THESIS

HAEMOSPORIDIAN PARASITES OF BARRED OWLS (*STRIX VARIA*) AND NORTHERN SPOTTED OWLS (*S. OCCIDENTALIS CAURINA*): INVESTIGATING THE EFFECTS OF AN INVASIVE SPECIES ON PARASITE TRANSMISSION AND COMMUNITY DYNAMICS

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ABSTRACT

HAEMOSPORIDIAN PARASITES OF BARRED OWLS (*STRIX VARIA*) AND NORTHERN SPOTTED OWLS (*S. OCCIDENTALIS CAURINA*): INVESTIGATING THE EFFECTS OF AN INVASIVE SPECIES ON PARASITE TRANSMISSION AND COMMUNITY DYNAMICS

Although the barred owl (*Strix varia*) was historically limited to eastern sections of North America, its range has steadily expanded westward over the past century. Currently the barred owl's range entirely overlaps the range of the federally threatened northern spotted owl (*Strix occidentalis caurina*) in the Pacific Northwest. Invasive species have been known to drastically impact a region's native species by altering the parasite communities among those species, yet little is known about the parasites of barred and northern spotted owls in particular. The purpose of this study was to determine if and to what extent avian blood parasite assemblages of barred and northern spotted owls have changed as a result of the range expansion by barred owls. Blood samples were collected from sympatric northern spotted and barred owls in northwestern California, as well as from barred owls from seven regions across the barred owl's historic range. I screened samples for blood parasites belonging to the genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, and I analyzed bird infection status and intensity using a combination of PCR and microscopy techniques. Additionally, a section of mitochondrial DNA was sequenced from all samples in which I detected *Haemoproteus* or *Plasmodium* parasites, and I used these sequence data to calculate parasite haplotype richness, haplotype diversity, and similarity of *Haemoproteus* and *Plasmodium* assemblages.

Using these five metrics I evaluated predictions of four hypotheses describing how biological invasions might affect parasite assemblages of invasive and native hosts: the Enemy Release (i.e., hosts benefit from a loss of parasites in their invasive range), Enemy of My Enemy (i.e., invasive hosts introduce parasites to naïve native hosts), Parasite Spillback (i.e., invasive hosts act as a new reservoir to native parasites), and Increased Susceptibility (i.e., native hosts introduce parasites to naïve invasive hosts) Hypotheses.

Analyses of *Leucocytozoon* spp. indicated that the population from which the samples were collected (i.e., eastern barred or western barred) was not important in determining a barred owl's infection status, which offered little support for the Enemy Release Hypothesis (ERH) in the context of *Leucocytozoon* parasites. However, population was an important explanatory variable in determining a barred owl's infection status, parasite richness, and parasite diversity in analyses of *Haemoproteus* haplotypes, offering strong support for the ERH in the context of this genus of parasite. These findings suggest that barred owls may be released from the costs associated with some, but not all, parasite infections in the Pacific Northwest. Additional analyses of *Haemoproteus* haplotypes allowed me to detect a phylogeographic pattern in which one haplotype was common in both barred and northern spotted owls throughout North America, three haplotypes appeared to be isolated to the barred owl's historic range, while a fifth haplotype was notably divergent from all of the other detected haplotypes and seemingly isolated to California owls. Furthermore, probability of infection analyses indicated that host population (i.e., western barred or northern spotted) was an important explanatory variable in determining parasite diversity and a bird's infection status. These findings offer some support for the Parasite Spillback Hypothesis, suggesting that barred

owls may be contributing to higher parasite prevalence among northern spotted owls by serving as an added reservoir host to northern spotted owl populations. *Plasmodium* spp. infections were rare among both barred and northern spotted owls, and I found no evidence that the barred owl range expansion has yet impacted the occurrence of *Plasmodium* spp. within northern spotted owls. Overall, this study demonstrates the complexity of host-parasite relationships and suggests that differences in parasite ecology across genera play an important role in determining whether or not parasites will persevere and be transmitted across invasive and native host populations. In addition, this study has identified a number of blood parasite haplotypes infecting barred and northern spotted owls, yet many questions still remain regarding the true cost of these parasite infections among barred and northern spotted owls and the implications of these infections for northern spotted owl conservation and management.

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As I reflect on my Master's career at Colorado State University, I am reminded of the quote by Sir Isaac Newton, "If I have seen further it is by standing on the shoulders of giants." While I by no means place my M.S. work at the same level of ingenuity and influence as Newton's, the people that have provided me with guidance and encouragement over the past several years are, in my opinion, nothing less than giants and deserve my heartfelt thanks. First and foremost, I would like recognize my advisors Drs. Alan Franklin and Kate Huyvaert for helping me mature into the researcher that I am today. In addition to providing me with generous financial support throughout my M.S. career, Dr. Franklin has extended countless gestures of intellectual and emotional support ranging from buying my cup of morning coffee to sharing his decades-worth of experience on northern spotted owl ecology. Furthermore, I would like to thank Dr. Franklin for introducing me to my major faculty advisor, Dr. Huyvaert. Even when her other research obligations took her thousands of miles away, Dr. Huyvaert's steadfast efforts to respond to my questions and thesis drafts in any way possible demonstrated her immense dedication to my education and research. I can unabashedly say that Dr. Franklin and Dr. Huyvaert's attitudes toward scientific research and their accomplishments in the field have been an inspiration for my own career, and it has been an honor to have worked with them.

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the actual number years ago. For those who have worked beside or been taught by Dr. Diller, they know how lucky I was to have the pleasure of tromping around the forest with such a hard-working and compassionate scientist.

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INTRODUCTION

Parasites can have profound impacts on natural systems by affecting both the ecology and evolution of host populations (Anderson and May, 1978; Scott and Dobson, 1989; Hudson and Greenman, 1998). When parasite communities change in host populations, cascade effects can lead to population-wide changes among other host and parasite species present in the community (Collinge et al., 2008), such as through the loss of native parasites (e.g., Torchin et al., 2001), the introduction of novel parasites (e.g., van Riper et al., 1986), or a shift in the abundance of natural parasites (e.g., Fiorello et al., 2004). As anthropogenic impacts on natural environments increasingly affect parasite and disease dynamics among wildlife populations (Scott, 1988; Daszak et al., 2000), parasites have become an important concern in wildlife conservation and management (Lafferty and Gerber, 2002; Altizer et al., 2003).

Invasive species are another key concern because they are a leading contributor to biodiversity loss worldwide (Clavero and Garcia-Berthou, 2005). Invasive species can lead to declines in native populations through both direct (e.g., predation [Wiles et al., 2003]) and indirect (e.g., competitive exclusion [Holway, 1999]) pathways (Mooney and Cleland, 2001). In addition, invasive species can impact parasite community dynamics in their non-native ranges, which in turn affects competitive interactions among invasive and native host species (Perkins et al., 2008). Theory predicts that ecological and phylogenetic similarities of both hosts and parasites facilitate parasite transmission between invasive and native hosts (Lebarbenchon et al., 2007), yet the impacts caused by changes in parasite communities are often less well-understood than other interactions between invasive and native host species.

Four general hypotheses have been proposed to explain how biological invasions can affect communities of "natural" parasites, which are those found in an invasive host species' native range, and "native" parasites, which are those already present in the invasive host species' introduced or expanded range (Colautti et al., 2004). The first of these hypotheses (the Enemy Release Hypothesis) predicts how the composition, prevalence, and intensity of natural parasite species of invasive hosts may change as a result of the invasion process, while the other three hypotheses (the Enemy of My Enemy, Parasite Spillback, and Increased Susceptibility Hypotheses) examine the dynamics of parasite transmission across interspecific hosts once an invasive host species is established in a novel area.

The **Enemy Release Hypothesis (ERH)** (Williamson, 1996; Crawley, 1997) posits that freedom from parasites provides an invasive host both energetic and competitive advantages in novel areas where hosts have been introduced (Roche et al., 2010). Populations of species invading a new area are generally founded by only a few individuals, which are likely infected with only a subset of the parasites from larger source populations of hosts (Shaw and Dobson, 1995; Colautti et al., 2004). In addition, abiotic (e.g., climate) and biotic (e.g., vector abundance) differences between native and introduced environments can disrupt the life cycles of natural parasites, which can cause hosts to escape infections from such parasites in introduced ranges (Phillips et al., 2010). As a result, invasive host populations tend to be infected with fewer parasite species in their introduced or expanded ranges compared to their native ranges (Torchin et al., 2003; Lebarbenchon et al., 2007). For example, both infection prevalence and parasite species richness are consistently lower in populations of European green crabs (*Carcinus maenus*) in introduced than native regions, which may partially explain

why European green crabs are larger and exhibit a greater biomass in regions where they have been introduced (Torchin et al., 2001).

While lower parasite diversity, prevalence, and infection intensity have been observed across a variety of invasive species occupying new ranges (e.g., Torchin et al., 2003; Ishak et al., 2008; Phillips et al., 2010), the ERH does not suggest that invasive species escape infection with natural parasites entirely. The concept of parasite "spillover" describes situations where parasites that accompany invasive host species are transmitted to new host species in the new environment occupied by the invader (Kelly et al., 2009). If a naïve host lacks the defense mechanisms necessary to deal with these novel parasites, the host population may be impacted severely, including through host mortality and other effects leading to population declines (Lebarbenchon et al., 2007; van Riper et al., 1986; 2002). Furthermore, invasive species may also benefit through apparent competition when the negative impacts of an introduced parasite are considerably higher among an area's native species than the parasite's natural hosts (Settle and Wilson, 1990; Colautti et al., 2004). These concepts have been encapsulated in the **Enemy of my Enemy Hypothesis (EEH)** (Sabelis et al. 2001), where negative impacts may arise from relatively higher infection prevalence, mean intensity, and a greater number of parasite species in native than invasive species. An example of the EEH is the introduction of a parapoxvirus by non-native gray squirrels (*Sciurus carolinenis*) in Great Britain, which has led to the sharp decline in the abundance of the congeneric native red squirrel (*S. vulgaris*) (Tompkins et al., 2002; 2003). While the virus has little effect on gray squirrel populations, it is highly pathogenic in red squirrels, providing gray squirrels a competitive advantage over sympatric

populations of red squirrels for shared resources (Tompkins et al., 2002; 2003; Lebarbenchon et al., 2007).

In addition to introducing parasites to naïve members of new ecological communities, invasive species may become novel, competent hosts for native parasites within these communities and indirectly increase prevalence in native hosts through parasite "spillback" (Kelly et al., 2009; Dobson, 2004). The **Parasite Spillback Hypothesis (PSH)** predicts that new reservoir hosts in a community, such as invasive host species, will increase native species' exposure to parasites, thus increasing the overall proportion of infected native individuals. For example, *Toxoplasma gondii* is a native parasite of wild felids in South America, but it can also infect domestic cats (Fiorello et al., 2004). As domestic cat populations have increased in South America, so has the proportion of wild felids infected with *T. gondii*, suggesting that domestic cats are acting as important reservoirs for the spillback of *T. gondii* into native felid populations (Lehmann et al., 2006; Kelly et al., 2009).

If populations of species invading a new area are founded by only a few individuals, invasive species may experience population bottlenecks that result in the loss of genetic variation (Colautti et al., 2004). Because some immune defense mechanisms rely on high genetic diversity at Major Histocompatibility Complex loci (Potts and Slev, 1995), invasive host species that experience a genetic bottleneck may be more susceptible to parasitic infections relative to populations not experiencing a bottleneck. More importantly, invasive species may act as naïve hosts to native parasites. Similar to the negative impacts of parasite spillover on native host populations, infections with novel parasites in invasive species may exert deleterious effects on their components of fitness, such as survival (Colautti et al., 2004). I

referred to this idea as the **Increased Susceptibility Hypothesis (ISH)**, which predicts that invasive species will be more vulnerable to parasites in new areas they invade relative to host populations in the species' historic range, which in turn may place invasive hosts at a competitive disadvantage with native hosts (Colautti et al., 2004). An example of the ISH has occurred in the Great Lakes—St. Lawrence River system, where a parasitic water mold infects populations of the exotic amphipod *Echinogammarus ischnus* at higher prevalence and intensity than native amphipods (Kestrup et al., 2010). While *E. ischnus* has often replaced native amphipods in many regions of the river system, the maintenance of abundant native amphipod populations in the upper St. Lawrence River is attributed to the presence of the parasitic water mold and its regulatory effects on *E. ischnus* populations (Kestrup et al., 2010).

Predictions of the ERH, EEH, PSH, and ISH are not mutually exclusive of one another. For example, an invasive host may lose natural parasites as it becomes established in new ranges as predicted by the ERH, but this loss does not exclude those invasive hosts from becoming infected with new parasites in invasive ranges as predicted by the PSH and ISH. When examining predictions of these four hypotheses, it is important to understand where along the invasion timeline parasite losses or acquisitions can occur. Because the ERH focuses on events that happen as a host leaves its native range while the EEH, PSH, and ISH focus on events that happen once an invasive host has become established in a new ecological system, predictions of the EEH, PSH, and ISH should take into account whether or not the invasive host population has lost natural parasites over the course of the invasion process. Furthermore, prevalence data alone may not allow us to distinguish infections of newly acquired parasites from those of

natural parasites, especially if a substantial proportion of the natural parasites were lost from host populations during the invasion process.

In this study, I tested predictions of the ERH, EEH, PSH, and ISH on avian blood parasites among native northern spotted owls (*Strix occidentalis occidentalis*) and sympatric invasive barred owls (*Strix varia*) in northwestern California. Distributed throughout the Pacific Northwest, the northern spotted owl was listed as threatened under the Endangered Species Act in 1990 (U.S. Fish and Wildlife Service, 1990) and subsequently became one of the most extensively studied birds in North America (Gutiérrez, 2008). The barred owl historically occurred from south-central Mexico north through the southern United States and into eastern North America (Figure 1A; Johnsgard, 1988). In the early 1900s, the barred owl range began expanding westward to British Columbia and then south through the Pacific Northwest; the species reached northern California by 1981 (Figure 1B; Grant, 1966; Taylor and Forsman, 1976; Dark et al., 1998).

The barred owl poses a competitive threat to northern spotted owl populations because of the ecological and phylogenetic similarities between the two species (Gutiérrez et al., 2007). Both species are large, long-lived, non-migratory, and nocturnal raptors that occupy forested habitats and have adapted relatively similar hunting and reproductive strategies (but see Gutiérrez et al., 2007, for subtler differences influencing competitive dynamics between the two species). Northern spotted/barred owl hybrids and subsequent backcrosses have been reported on numerous occasions (Kelly and Forsman, 2004; Funk et al., 2007), which is suggestive of the two species' close phylogenetic relationship despite their historic isolation. These similarities between barred and northern spotted owls, combined with our extensive

knowledge of northern spotted owl life history and population ecology, provide an excellent opportunity to test predictions of the ERH, EEH, PSH, and ISH and to evaluate the relevance of these hypotheses to northern spotted owl management strategies. In addition, the seemingly natural (i.e., absent of direct human assistance) expansion of barred owls allows for the northern spotted/barred owl system to inform studies on parasite transmission in other ecological systems where natural range expansions are underway. Range expansions are likely to become increasingly prevalent due to both international trade and climate change, which is altering habitat suitability for host species on a global scale (Parmesan, 2006).

Avian blood parasites of the genera *Haemoproteus, Plasmodium,* and *Leucocytozoon* comprise a diverse group of vector-borne blood parasites that have been utilized extensively to model host-parasite interactions in birds (Atkinson and van Riper, 1991; Hellgren et al., 2004). Based on blood smears, two morphologically distinct species of *Haemoproteus* (subgenus *Parahaemoproteus*), *H. noctuae* and *H. syrnii*, and one species of *Leucocytozoon*, *L. ziemanni*, commonly infect both northern spotted and barred owls in their native ranges (Table 1). Given that these species of blood parasites are common across North America, it is unlikely that 1) the barred owl would escape such parasites over the course of the range expansion as predicted by the ERH, 2) the barred owl could introduce novel morpho-species of blood parasites to the northern spotted owl range as predicted by the EEH, or 3) the barred owl would become infected with novel blood parasite species as predicted by the PSH and ISH. However, recent genetic sequence analyses of blood parasites suggest that thousands of undescribed cryptic species have similar morphology with known blood parasite taxa (Bensch et al., 2004; Martinsen et al., 2008; Ishak et al., 2008); therefore, the molecular analyses outlined in this

study have the potential to reveal the existence of such cryptospecies, as well as the geographic distribution and evolutionary history of such cryptospecies.

In addition, genetic analyses of host specificity and the geographic distribution of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* haplotypes among barred and northern spotted owls have the potential to help elucidate the role that vectors play in parasite transmission between these host species. Parasites from all three genera are similar in that they undergo sexual reproduction in dipteran vectors, but they are closely associated with three separate families of vectors with notably different life history strategies and habitat and feeding preferences. Although little is known about the exact species, distribution, and abundance of blood parasite vectors of owls in North America, it is generally accepted that "biting midges" (*Culicoides* spp.), culicine mosquitoes (*Aedes* spp. and *Culex* spp.), and black flies (*Simulium* spp.) serve as vectors for *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* parasites, respectively (Remple, 2004; Valkiūnas, 2005). All three of these vector families require moist habitats for development during immature life stages, but immature *Culicoides* spp. and culicine mosquitoes are adapted to a wide range of habitats that provide a source of free water or moisture (including streams, marshes, saturated soil, tree holes, rotting fruit, and other vegetation [Mellor et al., 2000; Borkent, 2005; Eldridge, 2005]), while immature *Simulium* spp. develop in running water (Alder, 2005). Because parasite prevalence and transmission success has been shown to vary with vector presence and abundance (Deviche et al., 2001), differences in the biology of these three vector families may result in varying transmission success and prevalence of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp. among barred

owls across its range expansion corridor, as well as between barred and northern spotted owls in the Pacific Northwest.

Infection with blood parasites may decrease overall host body condition (Dawson and Bortolotti, 2000) and reproductive success (Merino et al., 2000), especially during stressful periods. Therefore, the potential for the barred owl range expansion to alter blood parasite assemblages and disease dynamics of both barred and northern spotted owls has raised concerns over the parasite-mediated conservation implications of this range expansion for northern spotted owls (Ishak et al., 2008; U.S. Fish and Wildlife Service, 2011). Ishak et al. (2008) observed significantly higher prevalence of blood parasites in barred owls from parts of their historic range compared to invading barred owls in the Pacific Northwest, lending some support for the ERH. However, these results were based on a limited sample of eastern barred owls (*n* = 18) and other competing hypotheses were not explicitly examined. Additionally, Ishak et al. (2008) found that barred owls from both their historic and expanded ranges were infected with *Plasmodium* haplotypes (defined as "lineages" by Ishak et al., 2008), as was a single northern spotted owl, a finding which had not been previously documented (Gutiérrez, 1989; Greiner et al., 1975). Ishak et al. (2008) suggested that this *Plasmodium* detection in a spotted owl could have been caused by parasite spillover from the invading barred owl population but, again, hypotheses predicting spillover were never formally evaluated.

Using a combination of molecular and microscopy techniques, I compared the probability of infection of *Leucocytozoon* spp. and *Haemoproteus* and *Plasmodium* haplotypes among northern spotted owls and barred owls in their invasive and native ranges. I also compared parasite haplotype richness, haplotype diversity, and parasite assemblage similarity

of *Haemoproteus* and *Plasmodium* haplotypes among these owl populations. Finally, I compared *Haemoproteus* infection intensity among these three owl populations. Based on how the ERH, EEH, PSH, and ISH predict parasite community metrics to change over geographic space and among owl populations, I evaluated if and to what extent barred owl range expansion has altered blood parasite assemblages of invasive barred and native northern spotted owls. In doing so, my objective was to address some of the substantial information gaps regarding pathogens and ecological interactions between barred and northern spotted owls outlined in the U.S. Fish and Wildlife Service's Revised Recovery Plan for the Northern Spotted Owl (2011), as well as gain a better overall understanding of the general patterns and principles shaping parasite transmission between invasive and native hosts in the context of host range expansion.

METHODS

To evaluate the four hypotheses, I estimated five parasite metrics (haplotype richness, haplotype diversity, assemblage similarity, probability of infection, and infection intensity) in three populations of owls: northern spotted owls in their native geographic range in northwestern California, barred owls in their non-native range and in sympatry with northern spotted owls in northwestern California (referred to as "western barred owls"), and barred owls in their historic range in the eastern U.S. (referred to as "eastern barred owls").

Parasite haplotype richness and diversity were analogous to metrics used to describe parasite communities in other studies (Poulin and Morand, 1999; Poulin and Morand, 2004) except that I used haplotypes instead of species. I considered a haplotype as a unique DNA

sequence that is inherited from one generation to the next (Posada and Crandall, 2001). Therefore, sequences differing by at least one base pair of the region of the cytochrome *b* gene that I sequenced (see below) were classified as belonging to two distinct haplotypes. Given this definition, I defined parasite haplotype richness as the number of unique parasite haplotypes present in a single host population (Poulin and Morand, 2004). Parasite haplotype diversity was a combination of the number of parasite haplotypes present (i.e., richness) and the number of birds infected with each parasite haplotype (i.e., evenness) of a single host population (Poulin and Morand, 2004). I described parasite assemblage similarity as the total number of parasite haplotypes present in both host populations when comparing two populations of owls (referred to as "shared haplotypes"). I used two metrics of the probability of infection. First I calculated the probability that an owl was infected with a parasite of a given genus (*Haemoproteus, Plasmodium,* or *Leucocytozoon*), regardless of haplotype. Second I tabulated the probability that an owl was infected with parasite haplotypes shared by different host populations. In both cases, I focused my analyses on whether or not the host population (i.e., northern spotted, western barred, or eastern barred owl) was an important variable in predicting probability of infection. Finally, I estimated infection intensity as the proportion of infected blood cells divided by the total number of blood cells examined in a blood smear from an individual host, and, again, I focused my analyses on the importance of host population for predicting infection intensity among the various populations of interest.

Predictions

Enemy Release Hypothesis (ERH)

Under the ERH, western barred owls should host fewer parasites than eastern barred owls due to both the absence of natural parasites and the tendency for invasive species to become infected with few to no native parasites in their expanded range. If the ERH were correct, I first predicted that western barred owls would have lower parasite haplotype richness and diversity than eastern barred owls (Table 2). For this outcome to occur, natural parasites should have been lost as the barred owl population expanded westward and the number of native parasite haplotypes successfully infecting barred owls in the expanded range (new parasites) should be less than the number of natural parasite haplotypes that were lost.

I also predicted that eastern and western barred owls would share some, but not all, parasite haplotypes due to the loss of eastern parasite haplotypes in the western barred owl population. I predicted that western barred owls would be less likely to be infected with blood parasites overall than eastern barred owls (Table 2). However, I predicted that western and eastern barred owls would have similar probabilities of infection for shared parasite haplotypes. I based this latter prediction on the concept that host-parasite dynamics should not drastically change in a host's invasive range if the host was already well-adapted to a specific parasite haplotype. Finally, I predicted that the infection intensity of shared haplotypes would be similar in both eastern and western barred owl populations because western barred owls are expected to be well-adapted to infection with parasites shared with eastern barred owls (Table 2).

Enemy of My Enemy (EEH), Parasite Spillback (PSH), and Increased Susceptibility (ISH) Hypotheses

Predictions for the EEH, PSH, and ISH compare parasite community metrics between northern spotted and western barred owl populations. However, these "post-invasion" hypotheses must take into account whether barred owls escaped natural parasites over the course of the range expansion process under the ERH because they focus on parasite transmission events that occur in the barred owl's invasive range. Therefore, I constructed two sets of predictions for each of these three hypotheses: one set under the condition that predictions of the ERH were met (Table 3), and another set under the condition that ERH predictions were not met (Table 4). In addition, EEH, PSH, and ISH predictions will vary depending on whether or not northern spotted and barred owls are similarly susceptible to parasites in their respective native ranges. Given the two host species' ecological and phylogenetic similarities (Gutiérrez et al., 2007), I predicted that northern spotted and barred owls would be similarly susceptible to avian blood parasites in their respective native ranges, although they would not necessarily be infected with the exact same parasite haplotypes given the phylogeographic isolation of some blood parasites across North America (Kimura et al., 2006). Both sets of predictions for the post-invasion hypotheses were constructed under the condition that this prediction about host susceptibility was met.

Under the EEH, natural parasites that have accompanied invading barred owls into the northern spotted owl range will have subsequently been transmitted to naïve northern spotted owl populations. If both the ERH and EEH are correct, I predicted that parasite haplotype richness and diversity would be greater in northern spotted owls compared to western barred

owls (Table 3). Furthermore, I predicted that 1) a portion of the parasite haplotypes detected in northern spotted owls would also be detected in sympatric barred owls, and that these shared parasite species would have been introduced by invading barred owls, 2) northern spotted owls would have an overall higher probability of infection than western barred owls with shared parasite haplotypes, and 3) infection intensity of these shared parasite haplotypes would be higher among northern spotted owls than western barred owls because naïve northern spotted owls should be more heavily impacted by these introduced parasites than invasive barred owls (Table 3). If conditions of the ERH were not met, predictions for the EEH differed in that northern spotted owls would additionally have an overall higher probability of infection than western barred owls with both the total and shared number of parasite haplotypes (Table 4).

The PSH predicts that invading barred owls in the northern spotted owl range have become new reservoir hosts for parasites native to northern spotted owl hosts, which translates to higher prevalence of these parasites in northern spotted owl populations by increasing the likelihood that a northern spotted owl will be infected by such parasites (Table 3). If both the ERH and PSH are correct, I could not predict how parasite haplotype richness or diversity would compare between northern spotted and western barred owls because these metrics depend on how many natural parasite haplotypes western barred owls lost and how many native haplotypes they acquired. However, I predicted that 1) parasite haplotypes shared between northern spotted and western barred owls would have originated from northern spotted owls and, therefore, northern spotted owls would have a higher probability of infection with these shared parasite haplotypes than western barred owls, and 2) infection intensity

would be similar between the two populations (Table 3). If conditions of the ERH were not met, and assuming that northern spotted and barred owls were similarly susceptible to avian blood parasites in their respective native ranges, I predicted under the PSH that novel infections among western barred owls would result in greater parasite haplotype richness and diversity than northern spotted owls, but the addition of a new reservoir host would result in overall higher blood parasite infection prevalence (measured as probability of infection) in northern spotted owls (Table 4).

Under the ISH, parasites native to northern spotted owl populations have been transmitted to invasive barred owls. If both the ERH and ISH are correct, I could not predict how parasite haplotype richness or diversity would compare between northern spotted and western barred owls for the same reasons as under the PSH. Similar to the PSH, I predicted that parasite haplotypes shared between northern spotted and western barred owls would have originated from northern spotted owl populations. Because barred owls are naïve hosts to these native parasites, I predicted that western barred owls would have a higher probability of infection and higher intensity infections of shared parasite haplotypes than northern spotted owls (Table 3). If conditions of the ERH are not met I predicted under the ISH that parasite haplotype richness, parasite haplotype diversity, probability of infection, and infection intensity would be greater among western barred owls compared to northern spotted owls (Table 4).

Sample Collection

Northern Spotted and Western Barred Owls

From 2008 to 2012, northern spotted owl and western barred owl samples were collected from four different areas in northwestern California that were being surveyed for either one or both owl species: the Northwest California study area (NWC), the Hoopa Valley Tribe Reservation (Hoopa), Green Diamond Resource Company lands (GDRC), and the Weaverville study area (Figure 2). The initial focus was on the NWC and the other three study areas were selected as additional sampling sites because of their proximity to the NWC study area. All four of these study areas were located at the barred owl's invasion front, which is where the effects of host range expansion on parasite assemblages are expected to be most pronounced (Phillips et al., 2010) and, thus, an ideal location for testing predictions of the ERH, EEH, PSH, and ISH.

The NWC study area was subdivided into a Willow Creek "density" study area (WCSA) and 9 regional areas (Franklin et al., 2000). The WCSA encompassed 292 km^2 just south of Willow Creek, Humboldt County, and the entire area was surveyed for both barred and northern spotted owls each year. The regional areas, each with 1-6 spotted owl territories, collectively encompassed approximately 1,784 km^2 throughout Siskiyou, Trinity, Humboldt and Mendocino Counties and were typically surveyed only for northern spotted owls, although barred owls were detected during annual spotted owl surveys. The majority of the NWC study area consisted of federal land, where little to no logging had occurred since the owl was federally-listed in 1990. The NWC study area experienced cool, wet winters and hot, dry summers (Franklin et al., 2000), and elevations ranged from 200 m to 1700 m above sea level.

Below 1200 m, forests were dominated by Douglas fir (*Pseudotsuga menziesii*) mixed with tanoak (*Lithocarpus densiflorus*), madrone (*Arbutus menziesii*), and canyon live oak (*Quercus chrysolepis*). Above 1200 m, forests were dominated by white fir (*Abies concolor*) and pines (*Pinus* spp.).

The Hoopa study area was located on Hoopa Tribal lands 18 km north of Willow Creek, encompassing 356 km^2 of predominately managed timberland. Hoopa was similar to the NWC in climate and forest structure. Past survey efforts on the Hoopa study area focused only on northern spotted owls, but barred owls have been detected during these surveys and barred owl surveys are currently being implemented in this area.

The Weaverville study area consisted of commercial forest lands owned by Sierra Pacific Industries (SPI) and was located near the town of Weaverville, Trinity County, approximately 89 km east of Willow Creek. The elevation at sample sites ranged from 700 m to 1000 m in mixed conifer forests dominated by Ponderosa pine (*Pinus ponderosa*), sugar pine (*Pinus lambertiana*), white fir, and Douglas fir. This study area was drier than Hoopa and NWC but with similar temperature patterns to these study areas in the winter and summer. The Weaverville study area was only surveyed for northern spotted owls, although barred owls were occasionally detected during these surveys.

The majority of the GDRC study area was within 32 km of the Pacific coast, although small sections of GDRC land were as far as 85 km inland. Because of its proximity to the coast, GDRC land experienced milder temperatures and higher year-round precipitation than the other study areas (Ting, 1998). The GDRC study area encompassed approximately 1,265 km^2 of private commercial timberland in Humboldt and Del Norte counties, with elevations ranging

from sea level to approximately 900 m. Forests were dominated by redwood (*Sequoia sempervirens*) and Douglas fir mixed with hardwoods such as tanoak, big-leaf maple (*Acer macrophylum*), madrone, California bay (*Umbellularia californica*), and red alder (*Alnus rubra*). The GDRC land was surveyed for both barred and spotted owls each year. Since 2009 barred owls have been actively removed from treatment areas of GDRC land as part of an experiment designed to test the efficacy of barred owl removal as a strategy for managing northern spotted owl populations.

As part of long-term demography studies on northern spotted owls, owls of both species were captured on all study areas between April and September. Intensive blood sampling occurred between 2010 and 2012, during which time I attempted to collect all of three sample types (see description below) from each captured bird. All birds sampled prior to 2010 were located on the NWC study area and sample type varied depending on the year a bird was captured (Table 5).

Owls were captured using snare poles, noose poles, baited Dho gaza nets, baited mist nets, pan traps, or by hand (Clark, 1981; Forsman, 1983). All captured owls were marked with both U.S. Fish and Wildlife Service bands and unique color bands used for mark-recapture purposes as part of the demography studies. After a bird was banded and measured, blood was collected via brachial venipuncture.

On the GDRC study area, blood samples were also collected from barred owls killed during the removal experiment, using blood drawn from the brachial vein from carcasses within ten minutes of the bird's death. Four barred owls were also captured and sampled just outside of GDRC boundaries as part of radio-telemetry study by the National Council for Air and Stream

Improvement, Inc. (NCASI). These birds were included in the GDRC group because they were in areas similar in geographic location, habitat, and climate to GDRC. Finally, four barred owls were also captured and sampled from British Columbia, Canada, as part of the radio-telemetry study by NCASI.

Sex and age were recorded for each captured bird, as well as UTM coordinates of the capture location using a GPS. In most cases, sex and age were determined from field observations during either the capture visit or another visit to the owl's territory by demography crew members. However, sex was determined in some cases through PCR-based tests performed by Zoogen, Inc. (Davis, California) using whole blood stored on filter paper or in 70% ethanol. Location information was subsequently imported into ArcMap 10 (ESRI, Redlands, California) to calculate the distance (kilometers) of each owl's sampling location to the coast.

Eastern Barred Owls

Blood samples were collected year-round from March 2011 through May 2012 by eight raptor rehabilitation centers located throughout the barred owl's historic range (Table 6; Figure 3). In most cases (n = 155), samples were collected during routine examinations performed at the time of a bird's admission. In some cases (n = 20), samples were collected from resident birds that had been in captivity for up to 3 years. Sex, age, capture location (denoted as the closest city to which the bird was found), and a description of the bird's overall condition were recorded at the time of sample collection.

Sample Types

Three types of samples were collected: thin blood smears, whole blood stored on lysis buffer solution, and blood on Whatman filter paper or FTA cards (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Thin blood smears were made on microscope slides in the field and air dried. Once dried, smears were fixed and stained with a modified Wright-Giemsa stain (Dip Quick, Jorgensen Laboratories Inc., Loveland, CO).

Blood samples on paper were prepared by placing a drop (≈15µL) of blood on pre-cut strips of Whatman filter paper, dried, and stored in 1.5mL Eppendorf tubes at room temperature until processed in the laboratory. In 2011, I replaced the filter paper method with Whatman FTA cards. One to five drops of blood were placed on each FTA card, dried, and stored in envelopes at room temperature until processed.

Blood samples in lysis buffer were prepared by placing 1-2 drops of blood (≈10 µL blood/100 µL buffer) in 200µL of a solution of 1M Tris pH 8.0, 0.5M EDTA pH 8.0, 5M NaCl, and 10% SDS, which was identical to the solution described by Longmire et al. (1997) except 1 M Tris-HCl was used instead of 2 M Tris-HCl. I stored these samples at room temperature until subsequent processing.

Laboratory Analyses

Infection Status

To test for the presence of *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp. in blood samples, I extracted genomic DNA from paper and buffer samples using a DNeasy extraction kit (Qiagen, Valencia, California) following manufacturer's instructions. I then

followed the nested PCR protocol and primer sets described by Hellgren et al. (2004), which targets 480 base pairs of the cytochrome *b* region of the parasites' mitochondrial DNA genome. The cytochrome *b* region is widely used in phylogenetic studies of blood parasites because nucleotide changes in this region occur at a rate appropriate for detecting intraspecific variation among parasites (Farias et al., 2001; Hellgren et al., 2004).

For the first round of PCR, I used the following primers: HaemNFI: 5'-CAT ATA TTA AGA GAA ITA TGG AG-3' and HaemNR3: 5'-ATA GAA AGA TAA GAA ATA CCA TTC-3', and PCR was performed using the following conditions: 25 µL reaction mixtures that contained 0.6 µM of each primer, one Illustra ® Puretaq ® Ready-to-Go PCR Bead (GE Healthcare, Piscataway, NJ) and 2 µl of extracted DNA which served as template. The cycling profile consisted of an initial denaturation at 94°C for 3 min, followed by 20 cycles of 94°C denaturation for 30 sec, 50°C annealing for 30 sec, and 72°C extension for 45 sec. The samples then underwent a final extension at 72°C for 10 min. I adjusted these PCR conditions as needed depending on the quality and quantity of the extracted DNA. Additionally, I included multiple positive and negative controls in each PCR run (up to a plate of 48 reactions). I used aliquots of DNA from samples with DNA sequences matching those from known avian *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp. as positive controls and aliquots of purified water as negative controls.

For the second round of PCR, I used 2µL of the first PCR product and two sets of primers: one set to amplify the target region of the *Haemoproteus* and *Plasmodium* spp. cytochrome *b* genes, and another set to amplify the target region of the *Leucocytozoon* spp. cytochrome *b* gene. For *Haemoproteus* and *Plasmodium* spp., I used the following primers from Bensch et al. (2000) and Waldenström et al. (2004): HaemF: 5'-ATG GTG CTT TCG ATA TAT

GCA TG-3' and HaemR2: 5'-GCA TTA TCT GGA TGT GAT AAT GGT-3'. For *Leucocytozoon* spp., I used primers from Hellgren et al. (2004): HaemFL: 5'-ATG GTG TTT TAG ATA CTT ACA TT-3' and HaemR2L: 5'-CAT TAT CTG GAT GAG ATA ATG GIG C-3'. I used 1µL of the first PCR product for PCR of *Haemoproteus* and *Plasmodium* spp., and the remaining 1µL for PCR of *Leucocytozoon* spp. I performed these two PCRs in separate 25µL mixtures with the same conditions as the first round of PCR. The cycling profile for the second round of PCR was identical to the first round of PCR, except that it was run for 35 cycles instead of 20 cycles.

I ran 2µL of the final PCR products on a 2% agarose gel followed by ethidium bromide staining, UV visualization, and digital imaging. Aliquots of a 100 base pair size marker ("ladder"; New England BioLabs, Ipswich, Massachusetts) were included in at least one lane per row on each gel for size comparison. Positive samples were identified by the presence of a band of moderate to bright intensity at approximately 480 base pairs in size on gels (Appendix A).

To discern whether a bird was infected with *Haemoproteus* or *Plasmodium* spp., samples considered positive using the initial screening protocol as outlined above were selected for DNA sequencing. Excess primers and unincorporated nucleotides from PCR products were removed by adding 1µL of Exosap-IT® reagent (USB Corporation, Cleveland, Ohio) to 20µL of each HaemF/R2 PCR product on a thermal cycler at 37°C for 15 min, followed by 80°C for 15 min. Cleaned PCR products were cycle sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California) under the following conditions: 10µL reactions containing 1µM of either HaemF or HaemR2 primer, 0.25µl of BigDye® Terminator, 2.275µl of BigDye® 5X Sequencing Buffer, and 1µL of cleaned PCR product. The cycling profile consisted of an initial denaturation at 96°C for 1 min, followed by 35 cycles

of 96°C for 30 sec, 50°C for 30 sec, and 60°C for 4 min. Unincorporated dye terminators and salts from sequence products were then removed using a PrepEase® DNA Clean-Up Kit (USB Corporation, Cleveland, Ohio) following manufacturer's instructions. Final sequencing products were visualized on an ABI Prism 3130 genetic analyzer (Applied Biosystems Inc., Foster City, California). To assign each sequence to a parasite genus, sequences were compared with published *Haemoproteus, Plasmodium,* and *Leucocytozoon* sequences in GenBank (Benson et al., 2006) using the National Center for Biotechnology Information nucleotide Basic Local Alignment Search Tool (BLAST).

DNA sequencing from 18 individuals using the HaemF/R2 primers produced clean *Leucocytozoon* sequences rather than the expected *Haemoproteus* or *Plasmodium* sequences. In these instances, I suspected that neither *Haemoproteus* nor *Plasmodium* DNA was present and the positive bands on the agarose gel were a product of non-specific HaemF/R2 binding to *Leucocytozoon* DNA (i.e., false positives [Cosgrove et al., 2006]). Blood smears from these individuals were scanned at 500X and 1000X magnification under oil immersion, and in every instance only *Leucocytozoon* parasites were detected. Given this evidence, I scored these individuals as "Type I Apparent Misclassifications" and removed them from the *Haemoproteus/Plasmodium* datasets used for calculating parasite metrics (see below). Similarly, multiple sequencing attempts failed for 7 samples, which had tested positive for *Haemoproteus* or *Plasmodium* spp. with the initial PCRs, but no evidence of blood parasite infection (*Haemoproteus, Plasmodium*, or *Leucocytozoon* spp.) was detected through microscopy. Therefore I scored these 7 birds as "Type II Apparent Misclassifications" and removed them from the *Haemoproteus/Plasmodium* datasets. Because I did not attempt to

sequence presumptive *Leucocytozoon* positives, I assumed that all *Leucocytozoon* positives in my initial PCR screen were true positives, providing that the negative and positive controls performed as expected.

Thus, ultimately, the criteria used to assign *Haemoproteus/Plasmodium* spp. infection status differed slightly from that used to assign *Leucocytozoon* spp. infection status. An owl's *Haemoproteus/Plasmodium* spp. infection status was determined by a combination of the initial PCR screen and confirmed through DNA sequences. That is, samples identified as positive with the initial PCR and for which I generated a DNA sequence classified as *Haemoproteus* spp. or *Plasmodium* spp. were marked as "infected with *Haemoproteus* spp." or "infected with *Plasmodium* spp.", respectively; negative PCR's were marked as "not infected". *Leucocytozoon* spp. infection status was determined by whether a band was present (i.e., infected) or absent (i.e., not infected) on a gel after the initial PCR screen (Appendix B).

Haplotype Assignment and Phylogenetic Analysis

Sequences were aligned and edited using Sequencher v4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences differing by one or more base pairs were scored as unique haplotypes. Because only one base pair difference was required to classify a sequence as a unique haplotype, I excluded those with one or more ambiguous peaks (e.g., polymorphisms or weak peaks) from any analyses involving haplotype assignment.

Edited and aligned sequences were compared with all published sequences in both GenBank and MalAvi (Bensch et al., 2009) databases to evaluate 1) whether any of the haplotypes detected had been reported as being detected in other birds, and 2) how genetically

similar the unique haplotypes were to published sequences. I downloaded all of the highest-scoring parasite sequences from GenBank that matched the sequences detected in this study by 98% or higher identity. In some cases, I excluded sequences that had 98% or higher identity but a lower maximum score reported by GenBank than sequences with 97% or lower identity. I chose this threshold because blood parasites have an mtDNA evolution rate estimated between 0.1% and 1.3% per million years (Bensch et al., 2013); therefore, sequences falling below the threshold represent taxonomic groups of parasites too distantly related to provide resolution relevant to this study. I aligned all downloaded sequences using Sequencher v4.10.1 and constructed a haplotype table showing polymorphic sites using DNADiffer (Ritland, 2012).

Phylogenetic relationships among *Haemoproteus* and *Plasmodium* haplotypes were evaluated by initially constructing a maximum likelihood tree in PAUP* 4.0b10 (Swofford, 2002) using a midpoint rooting method (Farris, 1972; Hess and De Moraes Russo, 2007). This tree included *Haemoproteus* and *Plasmodium* sequences from Ishak et al. (2008) that were downloaded from GenBank (Accession Numbers EU627791 and EU627827-EU627845). To decrease computing time, the dataset consisted of only nonredundant haplotypes. Using Akaike's Information Criteria corrected for small samples sizes (AICc) reported in the program jModeltest 2.1.3 (Guindon and Gascuel, 2003; Darriba et al., 2012), the most likely model of base pair substitution was a TIM2 + G model (base frequencies = 0.29, 0.11, 0.13; number of substitution sites = 6; rate matrix = 17.31, 13.55, 17.31, 1.0; gamma shape distribution = 0.19). I performed a maximum likelihood heuristic search, enforcing the model parameters with branch swapping by stepwise addition, 100 repetitions of random addition sequences, and a TBR branch-swapping algorithm with a reconnection limit of 8 and 10,000,000 rearrangements per

replicate. A neighbor joining tree served as the starting tree in this analysis, and bootstrapping to assess nodal support was conducted with 1,000 replicates.

Ultimately, the maximum likelihood search yielded 3 trees. Because differences among these trees were between unsupported tip taxa, the overall inferences did not vary across trees; therefore, I reported the first of the 3 trees rather than constructing a consensus tree. The final tree was edited using the software FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).

I also used a haplotype network approach to analyze relationships among detected *Haemoproteus* haplotypes because such an approach is better suited than phylogenetic trees for analyzing intraspecific datasets with low genetic divergence and potentially non-hierarchical relationships among haplotypes (Posada and Crandall, 2001). I constructed a median-joining (MJ) network (Bandelt et al., 1995) using all of the *Haemoproteus* sequences detected in this study as well as *Haemoproteus* haplotypes that were 1) detected in birds from North America, and 2) at least 98% identical to my haplotypes. Ultimately, very few haplotypes met these criteria; therefore, the only additional haplotypes included in the haplotype network were four haplotypes from Ishak et al. (2008; EU627834, EU627836, EU627839, and EU627840) and one haplotype from Ricklefs and Fallon (2002; AF65589). I used NETWORK v4.6 (Fluxus Technology, Suffolk, United Kingdom) to estimate the most parsimonious network. Lastly, following criteria outlined by Ricklefs et al. (2005), I defined evolutionary lineages as groups including two or more haplotypes that were separated from each other by two mutations or fewer (< 0.6% sequence divergence) and I included these lineage demarcations in the final haplotype network.

Infection Intensity

To measure infection intensity of PCR-positive samples, I examined blood smears using an Olympus BX43 microscope with a DP72 digital camera and i-Solution Lite image analysis software (IMT i-Solution Inc., Burnaby, British Columbia, Canada). I took photographs of 25 fields within a 2 cm^2 area of the slide at 1000X magnification under oil immersion. When possible, I used a random selection protocol (Appendix C) to select the fields that I photographed. In cases where smears were in less than ideal condition, I moved across the 2 cm^2 area in an S-shaped pattern and took pictures of the first 25 fields that were of suitable condition for analysis (i.e., monolayer with few damaged cells and little debris). Samples with fewer than 1,000 erythrocytes across 25 fields were excluded from infection intensity analyses.

Using the Manual Tag tool in i-Solution Lite, I counted the total number of erythrocytes in each field, as well as the number of erythrocytes infected with *Haemoproteus*, *Plasmodium*, or *Leucocytozoon* spp. Infection intensity was estimated by dividing the number of infected erythrocytes by the total number of erythrocytes counted in the 25 fields and expressed as the proportion of infected erythrocytes in statistical analyses.

Statistical Analyses

Competing hypotheses were expressed as statistical models where parasite haplotype richness, haplotype diversity, infection status (i.e., the probability of being infected), and infection intensity were response variables (Table 7). In testing predictions for each of the four hypotheses, I conducted separate analyses for each response variable for *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* spp. Because I did not obtain *Leucocytozoon* sequence data,

analyses of *Leucocytozoon* parasites focused only on infection status. Similarly, due to an observed low *Plasmodium* prevalence, analyses of *Plasmodium* parasites focused only on descriptive haplotype richness, diversity, and assemblage similarity metrics. Analyses of *Haemoproteus* haplotypes incorporated all of the response variables listed above.

Development of Datasets

The number of individuals included in analyses of a single metric varied depending on 1) the owl populations of interest under a given hypothesis, 2) whether juveniles were included, 3) whether an analysis focused on all sampled individuals or only infected individuals, 4) the parasite genus of interest, and 5) the availability of clean sequence data. The exact number of individuals included in an analysis is reported in the Results section, below.

I used two different datasets to test ERH predictions. Both datasets consisted of all sampled adult barred owls from both eastern and western populations but juvenile barred owls were excluded from the probability of infection and infection intensity analyses because I obtained only one juvenile barred owl sample from the species' western range, and differences in parasite susceptibility of juvenile and adult hosts (Sol et al., 2003) may introduce bias in estimates. The dataset used for testing the prediction that northern spotted owls and barred owls are similarly susceptible to owl blood parasites in their respective native ranges consisted of all sampled northern spotted and eastern barred owls, regardless of age. The dataset used to test predictions of the post-invasion hypotheses consisted of northern spotted and western barred owls. Juvenile owls were excluded from the dataset for the probability of infection and

infection intensity analyses under post-invasion hypotheses for the same reasons that they were excluded from ERH analyses.

Due to the potential bias induced by unnaturally high intensity infections of rehabilitation birds, I excluded infection intensity analyses when examining the prediction for the ERH. In addition, analyses of *Plasmodium* and *Haemoproteus* parasites excluded individuals marked as "apparent misclassifications" through DNA sequencing. Finally, birds with ambiguous or multiple *Haemoproteus* haplotype infections were excluded from the dataset used to estimate haplotype richness and diversity.

Parasite Haplotype Richness and Diversity

I used infection status and haplotype assignment data generated from my initial PCR screen and subsequent DNA sequencing to estimate parasite haplotype richness and diversity for each population. Because sampling effort and low detection probability of rare haplotypes/species are often concerns in parasite studies (Poulin and Morand, 2004), I used the bias-corrected Chao 2 estimator to estimate total haplotype richness expected within a population (S_{Chao2}). This estimator accounts for potentially missing haplotypes based on the occurrence of rare haplotypes observed in the sample (Colwell and Coddington, 1994; Chao, 2005). The Chao 2 estimator is a nonparametric, incidence-based method that has been evaluated specifically to develop estimates of parasite richness (Poulin, 1998; Walther and Morand, 1998; Chao, 2005) and was calculated as:

$$S_{Chao2} = S_{obs} + \left(\frac{n-1}{n}\right)\left(\frac{Q_1(Q_1-1)}{2(Q_2+1)}\right)$$

where S_{obs} is the number of haplotypes observed among individuals in the sample set, n is the total number of individuals examined, Q_1 ia the number of haplotypes that occur in one bird only, and Q_2 is the number of haplotypes that occurred in two birds. Additionally, 95% confidence intervals for the Chao 2 estimator were calculated as:

$$\text{Lower 95\% Bound} = S_{obs} + \frac{T}{K}$$

$$\text{and Upper 95\% Bound} = S_{obs} + TK$$

where: $T = Chao_2 - S_{obs}$ and $K = exp\left\{1.96\left[log\left(1+\frac{var(S_{Chao2})}{T^2}\right)\right]^{1/2}\right\}$.

Parasite haplotype diversity describes parasite communities in terms of both parasite haplotype richness and the relative evenness of the distribution of parasite haplotypes among infected hosts (Bush et al., 1997). I calculated parasite haplotype diversity using two measures. The first measure was the Shannon Index (Shannon and Weaver, 1962; Sanders, 1968), which emphasizes the haplotype richness component of diversity and is expressed as:

$$H' = -\sum p_i ln\, p_i$$

where p_i is the proportion of birds infected with the ith parasite haplotype. I estimated a bootstrap standard deviation of the Shannon Index that was based on variation in sample order among 1000 randomizations.

The second measure used was the Simpson (1949) Index, which estimates the probability that any two individuals drawn at random from an infinitely large sample belong to the same haplotype using the following:

$$D = \sum p_i^2$$

Similar to the Shannon Index, I computed a bootstrap standard deviation of the Simpson Index based on variation in sample order among 1000 randomizations. Unlike the Shannon Index, the Simpson Index emphasizes the evenness component of diversity because it is weighted by the more common haplotypes (Bush et al., 1997; Magurran, 2004). For ease of interpretability, I reported the inverse of the Simpson Index (1/D), where higher reported index values correspond to higher parasite diversity (Magurran, 2004).

All richness and diversity measures described above were calculated using the software EstimateS (Colwell, 2005). Due to the small number of *Plasmodium* infections observed in my samples, I chose to only use the richness and diversity index estimators with data on *Haemoproteus* haplotypes. For both parasite haplotype richness and diversity analyses, I determined that two populations of owls had statistically different richness and diversity values if their 95% confidence intervals did not overlap the means of the other group.

The Shannon and Simpson indices are two nonparametric diversity measures commonly used to quantify species diversity in ecological studies, but because I was describing the blood parasite assemblages at the haplotype level, I referred to diversity as "haplotype diversity." It is important to note that this is a different usage of the term "haplotype diversity" that is typically used in genetic studies, which is defined as the probability that two random sequences in a sample are different, and is calculated using Nei's (1987) equation:

$$H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{h} p_i^2\right)$$

where n is the number of sequences, h is the number of haplotypes, and p_i is the relative frequency of haplotype i. Because n in this equation traditionally does not account for

uninfected hosts (i.e., hosts with no parasite sequences to report), I determined that the Shannon and Simpson indices would more appropriately describe the diversity of parasite assemblages infecting each owl population and did not report Nei haplotype diversity indices. However, I used Nei's formula for nucleotide diversity was of some interest because it quantified the average number of nucleotide differences per site between two sequences:

$$\pi_x = \frac{n}{n-1}\sum x_i x_j \pi_{ij}$$

where x_i is the frequency of the *i*th sequence in the population, x_j is the frequency of the *j*th sequence, and π_{ij} is the proportion of different nucleotides between sequences *i* and *j* (Nei and Li, 1989). I calculated nucleotide diversity within each owl population using the software DNAsp (Librado and Rozas, 2009).

Probability of Infection and Infection Intensity

Statistical models for probability of infection and infection intensity were analyzed using SAS v.9.3 software (SAS Institute Inc., 2011). I used logistic regression (PROC LOGISTIC) to estimate probability of infection, where infection status ('infected' or 'not infected') was the binary response variable. I used generalized linear models (PROC GENMOD) to estimate infection intensity where infection intensity (the number of erythrocytes examined) was the continuous response variable.

Ecological Variables.—Model sets included up to six biologically relevant ecological variables as explanatory variables (Table 8). The suite of variables included in a given model set depended on which populations of owls I was comparing and the hypotheses of interest. All model sets used to test ERH predictions included population (*PO*, eastern or western barred) as

an explanatory variable. Additionally, host sex has been documented to influence blood parasite infection among birds (McCurdy et al., 1998); therefore, I included sex (*SX*, male or female) as a variable in this model set.

Competing models used to evaluate the prediction that northern spotted owls and barred owls are similarly susceptible to owl blood parasite in their respective native ranges included population (northern spotted or eastern barred), sex, and age (*AG*, juvenile or adult) as explanatory variables.

All model sets used to test the post-invasion hypotheses included population and sex as explanatory variables. Additionally, model sets included a sampling site's management intensity level (*MG*, where owls captured on GDRC, Hoopa, and Weaverville lands = "high intensity" sites and owls captured on NWC lands = "low intensity" sites), distance to the coast (*DC*, kilometers), and the natural log of distance to the coast (*LnDC*). Management intensity level was included as an explanatory variable because blood parasite prevalence and infection intensity have been previously correlated with disturbance levels (e.g., Patz et al., 2000; Bonneaud et al., 2009). I included *DC* and *LnDC* as explanatory variables because of the temperature and precipitation differences between coastal and inland sites (see "Study Area" section above) that could impact vector abundance (Young et al., 1993; Mellor et al., 2000) and parasite reproduction (Valkiūnas, 1996) and, thus, parasite prevalence among and infection intensity within hosts.

Model Selection and Multi-Model Inference.—In evaluating multiple competing hypotheses with both the logistic regression and generalized linear modeling approaches, I used an information-theoretic approach (Burnham and Anderson, 2002) to select appropriate

models for inference. Due to limited sample size, I used a bias-corrected version of Akaike's Information Criteria, AICc (Hurvich and Tsai, 1995; Burnham and Anderson, 2002), to objectively rank models. In practice, models were ranked and compared using ΔAICc (the difference between each model, *i*, and the top-ranked model) and Akaike weights, respectively (Burnham and Anderson, 2002). I also used R^2 values as a measure of the proportion of variation in the data explained by each model. In the logistic regression models, I used a maximum re-scaled R^2 (SAS Institute Inc., 2011), and in the linear regression models I used values from general linear models (PROC GLM in SAS) as an approximation of R^2.

To account for model selection uncertainty, I model averaged parameter estimates (β_i) and their sampling variances across all models in a given model set (Burnham and Anderson, 2002). In some cases, such as for the effects of owl population, the parameters were included in all the models of a given model set. I used the model-averaged estimates of sampling variances to compute 95% confidence intervals (CI) for the model-averaged parameter estimates. I also reported estimates of the effects of other important variables when competing models with non-trivial Akaike weights included these variables. The relevance of parameter estimates was assessed based on whether 95% confidence intervals overlapped zero.

RESULTS

I analyzed blood samples from a total of 357 owls (127 northern spotted, 55 western barred, and 175 eastern barred). Of these samples, 97.6% (95% CI = 94.9, 100%) of northern spotted owls were infected by *Leucocytozoon* spp. in my initial PCR screen, while western and

eastern barred owls had similar prevalence of infection (52.7%, 95% CI = 39.6, 65.8% and 51.4%, 95% CI = 44.0, 58.8%, respectively). Twenty five samples were excluded from *Haemoproteus* and/or *Plasmodium* analyses due to apparent misclassifications. Additionally, sequences generated from eight eastern barred owls had a large number of polymorphisms. Because I could not discern whether these individuals were infected with *Haemoproteus* spp., *Plasmodium* spp., or both, I excluded them from *Haemoproteus* and/or *Plasmodium* analyses. Of the remaining samples analyzed for *Haemoproteus* and/or *Plasmodium* spp. (*n* = 112 northern spotted, 54 western barred, and 158 eastern barred owls), 1.9% (95% CI = 0, 4.1%) of eastern barred owls tested positive for *Plasmodium* spp. infections, but no *Plasmodium* spp. infections were detected in northern spotted and western barred owls. Northern spotted owls had slightly lower prevalence of *Haemoproteus* haplotype infection than eastern barred owls (79.5%, 95% CI = 72.1, 86.9% and 89.9%, 95% CI = 85.2, 94.6%, respectively), while western barred owls had low prevalence of *Haemoproteus* haplotype infection (33.3%, 95% CI = 20.8, 45.8%). Seventy-seven percent (95% CI = 69.0, 84.6%) of northern spotted, 16.7% (95% CI = 6.7, 26.7%) of western barred, and 47.5% (95% CI = 39.7, 55.3%) of eastern barred owls were co-infected with *Leucocytozoon* and *Haemoproteus* spp., while only one eastern barred owl was co-infected with *Leucocytozoon* and *Plasmodium* spp.

Across samples from northern spotted, western barred, and eastern barred owl, 478 base pairs were sequenced from a total of 3 owls with putative *Plasmodium* spp. infections and 211 owls with putative *Haemoproteus* spp. infections. Of these 214 infections, I detected 2 unique *Plasmodium* haplotypes (representing 2 unique *Plasmodium* lineages) and 5 unique *Haemoproteus* haplotypes (representing 4 unique *Haemoproteus* lineages). Including the

unique haplotypes detected in Ishak et al. (2008), the dataset used to construct the maximum likelihood tree consisted of 24 operational taxonomic units with 22 parsimony-uninformative variable sites and 96 parsimony-informative variable sites. In this tree with only nonredundant haplotypes, sequence divergence was only slightly higher across *Plasmodium* haplotypes (uncorrected p-distance = 0.055) than across *Haemoproteus* haplotypes (uncorrected p-distance = 0.051). Details on host breadth, geographic range, and relevance to the ERH, EEH, PSH, and ISH are described below.

Leucocytozoon

Northern Spotted Owls vs. Eastern Barred Owls

The analysis to test the prediction that northern spotted and barred owls are similarly susceptible to owl *Leucocytozoon* spp. in their respective native ranges included data from 127 northern spotted and 158 eastern barred owls (*n* = 285 total). Each of the top five models had an Akaike weight between 0.164 and 0.220, and these models collectively accounted for 94.1% of the cumulative Akaike weight (Table 9). Model-averaged estimates of the population effect suggested that eastern barred owls had a lower probability of infection with *Leucocytozoon* spp. than northern spotted owls ($\widetilde{\beta}$ = -1.93, 95% CI = -2.56, -1.30). Model-averaged estimates of sex and age effects indicated that females had a slightly higher probability of being infected than males ($\widetilde{\beta}$ = 0.155, 95% CI = 0.030, 0.280), and adults had a slightly higher probability of being infected than juveniles ($\widetilde{\beta}$ = 0.176, 95% CI = 0.001, 0.343). Based on the top-ranked model in which population was the only explanatory variable, northern spotted owls had

almost twice the probability of being infected than eastern barred owls (Figure 4). All of the models tested accounted for 39.3% to 41.5% of the variation in the data (Table 9).

Eastern vs. Western Barred Owls

The analysis to test predictions of the Enemy Release Hypothesis for *Leucocytozoon* spp. of barred owls included data from 121 eastern and 54 western barred owls (*n* = 175 total). In this model set, the intercept-only model was the top-ranked model, and models that included range or sex were not as heavily weighted as the top-ranked model (Table 10). These results indicated that neither population nor sex was important in determining a barred owl's infection status. Based on the intercept-only model, both eastern and western barred owls had similar probabilities of infection (0.537, 95% CI = 0.463, 0.610).

Northern Spotted vs. Western Barred Owls

I included data from 59 northern spotted and 50 western barred owls (*n* = 109 total) in the logistic regression analysis used to test predictions of the post-invasion hypotheses. Modeling of infection probability at the species level did not result in a clear top-ranked model, with no model carrying an Akaike weight greater than 0.17 (Table 11). No additional variables were repeatedly found among the top models aside from population, which was included in all of the models tested and therefore was expected to be repeatedly found among the top models. A model-averaged estimate of the population effect indicated that western barred owls had a lower probability of being infected with *Leucocytozoon* spp. than northern spotted owls ($\widetilde{\beta}$ = -2.03, 95% CI = -3.12, -0.93). Based on the top-ranked model where population was

the only explanatory variable, northern spotted owls had almost twice the probability of infection with *Leucocytozoon* spp. than western barred owls (Figure 5). All of the models evaluated accounted for 44.7% to 47.4% of the variation in the data (Table 11).

Plasmodium

I detected two *Plasmodium* haplotypes among the 357 owls that were screened. Two eastern barred owls were infected with one haplotype (P1 in Figure 6; Appendix D), while another eastern barred owl was infected with the second haplotype (P2 in Figure 6; Appendix D). Both haplotypes were identical to sequences published on GenBank which included data from over 18 avian species across North and South America, Asia, Africa, and Europe (Figure 6; Appendix E). I did not detect a *Plasmodium* haplotype in any western barred or northern spotted owl. Due to the low number of infected birds, I did not perform any statistical analyses of infection probability or intensity of *Plasmodium* parasites.

Haemoproteus

Northern Spotted Owls vs. Eastern Barred Owls

The analysis to test the prediction that northern spotted and barred owls are similarly susceptible to *Haemoproteus* parasites included data from 112 northern spotted and 143 eastern barred owls (*n* = 255 total). The top three models accounted for 87.4% of the cumulative Akaike weight, and all three models included population and sex as explanatory variables (Table 12). Model-averaged estimates of population indicated that eastern barred owls had a higher probability of being infected with *Haemoproteus* haplotypes than northern

spotted owls ($\widetilde{\beta}$ = 0.393, 95% CI = 0.014, 0.774). A model-averaged estimate of the effect of sex indicated that females had a lower probability of being infected with *Haemoproteus* haplotypes than males ($\widetilde{\beta}$ = -0.538, 95% CI = -0.933, -0.144). Using the top-ranked model that included population, sex, age, and a sex by age interaction as explanatory variables, population and age appeared to have a strong effect on the probability of infection among female owls, with adult female eastern barred owls being about one and a half times more likely to be infected with *Haemoproteus* parasites than juvenile female northern spotted owls (Figure 7). On the other hand, effects of population and sex only differed by a few percentage points among male owls (Figure 7), which suggested that parasites appear to have differential effects among male and female avian hosts. Top-ranked models explained between 9% and 12% of the variation in the data (Table 12).

Eastern vs. Western Barred Owls

Analyses of *Haemoproteus* haplotype richness, haplotype diversity, and assemblage similarity to test predictions of the ERH consisted of data from 135 eastern and 53 western barred owls (*n* = 188 total). I detected four unique *Haemoproteus* haplotypes among the eastern barred owls I sampled, three of which did not match any sequences previously detected and published on GenBank or MalAvi. The first of these three new haplotypes (H1 in Tables 13-14; Figures 6 and 8; Appendix D) was detected in 17 barred owls from states in the Midwest and Northeast United States. The second haplotype (H2 in Tables 13-14; Figures 6 and 8; Appendix D) was found in only one barred owl from Alabama. Because H2 differed from the first haplotype by one base pair, I considered H1 and H2 as belonging to the same putative

evolutionary lineage. The third haplotype (H3 in Tables 13-14; Figures 6 and 8; Appendix D) was also found in only one barred owl from Alabama and matched a haplotype found in a barred owl from Wisconsin in a previous study (Ishak et al., 2008). The majority of eastern barred owls were infected with the fourth haplotype (H4 in Tables 13-14; Figures 6 and 8), which matched a *Haemoproteus* sequence detected in both a barred and a great horned owl from Florida (Appendix E) and differed by one base pair from another sequence detected in several other owl species across the world (Tables 13-14; Figures 6 and 8; Appendix E).

I detected two unique *Haemoproteus* haplotypes among the western barred owls I sampled. The first haplotype was the same haplotype detected in the majority of eastern barred owls (H4). The second haplotype (H5) was detected in only one barred owl from California and matched a *Haemoproteus* sequence detected in a California spotted owl (*S. o. occidentalis*) in a previous study (Tables 13-14; Figures 6 and 8; Appendices D and E). H5 differed from all other haplotypes by at least 13 base pairs, suggesting that it is distantly related to these other haplotypes.

With the exception of five *Haemoproteus* haplotypes found in owls (see "Haplotype Assignment" section, above), and two haplotypes found in quail (Pacheco et al., 2011), no *Haemoproteus* haplotypes from North America matched the haplotypes detected in this study by 98% or above (Tables 13 and 14), despite the fact that over 20 studies have examined avian blood parasites in over 105 avian host species on this continent (MalAvi, 2008). Furthermore, with the exception of a *Haemoproteus* haplotype detected in an owl from Turkey, none of the previously documented *Haemoproteus* haplotypes would be considered the same lineage as the haplotypes detected in this study based on the criteria of 0.6% or less divergence (Tables 13

and 14). These results suggest that the *Haemoproteus* haplotypes infecting barred and northern spotted owls are host specialists that are restricted to owls.

Using the Chao 2 estimator to account for undetected rare species, the estimated number of *Haemoproteus* haplotypes was 5 (95% CI = 4, 17) infecting eastern barred owls and 2 (95% CI = 2, 2.03) infecting western barred owls. The estimated Shannon diversity index (Figure 9) for eastern barred owls (0.47, 95% CI = 0.46, 0.48) was more than twice that for western barred owls (0.19, 95% CI = 0.15, 0.23), while the estimated Simpson diversity index (Figure 10) was also higher for eastern barred owls (1.35, 95% CI = 1.32, 1.38) than for western barred owls (1.14, 95% CI = 1.10, 1.18). Among infected owls, nucleotide diversity was approximately twice as high in eastern (π_x = 0.00668) than western (π_x = 0.00343) barred owls. These indices suggested that barred owl populations in their native range were infected with a higher richness and diversity of *Haemoproteus* haplotypes compared to barred owl populations in California.

Analysis of infection probability included data from 115 eastern and 52 western barred owls (*n* = 167 total). The top-ranked model carried an Akaike weight of 0.644 and included population as the only explanatory variable (Table 15). A model-averaged estimate of the population effect indicated that population contributed to a higher probability of infection with *Haemoproteus* haplotypes among eastern barred owls compared to western owls ($\widetilde{\beta}$ = 1.552, 95% CI = 1.114, 1.990). The top-ranked model explained 45% of the variation (Table 15) and predicted that eastern barred owls had almost three times the probability of infection than western barred owls (Figure 11).

The second logistic regression analysis included data from 99 eastern and 52 western barred owls (*n* = 151 total) and tested the probability that a barred owl was infected with the shared haplotype H4. Similar to logistic regression analyses that tested the probability that a barred owl was infected with any *Haemoproteus* haplotype, the top-ranked model had an Akaike weight of 0.670 and included population as the only explanatory variable (Table 16). A model-averaged estimate of the population effect indicated that population contributed to a higher probability of infection with haplotype H4 among eastern barred owls compared to western barred owl ($\widetilde{\beta}$ = 1.031, 95% CI = 0.095, 1.966). The top-ranked model explained 26% of the variation (Table 16) and predicted that eastern barred owls had two and a half times the probability of infection than western barred owls (Figure 12).

Northern Spotted vs. Western Barred Owls

Haemoproteus haplotype richness, haplotype diversity, and assemblage similarity analyses that tested predictions of the post-invasion hypotheses consisted of data from 147 owls from California (98 northern spotted; 49 barred). I detected two unique haplotypes in northern spotted owls. These were the same haplotypes that I detected in western barred owls (H4 and H5; see above). However, 10 (10.2%) northern spotted owls were infected with H5 (i.e., the California-specific haplotype) compared to one (2.0%) western barred owl (Table 14; Figure 8). Chao 2 estimates and confidence intervals did not differ from the raw number of haplotypes detected (northern spotted owls = 2 haplotypes, 95% CI = 1.99, 2; western barred owls = 2 haplotypes, 95% CI = 2, 2.05). Estimated Shannon and Simpson diversity indices were both much lower for western barred owls and barred owls in California than northern spotted

owls (Figures 9 & 10). In addition, nucleotide diversity was approximately twice as large in the northern spotted owl population (π_x = 0.0064) than sympatric barred owls from California (π_x = 0.0037). These indices suggest that *Haemoproteus* haplotype richness was similar between northern spotted and western barred owls, but *Haemoproteus* haplotype diversity was greater in northern spotted owls than western barred owls in general and those specifically in California.

Analysis of infection probability included data from 105 adult owls from California (56 northern spotted; 49 western barred). The top-ranked model carried an Akaike weight of 0.214 and included population and distance to coast as explanatory variables (Table 17). Model-averaged estimates of the population and distance to coast effects indicated that western barred owls had a lower probability of infection with *Haemoproteus* haplotypes than northern spotted owls ($\widetilde{\beta}$ = -0.947, 95% CI = -1.592, -0.302) while the probability of an owl being infected by *Haemoproteus* spp. increased as distance from the coast increased ($\widetilde{\beta}$ = 0.024, 95% CI = 0.010, 0.038; Figure 13). The top-ranked model explained 43.8% of the variation (Table 17).

On average, 2431 (95% CI = 2328, 2534) erythrocytes were examined per bird for infection intensity analyses. Generalized linear models of infection intensity included data from 47 adult owls from California (34 northern spotted, 13 western barred). The top-ranked model was an *a posteriori* model that included distance to coast as its only explanatory variable, with an Akaike weight of 0.333 (Table 18). Additionally, distance to coast was included in 11 out of the 18 top-ranked models. A model-averaged estimate of distance to coast indicated that infection intensity increased as distance from the coast increased ($\widetilde{\beta}$ = 0.0002, 95% CI = 0.0001, 0.0004). A model-averaged estimate of the population effect indicated that infection intensity

was not an important explanatory variable ($\widetilde{\beta}$ = -0.0003, 95% CI = -0.004, 0.0032). The top-ranked model explained 20.0% of the variation (Table 18) and suggested that owls sampled further inland had almost 5 times the number of infected cells than those samples near the coast (Figure 14).

DISCUSSION

In this study, I compared five metrics of blood parasite assemblages in northern spotted, western barred, and eastern barred owls in order to test predictions of four hypotheses that describe how host range expansion can affect parasite assemblages of both native and invasive host populations. Birds from all three populations were infected with blood parasites, ranging from 33.3% to 97.6% sample prevalence. Overall, the prevalence of blood parasites was highest in northern spotted owls and lowest in sympatric western barred owls. I detected all three genera of blood parasites among the populations that I sampled, but *Plasmodium* parasites were much less common than *Leucocytozoon* and *Haemoproteus* parasites. I found mixed support for the hypotheses that I examined in this study, especially when compared across blood parasite genera. Some of these findings exhibited similar patterns to those of past studies on blood parasites in both the northern spotted/barred owl system and other avian systems, yet other findings were unique to my study. These results can be partially explained by acknowledging the differences between this and past studies, as well as by examining my results in the greater context of vector ecology, host behavior, and host susceptibility.

Leucocytozoon

Estimates of *Leucocytozoon* spp. infection probability demonstrated no support for the Enemy Release, Parasite Spillback, or Increased Susceptibility Hypotheses and moderate support for the Enemy of My Enemy Hypothesis (Table 19). I found a negligible difference between infection probability of eastern and western barred owls, which failed to support the Enemy Release Hypothesis prediction that western barred owls would have a lower probability of infection with *Leucocytozoon* spp. compared to eastern barred owls. These results exhibited a similar pattern to that detected in Ishak et al. (2008) where *Leucocytozoon* spp. prevalence was 17% and 12% among eastern and western barred owls, respectively; however, the fact that *Leucocytozoon* spp. prevalence was almost three times higher among the barred owls in my study was surprising. Prevalence of *Leucocytozoon* parasites varies among populations of a single species of avian host based on sampling location (e.g., Paperna et al, 2005); therefore, differences in sample size and sample locations may explain some of the discordance between results. For example, Ishak et al. (2008) sampled 18 barred owls from Minnesota, Wisconsin, and Texas, while my eastern barred owl dataset was composed of 121 owls from 12 eastern states (including Minnesota and Wisconsin, but not Texas). Furthermore, Ishak et al. (2008) sampled 26 barred owls from California, Oregon, and Washington while samples from 54 barred owls from northwest California comprised my dataset.

Loss of natural parasites as predicted by the ERH occurs under the premise that the parasite life cycle is disrupted by some change in either the physical or biological environment in a host's invasive range (Phillips et al., 2010). For *Leucocytozoon* spp. blood parasites, possible disruptions include a change in the presence or abundance of ornithophilic simuliid fly

vectors, as well as host introduction to environments where the surrounding temperature is outside of the optimal range for *Leucocytozoon* spp. reproduction in these vectors. The dearth of support for the ERH for *Leucocytozoon* spp. in this study suggests that no such disruptions in the parasite life cycle occurred during the range expansion process, which in turn suggests that suitable habitat and vector abundance of the *Leucocytozoon* spp. infecting barred owls occur throughout the barred owl's historic and expanded range. These results corroborate the notion that *Leucocytozoon* parasites and their vectors are well-adapted to the low temperatures of the Northern Holarctic, and may partially explain why a high prevalence of birds infected with *Leucocytozoon* spp. occurs in this ecozone across North America (Valkiūnas, 1996).

Given no support for the ERH, I predicted that eastern barred owls would exhibit a greater probability of being infected with *Leucocytozoon* spp. than western barred owls under either the Parasite Spillback or Increased Susceptibility Hypotheses. Again, results of my analyses comparing infection probability of eastern and western barred owls did not support this prediction. A critical component of both the PSH and ISH is that an invasive host acquires novel infections by parasites native to the invasive range (Kelly et al., 2009). However, if *Leucocytozoon* spp. are common and ubiquitous across North America, the conditions necessary for either the PSH or ISH to occur among invasive barred owl populations may not exist because there may not be many *Leucocytozoon* spp. isolated to the western half of North America to which barred owls would be naïve.

Northern spotted owls had a higher probability of being infected with *Leucocytozoon* spp. than both eastern and western barred owls. These results can be explained by one of two general mechanisms. First, northern spotted and barred owls may not be similarly susceptible

to *Leucocytozoon* spp. Differences in host susceptibility may be driven by differences in biotic factors, such as host immune system, host behavior, habitat, and vector behavior (Tella et al., 1999; Anderson and DeFoliart, 1961; Hale and Briskie, 2007), or abiotic factors, such as climate (Bonneaud et al., 2009; Lacorte et al., 2013). Garvin and Remsen (1997) posited that parasite prevalence differences between avian host species may be driven by interspecific differences in host behavior, such as nesting and foraging that influence a host species' exposure to vectors at the microhabitat scale. Wiens (2012) documented that barred owls in the Pacific Northwest use all available forest types more evenly than do sympatric northern spotted owls, and that they had stronger associations with flat, riparian areas compared to northern spotted owls. These differences in habitat use may contribute to the observed higher prevalence of *Leucocytozoon* spp. in northern spotted owls than western barred owls in my study, but the trend is opposite than what would be expected based on prior knowledge of host-vector interactions and factors driving host susceptibility. Specifically, one would predict higher prevalence of *Leucocytozoon* spp. in barred owls given that host populations occupying a wider range of habitats should be exposed to a more diverse group of vectors and parasites, and that the simuliid fly vectors of *Leucocytozoon* spp. requires moist habitats for reproduction. However, because simuliid flies reproduce in running water (Forrester and Greiner, 2008), it is possible that these vectors are more abundant in areas with steeper terrain (and, thus, more rapidly flowing water), which would cause northern spotted owls to be more consistently exposed to such vectors. A survey of vector abundance at the microhabitat scale in northwestern California may help elucidate components of this host-vector-parasite relationship.

Hellgren et al. (2008) documented that simuliid fly vectors have stronger associations with some avian groups, such that *Leucocytozoon* spp. prevalence is expected to be higher among avian hosts that are preferentially fed upon by simuliid flies. Therefore, another explanation under the "host susceptibility" category is that simuliid fly vectors may preferentially feed on northern spotted owls over barred owls, thus increasing the likelihood that northern spotted owls become exposed to *Leucocytozoon* parasites.

I also found some support for the Enemy of My Enemy Hypothesis, which predicts that northern spotted owls have a higher probability of being infected with *Leucocytozoon* spp. due to the introduction of novel *Leucocytozoon* spp. by invading barred owls. Without *Leucocytozoon* DNA sequence data, I could not evaluate whether any *Leucocytozoon* haplotypes found in western owl populations originated from eastern barred owl populations, nor could I assess the prevalence and intensity of these infections among northern spotted owls. However, Gutiérrez (1989) found that 91% (95% CI = 73, 100%) of northern spotted owls he sampled were infected with *Leucocytozoon* spp. prior to when barred owls became well-established in northwestern California, which is only slightly lower than the observed 97% prevalence documented in this study. If barred owls have introduced novel *Leucocytozoon* parasites to northern spotted owls, the EEH predicts that the probability of infection should be greater in post-invasion northern spotted owl populations than pre-invasion populations. Given that past and present northern spotted owl populations appear to have a high prevalence of *Leucocytozoon* parasites, it seems more likely that differences in host susceptibility are driving differences in probability of *Leucocytozoon* spp. infection between northern spotted and barred owls than spillover of *Leucocytozoon* parasites from invasive barred owl populations.

Plasmodium

With *Plasmodium* spp., I found some qualitative support for the Enemy Release Hypothesis (Table 19), but this conclusion is limited due to the low prevalence of *Plasmodium* spp. in the individuals that I sampled. I detected two *Plasmodium* haplotypes among 3 eastern barred owls and no *Plasmodium* haplotypes among western barred owls, which supports the ERH predictions that western barred owls will have lower haplotype richness and diversity in their introduced range than their native range. Given that I did not find *Plasmodium* spp. in either northern spotted or western barred owls, I could not assess predictions of the Enemy of My Enemy, Parasite Spillback, or Increased Susceptibility Hypotheses.

Despite limited infections with *Plasmodium* haplotypes, several broad conclusions can be inferred from my results. GenBank and MalAvi searches showed that one of the two haplotypes detected in my eastern barred owl samples (P1) has been found in over 15 species of bird (all passerines or raptors) sampled from sites across North and South America (Figure 6; Appendix D). Furthermore, P1 has been detected in a passerine from a region of northern California that does not currently overlap the barred owl's range (Kimura et al, 2006); this supports the idea that this *Plasmodium* haplotype is relatively rare yet cosmopolitan, and that *Plasmodium* spp. are more capable of infecting a broader range of vertebrate hosts compared to parasites belonging to other genera of blood parasites (Beadell et al., 2004). In concluding that this *Plasmodium* haplotype is rare, there is also support for the ERH prediction that rare parasites will be lost from invading host populations as a host species invades new regions (Coulatti et al., 2004) because it was detected in eastern but not western barred owls. *Plasmodium* spp. have been observed to have the greatest impact on avian populations in cases

where hosts were entirely naïve to the parasite (e.g., van Riper, 1986), which is not likely the case for northern spotted owls and this haplotype given the likelihood that this haplotype occurs in the range of the northern spotted owl at low prevalence.

The second *Plasmodium* haplotype detected in my study in a barred owl from Minnesota (P2) has been detected in several avian hosts in eastern North America, Asia, Africa, and Europe, but it has not been detected in the Pacific Northwest (Figure 6; Appendix E). It is unclear if these findings are because this haplotype is currently restricted to the eastern half of the US or because of limited sampling in the west. Regardless, my results offer no evidence that either of the two *Plasmodium* haplotypes detected in my study are having widespread negative impacts in northern spotted owl populations given their rarity.

Haemoproteus

Results from my analyses of *Haemoproteus* haplotype infections in northern spotted and barred owls provided strong support for the Enemy Release Hypothesis, mixed support for the Enemy of My Enemy and Parasite Spillback Hypotheses, and very little support for the Increased Susceptibility Hypothesis (Table 19). Northern spotted owls were less likely to be infected with *Haemoproteus* haplotypes than eastern barred owls, which suggests that northern spotted and eastern barred owls did not have similar susceptibility to infection with *Haemoproteus* in their native ranges, even though as hosts they may have ecological and phylogenetic similarities such as occupying forested areas, hunting strategies, and a congeneric relationship. However, this does not contradict the results of my other analyses because I observed that western barred owls were considerably less likely to be infected with

Haemoproteus haplotypes than either eastern barred or northern spotted owls. The lower infection probability in western barred compared to eastern barred owls supports the ERH prediction that infection prevalence is lower in a host species' invasive range than its native range. Additionally, I detected lower haplotype richness and diversity in western than eastern barred owls, both of which support the ERH prediction that host populations escape native parasites when invading ecological communities in which those native parasites have yet to adapt. Finally, the ERH predicts that as a host species invades new regions, rare parasites will be lost from invading host populations while common, generalist parasites will persist among host populations (Coulatti et al., 2004). Results from my phylogenetic analyses support this prediction as well: eastern and western barred owls shared one *Haemoproteus* haplotype, and in both owl populations this haplotype comprised the majority (63% eastern barred; 94% western barred) of *Haemoproteus* infections. This same haplotype was also found in the majority (87%) of the northern spotted owls that tested positive for *Haemoproteus* spp., and a closely related (one base pair different) haplotype has been found in owls from North America, Africa, and Europe (Ishak et al., 2008), suggesting this is a common, cosmopolitan haplotype.

Interestingly, the finding that western barred owls had a lower probability of infection of the H4 haplotype contradicts my prediction that probability of infection should be similar among eastern and western barred owls for shared haplotypes. It is possible that this difference between barred owl populations was driven by the fact that all of my eastern barred owl samples were obtained from birds in rehabilitation centers (i.e., potentially immune-compromised birds), which could lead to a positive bias in my estimates of prevalence in the eastern barred owl population. However, this conclusion contradicts results from previous

studies that showed negligible difference in blood parasite prevalence between wild-caught and rehabilitation birds (Tella et al., 1999; Krone et al., 2001). It is also unlikely that the observed difference in probability of infection of H4 was driven by the fact that this haplotype is naturally rarer in the Pacific Northwest, given that a large number of northern spotted owls were infected with this same haplotype. Alternative explanations for the observed difference in probability of infection between eastern and western barred owls are 1) western barred owls may have new behavioral adaptations and habitat associations that have decreased their exposure to *Haemoproteus* vectors, 2) western barred owls may have relatively higher MHC diversity than their eastern counterparts, resulting in lower susceptibility to these avian blood parasites, or 3) a combination of the first two explanations. Future studies that compare behavioral adaptations, habitat associations, and genetic variation of immune-regulating genes may help us to assess the validity of these explanations, as well as broaden our understanding of the biological changes that can occur within a species throughout the range expansion process.

The observed higher prevalence in *Haemoproteus* haplotypes in eastern barred owls than western barred owls is similar to the pattern observed by Ishak et al. (2008) and supports the notion that suitable habitat for and/or abundance of *Haemoproteus* vectors is more heterogeneous and fragmented than that of *Leucocytozoon* vectors across North America. *Haemoproteus* parasites require warm temperatures for development in biting midge vectors (Valkiūnas, 1996). It is possible that the high elevations and associated cooler temperatures of the Rocky and Cascade Mountain ranges are hindering rarer *Haemoproteus* haplotypes from accompanying barred owl hosts to invasive ranges in the west because of the parasites'

dependence on these warmer temperatures. Therefore, owls inhabiting the cooler temperatures of the Rocky and Cascade mountain ranges may harbor low densities of *Haemoproteus* haplotype vectors, which, in turn, may lower the likelihood that owls encounter *Haemoproteus* parasites in these ranges. Future studies that sample barred owls from the invasion corridor along southern Canada would allow for a better test of these hypotheses.

Given support for the ERH, I could not make predictions about how *Haemoproteus* haplotype richness or diversity would compare between northern spotted and western barred owls under the PSH or ISH. However, I predicted similar or greater haplotype richness and diversity in northern spotted owls than western barred owls under the EEH. I found that *Haemoproteus* haplotype richness was similar in both northern spotted and western barred owls, and haplotype diversity was greater in northern spotted owls than western barred owls. Under the EEH, I also predicted that infection probability and intensity with shared haplotypes would be higher in northern spotted owls than western barred owls, and my results support these predictions as well. However, under the EEH I also predicted that the haplotypes shared by northern spotted and western barred owls originated from the barred owl's eastern range. Analyses to assess evolutionary relationships did not support this last prediction; of the two *Haemoproteus* haplotypes detected in northern spotted and western barred owls, one appears to be common and cosmopolitan (H4; see above), while the other was detected only in California (H5; Table 14; Figures 6 and 8) and not in the eastern barred owl range.

The California-specific haplotype is noteworthy because it has not been documented in other phylogenetic studies (e.g., Perkins and Schall, 2002; Ricklefs and Fallon, 2002) and it is genetically distant from *Haemoproteus* haplotypes detected in previous studies. Discovery of

this haplotype lends support to predictions of both the PSH and ISH because it suggests that barred owls may be acquiring new *Haemoproteus* haplotypes as they expand their range. The ISH also predicts that barred owls will be more negatively impacted by the parasite infections gained from range expansion relative to northern spotted owls, which would be supported indirectly by detection of a higher prevalence and more intense infections among western barred owls compared to northern spotted owls. My results do not support either of these predictions because I observed that northern spotted owls were more likely to be infected with *Haemoproteus* haplotypes than western barred owls. In addition, while infection intensities were more variable among northern spotted owls compared to western barred owls, they did not differ statistically between the two host populations.

Observed infection probabilities supported the PSH prediction that infection probability would be higher in northern spotted owls compared to western barred owls, and infection intensity analyses support the PSH prediction that infection intensity should be similar between the two host species. This evidence, coupled with the lack of evidence for the EEH through phylogenetic analyses, supports the notion that parasite spillback may occur more commonly than parasite spillover in ecological systems with invasive hosts and motivates future studies on this "largely overlooked" concept (Kelly et al., 2009). One such future study directly applicable to the northern spotted and barred owl system would be to monitor parasite prevalence of northern spotted owls as barred owls are experimentally removed from certain areas throughout the northern spotted owl's range, as proposed by U.S. Fish and Wildlife Service (2012). If barred owls are indeed contributing to higher parasite prevalence among northern spotted owls by acting as an added reservoir host, I predict that over time parasite prevalence

will decrease in northern spotted owls as barred owls are removed. If higher parasite prevalence is negatively impacting northern spotted owl survival and reproduction as predicted by the PSH (Kelly et al., 2009), I predict that northern spotted owl survival and reproduction in barred owl removal areas will subsequently increase due to an overall increase in the proportion of uninfected northern spotted owls in these areas.

The Parasite Life Cycle and Range Expansion

Distance of a sampling location to the coast (as both a linear and a log-linear effect) was a strong effect for both northern spotted and barred owls in the models that I tested for *Haemoproteus* infection probability and intensity. Conversely, I did not find a strong effect of distance to the coast on *Leucocytozoon* spp. prevalence. These results further support the notion that habitat attributes such as temperature, precipitation, and forest structure affect parasite dynamics of host populations, partially through effects of habitat on vector abundance (Tella et al., 1999). These results also support the notion that such habitat attributes may differentially affect the occurrence and intensity of infections with blood parasites of different genera when such parasites and their associated vectors have adapted to different abiotic and biotic conditions. This concept is particularly important when identifying parasites posing a threat to northern spotted owl conservation.

The fact that barred owl establishment in the Pacific Northwest is a result of range expansion rather than direct introduction is another important component for assessing the degree of the threat that avian blood parasites may impose on northern spotted owls. When an avian host and its parasites are directly introduced into a system, such as with non-native

host bird species and *Plasmodium relictum* in Hawaii (van Riper et al., 1986), parasite and vector habitat suitability of the geographic space between an invasive host's native and non-native range has little to no impact on whether a parasite will persist in an invasive host population as long as suitable vectors and environmental conditions are present in the invasive range. In the case of range expansions, once the parasite transmission cycle is broken due to unfavorable abiotic or biotic conditions along the range expansion route, the reestablishment of host-parasite associations will either be severely delayed (e.g., Phillips et al., 2010) or altogether lost along the host's invasion front.

The introduction of novel *Plasmodium* and *Haemoproteus* haplotypes may not be a large threat to northern spotted owl populations for two reasons. First, *Plasmodium* and *Haemoproteus* spp. appear to be naturally less common and their distribution is more fragmented in colder climates of North America in general (Valkiūnas, 1996). Second, eastern blood parasites would have to be transmitted across barred owl populations occupying colder regions of southern Canada where parasites of neither genus may reproduce well and consequently become lost from host populations. On the other hand, if barred owls are acting as added reservoir hosts of *Haemoproteus* and *Plasmodium* parasites in the Pacific Northwest, they may still exert parasite-mediated apparent competition on northern spotted owls by increasing the likelihood that northern spotted owls will encounter blood parasites native to the Pacific Northwest that were previously rare. This is supported by my finding of some evidence for parasite spillover among *Haemoproteus* haplotypes (where 79.5% of northern spotted and 33.3% of barred owls were infected with *Haemoproteus* parasites) in this study. Because of their ability to reproduce in their vectors at colder temperatures than

Haemoproteus and *Plasmodium* parasites, *Leucocytozoon* spp. may be better adapted to survive throughout the barred owl's entire expanded range, and haplotypes that were isolated to eastern North America prior to range expansion may be a plausible threat to northern spotted owls.

The True Cost of Parasitism?

I found that northern spotted owls were more likely to be infected with *Leucocytozoon* spp. and *Haemoproteus* haplotypes than sympatric barred owls. While I concluded that the underlying mechanisms driving these results differed between parasite genera, the effects of parasitism on host population health remain a valid concern for northern spotted owls. My study did not directly evaluate if and to what extent parasite infection status and intensity influence northern spotted owl and barred owl host fitness; however, *Plasmodium* parasites are the only genera of the three genera examined in this study that are considered highly pathogenic (Remple, 2004), and *Plasmodium* parasites do not appear to be spreading rapidly through northern spotted owl populations based on this study. *Haemoproteus* and *Leucocytozoon* parasites alone are thought to be relatively innocuous to their avian hosts (Remple, 2004) but can become pathogenic when coupled with additional stressors such as reproduction (Korpimaki et al., 1993) and low food availability (Appleby et al., 1999). Given that competition from the barred owl is likely a cause of stress among northern spotted owls, blood parasite infections have the potential to become pathogenic in northern spotted owls in sympatry with barred owls. Negative effects of pathogenic blood parasites can decrease overall

body condition (Dawson and Bortolotti, 2000) and reproductive success (Merino et al., 2000), which in turn may decrease northern spotted owl fitness over time.

I found strong support for the Enemy Release Hypothesis in *Haemoproteus* parasites of invasive barred owls, but the question of the true cost of *Haemoproteus* infections also has implications for invasive barred owl fitness. If *Haemoproteus* parasites are relatively innocuous to their barred owl hosts, the loss of *Haemoproteus* parasites among western barred owls may not have much biological relevance. Nevertheless, these results demonstrate an important pattern that may be occurring among more cost-demanding parasites that I did not examine in this study. To address this issue, future studies should include more comprehensive screening of barred owl parasites and pathogens. In addition, I echo Ishak et al.'s (2008) suggestion that follow-up studies should evaluate the relationship of infection status with immunological competency, estimated survival and reproductive rates for infected compared to uninfected birds, and competitive interactions of both northern spotted and barred owls.

Finally, in this study I compared parasite haplotype diversity under the assumption that host populations infected with a lower diversity of parasites were more immunologically competent than host populations infected with a higher diversity of parasites. Hudson et al. (2006) argue the contrary, in that high parasite diversity is an indicator of ecosystem health because high parasite diversity is often a result of long chains of multispecies connections that can only be present in healthy ecosystems. I detected a higher diversity of *Haemoproteus* haplotypes among northern spotted owls than western barred owls, and Ishak et al. (2008) reported a high diversity of *Leucocytozoon* lineages among northern spotted owls relative to *Leucocytozoon* assemblages of other owl species across the world. If the blood parasite

infections among northern spotted owls are a result of host-vector-parasite interactions that have co-evolved over a long period of time, then my study suggests that *Haemoproteus* and *Leucocytozoon* infections may be benign if not beneficial in northern spotted owls. Svensson-Coelho et al. (2013) found that avian host species with a high prevalence of *Haemoproteus* showed low prevalence of *Plasmodium* and vice versa. One explanation for this observed pattern is that infection of parasites from one genus may inhibit infection of parasites from the other genus. In the context of my study system, it is possible that northern spotted owls have adapted to high *Haemoproteus* prevalence as part of a defense mechanism against more virulent *Plasmodium* parasites. Future studies on this concept in northern spotted owls would help elucidate both the role that blood parasites have on northern spotted owl fitness and the complex relationships between blood parasites and avian hosts in general.

Table 1. Summary of previous studies examining the prevalence of haemosporidian parasites of three genera, *Haemoproteus* (*H*.), *Plasmodium* (*P*.), and *Leucocytozoon* (*L*.) in northern spotted (*Strix occidentalis caurina*) and barred (*S. varia*) owls.

		% Infected					
Species & Location	***n***	**Total**	***H*.**	***P*.**	***L*.**	**Detection Method**	**Citation**
Strix o. caurina							
CA	22	100	50	0	95	Microscopy	Gutiérrez (1989)
CA & OR	36	*unk*	61	14	61	Microscopy	Clark et al. (2005)
CA, OR, WA	63	52	25	2	40	DNA Sequencing & Microscopy	Ishak et al. (2008)
Total	**121**	**64**	**40**	**5**	**56**		
Strix varia							
Eastern N. America	4	75	50	75	75	Microscopy	Greiner et al. (1975)*
USA – Mid Atlantic	1	0	0	0	0	Microscopy	Kirkpatrick & Lauer (1985)
OK	9	78	67	0	55	Microscopy	Kocan et al. (1977)
MN, WI, TX	18	61	33	33	6	DNA Sequencing & Microscopy	Ishak et al. (2008)
CA, OR, WA	26	15	4	8	8	DNA Sequencing & Microscopy	Ishak et al. (2008)
FL & Southern GA	54	6	n/a	6	n/a	Microscopy	Telford et al. (1997)
FL	28	68	68	11	0	Microscopy	Forrester et al. (1994)
LA	21	90	10	0	90	Microscopy	Olsen & Gaunt (1985)
USA – Mid Atlantic	5	20	20	0	0	Microscopy	Williams & Bennett (1978)
GA	1	100	n/a	100	n/a	Microscopy	Telford et al. (1992)
Total	**167**	**41**	**22**	**11**	**18**		

* Greiner et al. (1975) provided a summary of all avian hematozoan studies in North America prior to 1975, including results from Wetmore (1941) and Hart (1949). The parasite counts in this table differ from those reported by Ishak et al. (2008) because Ishak et al. counted the Wetmore and Hart studies twice in their Table 1.

Table 2. Summary of parasite community parameters evaluated, and the predicted outcomes under the Enemy Release Hypothesis using data collected from barred owl populations in their historic range in eastern North America (BOE) and in their invaded range in western North America (BOW). Separate analyses were performed for each parameter and prediction for each genus of blood parasite examined (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*). Parameters actually evaluated for each genus given the final data are denoted with a "§" under each respective genus column.

		Genus of Avian Blood Parasite		
Parameter	**Prediction**	*Haemoproteus*	*Plasmodium*	*Leucocytozoon*
Haplotype Richness	$BO_E > BO_W$	§	§**	
Haplotype Diversity	$BO_E > BO_W$	§	§**	
Haplotype Assemblage Similarity	BO_E and BO_W share some haplotypes, but cannot share all haplotypes	§	§**	
Pr(inf)* – All Haplotypes	$BO_E > BO_W$	§		§
Pr(inf) – Shared Haplotypes	$BO_E = BO_W$	§		
Infection Intensity – Shared Haplotypes	$BO_E = BO_W$	§		

* Pr(inf) = probability of infection

** Indicates parameters that were evaluated descriptively (versus statistically) given low number of owls infected with parasites of a given genus

Table 3. Summary of parasite community parameters evaluated and the predicted outcomes under the Enemy of My Enemy (EEH), Parasite Spillback (PSH), and Increased Susceptibility (ISH) Hypotheses, using data collected from northern spotted owls (SO) in their native range and barred owls (BO_W) in their invaded range in northwestern California. Predictions were made assuming that both owl species are similarly susceptible to owl blood parasite infections in their native ranges ($SO \approx BO_E$) and that the Enemy Release Hypothesis is supported. Separate analyses were performed for each parameter and prediction for each genus of blood parasite examined (*Haemoproteus, Plasmodium,* and *Leucocytozoon*). Parameters actually evaluated for each genus given the final data are denoted with a "§" under each respective genus column.

				Genus of Avian Blood Parasite		
Metric	**EEH**	**PSH**	**ISH**	*Haemoproteus*	*Plasmodium*	*Leucocytozoon*
Haplotype Richness	$SO > BO_W$	*Cannot predict*	*Cannot predict*	*§*	*§***	
Haplotype Diversity	$SO > BO_W$	*Cannot predict*	*Cannot predict*	*§*	*§***	
Haplotype Assemblage Similarity	SO and BO_W share some haplotypes, originating from BO_W populations	SO and BO_W share some haplotypes, originating from SO populations	SO and BO_W share some haplotypes, originating from SO populations	*§*	*§***	
Pr(inf)* – All Haplotypes	*Cannot predict*	*Cannot predict*	*Cannot predict*	*§*		*§*
Pr(inf) – Shared Haplotypes	$SO > BO_W$	$SO > BO_W$	$SO < BO_W$	*§*		
Infection Intensity – Shared Haplotypes	$SO > BO_W$	$SO = BO_W$	$SO < BO_W$	*§*		

* Pr(inf) = probability of infection

** Indicates parameters that were evaluated descriptively (versus statistically) given low sample sizes

Table 4. Summary of parasite community parameters evaluated and the predicted outcomes under the Enemy of My Enemy (EEH), Parasite Spillback (PSH), and Increased Susceptibility (ISH) Hypotheses, using data collected from northern spotted owls (SO) in their native range and barred owls (BO_W) in their invaded range in northwestern California. Predictions were made assuming that both owl species are similarly susceptible to owl blood parasite infections in their native ranges ($SO \approx BO_E$) and that the Enemy Release Hypothesis is not supported. Separate analyses were performed for each parameter and prediction for each genus of blood parasite examined (*Haemoproteus, Plasmodium,* and *Leucocytozoon*). Parameters actually evaluated for each genus given the final data are denoted with a "§" under each respective genus column.

				Genus of Avian Blood Parasite		
Metric	**EEH**	**PSH**	**ISH**	*Haemoproteus*	*Plasmodium*	*Leucocytozoon*
Haplotype Richness	$SO > BO_W$	$SO < BO_W$	$SO < BO_W$	§	§**	
Haplotype Diversity	$SO > BO_W$	$SO < BO_W$	$SO < BO_W$	§	§**	
Haplotype Assemblage Similarity	SO and BO_W share some haplotypes, originating from BO_W populations	SO and BO_W share some haplotypes, originating from SO populations	SO and BO_W share some haplotypes, originating from SO populations	§	§**	
Pr(inf)* – All Haplotypes	$SO > BO_W$	$SO > BO_W$	$SO < BO_W$	§		§
Pr(inf) – Shared Haplotypes	$SO > BO_W$	$SO > BO_W$	$SO < BO_W$	§		
Infection Intensity – Shared Haplotypes	$SO > BO_W$	$SO = BO_W$	$SO < BO_W$	§		

* Pr(inf) = probability of infection

** Indicates parameters that were evaluated descriptively (versus statistically) given low sample sizes

Table 5. Summary of northern spotted and western barred owl samples collected in the Pacific Northwest for comparisons of avian blood parasite assemblages among barred and northern spotted owls across North America. Samples are separated by year collected and study area.

Year	Owl Species	Study Area	Sample Type	
			Whole Blood and Smear	Whole Blood Only
2008	Northern Spotted	NWC*	0	19
	Barred	NWC	0	4
2009	Northern Spotted	NWC	0	24
2010	Barred	NWC	5	0
		GDRC**	13	1
		Canada	4	0
	Northern Spotted	NWC	22	7
		GDRC	6	0
2011	Barred	NWC	4	0
		GDRC	9	1
		Hoopa	2	0
	Northern Spotted	NWC	14	4
		GDRC	12	0
		Hoopa	7	0
		Weaverville	7	0
2012	Barred	NWC	3†	0
		GDRC	4	0
		Hoopa	4	0
	Northern Spotted	NWC	3	1
Total	**Barred**		**47**	**6**
Total	**Northern Spotted**		**71**	**55**

* NWC = Northwest California Study Area

** GDRC = Green Diamond Resource Company Study Area

† One sample was collected from a freshly killed barred owl on a road in the NWC study area.

Table 6. Numbers of blood samples collected from barred owls admitted to rehabilitation centers used to test predictions of the Enemy Release Hypothesis about blood parasites of barred and northern spotted owls in North America. Rehabilitation centers were located throughout the barred owl's historic range in the eastern USA (see Figure 3).

Rehabilitation Center	**Location**	***n***
Avian Conservation Center – The Center for Birds of Prey	Awendaw, South Carolina	55
WildCare Foundation	Noble, Oklahoma	11
Avian Haven	Freedom, Maine	15
Carolina Raptor Center	Huntersville, North Carolina	10
Audubon of Florida – Center for Birds of Prey	Maitland, Florida	31
The Raptor Center	Saint Paul, Minnesota	25
Tri-State Bird Rescue & Research	Newark, Delaware	12
Alabama Raptor Center	Pelham, Alabama	16
Total		**175**

Table 7. Description of response variables used to compare predictions of the Enemy Release, Enemy of My Enemy, Parasite Spillback, and Increased Susceptibility hypotheses in the context of barred and northern spotted owl blood parasites in North America.

Response Variable	Variable Type	Sampling Unit	Description	Statistical Analysis
Parasite Haplotype Richness	Continuous	Population	Number of haplotypes observed across each sampled population (northern spotted, western barred, or eastern barred) sampled	Chao 2 Richness estimator (with 95% CI)
Parasite Haplotype Diversity	Continuous – Index	Population	Index combining the number of haplotypes observed across each sampled population (richness) and the number of individuals infected with each haplotype (evenness)	Shannon and Simpson Diversity Indices (with 95% CI)
Parasite Assemblage Similarity	Continuous	Population	Description of the shared haplotypes found in two populations of interest, as well as the haplotypes' relationships among previously detected blood parasites	Descriptive
Infection Status – All Haplotypes	Categorical	Individual	Whether a bird was infected (1) or not infected (0) with a blood parasite from the genus of interest	Logistic Regression
Infection Status – Shared Haplotypes	Categorical	Individual	Whether a bird was infected (1) or not infected (0) with a blood parasite from the haplotype of interest	Logistic Regression
Infection Intensity	Continuous - proportion	Individual	Number of infected erythrocytes ÷ total number of erythrocytes examined	Generalized Linear Model

Table 8. Description of explanatory variables used in analyses of probability of infection and infection intensity for testing predictions of the Enemy Release, Enemy of My Enemy, Parasite Spillback, and Increased Susceptibility Hypotheses in barred and northern spotted owls across North America.

Variable	Variable Acronym	Variable Type	Rationale
Population	PO	Categorical (Northern Spotted, Western Barred, and Eastern Barred)	Ecological and phylogenetic species differences may lead to differences in vector exposure and immunocompetence. Parasite community dynamics may also differ between species sampled from its native versus expanded range, following predictions of the ERH, EEH, PSH, and/or ISH
Sex of Bird	SX	Categorical (Male versus Female)	Males and females may vary in behavior and reproductive stressors, leading to variations in vector exposure and immunocompetence
Age of Bird	AG	Categorical (Juvenile versus Adult)	Probability of exposure to blood parasites increases with time (e.g., age of individual)
Management Intensity Within an Owl's Territory	MG	Categorical (Low versus High)	Differences in management intensities lead to different environmental stressors, which in turn affect a bird's immunocompetence
Distance of Capture Location to the Coast	DC	Continuous	Habitat and climate differences between coastal and inland sites may support different vector communities
Natural Log of Capture Location to the Coast	LnDC	Continuous	Same as DC but the effect may asymptote as distance increases

Table 9. Ranking of *a priori* models used to examine the probability that northern spotted (*n* = 127) and eastern barred (*n* = 158) owls from their native ranges were infected with *Leucocytozoon* spp. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO	247.33	2	251.37	0.00	0.22	0.39
PO + SX + AG + SX*AG	241.40	5	251.61	0.24	0.20	0.42
PO + SX	245.58	3	251.67	0.29	0.19	0.40
PO + AG + SX	243.76	4	251.90	0.53	0.17	0.41
PO + AG	245.88	3	251.97	0.59	0.16	0.40
PO + AG + PO*AG	245.87	4	254.01	2.64	0.06	0.40
Intercept-only	338.36	1	340.37	89.00	0.00	--

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 10. Ranking of *a priori* models used to examine the probability that eastern (*n* = 121) and western (*n* = 54) barred owls were infected with *Leucocytozoon* spp. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
Intercept-only	241.64	1	243.66	0.00	0.63	--
PO	241.64	2	245.70	2.05	0.23	0.00
PO + SX	241.05	3	247.19	3.53	0.11	0.00
PO + SX + PO*SX	240.83	4	249.06	5.40	0.04	0.01

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 11. Ranking of *a priori* and *a posteriori* models used to analyze the probability that northern spotted (*n* = 59) and barred (*n* = 50) owls from California were infected with *Leucocytozoon* spp. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO	79.37	2	83.49	0.00	0.17	0.45
PO + SX	77.56	3	83.79	0.31	0.15	0.46
PO + LnDC†	78.45	3	84.67	1.19	0.10	0.46
PO + DC	78.76	3	84.99	1.51	0.08	0.45
PO + SX + LnDC†	76.71	4	85.09	1.61	0.08	0.47
PO + SX + DC	77.03	4	85.41	1.93	0.07	0.47
PO + MG	79.22	3	85.45	1.96	0.06	0.45
PO + SX + MG	77.46	4	85.84	2.35	0.05	0.47
PO + MG + LnDC†	78.31	4	86.69	3.21	0.03	0.46
PO + LnDC + PO*LnDC†	78.32	4	86.70	3.21	0.03	0.46
PO + MG + DC	78.70	4	87.08	3.60	0.03	0.45
PO + SX + MG + LnDC†	76.53	5	87.11	3.63	0.03	0.47
PO + DC + PO*DC	78.76	4	87.14	3.65	0.03	0.45
PO + SX + LnDC + PO*LnDC†	76.56	5	87.15	3.66	0.03	0.47
PO + SX + MG + DC	76.94	5	87.52	4.04	0.02	0.47
PO + SX + DC + PO*DC	77.02	5	87.60	4.11	0.02	0.47
PO + MG + LnDC + MG*LnDC†	77.24	5	87.82	4.33	0.02	0.47
PO + MG + DC + MG*DC	77.54	5	88.12	4.64	0.02	0.46
Intercept-only	117.39	1	119.43	35.94	0.00	--

† *a posteriori* model

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 12. Ranking of *a priori* models used to analyze the probability that northern spotted (*n* = 112) and eastern barred (*n* = 143) owls from their native ranges were infected with *Haemoproteus* spp. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO + SX + AG + SX*AG	196.70	5	206.94	0.00	0.33	0.12
PO + AG + SX	199.07	4	207.23	0.29	0.29	0.10
PO + SX	201.41	3	207.51	0.57	0.25	0.09
PO + SX + PO*SX	201.41	4	209.57	2.63	0.09	0.09
PO + AG	206.87	3	212.97	6.03	0.02	0.05
PO	209.75	2	213.79	6.86	0.01	0.03
PO + AG + PO*AG	206.79	4	214.95	8.01	0.01	0.05
Intercept-only	214.71	1	216.73	9.79	0.00	--

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 13. Nucleotide sequence variation at sites that vary among the five *Haemoproteus* haplotypes detected in northern spotted and barred owls sampled in this study (H1-H5), as well as all *Haemoproteus* haplotypes described in Ishak et al. (2008) and *Haemoproteus* haplotypes detected by other avian blood parasite studies that were 2% or less divergent from H1-H5 (based on GenBank search results). Number of avian hosts, host species, and sampling location for each haplotype or accession number are listed in Table 14.

	Nucleotide Site																																									
Haplotype or Accession Number	**8**	**14**	**20**	**23**	**26**	**27**	**32**	**35**	**36**	**44**	**45**	**69**	**70**	**71**	**74**	**95**	**99**	**102**	**103**	**104**	**107**	**109**	**116**	**122**	**125**	**134**	**146**	**147**	**170**	**179**	**182**	**194**	**203**	**206**	**209**	**218**	**221**	**244**	**252**	**254**
H4	T	A	T	T	A	T	A	T	T	C	T	T	C	A	T	T	T	A	C	T	T	T	T	T	T	T	A	T	C	A	C	C	T	T	T	T	T	A	G	T
H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	A	.	.	.	.	.	C	G	.	.	T	.	.	.	.	A	.	A	.	.	.
H2	-	-	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	A	.	.	.	.	.	C	G	.	.	T	.	.	.	.	A	.	A	.	A	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	A	T	.	.	A	.	A	.	.	.
AF465589	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU627834	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU627840	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	.	.	.	.	.	.	.	.
EU627839	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	.	.	A	.	A	.	.	.
EU627836	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	C	A	.	.	.	.	.
EU627829	C	.	C	C	T	C	T	.	C	T	C	G	T	.	.	.	.	G	T	.	A	.	C	A	.	.	T	C	.	.	A	T	C	C	A	A	A	C	.	C
EU627830	C	.	A	C	T	.	T	.	C	T	C	G	T	.	.	.	.	.	T	.	A	.	C	A	.	.	T	C	.	.	A	T	C	C	A	A	A	C	.	C
EU627838	.	.	C	.	T	C	T	.	C	T	C	G	T	.	.	.	.	G	T	.	A	.	C	.	.	.	T	C	.	.	T	T	C	C	A	A	A	C	.	C
JQ768232	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
EF607290	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AB604311	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU810722-24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
AY714134	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	C	.	.	.
EU810721	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
AY714138	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	.	.	.	.	.
EU810717	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.
GU251990	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
EU810716, DQ659592	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	T	A	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.
HM222464	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	G	.	A	.	.	.	.	.	.	.	.	A	T	.	.	A	.	.	.	.	.
AY714135	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.
GU085195	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	G	C	.	.	.	.	.	T	.	.	.	T	T	.	.	A	.	.	.	.	.
AF465592	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.
JX418192	-	-	A	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
JX418180	-	-	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	T	T	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	.
AB604310	.	.	A	.	.	.	.	.	.	.	.	.	.	T	C	.	C	.	T	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	A	.	.	.	.	.
JN792174, AY714137	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	A	.	.	.	.	.
HQ724294, HQ724292	A	.	.	.	.	.	.	C	.	T	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.
GQ404559	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	.
JQ988459	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	G	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.

Table Continued…

Haplotype or Accession Number	Nucleotide Site																																							
	266	272	275	276	284	290	293	299	300	302	305	306	314	335	341	342	359	368	377	378	403	420	431	433	434	438	443	444	446	449	451	452	453	455	458	464	469	473	476	477
H4	A	C	T	A	T	A	T	T	A	A	T	T	T	T	T	T	T	A	A	G	G	A	T	G	T	A	A	G	A	A	A	T	T	A	T	T	T	T	A	T
H1	.	.	.	G	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H2	.	.	.	G	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H3	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
H5	.	.	.	G	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	C	.	.	.	.	.	.	.	.
AF465589	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EU627834	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
EU627840	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
EU627839	.	.	.	G	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	C	.	.	.	.	.	.	.	.
EU627836	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	C
EU627829	.	.	.	G	C	.	C	.	.	.	C	C	C	C	.	G	.	.	.	.	.	G	.	A	.	G	.	.	C	.	T	C	.	.	A	.	.	A	T	C
EU627830	.	.	.	G	C	.	C	.	.	.	C	.	C	C	.	C	.	.	.	.	.	G	.	A	.	G	.	.	C	.	T	C	.	.	A	.	.	.	T	C
EU627838	.	.	.	G	C	.	C	.	.	.	C	C	C	C	.	.	C	.	.	.	.	G	.	A	.	G	.	.	C	.	T	C	.	.	A	.	.	A	T	C
JQ768232	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF607290	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AB604311	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
EU810722-24	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.
AY714134	.	.	.	G	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	-
EU810721	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.
AY714138	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	.	.
EU810717	.	.	.	G	.	T	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
GU251990	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	C	.	.	.	C	.	.	.	.
EU810716, DQ659592	.	.	.	G	.	T	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
HM222464	G	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	-
AY714135	.	.	.	G	.	.	.	C	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	-
GU085195	.	.	.	.	.	.	C	.	G	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.
AF465592	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	N	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	-
JX418192	.	.	.	G	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JX418180	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	G	.	.	.	.	.	C	C	.	.	.	.	.	.	-
AB604310	.	.	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.
JN792174, AY714137	.	.	C	G	.	.	.	.	G	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	-
HQ724294, HQ724292	.	.	.	G	.	C	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	-
GQ404559	.	T	.	G	.	.	.	C	G	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	.	.	C
JQ988459	G	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.

Table 14. List of number of hosts, host species, and sampling location for the *Haemoproteus* haplotypes included in Table 13.

Haplotype or Accession Number	*n*	Host Species & Location
H4	181	Northern Spotted Owl (65 CA), Barred Owl (15 CA, 1 British Columbia, 9 MN/WI, 4 DE/MD/PA, 42 NC/SC/GA, 25 FL, 10 AL, 10 OK)
H1	17	Barred Owl (4 MN, 6 ME, 4 DE, 3 NC/SC)
H2	1	Barred Owl (AL)
H3	1	Barred Owl (AL)
H5	11	Northern Spotted Owl (10 CA), Barred Owl (1 CA)
AF465589	2	Barred Owl, Great Horned Owl (SE USA)
EU627834	54	Northern Spotted Owl (5 CA, 8 OR, 2 WA), California Spotted Owl (27 CA), Barred Owl (1 CA, 1 TX, 3 MN), Great Horned Owl (6 CA), African Wood Owl (1 Africa)
EU627840	1	Barred Owl (WI)
EU627839	1	California Spotted Owl
EU627836	1	Long Eared Owl (CA)
EU627829	1	Barn Owl (CA)
EU627830	1	Barn Owl (CA)
EU627838	1	Barn Owl (CA)
JQ768232	1	"Owl" (Turkey)
EF607290	*unk*	Tawny Owl (Germany)
AB604311	*unk*	Humboldt Penguin (Japan – zoo)
EU810722-24	*unk*	Woodhouse Antpecker, Pale Breasted Illadopsis, Dwarf Kingfisher (Gabon)
AY714134	*unk*	Common Paradise Kingfisher (Papua New Guinea)
EU810721	*unk*	Blue-breasted Kingfisher (Gabon)
AY714138	*unk*	Yellow-billed Kingfisher (Papua New Guinea)
EU810717	*unk*	Shining Blue Kingfisher (Gabon)
GU251990	*unk*	Eurasian Teal
EU810716, DQ659592	*unk*	White-bellied Kingfisher (Gabon)
HM222464	*unk*	Broad-billed Tody (Hispaniola)
AY714135	*unk*	Common Paradise Kingfisher (Papua New Guinea)
GU085195	*unk*	Grey-headed Woodpecker (Bulgaria)
AF465592	*unk*	Asian Koel
JX418192	*unk*	Phillipine Scops Owl (Phillipines)
JX418180	*unk*	Spotted Wood Kingfisher (Phillipines)
AB604310	*unk*	Magellanic Penguin (Japan – zoo)
JN792174, AY714137	*unk*	Beautiful Fruit Dove, Superb Fruit Dove (Papua New Guinea)
HQ724294, HQ724292	*unk*	Gamble's Quail, Masked Bobwhite Quail (USA)
GQ404559	*unk*	Christmas Frigatebird (Australia)
JQ988459	*unk*	Buff-tailed Sicklebill (Peru)

Table 15. Ranking of *a priori* models used to analyze the probability that eastern (*n* =115) and western (*n* = 52) barred owls were infected with *Haemoproteus* spp., regardless of haplotype. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO	134.277	2	138.350	0.000	0.644	0.45
PO + SX	134.217	3	140.364	2.014	0.235	0.45
PO + SX + PO*SX	133.443	4	141.690	3.340	0.121	0.45
Intercept-only	196.594	1	198.618	60.268	0.000	--

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 16. Ranking of *a priori* models used to analyze the probability that infected eastern (*n* = 99) and western (*n* = 52) barred owls were infected with a *Haemoproteus* haplotype found in both the eastern and western range. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO	169.08	2	173.16	0.00	0.67	0.26
PO + SX	169.01	3	175.01	2.02	0.24	0.26
PO + SX + PO*SX	168.97	4	176.97	4.09	0.09	0.26
Intercept-only	201.44	1	203.47	30.31	0.00	--

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 17. Ranking of *a priori* and *a posteriori* models used to analyze the probability that northern spotted (*n* = 56) and western barred (*n* = 49) owls from California were infected with *Haemoproteus* spp. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
PO + DC	100.18	3	106.42	-3.18	0.21	0.44
PO + LnDC†	101.24	3	107.47	-2.13	0.13	0.43
PO + MG + DC	99.73	4	108.13	-1.47	0.09	0.44
PO + SX + DC	99.81	4	108.21	-1.40	0.09	0.44
PO + MG + DC + MG*DC	97.95	5	108.56	-1.05	0.07	0.46
PO + DC + PO*DC	100.18	4	108.58	-1.02	0.07	0.44
PO + SX + LnDC†	100.93	4	109.33	-0.28	0.05	0.43
PO + LnDC + PO*LnDC†	100.97	4	109.37	-0.23	0.05	0.43
PO + MG + LnDC†	101.21	4	109.61	0.00	0.04	0.43
PO + SX + DC + PO*SX	99.27	5	109.87	0.27	0.04	0.45
PO + SX + MG + DC	99.39	5	109.99	0.39	0.04	0.45
PO + SX + DC + PO*DC	99.80	5	110.41	0.80	0.03	0.44
PO + MG + LnDC + MG*LnDC†	100.33	5	110.94	1.33	0.02	0.44
PO + SX + LnDC + PO*SX†	100.41	5	111.02	1.41	0.02	0.44
PO + SX + LnDC + PO*LnDC†	100.65	5	111.26	1.65	0.02	0.43
PO + SX + MG + LnDC†	100.87	5	111.48	1.87	0.02	0.43
PO + MG	109.06	3	115.30	5.69	0.00	0.36
PO	111.28	2	115.40	5.79	0.00	0.34
PO + MG + PO*MG	108.64	4	117.04	7.44	0.00	0.36
PO + SX + MG	108.78	4	117.18	7.57	0.00	0.36
PO + SX	111.14	3	117.37	7.77	0.00	0.34
PO + SX + MG + PO*SX	108.30	5	118.91	9.30	0.00	0.36

Table Continued…

Table Continued…

PO + SX + MG + PO*MG	108.38	5	118.99	9.38	0.00	0.36
PO + SX + PO*SX	110.76	4	119.16	9.55	0.00	0.34
Intercept-only	141.33	1	143.37	33.77	0.00	--

† *a posteriori* model

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 18. Ranking of *a priori* and *a posteriori* models used to analyze *Haemoproteus* infection intensity among northern spotted (*n* = 34) and western barred (*n* = 13) owls from California that tested positive for a *Haemoproteus* spp. infection through DNA sequencing. Explanatory variables included in the models are described in Table 7.

Model	-2lnL	K	AICc*	ΔAICc**	Akaike Weight***	R^2
DC†	-250.33	3	-243.77	0.00	0.33	0.20
MG + DC†	-251.43	4	-242.48	1.29	0.17	0.22
PO + DC	-250.82	4	-241.87	1.90	0.13	0.21
PO + MG + DC	-251.89	5	-240.43	3.35	0.06	0.23
PO + DC + PO*DC	-251.48	5	-240.01	3.76	0.05	0.22
PO + MG + LnDC†	-251.20	5	-239.74	4.03	0.04	0.22
PO + SX + DC	-250.82	5	-239.36	4.41	0.04	0.21
PO + LnDC†	-247.56	4	-238.61	5.17	0.03	0.16
PO + LnDC + PO*LnDC†	-249.57	5	-238.11	5.66	0.02	0.19
PO + MG + DC + MG*DC	-252.02	6	-237.92	5.85	0.02	0.23
PO + MG + DC + MG*LnDC†	-251.96	6	-237.86	5.91	0.02	0.23
PO + SX + MG + DC	-251.94	6	-237.84	5.93	0.02	0.23
PO + SX + DC + PO*DC	-251.49	6	-237.39	6.38	0.01	0.22
PO + SX + MG + LnDC†	-251.22	6	-237.12	6.65	0.01	0.22
PO + SX + DC + PO*SX	-250.91	6	-236.81	6.97	0.01	0.21
PO + SX + LnDC†	-247.60	5	-236.14	7.63	0.01	0.16
PO + SX + LnDC + PO*LnDC†	-249.58	6	-235.48	8.30	0.01	0.19
PO	-241.99	3	-235.43	8.34	0.01	0.05
Intercept-only	-239.62	2	-235.35	8.42	0.00	--
MG†	-240.95	3	-234.39	9.38	0.00	0.03
PO + MG	-243.20	4	-234.25	9.52	0.00	0.07
PO + SX + LnDC + PO*SX†	-247.81	6	-233.71	10.06	0.00	0.16
PO + SX	-242.01	4	-233.06	10.72	0.00	0.05

Table Continued…

Table Continued…

PO + MG + PO*MG	-243.67	5	-232.21	11.57	0.00	0.08
PO + SX + MG	-243.35	5	-231.89	11.89	0.00	0.08
PO + SX + PO*SX	-242.02	5	-230.55	13.22	0.00	0.05
PO + SX + MG + PO*MG	-243.81	6	-229.71	14.06	0.00	0.09
PO + SX + MG + PO*SX	-243.36	6	-229.26	14.52	0.00	0.08

† *a posteriori* model

* AICc = small sample size corrected version of Akaike's Information Criterion

** ΔAICc = difference in AICc between a given model and the top-ranked model

*** Akaike Weight = probability that a given model is the best supported model given the model set and the data

Table 19. Relative support for the Enemy Release (ERH), Enemy of My Enemy (EEH), Parasite Spillback (PSH), and Increased Susceptibility (ISH) Hypotheses based on estimates of parasite haplotype richness, haplotype diversity, assemblage similarity, infection probability, and infection intensity for barred and northern spotted owls sampled in North America.

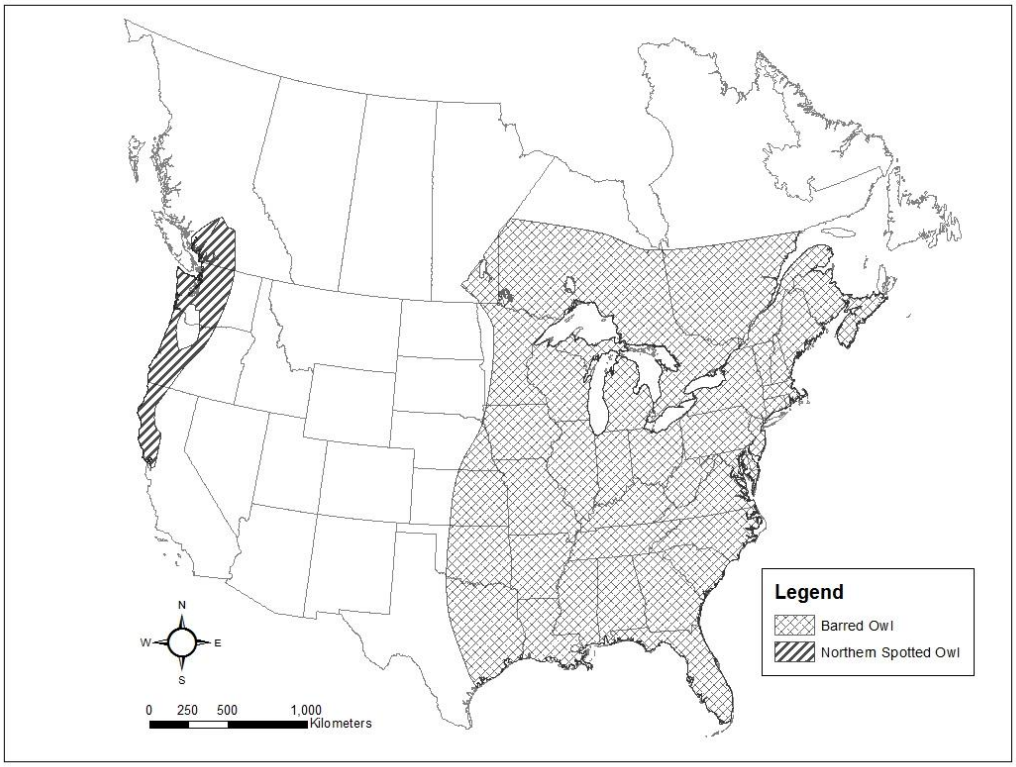
Metric	ERH	EEH	PSH	ISH
Leucocytozoon				
Pr(inf)† – all haplotypes	0*	+ +	- -	- -
Plasmodium				
Haplotype Richness	+**	*did not measure*	*did not measure*	*did not measure*
Haplotype Diversity	+**	*did not measure*	*did not measure*	*did not measure*
Assemblage Similarity	+**	*did not measure*	*did not measure*	*did not measure*
Haemoproteus				
Haplotype Richness	+ +	0	*cannot predict*	*cannot predict*
Haplotype Diversity	+ +	+ +	*cannot predict*	*cannot predict*
Assemblage Similarity	+ +	0	+	+
Pr(inf) – All Haplotypes	+ +	*cannot predict*	*cannot predict*	*cannot predict*
Pr(inf) – Shared Haplotypes	0	+ +	+ +	- -
Infection Intensity	*did not measure*	0	+ +	0

† Pr(inf) = probability of infection

* (+ +) indicates strong support for the hypothesis, (+) indicates some support for the hypothesis, (0) indicates no substantial support for the hypothesis, (-) indicates a weak trend in the opposite direction that was predicted, and (- -) indicates a strong trend in the opposite direction that was predicted; for predictions, refer to Tables 1-3

** Metrics evaluated qualitatively (rather than quantitatively)

A



B

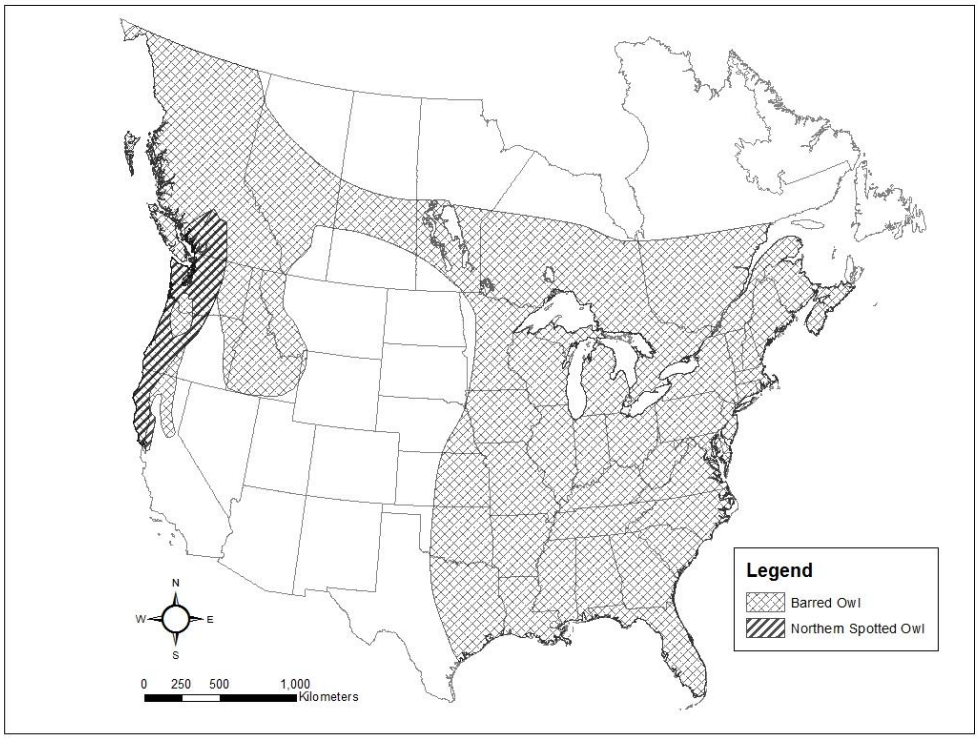


Figure 1. Geographic distribution in North America of northern spotted owls and barred owls in (A) their historic (per-1900s) range and (B) their present-day range. (Base GIS layer source: USGS National Atlas)

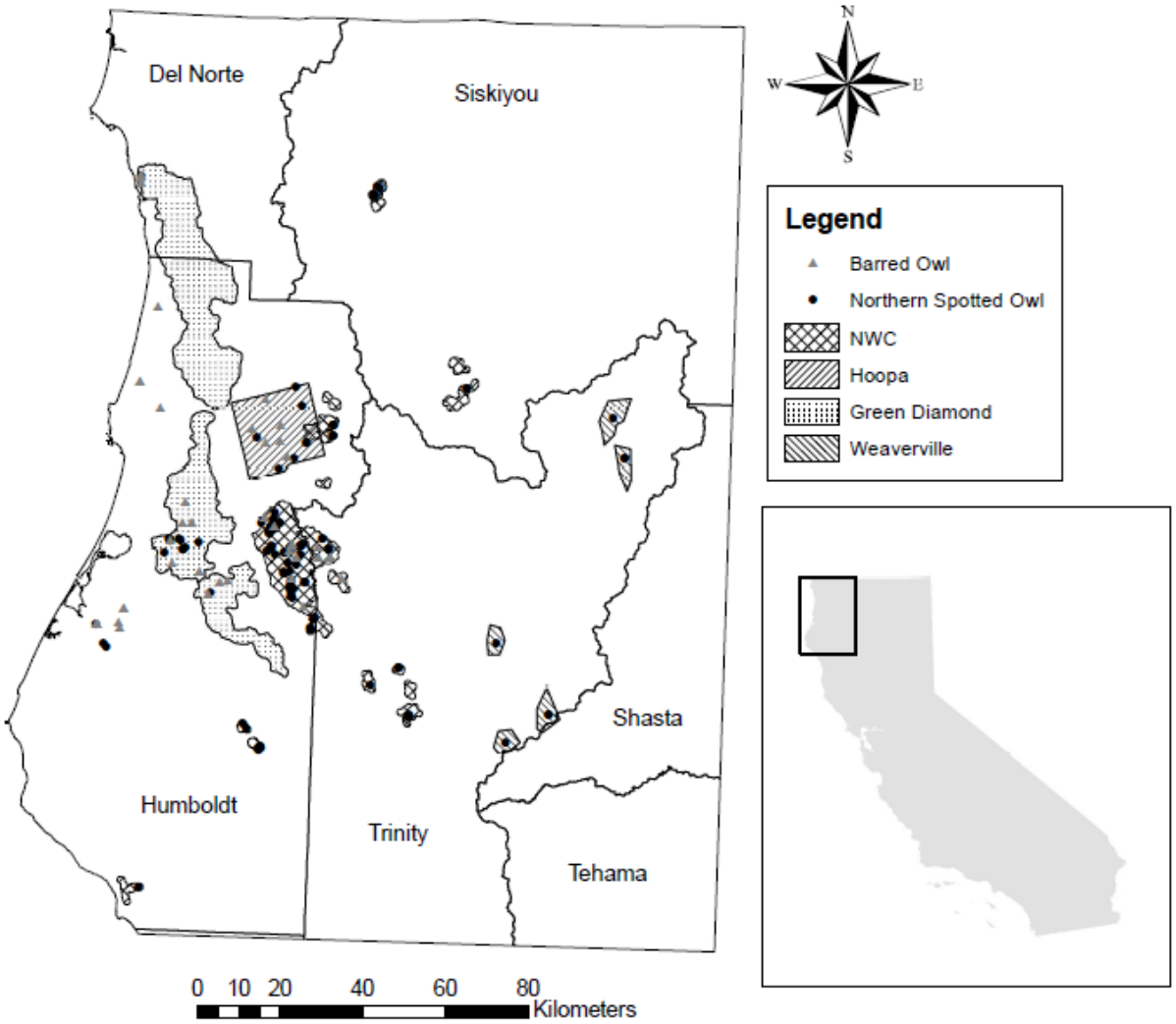


Figure 2. Locations of northern spotted and western barred owls sampled from four study sites in Northwestern California. (Map Credit: Jeremy Rockweit, Colorado State University)

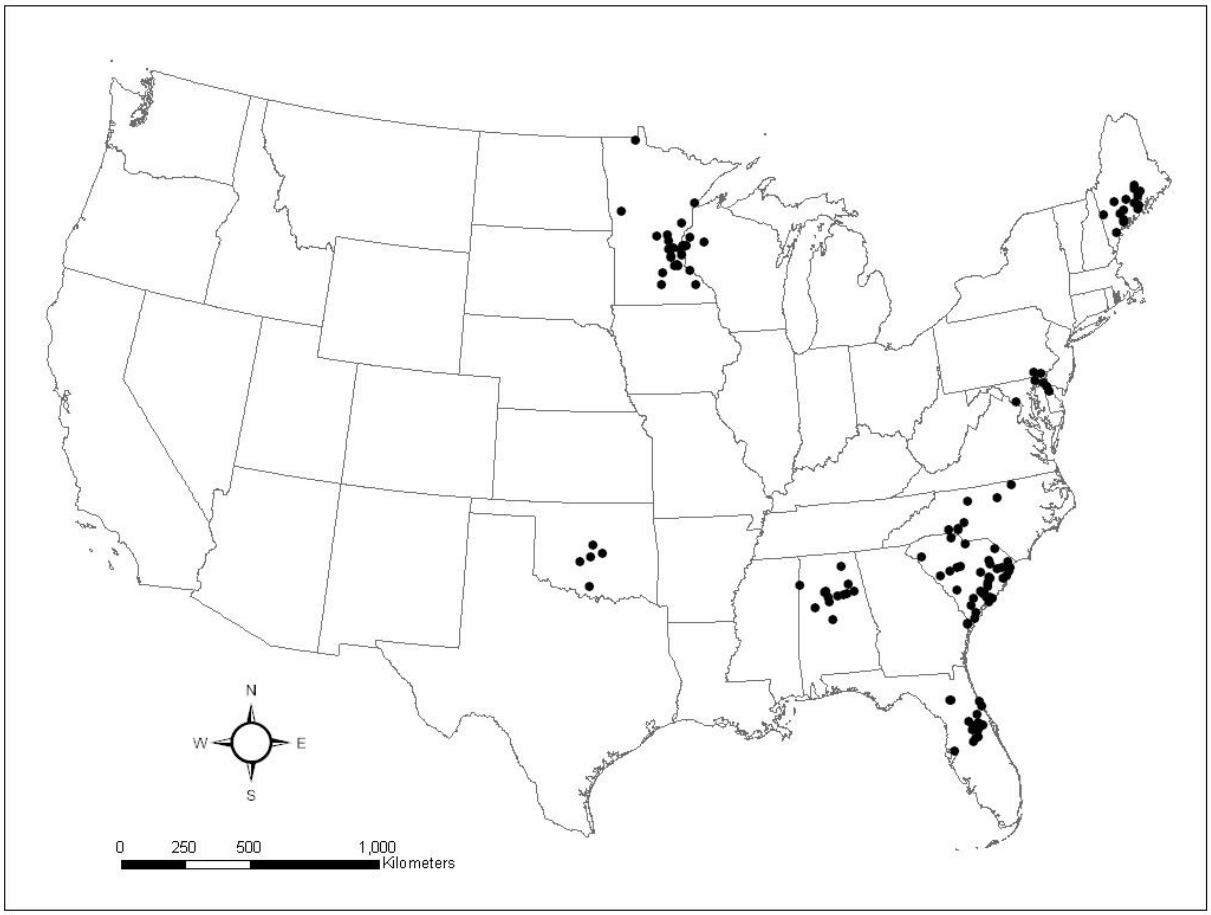


Figure 3. Locations of eastern barred owls sampled from raptor rehabilitation centers. (Base GIS layer source: USGS National Atlas)

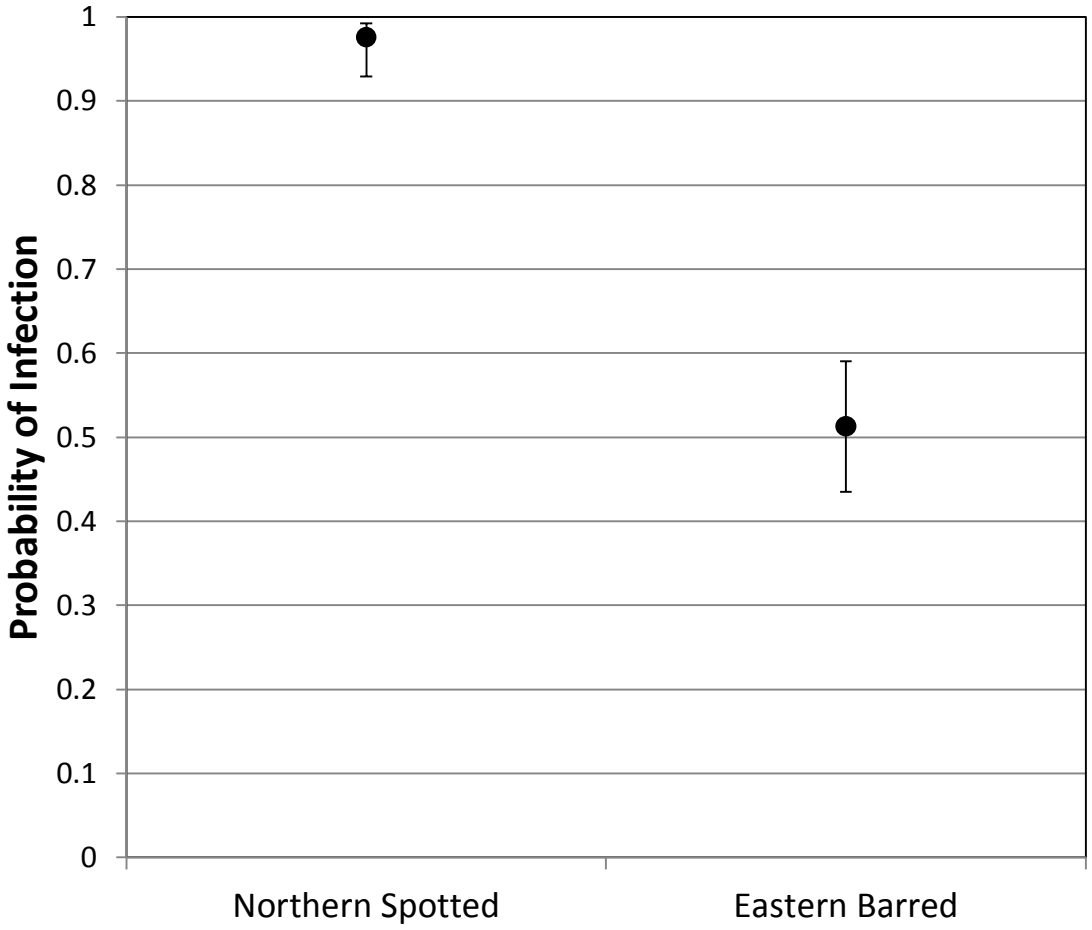


Figure 4. Predicted probabilities that northern spotted and eastern barred owls are infected with *Leucocytozoon* spp. in their native ranges in North America. Probabilities were estimated from a logistic regression model where owl population was the only explanatory variable (Pr(Inf) ≈ PO).

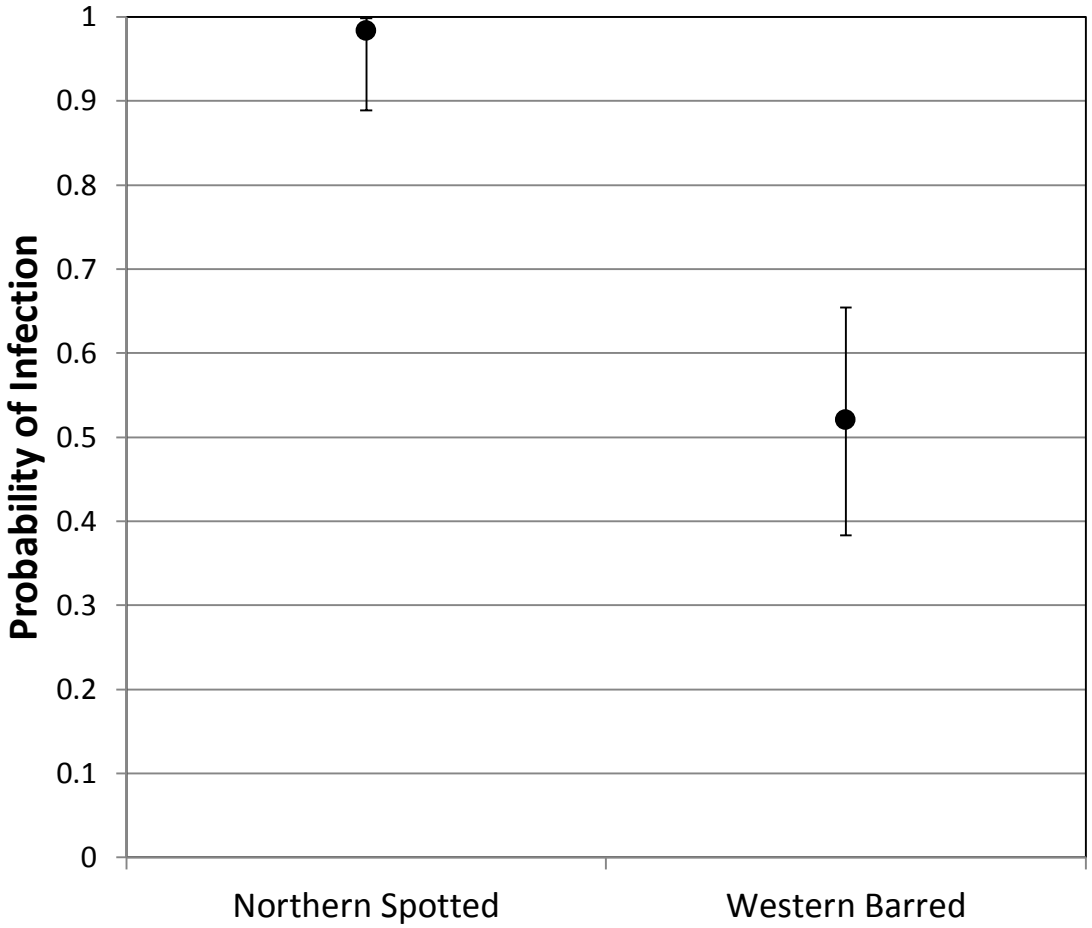


Figure 5. Predicted probabilities that northern spotted and western barred owls are infected with *Leucocytozoon* spp. in northwest California. Probabilities were estimated from a logistic regression model where owl population was the only explanatory variable (Pr(Inf) ≈ PO).

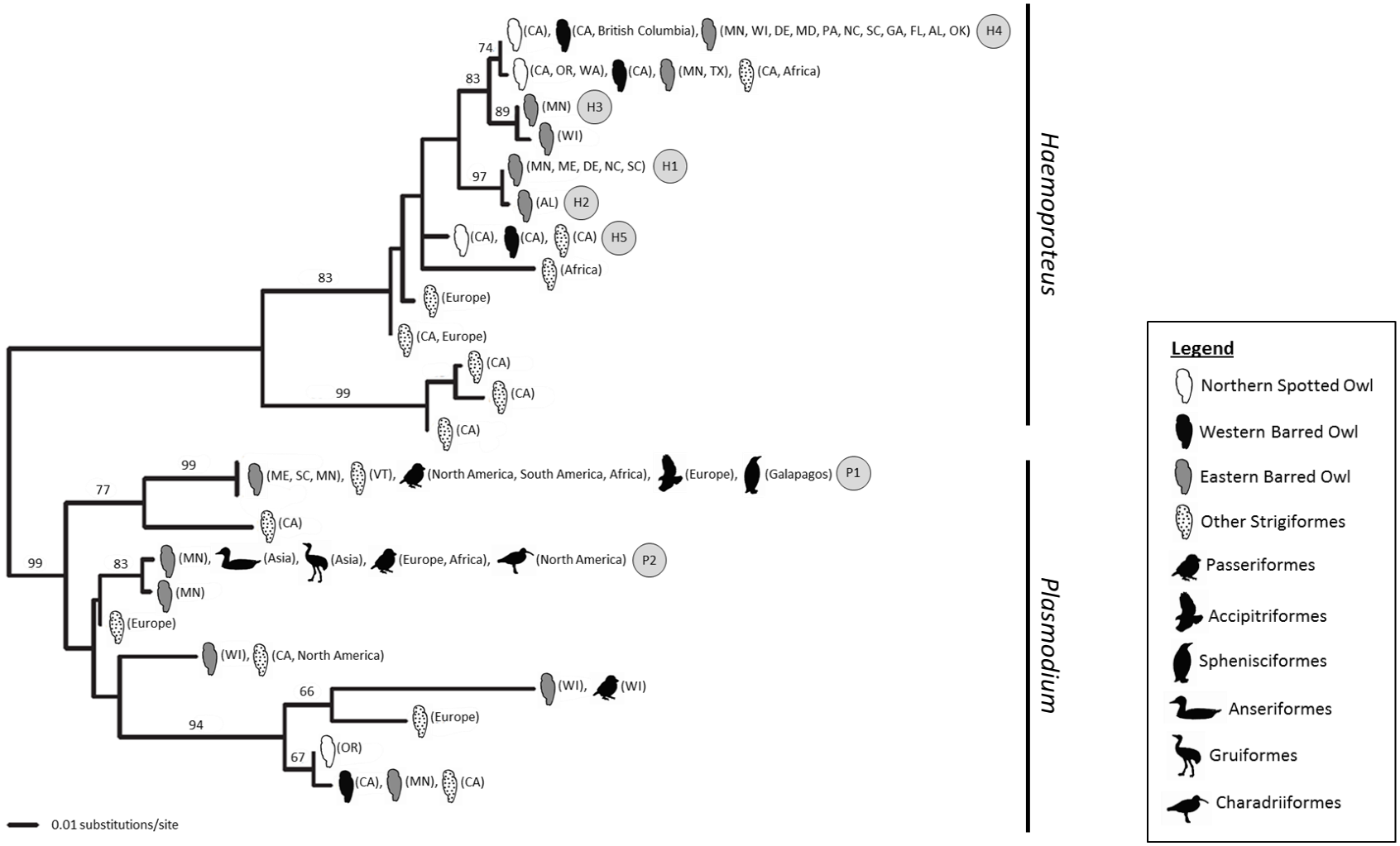


Figure 6. Maximum likelihood tree for *Plasmodium* and *Haemoproteus* haplotypes from this study and reported in Ishak et al. (2008). Each branch is labeled with the bird family in which each haplotype was found, as well as the location followed in parentheses. Branches labeled with a circled letter/number represent haplotypes detected in this study. Bootstrap values were derived using 1000 replicates and are shown to the left of each node.

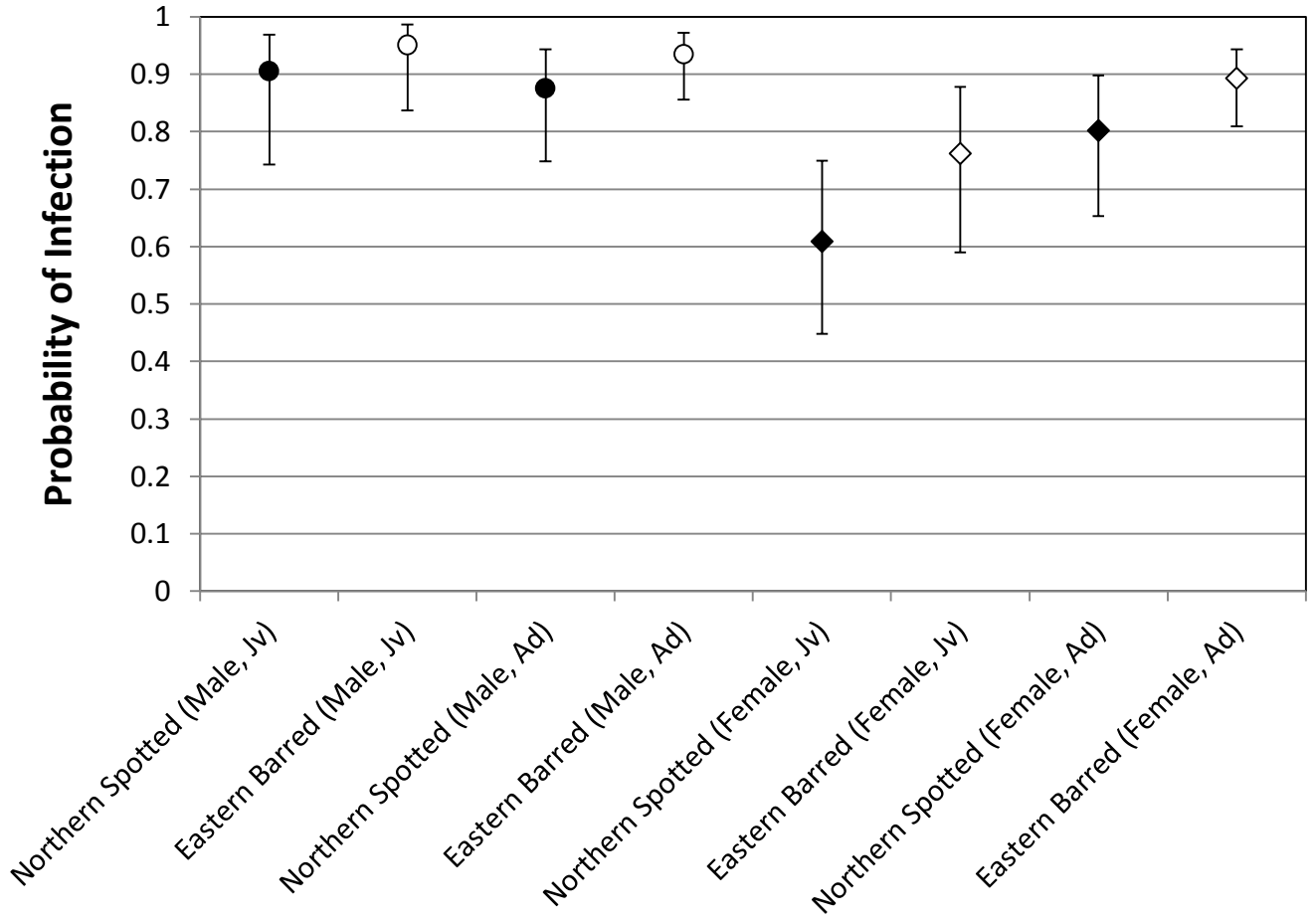


Figure 7. Predicted probabilities that northern spotted and barred owls are infected with *Haemoproteus* haplotypes in their native ranges in North America. Probabilities were estimated from a logistic regression model where owl population, sex, age, and an sex-age interaction were explanatory variables (Pr(Inf) ≈ PO + SX + AG + SX*AG).

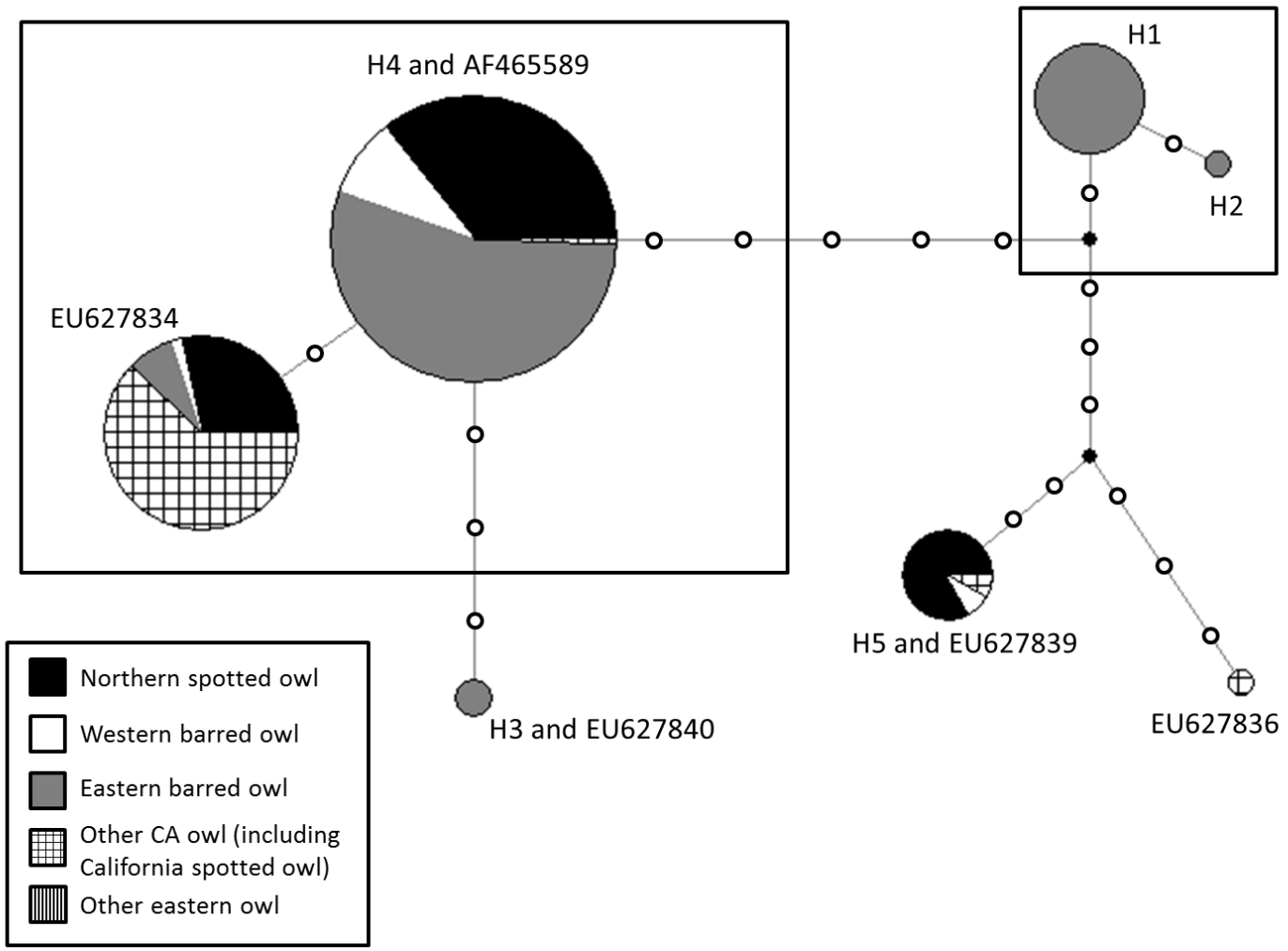


Figure 8. Haplotype network based on *Haemoproteus* haplotypes detected in barred and northern spotted owls sampled in this study and all *Haemoproteus* haplotypes reported in GenBank or MalAvi for samples from owls of North America that shared 98% or higher sequence agreement. Empty circles in the network indicate mutations, filled black circles are median vectors, and evolutionary lineages (i.e., groups of haplotypes separated by two or fewer mutations) are boxed.

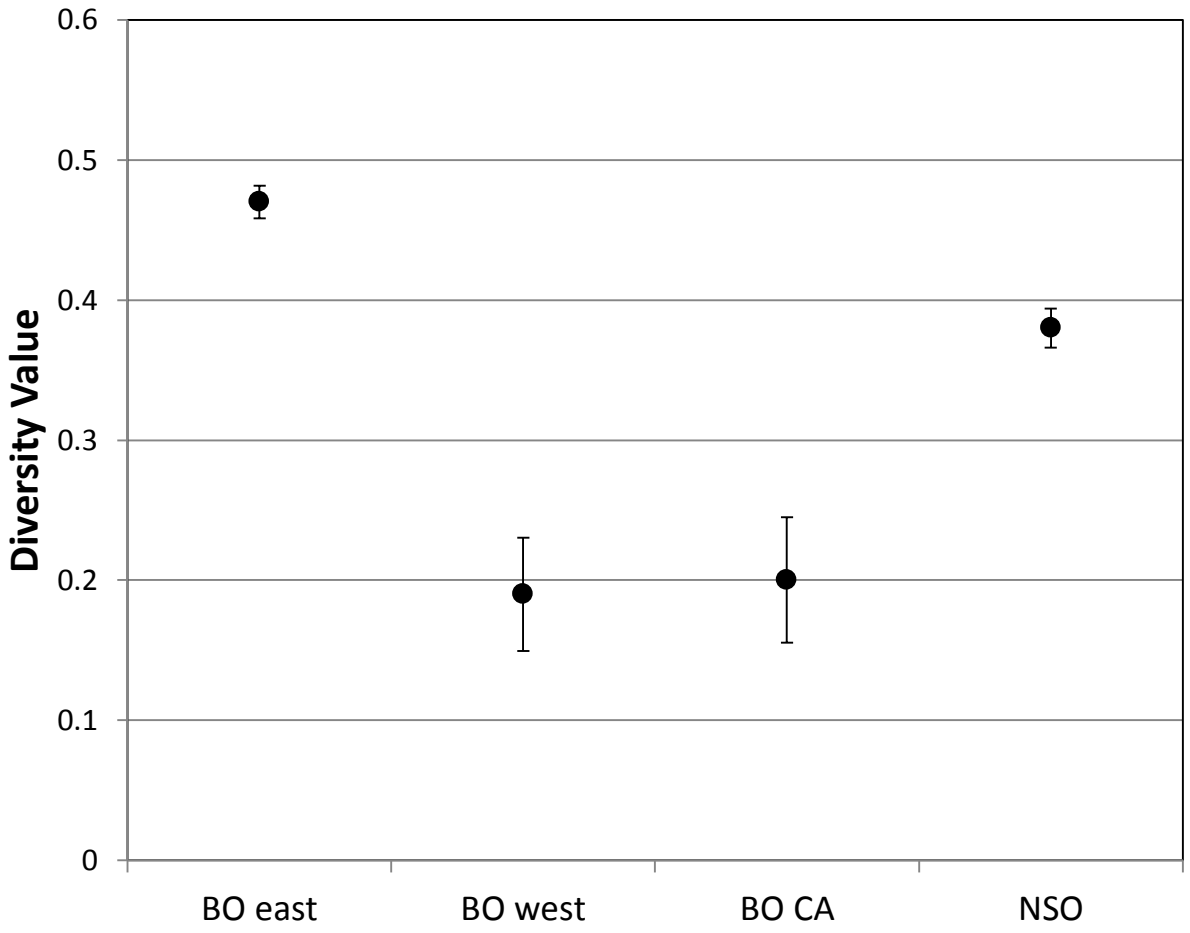


Figure 9. Estimates of Shannon diversity indices for *Haemoproteus* assemblages of eastern barred owls (*n* = 135), western barred owls (*n* = 53), barred owls from California (a subset of western barred owls; *n* = 49), and northern spotted owls (*n* = 98), along with 95% confidence intervals generated by running 1000 replicates with replacement.

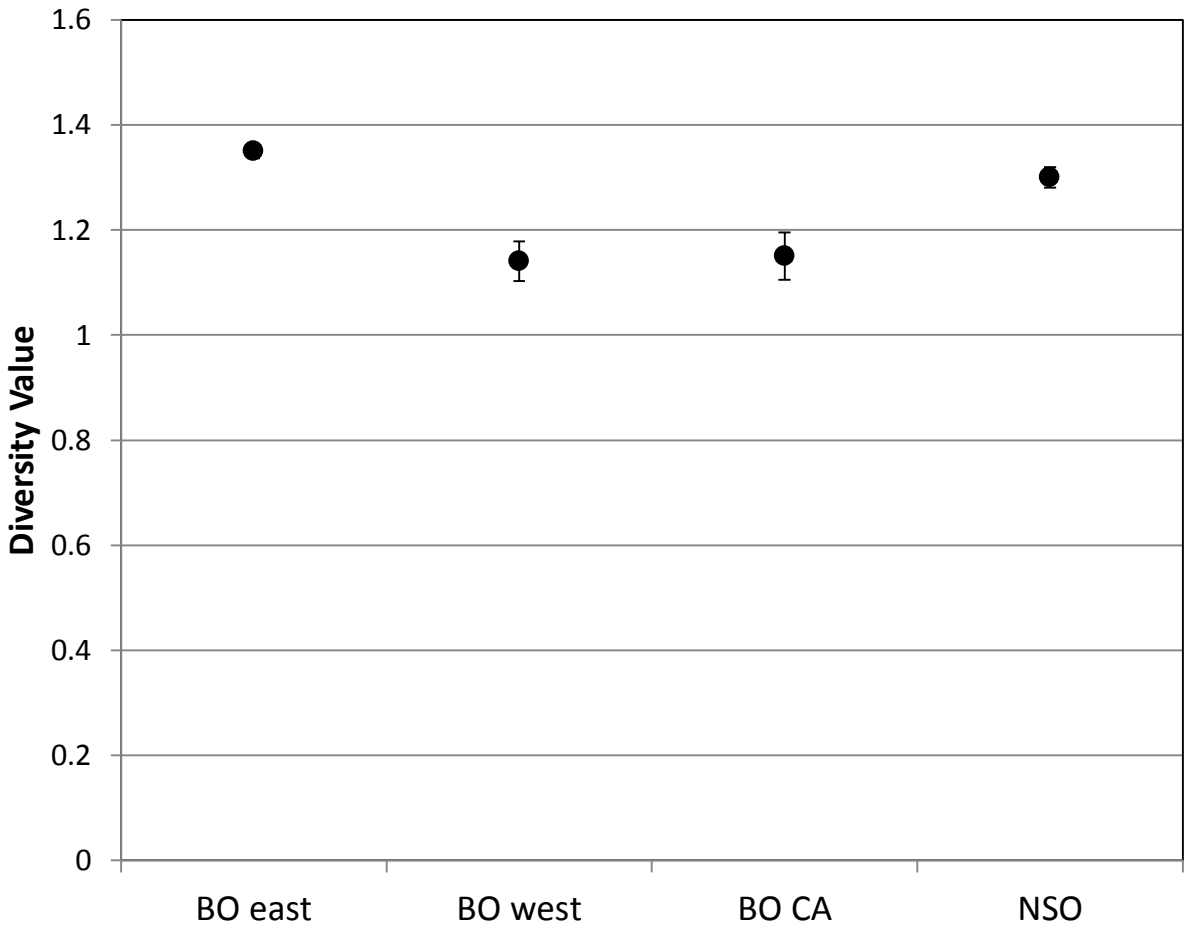


Figure 10. Estimates of Simpson diversity indices for *Haemoproteus* assemblages of eastern barred owls (*n* = 135), western barred owls (*n* = 53), barred owls from California (a subset of western barred owls; *n* = 49), and northern spotted owls (*n* = 98), along with 95% confidence intervals generated by running 1000 replicates with replacement.

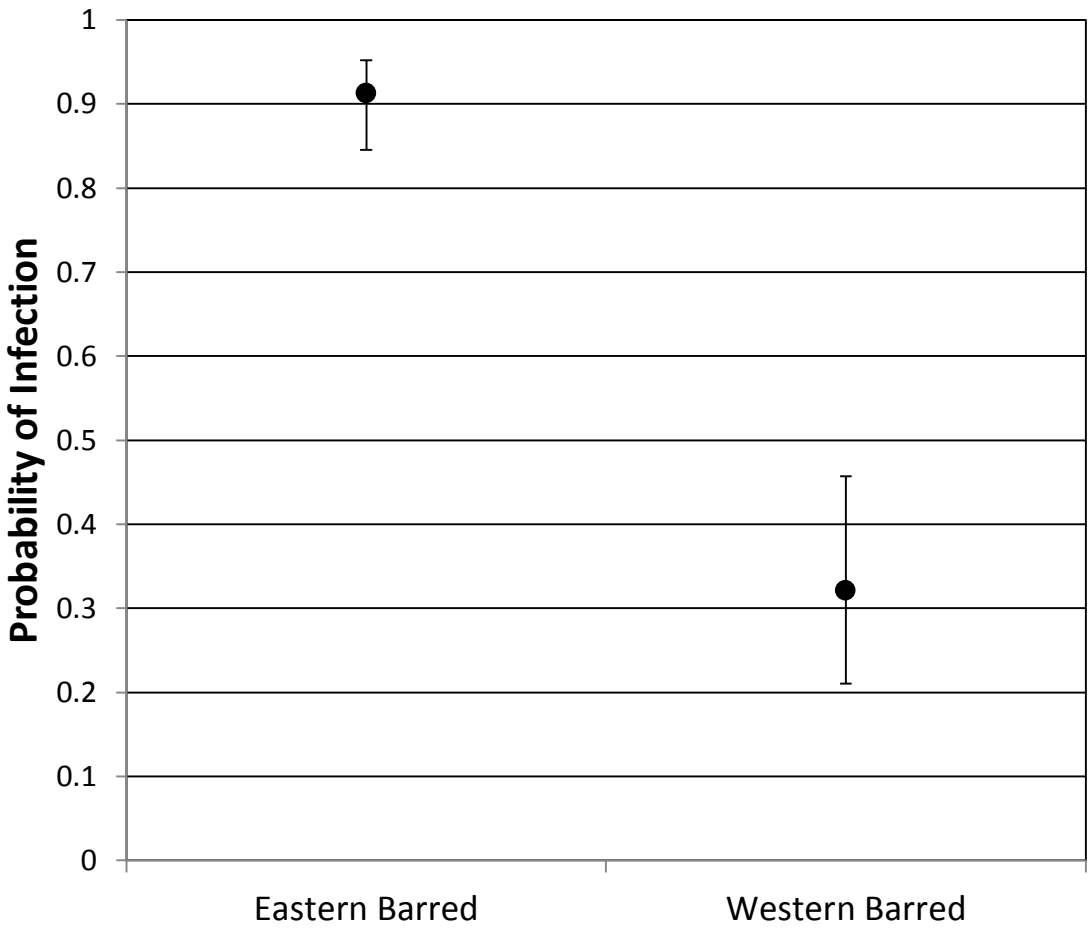


Figure 11. Predicted probabilities that eastern and western barred owls are infected with *Haemoproteus* spp., regardless of haplotype. Probabilities were estimated from a logistic regression model where owl population was the only explanatory variable (Pr(Inf) ≈ PO).

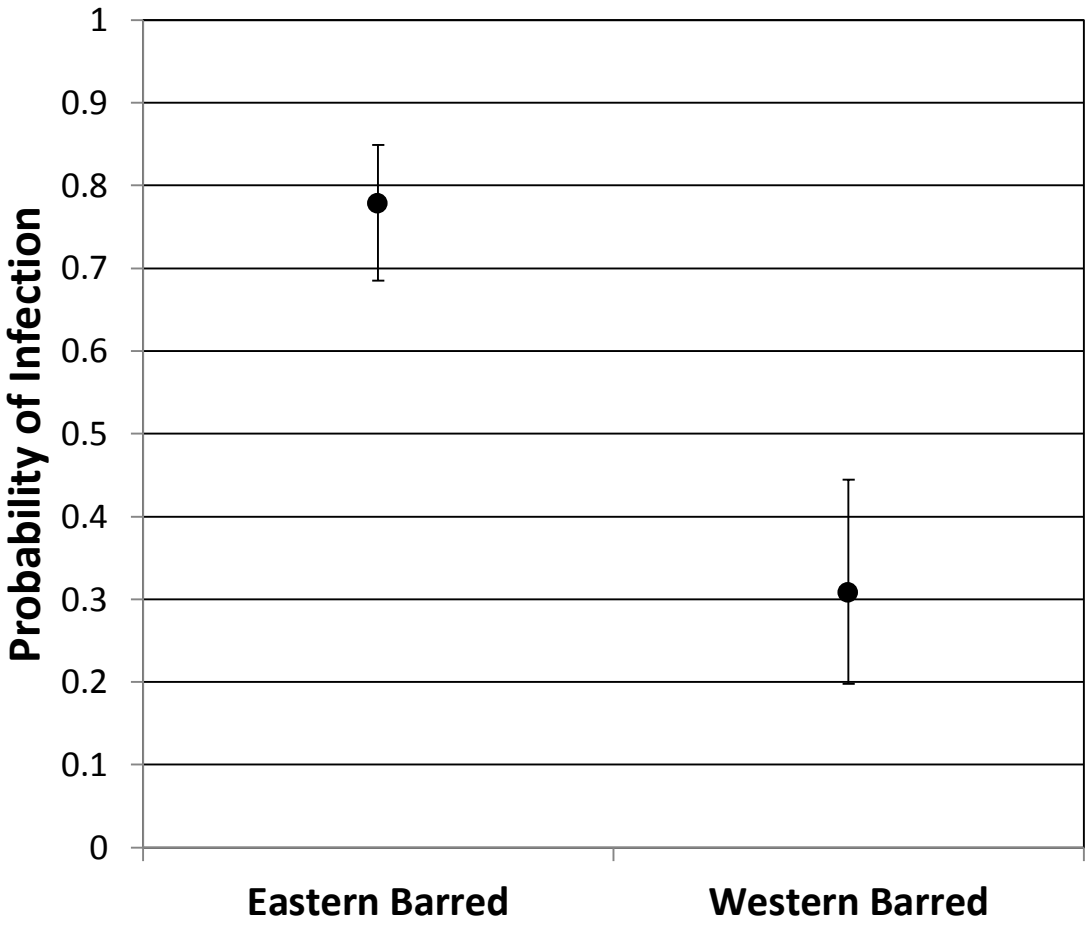


Figure 12. Predicted probabilities that eastern and western barred owls are infected with the shared *Haemoproteus* haplotype, H4. Probabilities were estimated from a logistic regression model where owl population was the only explanatory variable (Pr(Inf) ≈ PO).

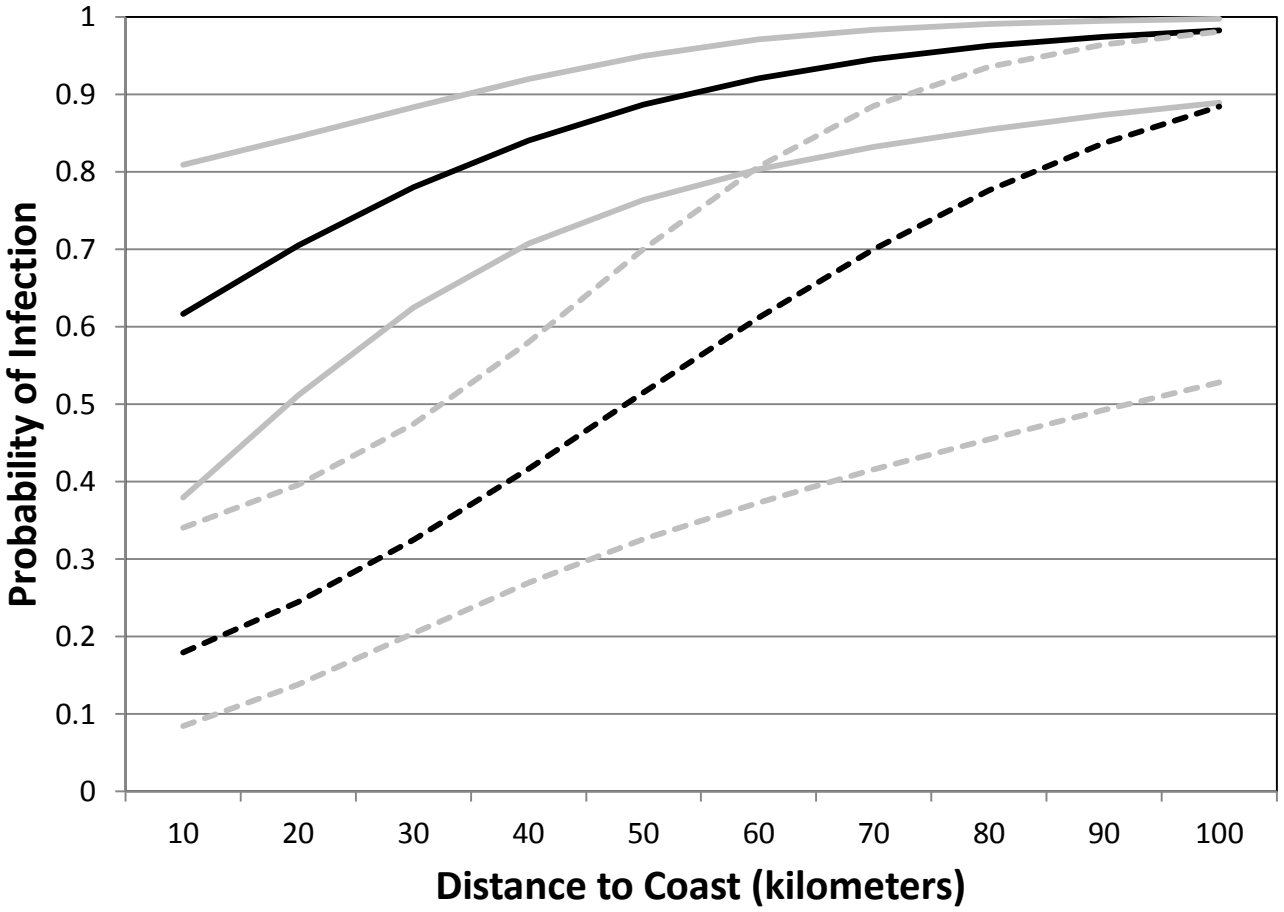


Figure 13. Predicted probabilities with 95% confidence intervals that a northern spotted (solid lines) and western barred (dashed lines) owls from northwest California were infected with *Haemoproteus* haplotypes. Probabilities were estimated from a logistic regression model where owl population and distance to the coast were explanatory variables (Pr(Inf) = PO + DC).

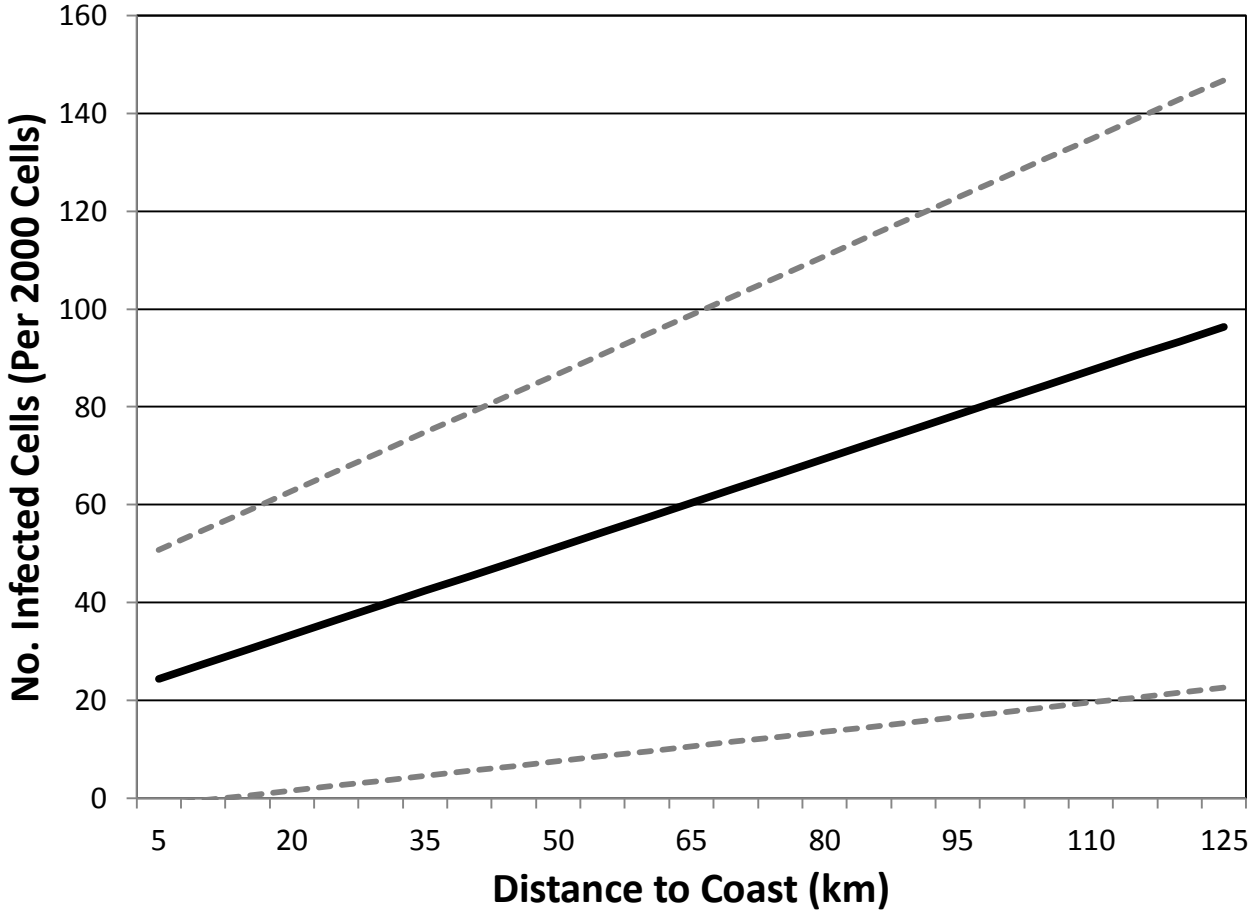


Figure 14. Predicted infection intensity (solid line) and 95% confidence intervals (dashed lines) for northern spotted and western barred owls, based on samples collected in northwest California. Predicted values were estimated from a generalized linear model in which distance to coast was the only explanatory variable (Intensity ≈ DC).

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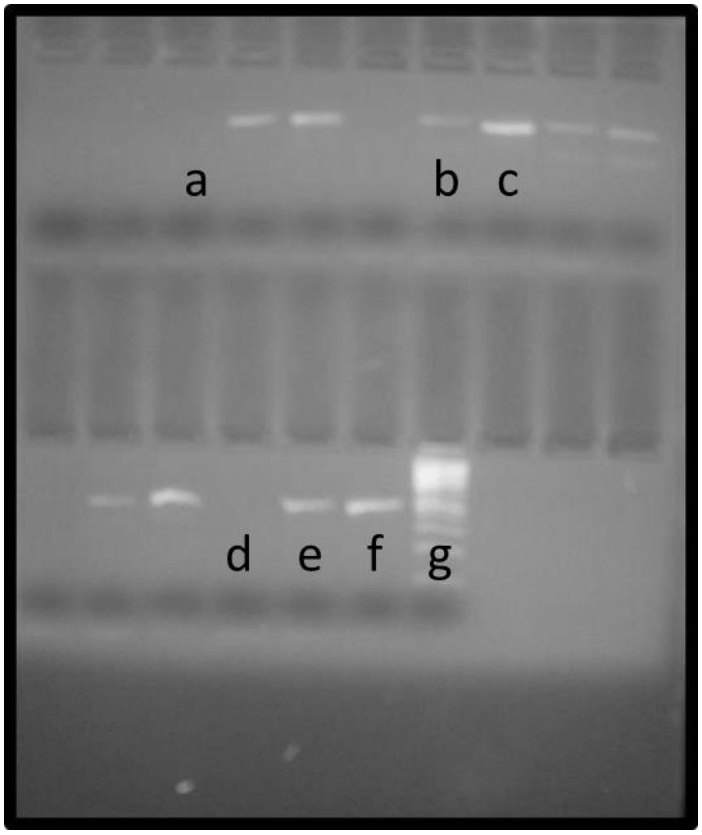
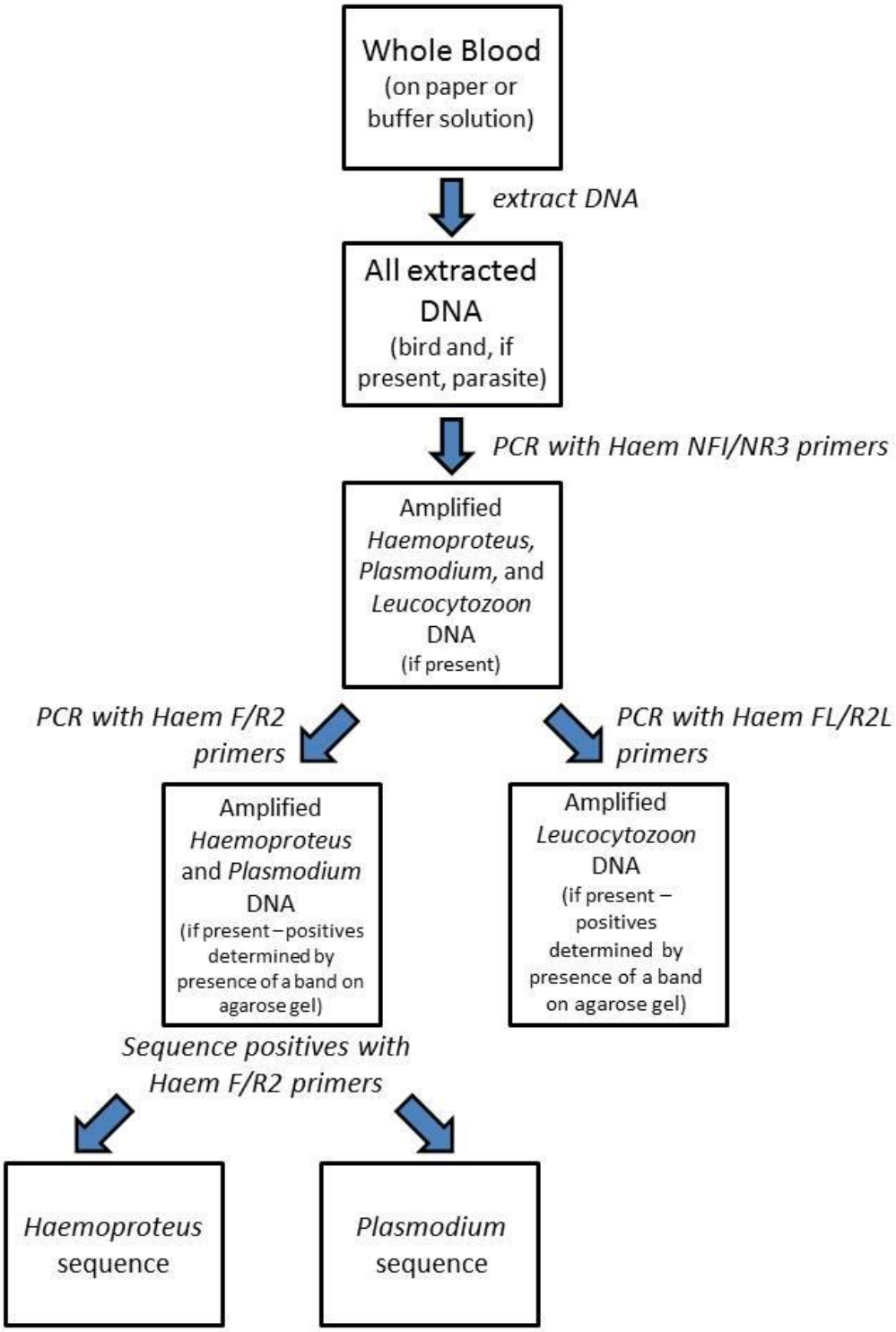
APPENDIX A

Ultra-violet visualization of PCR products on an agarose gel, used for an initial PCR screen to identify northern spotted and barred owls infected with *Haemoproteus* parasites. Primers (HaemF/R2 for *Haemoproteus* and *Plasmodium*; HaemFL/R2L for *Leucocytozoon*) amplified approximately 480 base pairs of parasite mitochondrial DNA. Labels correspond to the lane above the letter. Lane (a) indicates a bird that was not infected with either *Haemoproteus* or *Plasmodium* spp. Lanes (b) and (c) indicate samples that tested positive for either *Haemoproteus* or *Plasmodium* spp. based on the presence of a moderate (b) or bright (c) band. Lanes (d-g) indicate the negative control, positive *Haemoproteus* control, positive *Plasmodium* control, and 100 bp ladder, respectively. Subsequent sequencing of positive samples discerned whether a bird was infected with *Haemoproteus* or *Plasmodium*.



APPENDIX B

Flow chart of laboratory procedures used to determine the infection status of northern spotted and barred owls with avian blood parasites.



APPENDIX C

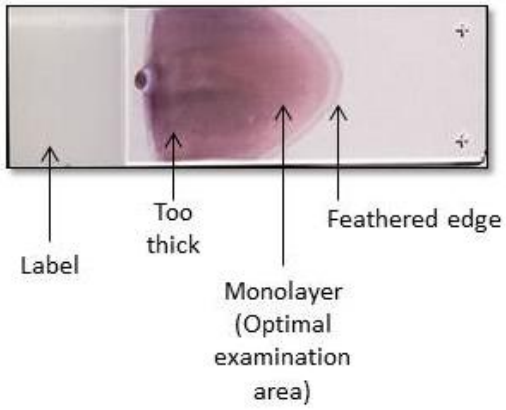
NSO/BO BLOOD PARASITES STUDY – MICROSCOPY PROTOCOL

1.0 PURPOSE

1.1 To provide a general lab procedure for detecting and measuring infection intensity of avian blood parasites from stained thin blood smears.

2.0 PROCEDURE

2.1 The optimal area of the smear to examine is located just before the feathered edge. This is the area of the smear where cells form a monolayer (cells are separated or barely touching, with little overlapping):

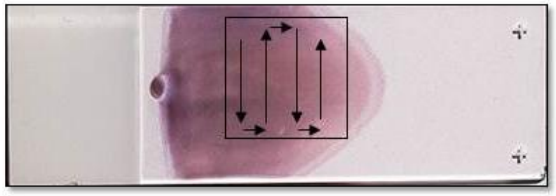


Place a drop of immersion oil in the center of the optimal area and cover with a coverslip. Allow the oil to distribute evenly beneath the coverslip without trapping air bubbles, and then place the slide on the microscope stage.

2.2 Initial scan: 10 minutes

2.2.1 Set the magnification to 500X by selecting the 50X objective lens. This will require placing a drop of oil on the cover slip and working the oil in between the objective lens and cover slip.

2.2.2 Scan the entire area of the smear that is covered by the cover slip (approximately 2 cm^2) for **10 min**. Begin in the top left corner of the cover slip and move in an S-shaped pattern across the slide:



2.2.3 Make notes about slide and overall cell condition, as well as any parasites (e.g., Leucocytozoon, Trypanosoma, Haemoproteus) detected on Microscopy Data Sheet, Section 1.

2.3 Intensity scan: 25 fields

2.3.1 Set the magnification to 1000X by selecting the 100X objective lens.

2.3.2 Select a field in the top left corner of the cover slip. Focus the lens and take a photo of the field using a digital microscope camera and imaging software. Save the photo using the following nomenclature: "number of photo in series"[underscore]"bird identification number". For example, the first photo from a bird with the identification number "PI0001CA00853" should be saved as "01_PI0001C00853".

2.3.3 Using a random number generator, choose a number from 2 to 4. Move down the number of field indicated by the random number generator. The final field is your next sampling field. Take a picture and save the photo as in step 2.3.2.

2.3.4 Repeat step 2.3.3 until the lens reaches the bottom of the cover slip. Move 3 fields to the right and begin moving back up the slide in randomly generated increments of 2-4 fields. When you reach the top of the cover slip, move right 3 more fields. This movement pattern will ensure that

2.3.5 Continue to move across the smear in this pattern (random number down or up; 3 fields to the right when the lens reaches the edge of the cover slip) until 25 fields have been examined.

2.4 Enumerating cells and parasites

2.4.1 Open a digital image of a field using iSolution Lite software.

2.4.2 Using the Manual Tag tool, tag the number of erythrocytes, number of leucocytes, and number of cells infected with each parasite (*Leucocytozoon, Haemoproteus, Plasmodium,* or unknown). Do not count cells that are not entirely within the field.

2.4.3 Select "Apply Vector" to the image and save the image in a "tagged" folder within the greater "bird identification number" folder.

3.0 DATA RECORDING

3.1 Data will be recorded on the Microscopy Data Sheet.

4.0 MATERIALS

4.1 Light microscope equipped with:

4.1.1 10X eyepiece
4.1.2 40X and 100X objective lenses
4.1.3 Digital microscope camera, such as an Olympus DP72

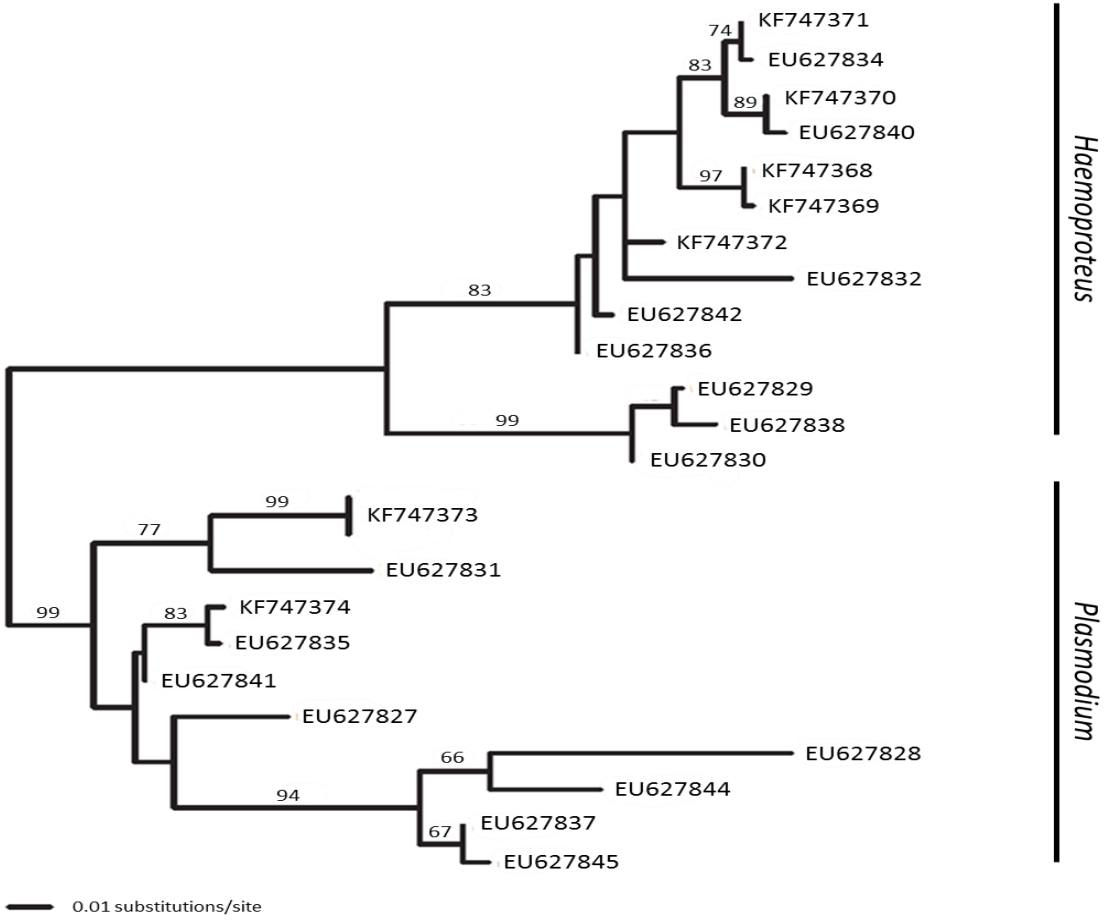
4.2 Immersion oil

4.3 Cover slips

4.4 Image capturing software, such as ProcRes CapturePro (Jenoptik)

APPENDIX D

Maximum likelihood tree for *Plasmodium* and *Haemoproteus* haplotypes from this study and reported in Ishak et al. (2008). ML tree is identical to the tree reported in Figure 6, except branches are labeled with GenBank accession numbers instead of bird family and location information. Although some haplotypes were identical to haplotypes detected in other studies, each branch is represented by a single accession number in this tree. For a list of additional accession numbers, see Appendix E. Bootstrap values were derived using 1000 replicates and are shown to the left of each node.



APPENDIX E

List of GenBank accession numbers for previously detected *Plasmodium* and *Haemoproteus* haplotypes that matched parasite haplotypes detected in northern spotted (*n* = 98), eastern barred (*n* = 138), and western barred (*n* = 53) owls sampled across North America, as well as the avian host, sampling location, and author citation of each detection. For the relationships among previously reported haplotypes and the other haplotypes detected among northern spotted, eastern barred, and western barred owls, refer to Figure 12.

Accession Numbers	Host	Location	Citation
P1 (KF747373)			
JX021463.1	Sedge Warbler	Nigeria	Lacorte et al., 2013
HF543660.1	Old World Kites	Spain	Perez-Rodriguez et al., 2013
GU252027.1	Black-and-white Warbler	North America	Outlaw and Ricklefs, 2010
GU252012.1	Cocoa Thrush	South America	Outlaw and Ricklefs, 2010
GQ395680.1	Galapagos Penguin	Galapagos Archipelago	Levin et al., 2009
GQ395654.1	Galapagos Penguin	Galapagos Archipelago	Levin et al., 2009
GQ395648.1	Galapagos Penguin	Galapagos Archipelago	Levin et al., 2009
GQ141594.1	White-eyed Vireo	North America	Outlaw and Ricklefs, 2010
GQ141574.1	Black-and-white Warbler	North America	Outlaw and Ricklefs, 2010
GQ141569.1	Cocoa Thrush	South America	Outlaw and Ricklefs, 2010
EU627843.1	Barred Owl	USA (Minnesota)	Ishak et al., 2008
n/a	House Finch	USA (Georgia, Idaho, Wisconsin, and California)	Kimura et al., 2006
n/a	Chesnut-capped Blackbird	Uruguay	Durrant et al., 2006
n/a	Song Sparrow	USA	Martinsen et al., 2007
n/a	Slate-colored Grosbeak	Guyana	Durrant et al., 2006
n/a	Buff-throated Saltator	Guyana	Durrant et al., 2006
n/a	Yellow-rumped Cacique	Guyana	Durrant et al., 2006

Table Continued…

Table Continued…

n/a	Northern Saw-whet Owl	USA (Vermont)	Martinsen et al., 2007
n/a	House Sparrow	Brazil and USA (Colorado, Michigan, and Missouri)	Marzal et al., unpublished
n/a	Blue-black Grosbeak	Guyana	Durrant et al., 2007
P2 (KF747374)			
AB741489.1	Mallard	Japan	Yoshimura et al., 2012 (unpublished)
AB741488.1	Mallard	Japan	Yoshimura et al., 2012 (unpublished)
AB741486.1	Mallard	Japan	Yoshimura et al., 2012 (unpublished)
AB601441.1	Red-crowned Crane	Japan	Yoshimura et al., 2012 (unpublished)
AF495574.1	Sedge Warbler	Nigeria	Lacorte et al., 2013
n/a	Pectoral Sandpiper	USA (Alaska)	Yohannes et al., 2009
n/a	Great Reed Warbler	Sweden	Bensch et al., 2007
H4 (KF747371)			
AF465589.1	Barred Owl	USA (Florida)	Ricklefs and Fallon, 2002
AF465589.1	Great Horned Owl	USA (Florida)	Ricklefs and Fallon, 2002
H4 – 1 bp difference			
EU627834.1	Barred Owl	USA (Minnesota)	Ishak et al., 2008
EU627834.1	Barred Owl	USA (California)	Ishak et al., 2008
EU627834.1	Barred Owl	USA (Texas)	Ishak et al., 2008
EU627834.1	African Wood Owl	Africa	Ishak et al., 2008
EU627834.1	California Spotted Owl	USA (California)	Ishak et al., 2008
EU627834.1	Northern Spotted Owl	USA (California)	Ishak et al., 2008

Table Continued…

Table Continued…

EU627834.1	Northern Spotted Owl	USA (Oregon)	Ishak et al., 2008
EU627834.1	Northern Spotted Owl	USA (Washington)	Ishak et al., 2008
EU627834.1	Great Horned Owl	USA (California)	Ishak et al., 2008
H5 (KF747372)			
EU627839.1	California Spotted Owl	USA (California)	Ishak et al., 2008