN-NT
GF	July 15	C / CPN-T	B / CPN-NT	D / CAN-NT	A / CAN-T
GF	July 16	D / CAN-T	A / CPN-NT	C / CAN-NT	B / CPN-T
GF	July 17	A / CPN-T	C / CAN-T	B / CAN-NT	D / CPN-NT
GF	July 21	B / CPN-NT	D / CAN-NT	A / CAN-T	C / CPN-T
GF	July 22	D / CAN-T	C / CAN-NT	A / CPN-NT	B / CPN-T
AT	May 21	B / CPN-NT	A / CAN-T	D / CAN-NT	C / CPN-T
AT	May 22	B / CAN-NT	D / CPN-NT	C / CAN-T	A / CPN-T
AT	May 23	A / CAN-NT	B / CAN-T	D / CPN-T	C / CPN-NT
AT	May 24	C / CAN-NT	A / CPN-NT	B / CPN-T	D / CAN-T
AT	May 25	C / CAN-T	B / CAN-NT	A / CPN-T	D / CPN-NT
AT	May 26	D / CAN-NT	C / CPN-T	B / CPN-NT	A / CAN-T
AT	May 27	A / CAN-NT	D / CPN-T	C / CPN-NT	B / CAN-T
AT	May 28	D / CAN-T	C / CAN-NT	A / CPN-NT	B / CPN-T

Table 6. Results of multiple logistic regressions estimating the probability of capturing at least one female (per night per site), on temperature and treatment (fixed effects) with site (random effect) for each of the three study species. The reference treatment was CAN-NT Treatments are defined in Table 4.

Species	Predictor		Coefficient	X²	p-value
Gray Treefrog	Treatment			3.264	0.037
		CPN-T	0.109		
		CAN-T	-0.218		
		CPN-NT	0.390		
		CAN-NT	0		
	Temperature		0.344	7.164	0.007
Green Frog	Treatment			4.917	0.006
		CPN-T	-0.098		
		CAN-T	-0.590		
		CPN-NT	0.421		
		CAN-NT	0		
	Temperature			1.390	0.234
American Toad	Treatment			0.658	0.588
		CPN-T	-0.327		
		CAN-T	-0.099		
		CPN-NT	-0.365		
		CAN-NT	0		
	Temperature			0.802	0.620

Table 7. AIC values for significance tests of the treatment effect ("treat"; see Table 4 for treatments), for increasingly complex logistic regression models. The response variable was the capture of at least one female (per site per night). "temp" = temperature.

Gray Treefrog		
Model	treat	treat + temp
AIC	57.9	48.0
Green Frog		
Model	treat	treat + temp
AIC	70.7	67.3
American Toad		
Model	treat	treat + temp
AIC	55.2	47.9

Figure 5. Power spectra of the broadcast calls of male anurans used to attract females, recorded in WAV format (96 kHz, 24-Bit). Spectra were generated by fast Fourier transformation (FFT) of a single advertisement call. Red lines are the calls in the absence of traffic noise and black lines are the calls of the same individuals in the presence of broadcast traffic noise. A. gray treefrog. B. green frog. C and D. American toad.

Figure 6. Box trap (A - open position, B - closed position, C - closed in situ) and broadcast equipment (D) used to attract and capture three species of anuran in Eastern Ontario. The speaker broadcasting the male call was placed inside the screened portion of the trap and was therefore floating in the water. Individuals approached the trap (in the open position) by first sitting on the large flat area; to get closer to the recorded call they would cross over the raised lip (which was sloped towards the centre of the trap) and enter the central screened area. The screened area was open only at the top, and the lip around the top edge retained trapped individuals. Traps were placed such that the water line was level with the large flat area of the trap and therefore with the base of the screened lip.

Figure 7. Comparison of the power spectra of the field recordings in WAV format (black line) to the broadcast files in WMA format (red line). A: traffic noise. B: male green frog call.

Figure 8. Proportion of nights with a capture (with 95% confidence intervals) of three species of anuran in each of four experimental treatments (see Table 2 for definitions of treatments). Post-hoc pairwise comparisons, using the least significant difference method for logistic regression (fixed: treatment, temperature; random: site), revealed that the CAN-T treatment for gray treefrog and green frog differed significantly from all three other treatments ($p \leq 0.05$, indicated by * above CAN-T). There were no other significant pairwise differences.

4 Chapter: Culverts alone do not reduce road mortality in anurans

(Adapted from: Cunningham, G.M., E. Garrah, E. Eberhardt, and L. Fahrig. In Press. Culverts alone do not reduce road mortality in anurans. *Ecoscience*)

4.1 Chapter Summary

Roads are linked to population declines in amphibians, primarily due to road mortality. Culvert-type ecopassages, along with fencing to direct animals to the passage, have been used to mitigate these impacts. However, the effectiveness of the ecopassage itself, independent of the associated mitigation fencing, is largely untested. In regions with heavy snowfall, long-term maintenance of amphibian-proof fencing is extremely costly. Therefore, it is important to know whether ecopassages alone (without fencing) mitigate amphibian mortality. I used a Before-After-Control-Impact design to experimentally test the hypothesis that pre-existing drainage culverts of the type typically used to mitigate road effects on herpetofauna, mitigate anuran road mortality. Grates were installed at both ends of six culverts to exclude anurans from the culverts. At an additional four sites fencing was installed on either side of culverts on both sides of the road, to keep anurans off the road. Ten control culverts were left un-manipulated. Road kill surveys were conducted one year before treatments were installed and in each of two years after. I predicted that, if culverts alone mitigate mortality, road kill should increase following installation of the grates. If fencing is effective for mitigation, road kill should decrease following installation of fences. I found no evidence for the first prediction; culverts alone did not mitigate road kill effects. In contrast, there was a large decrease in mortality at fenced sites, relative to control sites, indicating that fencing is effective at mitigating road mortality. These results suggest that culverts alone do not reduce anuran mortality; to reduce mortality animals must be excluded from the road surface.

4.2 Introduction

Studies of the effects of roads on wildlife abundance have documented negative effects on large mammals, birds, reptiles, amphibians, and invertebrates (Fahrig and Rytwinski 2009 and references therein). Road effects on wildlife can result from direct mortality (Ashley and Robinson 1996, Romin and Bissonette 1996, Trombulak and Frissell 2000, Gibbs and Shriver 2002, Clevenger et al. 2001a, Clevenger et al. 2003, Glista et al 2008), habitat loss and degradation (Gibbs 1998, Underhill and Angold 1999), and isolation of populations (Lode 2000, van der Ree et al. 2007, Eigenbrod et al. 2008a, Shepard et al. 2008, Corlatti et al. 2009). These effects have been linked to reductions in wildlife populations.

Amphibian and reptile populations are often reduced near roads (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). For these species, the main cause of population declines near roads is likely road mortality. Results of previous studies suggest that amphibians and reptiles experience higher levels of road mortality than other taxa (Ashley and Robinson 1996, Smith and Dodd 2003, Glista et al. 2009). The ectothermic nature of reptiles and amphibians may cause them to be attracted to increased temperatures associated with road surfaces for thermoregulation, which increases the likelihood of individuals being killed by traffic (Dodd et al. 1989, Rosen and Lowe 1994). In addition, Mazerolle et al. (2005) found that frogs traversing roadways stop moving in response to oncoming traffic. This behaviour may increase the vulnerability of frogs to wildlife-vehicle collisions as it increases the time spent on the road (Andrews et al. 2008). Finally, amphibians are often found on roads during mass migration events (Forman and Deblinger 2000, Jochimsen et al. 2004, Andrews et al. 2008, Glista et al.

2009), which often take place at night, making detection of individuals by motorists difficult.

The most common proposed solution to road impacts is the construction of ecopassages, also known as wildlife crossings, to allow animals to cross safely above or below the road surface (Aresco 2003, Clevenger and Waltho 2005, Cramer and Bissonette 2006, Glista et al. 2009, Balmori and Skelly 2012, van der Grift et al. 2012). Use of ecopassages has been demonstrated for several taxa. For example, Clevenger et al. (2001a) found that mammals' use of culvert-type ecopassages increased in response to traffic volume. In a cross-taxa comparison of ecopassage use, Mata et al. (2008) found that larger bodied animals favoured more open passages while small mammals and herpetofauna favoured culvert-type structures (Mata et al. 2008). Culvert-type ecopassages designed for amphibians and reptiles have been installed in numerous locations across North America (Jackson and Griffin 1996, Jackson 1999, Dodd et al. 2004, Aresco 2005a, Cramer and Bissonette 2006), Europe (Puky 2003, Lesbarrères et al. 2004, Mata et al. 2005, 2008), and Australia (Norman et al. 1998, Taylor and Goldingay 2003, 2010). In most cases, fencing accompanies the ecopassage, to direct the animals to the culvert (Clevenger et al. 2001a,b, Dodd et al. 2004, Aresco 2005b).

Several studies have shown that amphibians and reptiles use culverts. In Spain, pre-existing drainage culverts under a railway line were used by snakes and lizards (Rodriguez et al. 1996). Also in Spain, Yanes et al. (1995) found that amphibians and reptiles used sub-road crossing structures of less than 1.2 m in height. Use of structures by amphibians appears to be tied directly to the environmental conditions within the culvert; preference was for high moisture content and natural substrates (Lesbarrères et

al. 2004, Mazerolle and Desrochers 2005, Semlitsch et al. 2012). Therefore, road mitigation sites for amphibians often coincide with sites where drainage culverts would be needed under the road, to allow passage of small streams. Recommendations for mitigation usually involve installation of large, flat-bottomed drainage culverts, as these are thought to be more likely to allow animal passage than the more typical round metal culverts (Dexel 1989, Dodd et al. 2004, Ontario Ministry of Transportation 2006, Merrow 2007).

However, the mere fact that animals use ecopassages such as culverts does not demonstrate that these passages effectively mitigate the impact of the road on the population. In the case of amphibians and reptiles, where road mortality is the main impact of concern, effective mitigation would require that the culverts actually reduce mortality rates; it is not sufficient to show that animals are in the culverts. Several studies show that culverts combined with fencing or barrier walls to keep animals off the road and direct them to the culverts, reduce road mortality. Schmidt and Zumbach (2008) found that barrier walls were critical for use of culverts by amphibians. Aresco (2005b) found that drift fencing in conjunction with drainage culverts significantly reduced turtle road mortality. Clevenger et al. (2001b) observed decreases in ungulate mortality in response to the installation of exclusion fencing and wildlife crossing structures. Dodd et al. (2004) found reduced mortality in amphibians, reptiles, and mammals following the installation of a 2.5 km barrier wall and eight culverts. Finally, a review of road mitigation studies on amphibians found high variation in culvert use among studies (Smith and Sutherland 2014), possibly linked to variation in the presence, type or length of fences or barrier walls.

While the studies above demonstrate mitigation effectiveness, they do not demonstrate an effect of the culvert itself, rather than the mitigation fencing or barrier walls, on mortality. In every case, fencing or a barrier wall, which not only led the animals to the culverts but also kept them off the road, accompanied the culverts. It is possible that the fencing or wall was entirely responsible for the reduced mortality observed in these studies and that the culvert did not play a role in the documented reduction. In parts of the world experiencing heavy snowfall, long-term maintenance of amphibian-proof mitigation fencing is very costly. Such fencing, being near the road, is easily damaged by the weight of direct snow accumulation and snow cleared from the road. Therefore, it is important to know whether culverts alone, without mitigation fencing, reduce anuran road mortality. To test this I took advantage of an existing set of large, flat-bottomed drainage culverts typical of the design used for amphibian road mitigation. To some of these I added screens to block access to the culverts by anurans. For others, fencing was added along the road on each side of the culvert on both sides of the road. I predicted that if culverts alone reduce road kill then road kill should increase at sites where anuran access to culverts has been blocked by screens. In contrast, if the apparent effect of culverts found in previous studies is due partly or even entirely due to the fencing, then road kill should decrease at sites where fencing is added.

4.3 Methods

The overall design of my experiment was to compare the number of road-killed anurans, before and after experimental manipulation with control sites (Before-After-Control-Impact experiment), on road sections within 100 m of each of 20 pre-existing concrete box drainage culverts (hereafter culverts) along a 24 km stretch of paved

highway with a 60-80 km/h speed limit. The experimental manipulation consisted of installing grating at both ends of six of the culverts, to keep anurans out of the culverts (GRATE, Figure 9A), and installing 90 m of drift fencing on either side of the culvert, along both sides of the road, at four other culverts, to keep anurans off the road and potentially direct them to the culvert openings (FENCE, Figure 9B, Figure 9C). I left the remaining 10 culverts in their original (CONTROL) state (no grates or fencing, Figure 9D). I predicted that, if the culverts alone mitigate road mortality, the number of road-killed anurans should increase at the GRATE sites (in comparison to CONTROLS) following installation of the grates, and if fencing is important for mitigation then the number of road-killed anurans should decrease at the FENCE sites (in comparison to CONTROLS) after installation of the fencing.

The Thousand Islands Parkway ("the Parkway", Figure 10) is a 37 km long, 2-lane portion of Provincial Highway 2 that begins approximately 12 km west of Brockville, Ontario and extends to the eastern edge of Gananoque, Ontario. Prior to the construction of Highway 401 (MacDonald-Cartier Freeway), Provincial Highway 2, including the Parkway, was the primary transportation corridor between Kingston and Brockville. Currently, it provides access to National Park facilities (St. Lawrence Islands National Park) as well as numerous privately owned parcels of land. My study area was comprised of a 24 km subset of the Parkway extending from the Thousand Islands Bridge, a United States-Canada border crossing to U.S. Interstate Highway 81, east to the intersection with Highway 401. Along this 24 km portion of the Parkway, the speed limit is 80 km/hour, with the exception of 1-km section where the speed limit is 60 km/hour (Figure 10).

The twenty concrete box culverts were selected to be as similar to each other as possible, to avoid confounding factors. To identify culverts for the study, in 2008 the locations of all 140 drainage culverts along the 24 km section of the Parkway (Figure 10) were recorded. I then applied a sequence of selection criteria to identify the culverts that were most likely to be used by anurans and were most similar to each other in size, shape, and site. The 140 culverts ranged in size from 25 to 200 cm, and in shape (circular or rectangular). I first narrowed my selection to rectangular culverts of 100–200 cm width ($n=41$), as anurans are known to preferentially use crossing structures with larger openings (Yanes et al. 1995, Aresco 2005a, Woltz et al. 2008) ($n = 41$). Woltz et al. (2008) suggest that dry culverts may be avoided by anurans, so I further reduced my culvert selection to only those that contained water throughout the survey period ($n = 30$). Finally, I selected 20 culverts that were not within 200 m of any other culvert, driveways or intersecting roadways, to reduce or avoid inter-site variation that could have confounded my results (Figure 10). The mean distances from the culverts to the closest wetlands were 56 m, 42 m, and 64 m, for the GRATE, FENCE, and CONTROL treatments respectively. I was unable to determine if any of the culverts had been replaced since the road was constructed in the late 1930s. A number of the culverts were marked with the inscription “The King’s Highway 1938”; I assumed they were all the same age.

In the summers of 2009 and 2010, six of the 20 culverts were randomly selected, and both openings of each culvert were covered with hardware cloth (6 mm mesh size) stretched across wooden frames (GRATE). These grates allowed water to pass through but blocked anurans from accessing the culvert (Figure 9A). Also in the summer of 2009

and 2010, at each of four randomly selected culverts I installed 0.9-m-high plastic silt fencing (FENCE), extending 90 m from either side of the culvert opening, on both sides of the road (Figure 9B, Figure 9C). The fencing height was based on Woltz et al. (2008) who found that barriers 0.9 m in height excluded 99% of anurans. The final 10 culverts were left unaltered as control sites (CONTROL; Figure 9D). Treatments were installed at the same locations in 2009 and 2010. Treatments were in place by the last week in June 2009, were removed in mid-October 2009, reinstalled in the third week in May 2010, and removed in mid-October 2010. They were not in place over winter to avoid damage from ice and snow; in any case, anurans in my area are not active during winter.

4.3.1 Anuran Mortality

Road kill surveys along the Parkway were conducted by bicycle and on foot three to four mornings weekly in each of 2008 (the year before the experimental manipulations), 2009, and 2010. Surveys were completed on the same dates each year to avoid potential confounding effects of season and weather with my treatments. During each survey, the location of each anuran found dead on the paved road surface or gravel shoulder (hereafter road) was recorded with a differentially corrected Global Positioning System (GPS) receiver (Trimble Nomad, mean accuracy 3-5 m) with a mobile GIS (Environmental Systems Research Institute (ESRI ArcPad, version 7.1), and then removed from the road. The gravel shoulder was included in the survey area to include individuals thrown from the road by the impact with a vehicle. While it was often possible to determine the species of anuran killed, the condition of most made it impossible to determine size, age or sex. I assumed that all anurans found dead on the road were killed by vehicular traffic. As some injured or killed individuals may have

been removed from the road by scavengers (Smith and Dodd 2003, Row et al. 2007), counts of the number of dead anurans were therefore considered conservative estimates of road mortality. I assumed that the probability of removal by scavengers was unrelated to treatment (GRATE, FENCE, and CONTROL).

4.3.2 Analyses

The total number of road-killed anurans within 100 m of each study culvert per year was the response variable in analyses. The fencing used in the four fenced sites extended 90 m from each side of the culvert opening, so the road kill surveys included 10 m of road beyond the ends of the fence. This was to ensure that I included the possibility of elevated road mortality at the fence ends in case anurans followed the fencing to the end and then attempted to cross the road. Road kill values were summed for each sampling site ($n = 20$) in a given year and then log transformed to improve homogeneity of variance. I used a linear mixed model using the restricted maximum likelihood method (REML) as recommended by McDonald et al. (2000). I conducted two analyses: one comparing road kill numbers at GRATE vs. CONTROL sites and the other comparing road kill numbers at FENCE vs. CONTROL sites. Predictors were treatment (GRATE vs. CONTROL or FENCE vs. CONTROL), year (2008 [Before], 2009 and 2010 [After]), and the interaction between year and treatment as fixed effects. The interaction effect between treatment and year in each analysis is the effect of interest to me since it tests my predictions that mortality should increase following installation of grates and decrease following installation of fencing. In the GRATE vs. CONTROL analysis I predicted an increase in mortality from Before (2008) to After (2009, 2010) at the GRATE sites relative to the changes over these same years at the CONTROL sites. In the FENCE vs.

CONTROL analysis I predicted a decrease in mortality from Before (2008) to After (2009, 2010) at the FENCE sites relative to the changes over these same years at the CONTROL sites. Finally, I tested for spatial autocorrelation of the residuals using Moran's I to ensure that it was valid to treat each site as an independent sample.

4.4 Results

Altogether, I identified 1761 dead anurans (726 in 2008, 454 in 2009, 581 in 2010). Road-killed anurans identified included bullfrog (*Lithobates catesbeianus*), leopard frog (*Lithobates pipiens*), green frog (*Lithobates clamitans melanota*), eastern American toad (*Anaxyrus americanus americanus*), gray treefrog (*Hyla versicolor*), mink frog (*Lithobates septentrionalis*), and wood frog (*Lithobates sylvaticus*). These are all the anuran species common to the survey area (Cunnington unpublished data), with the exception of the spring peeper (*Pseudacris crucifer*), which may have been present but is likely very difficult to detect due to its small size. Anuran road-kills occurred throughout the survey period, with an apparent peak in July (Appendix B).

Road-kill numbers did not vary significantly between years at the CONTROL ($F_{2,27} = 1.981$, $p = 0.157$) and GRATE ($F_{2,15} = 0.758$, $p = 0.486$) sites; however, there was a significant difference between years in the number of dead anurans at FENCE sites ($F_{2,9} = 4.55$, $p = 0.043$). In the GRATE vs. CONTROL analysis, there was no significant interaction effect between treatment and year, indicating no significant effect of the GRATE treatment on anuran road mortality (Figure 11, Table 8). In contrast, in the FENCE vs. CONTROL analysis, there was a significant interaction between treatment and year (Figure 11, Table 8) indicating that the effect of FENCE relative to CONTROL (i.e., treatment) depended on year. In particular, relative to the CONTROL sites, the

number of dead anurans was reduced by about 40% at the FENCE sites after the fencing was in place (2009, 2010) than before (2008) (Figure 3, coefficients in Table 8). Residual road kill numbers were not spatially autocorrelated (Moran's $I = 0.001$, $z = 0.68$, $p = 0.495$) suggesting that the sites could be considered independent samples.

4.5 Discussion

Results of my study indicate that culverts alone do not reduce anuran mortality. Placing grates over the openings of culverts to block anuran entry did not result in an increase in mortality of anurans on the roads. This suggests that the presence of a culvert does not reduce the likelihood that anurans attempt to cross over the road, and that anurans attempting to cross the road near a culvert do not detect the presence of the culvert and direct their movements towards it. This implies that culverts, at least concrete box culverts such as those I studied, should not be viewed as a means to mitigate road mortality on anurans.

On the other hand, culverts associated with fencing along both sides of the road did reduce anuran mortality. This result suggests that reducing anuran mortality on roads requires fencing (or some other means) to keep the anurans off the road. However, it would not be accurate to infer that simply adding fencing to culverts makes them effective; the same reduction in mortality might have been observed if I had installed the same length of fencing along road sections where no culvert was present. Future study should be directed towards determining if there are differences in efficacy in the use of fencing alone, versus culverts in conjunction with fencing, at reducing anuran road mortality.

Note that I detected a significant effect of the FENCE treatment even though this treatment had the smallest number of replicates (four). This indicates that my study had sufficient power to detect a treatment effect despite my relatively small sample sizes. I had six replicates of the GRATE treatment but did not detect any effect. It remains possible that culverts have a small impact on mortality, which I missed due to my low sample size. However, my results do indicate that if there is an effect of the culverts it is a very small effect, especially relative to the effect of the fencing.

My results have important implications for mitigation of road effects on amphibians. Most mitigation efforts currently assume that the primary focus should be on improving permeability of roads to animal movements; for that reason, the focus is on ecopassages such as culverts (Beebee 2013). This assumption may be valid in some situations, such as when road mortality is low (e.g. because the animals are able to avoid oncoming vehicles), and migration across the road is required for the animal to complete its life cycle (Jaeger et al. 2000). However, simulation studies suggest that road mortality usually has a much stronger impact than the movement barrier effect on both population persistence and genetic diversity (Jaeger and Fahrig 2004, Jackson and Fahrig 2011). My results suggest that fencing is effective at reducing road mortality in anurans, a group of species vulnerable to road mortality. I conclude that the first priority for mitigation should normally be installation of fencing or other structures that keep animals off the roads, thereby keeping them from being killed. This is critical for designing effective road mitigation in situations where choices must be made due to limited mitigation funds. For example, if a road runs for 2 km through a wetland, where anuran populations have declined due to road kill, a limited mitigation budget would likely be better spent on

fencing the entire 2 km to keep frogs off the road rather than spending most of the money on a few crossing structures with only limited fencing, thus leaving most of the road unfenced. If the road blocks access to a resource needed for the species to complete its life cycle (e.g., breeding habitat is on one side of the road while overwintering habitat is on the other side, then one or more ecopassages will need to be included along with the fencing). On the other hand, in some situations it may be feasible and less costly to create or enhance habitat such that both required habitat types exist on the same side of the road.

The implications of my results for mitigation of road mortality are particularly challenging in regions experiencing heavy snowfall, where long-term maintenance of amphibian-proof fencing is very costly. If fencing is susceptible to damage over the winter, it may be preferable to erect temporary fencing each spring and dismantle it each fall, so that the fencing can be re-used. However, this is also very costly particularly because the fencing needs to be buried to keep small animals from passing underneath. If a permanent solution to the road mortality is preferred, my results imply that development of new engineering solutions for keeping amphibians off roads should be a high priority. One notable example is the reconstruction of Highway 441, Florida, U.S.A., which passes through a 3.2 km section of prairie habitat. In response to concern over the extremely high road-kill numbers (Smith and Dodd 2003) on this section of road, the road was rebuilt such that the road bed was raised and walls were installed along the sides of the roadbed (below the road surface). The walls have a lip along the top so that most animals cannot climb onto the road. Since reconstruction of this section of road, mortality has been reduced by over 90% (excluding birds; Barichivich and Dodd 2002). There may also be other ways to keep animals from being killed on roads, besides

installation of fencing or other barriers. Closures of roads at specific times of year when large numbers of individuals attempt to cross the road can be effective. In Burlington, Ontario, Canada, a stretch of municipal roadway is closed for three weeks each spring to allow the annual migration of Jefferson Salamanders (*Ambystoma jeffersonianum*), an endangered species. In addition to closing existing roads during periods when species are most likely to migrate across them, the mortality imposed by new roads could be reduced by building them far from natural habitats such as wetlands. However, as impacts of roads on anurans extend as far as 1 km from the road (Eigenbrod et al. 2009), this strategy will be limited in many regions.

The results of my study may have differed if they were completed on high traffic vs. low traffic roads. On a very low traffic road, mortality is unlikely to be very high. Excluding individuals from the road surface through fencing would reduce the number of road-killed individuals; however, it may be difficult to detect an effect of any of the treatments due to low road-kill numbers associated with low traffic volume. Amphibian abundance is negatively correlated with traffic volume (Fahrig et al. 1995); lower amphibian abundance occurs in areas adjacent to high traffic volume roads. It has been suggested that the decreased abundance of amphibians is the result of high levels of road mortality associated with the heavily travelled roads (Lesbarrères and Fahrig 2012). If my study was conducted on a high traffic road, the local amphibian populations may have been reduced to levels that were so low that the effects of any mitigation measures could not be detected.

My finding that culverts alone do not reduce mortality of anurans cannot necessarily be extrapolated to other species or species groups. In particular, it may not

apply to animals that avoid moving onto the road surface, as road mortality in such species will be very low. Several studies have shown that small mammals avoid moving onto the road surface (Garland and Bradley 1984, Ford and Fahrig 2008, McGregor et al 2008, Brehme et al. 2013), and work by Clevenger et al. (2001a) suggests that culverts are often used by small mammals to cross roads. This implies that small mammals may move along the road edge searching for a means to cross without going over the road surface. Ecopassages may thus further reduce road mortality in these species. On the other hand, because roads appear to have little effect on small mammal abundances (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012), it is questionable whether ecopassages are needed for them. In contrast to small mammal behaviour, amphibians attempt to cross the road where their paths intersect it (Bouchard et al. 2009). Without the aid of fencing, amphibians would not locate a culvert unless it occurs directly in their path.

My findings suggest that I should not assume that the occurrence of animals in or on ecopassages actually demonstrates effectiveness at mitigating the effect of the road. More Before-After-Control-Impact (BACI) studies like the one presented here are required to determine effectiveness of these structures. Future studies would benefit from the inclusion of population level monitoring to determine the efficacy of ecopassages at mitigating the impacts of roads on wildlife populations. Given the cost of installing ecopassages, standard practice for monitoring their effectiveness should follow a BACI design (van der Grift et al. 2013). This would include measurements of road mortality, movement rate, and/or population size taken before the structures are installed, at both

the structure site and at control sites, and then these measurements should be repeated after the structures are installed.

I suggest it may be time to reconsider the appropriateness of the current road mitigation emphasis on crossing structures, at least for amphibians. Amphibian and reptile populations are known to be particularly vulnerable to the effects of roads, with road mortality likely the main mechanism (Fahrig and Rytwinski 2009, Rytwinski and Fahrig 2012). Because my results suggest fencing is much more effective than ecopassages at reducing mortality, I suggest that a realignment of road mitigation for amphibians (and possibly reptiles) towards fencing and other measures aimed at keeping animals off roads would be appropriate.

Table 8. Linear Mixed Model analyses using the Restricted Maximum Likelihood (REML) method on a Before-After-Control-Impact (BACI) study of the effects of GRATE and FENCE treatments on anuran mortality. Treatments were "GRATE" sites (n = 6) where grates were placed over culvert entrances such that anurans could not enter them, and "FENCE" sites (n = 4) where 90 m of fencing was placed along the road on either side of both culvert entrances. "CONTROL" (n = 10) are culvert sites where no grates or fencing were installed. Road kill surveys were conducted at all sites in 2008 before grates and fencing were in place, and again in 2009 and 2010 after grates and fencing were in place.

Comparison	Variable	β	F	p-value
Grate vs. Control	Treatment		0.065	0.799
	Control	0		0.923
	Grate	0.013		
	Year		0.475	0.627
	2008	0		
	2009	-0.187		0.063
	2010	0.069		0.513
	Treatment*Year		2.612	0.088
	Control*2008	0		
	Grate*2008	0		
	Control*2009	0		
	Grate*2009	0.222		0.170
	Control*2010	0		
	Grate*2010	-0.194		0.306
Fence vs. Control	Treatment		0.512	0.479
	Control	0		
	Fence	0.404		0.052
	Year		10.551	<0.001
	2008	0		
	2009	-0.187		0.112
	2010	0.093		0.461
	Treatment*Year		10.166	<0.001
	Control*2008	0		
	Fence*2008	0		
	Control*2009	0		
	Fence*2009	-0.438		0.051
	Control*2010	0		
	Fence*2010	-1.086		>0.001

Figure 9. Examples of GRATE (A), FENCE (B & C), and CONTROL (D) sites.

Figure 10. Locations of FENCE, GRATE and CONTROL sites along a 24Km section of the St. Lawrence Islands Parkway, ON.

Figure 11. Mean log number of dead anurans at FENCE, CONTROL, and GRATE sites before and after the treatments were applied. Error bars represent standard errors. Letters indicate significant differences (alpha = 0.05) between groups.

5 Chapter: General Discussion

The results of my work as presented in Chapters 2 and 3 suggest that anurans are able to compensate for potential negative effects of traffic noise on mate attraction. In Chapter 2, I reported my findings that individual male anurans are able to respond immediately to increased traffic noise by adjusting the characteristics of their vocalizations. Additionally, the altered vocalizations are more similar to those produced by anurans at sites with chronically high traffic noise. These results suggest that anuran species are able to employ a plastic vocalization response to contend with anthropogenic noise. Additionally, I found that some species did not alter their vocalizations in response to traffic noise.

Chapter 3 built on the results of Chapter 2 to test the hypothesis that alteration of mating calls by anurans compensates for effects of traffic noise on mate attraction. I found that (1) when male anurans alter their vocalizations in response to traffic noise, they are more successful in attracting mates than if they were to use an unaltered vocalization, and (2) altered vocalizations in the presence of traffic noise are just as successful at attracting mates as unaltered vocalizations in the absence of traffic noise. These results supported my hypothesis that the alteration of male mating calls in the presence of traffic noise completely compensates for the effect of traffic noise on mate attraction. For the species that did not alter their vocalizations in response to traffic noise, my results suggest that alteration does not occur as it is not required, because the unaltered call is effective in attracting mates in the presence or absence of traffic noise. In the anuran species I studied, my results indicate that when necessary to do so, male

anurans are capable of changing their calls to attract females in the presence of traffic noise, such that traffic noise has no effect on mate attraction.

The results shown in Chapter 2 indicate that some species of anurans are able to alter their vocalizations in response to traffic noise, and that these alterations can occur quickly; however, the costs of these alterations has not been fully examined. The general trend of the alterations observed in my thesis suggests that increases in dominant frequency of vocalizations are coupled with decreases in mean amplitude and call rate. These changes may represent a physiological tradeoff between the energetics of producing high frequency or loud vocalizations. Sounds produced at high frequencies have been shown to be more energetically costly than those produced at low frequencies, and the amplitude of a vocalization is positively correlated with energy expenditure (Parris 2002). It seems likely that the energetic costs associated with producing a high frequency vocalization can be offset by reducing the amplitude or number of calls produced; this approach may provide the means to produce an effective call without incurring additional energetic costs. Alternatively, alteration of the location in which individuals call from, may provide a means for producing an energetically neutral vocalization in response to changes in traffic noise. Given the reflective nature of water, species that call near the water surface may be able to produce longer range vocalizations than those that call at greater distances from water (Forrest 1994). Due to the nature of sound reverberation in water, amphibians may be able to produce louder vocalisations with no increased effort simply by adjusting the depth of water in which they call (Forrest 1994).

In Chapter 3 I have shown that the alteration of calling characteristics by male anurans in response to traffic noise compensates for the negative, masking effects of this

noise on mate attraction success. It is unlikely that the vocal plasticity of anurans documented in Chapters 2 and 3 is unlimited; there likely exists some critical threshold noise level that would result in conditions that cannot be compensated for. Recent work by Vargas-Salinas et al. (2014) concluded that behavioural alteration of calling activity may become ineffective in areas where traffic noise is continuous. Future work should be directed towards determining levels of traffic noise that represent thresholds beyond which anurans are unable to compensate.

Based on my work, traffic noise does not appear to be a factor contributing to the negative relationship between roads and amphibian populations. The results of Chapters 2 and 3 indicate that anurans are able to compensate for the presence of traffic noise and the potential for this noise to reduce their ability to attract mates. These results suggest that efforts directed at reducing traffic noise *per se* will not result in increases in surrounding amphibian populations and would likely be ineffective in mitigating the impacts of roads on amphibian populations. Assuming that my results can be generalized to anurans as a whole, it seems likely that the previously documented negative effects of roads on anuran populations are likely not caused by noise disturbance. In order to definitively rule out traffic noise as a component of the negative effects of roads on anurans, future research could be directed at designing a study that controls for mortality but varies traffic noise levels to determine if there is an effect of noise at the population level. This research would require a long term study beyond that which I have presented here in order to address annual variation in populations resulting from climatic factors.

In addition to soliciting changes in vocalizations, anthropogenic noise can result in impacts on wildlife in a number of ways. Individuals have been shown to exhibit

higher rates of vigilance after hearing conspecific alarm calls in areas with high levels of anthropogenic noise (Rabin et al. 2006). Additionally, assessment of predation risk may be impacted in noisy environments (Chan et al. 2010). Flight initiation distance is increased in some species of birds when confronted with elevated levels of anthropogenic noise Karp and Root (2009). Conversely, Krause and Godin (1996) suggested that anthropogenic noise may result in decreases in prey vigilance thereby allowing predators to take advantage of less wary prey. If this is true, noisy environments may lead to increased predation rates, at least for some species. In addition to changes in vigilance and predation risk, anthropogenic noise has been found to cause stress responses in some species. Decreases in body condition and weight in some wildlife have been linked to the presence of anthropogenic noise (Anderson et al. 2011). Additionally, noise induced stress was suggested as the likely cause of decreased foraging performance in some species (Purser and Radford 2011). While some species may be able to compensate for the impacts of anthropogenic noise, it is clear that noisy environments can have dramatic impacts on the species that inhabit them.

Following the results of Chapters 2 and 3 I turned my attention toward mitigation of road mortality, the more likely cause of the negative effects of roads on anuran abundance. The results in Chapter 4 suggest that culverts alone do not reduce anuran mortality. By excluding access to culverts, I found no corresponding increase in anuran road mortality. However, fencing installed on both sides of the road surface did result in a reduction in anuran mortality. My results suggest that reducing anuran mortality on roads requires a means of excluding anurans from the road surface.

The implications of my results for road mitigation efforts are considerable. Currently, mitigation efforts assume the primary focus should be on alleviating barrier effects of roads to animal movements and therefore focus is on culverts and other types of ecopassages (Beebee 2013). While movement across roads may be important for some species, simulation studies completed by Jackson and Fahrig (2011) suggest that road mortality has a greater impact on wildlife populations than barrier effects. Results of my work indicate that at a minimum, structures should be installed to exclude individuals from the road surface thereby reducing or removing all together the possibility of road mortality.

Regions that experience considerable seasonal precipitation either in the form of snow or rain, can provide difficult design constraints. Exclusion structures in regions of high snowfall must allow for snow removal to occur in such a way as to prevent snow from forming ramps over the structures or producing holes along the structure that will allow access to the road surface when snow has melted. Additionally, exclusion structures must allow natural drainage patterns to remain during rainfall events to avoid flooding of the road or surrounding communities. Exclusion structures must therefore be designed to address local climatic factors.

In addition to the applied nature of my work, the study presented in Chapter 4 demonstrates the application of the BACI study design in testing the efficacy of crossing structures, and their associated features, in mitigating road effects on wildlife. This study is added to a small number of others that have made use of pre-treatment data to determine the effectiveness of crossing structures (Romin and Bissonette 1996, Aresco 2005a, Clevenger et al. 2001b). The results of my work demonstrate the need to study

road mitigation measures to ensure they have the desired outcome; BACI style studies are ideally suited for this. Future studies would benefit from the inclusion of population level monitoring to determine the efficacy of ecopassages at mitigating the impacts of roads on wildlife populations. Given the cost of installing ecopassages, standard practice for monitoring their effectiveness should follow a BACI design (van der Grift et al. 2013).

The results of my work indicate that traffic noise is not a contributing factor to the negative relationship between roads and amphibian populations; instead, I hypothesize that mortality is a more likely driver of this relationship. Contrary to the currently applied approach, use of culverts per se does not reduce amphibian mortality on roads.

Amphibians must be excluded from the road surface to reduce road mortality. Future efforts should be directed at designing structures that exclude amphibians from the road surface, while addressing local constraints (e.g., precipitation, maintenance, etc.). There exists a continued need to refine our approach to transportation planning to one that understands the long-term ecological costs of roads and recognizes the need to mitigate these impacts.

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Appendix A: Raw capture data of amphibian trapping from 2008. For definitions of treatments, see Table 4.

Date	Site	Treatment	Temperature (°C)	No. Captures
Gray Treefrog				
May 30	A	CPN-T	18	0
May 30	B	CAN-NT	17.5	0
May 30	C	CAN-T	18.5	0
May 30	D	CPN-NT	18.5	0
May 31	A	CPN-NT	11	0
May 31	B	CPN-T	11	0
May 31	C	CAN-NT	10.5	0
May 31	D	CAN-T	10	0
June 1	A	CPN-T	15	1
June 1	B	CAN-NT	14	1
June 1	C	CAN-T	14	0
June 1	D	CPN-NT	13.5	0
June 2	A	CAN-NT	16	1
June 2	B	CAN-T	16	1
June 2	C	CPN-NT	16	1
June 2	D	CPN-T	15.5	1
June 4	A	CPN-NT	16	1
June 4	B	CPN-T	15.5	0
June 4	C	CAN-NT	15.5	1
June 4	D	CAN-T	14.5	3
June 9	A	CPN-NT	25	1
June 9	B	CPN-T	25	0
June 9	C	CAN-NT	24.5	2
June 9	D	CAN-T	24.5	1
June 11	A	CAN-T	17	1
June 11	B	CPN-NT	17	0
June 11	C	CPN-T	16.5	0
June 11	D	CAN-NT	16	1
June 12	A	CAN-NT	19	0
June 12	B	CAN-T	19	2
June 12	C	CPN-NT	19	1
June 12	D	CPN-T	18.5	0
June 16	A	CPN-NT	15.5	0
June 16	B	CPN-T	15.5	0
June 16	C	CAN-NT	15	0

Date	Site	Treatment	Temperature (°C)	No. Captures
June 16	D	CAN-T	15.5	0
June 23	A	CAN-T	16.5	0
June 23	B	CPN-NT	16.5	1
June 23	C	CPN-T	16	0
June 23	D	CAN-NT	15.5	1
June 24	A	CAN-NT	15.5	0
June 24	B	CAN-T	15.5	1
June 24	C	CPN-NT	14	0
June 24	D	CPN-T	14	0
June 25	A	CPN-NT	21.5	2
June 25	B	CPN-T	21.5	0
June 25	C	CAN-NT	21	2
June 25	D	CAN-T	21	1

Green Frog

July 1	A	CAN-T	22.5	1
July 1	B	CPN-NT	22.5	3
July 1	C	CPN-T	23	1
July 1	D	CAN-NT	23	4
July 2	A	CAN-NT	22	3
July 2	B	CAN-T	22	2
July 2	C	CPN-NT	22	2
July 2	D	CPN-T	22	0
July 4	A	CPN-NT	18.5	1
July 4	B	CPN-T	18.5	0
July 4	C	CAN-NT	19	1
July 4	D	CAN-T	19	1
July 7	A	CPN-T	23	0
July 7	B	CAN-NT	23	4
July 7	C	CAN-T	23.5	1
July 7	D	CPN-NT	23	2
July 8	A	CPN-NT	22.5	0
July 8	B	CPN-T	22.5	0
July 8	C	CAN-NT	22	0
July 8	D	CAN-T	21.5	0
July 9	A	CAN-NT	20	0
July 9	B	CAN-T	20	0
July 9	C	CPN-NT	19.5	0
July 9	D	CPN-T	19	0
July 14	A	CPN-T	20	0

Date	Site	Treatment	Temperature (°C)	No. Captures
July 14	B	CAN-NT	20	2
July 14	C	CAN-T	19	1
July 14	D	CPN-NT	19.5	1
July 15	A	CAN-T	19.5	0
July 15	B	CPN-NT	19.5	0
July 15	C	CPN-T	19	0
July 15	D	CAN-NT	18.5	0
July 16	A	CPN-NT	19	2
July 16	B	CPN-T	19	0
July 16	C	CAN-NT	19	2
July 16	D	CAN-T	19	1
July 17	A	CPN-T	22	0
July 17	B	CAN-NT	22	0
July 17	C	CAN-T	21.5	0
July 17	D	CPN-NT	21.5	0
July 21	A	CAN-T	20	2
July 21	B	CPN-NT	20	1
July 21	C	CPN-T	20	0
July 21	D	CAN-NT	19.5	2
July 22	A	CPN-NT	19.5	1
July 22	B	CPN-T	19.5	0
July 22	C	CAN-NT	20	1
July 22	D	CAN-T	19.5	0

American Toad

May 21	A	CAN-T	13	0
May 21	B	CPN-NT	12	0
May 21	C	CPN-T	13	0
May 21	D	CAN-NT	13	1
May 22	A	CPN-T	11.5	0
May 22	B	CAN-NT	11.5	0
May 22	C	CAN-T	12.5	1
May 22	D	CPN-NT	11.5	0
May 23	A	CAN-NT	11.5	0
May 23	B	CAN-T	11.5	1
May 23	C	CPN-NT	13	0
May 23	D	CPN-T	13	0
May 24	A	CPN-NT	13	0
May 24	B	CPN-T	13	0
May 24	C	CAN-NT	14	1

Date	Site	Treatment	Temperature (°C)	No. Captures
May 24	D	CAN-T	12.5	0
May 25	A	CPN-T	14.5	0
May 25	B	CAN-NT	14	1
May 25	C	CAN-T	14	1
May 25	D	CPN-NT	14	1
May 26	A	CAN-T	15.5	0
May 26	B	CPN-NT	15.5	1
May 26	C	CPN-T	16	1
May 26	D	CAN-NT	16	0
May 27	A	CAN-NT	14	0
May 27	B	CAN-T	13.5	0
May 27	C	CPN-NT	13	1
May 27	D	CPN-T	13.5	1
May 28	A	CPN-NT	9	1
May 28	B	CPN-T	9	1
May 28	C	CAN-NT	9	0
May 28	D	CAN-T	9	0

Appendix B. Summary of road killed anurans throughout the survey period. Data presented represent combined counts from all three years of survey (2008-2010) at all sites.