DISEASES AND PARASITES OF BIRDS: ECOLOGY AND EPIDEMIOLOGY IN A CHANGING WORLD

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Diseases and Parasites of Birds: Ecology and Epidemiology in a Changing World

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Department of Biology
Memorial University of Newfoundland

2007

ABSTRACT

Parasites, the diseases they cause, and their hosts together share a complex evolutionary history. In recent years, however, long-term host-parasite-disease associations have been disrupted primarily due to profound anthropogenic changes in the environment and emerging and re-emerging infectious diseases are recognized as important ecological forces. Birds serve as excellent models in the study of the ecology of parasites and diseases. In this study, I evaluated three aspects of avian disease dynamics. First, I reviewed the role of birds in the spread of highly pathogenic avian influenza (HPAI) of the subtype H5N1 from southeastern Asia. Although limited cases of HPAI have been recorded in wild birds, the overwhelming route of movement and geographic spread of the virus has been via poultry trade and related operations, contrary to scientific and media speculation. The role of wild birds in maintaining the disease in the wild remains unknown and requires considerable study. Control of poultry and human-wildlife-domestic animal interfaces needs to be strengthened to prevent the mixing, mutation and spread of such viruses.

Second, I evaluated the ecology of the Lyme disease spirochete, *Borrelia garinii*, in seabirds from eastern Canadian colonies. I record the first case of *B. garinii* from Gull Island, Newfoundland. Movement of this spirochete is

consistent with short-distance movements of seabirds and the presence of the seabird tick, *Ixodes uriae,* the vector of the spirochete. Identical strains occurring on both hemispheres have suggested long distance movement of the spirochete, however, current information does not offer a good explanation for trans-hemispheric exchange. The strains obtained from Gull Island were similar to eastern European strains, consistent with a hypothesized invasion of colonies in the North Sea from mainland Europe followed by a gradual northwestern movement, with seabirds dispersing into Northwest Atlantic seabird colonies. The timing of breeding of the host seabirds and the life cycle of *Ixodes uriae* are both very important, complex factors influencing the widespread distribution of *B. garinii* in the northern hemisphere.

Third, I examined changes in the endoparasite fauna of Common (*Uria aalge*) and Thick-billed Murres (*U. lomvia*) since the late sixties in the Northwest Atlantic. Species composition and relative abundance were very different in both species reflecting long-term change in the marine environment. I recorded the first case of *Alcataenia longicervica,* a tapeworm species endemic to the North Pacific basin, from murres in Newfoundland. The presence of this species in varying abundance in Coats Island, the Gannet Islands, Gull Island and Greenland reflects a possible route of invasion along

the Siberian, Kara and Laptev seas with infected intermediate hosts (euphausiids). Changes in distributions of these tapeworms in murres therefore reflect long-term change in the distribution, abundance and intermingling of Artic, Pacific and Atlantic krill.

COAUTHORSHIP STATEMENT

Chapter 2 (Avian influenza) emerged as a discussion paper as the Asian avian influenza crisis unfolded in the media, often as a misrepresentation of the scientific facts associated with outbreaks. I have co-authored this paper in *Waterbirds* with Ron C. Ydenberg and Ian Jones: (Muzaffar, S.B., Ydenberg, R.C. and Jones, I.L. 2006. Waterbirds and avian influenza: an ecological and evolutionary perspective for waterbird scientists. *Waterbirds* 29: 243-406). The ideas in this chapter are primarily my own. The coauthors have improved the paper by providing critical comments.

Chapter 3 (*Borrelia garinii* in seabirds) is the product of a collaboration with Peter Rand and his coworkers at the Vector Borne Disease Laboratory of the Maine Medical Center, Maine, U.S.A. The first record of *Borrelia garinii* is published (Smith, Jr., R.P., Muzaffar, S.B., Lavers, J., Lacombe, E.H., Cahill, B.K., Lubeczyk, C.B., Rand, P.W. Presence of *Borrelia garinii* in seabird ticks (*Ixodes uriae*) from the Atlantic coast of North America. *Emerging Infectious Diseases*). We collected ticks and shipped them to the Maine Medical Center for Lyme disease testing and Peter Rand's generously provided the prevalence data from the years 2005 and 2006 that I have used in this chapter for analyses of trends in the dispersal and movement of *Borrelia garinii*. The ideas presented in this chapter are my own and they have been refined by all

of the authors on the following invited paper that has been submitted: Muzaffar, S.B., Smith, Jr., R.P., Jones, I.L., Lavers, J., Lacombe, E.H., Cahill, B.K., Lubeczyk, C.B., Rand, P.W. Ecology of emerging Lyme Disease (*Borrelia garinii*) in seabird colonies of the Atlantic coast of North America. *Studies in Avian Biology*.

Chapter 4 (Questing ecology of *Ixodes uriae*) has been published (Muzaffar, S.B. and Jones, I.L. 2007. Activity periods and questing behavior of the seabird tick *Ixodes uriae* (Acari: Ixodidae) on Gull Island, Newfoundland: the role of puffin chicks. *Journal of Parasitology* 93: 258-264). The ideas in this paper are my own and have been improved by Ian L. Jones.

Chapter 5 (Endoparasites of auks) reports the presence of *Alcataenia longicervica* tapeworms in the North Atlantic. A paper suggesting the possible movement of this tapeworm from the North Pacific has been published (Muzaffar, S.B., Hoberg, E.P. and Jones, I.L. 2005. Possible recent range expansion of *Alcataenia longicervica* Hoberg 1984 parasitic to murres (*Uria* spp., family Alcidae) into the North Atlantic. *Marine Ornithology* 33: 189-191). The ideas in this paper are primarily my own and Eric P. Hoberg helped in confirming the identifications and refining the contents of the paper. This Chapter also deals with an extension of the published paper, analyzing the presence of the tapeworm in the seabird colonies in the North Atlantic and

provides insight into how the range expansion had occurred. This aspect of the study is my own and a second paper will be submitted shortly in which I hope to invite Eric P. Hoberg and Ian L. Jones as coauthors.

ACKNOWLEDGMENTS

My Ph.D. work has been a remarkable journey and I could not have completed it without the whole-hearted assistance from many people. For this I am grateful to all of them. I thank Ian L. Jones, my supervisor, who has been much more than just a supervisor: a mentor, a guide and a friend. I will cherish the discussions with him ranging from host-parasite interactions in an anthropogenic world to nuances of world politics. I thank Murray H. Colbo, who served in my supervisory committee and was available for stimulating discussions drawing on his vast experience with insects, parasites and diseases. He was instrumental in helping me direct my thoughts while developing and carrying out the studies. I am grateful to David C. Schneider, whose ability to make statistics lucid had made me enjoy the task of data analysis. He also helped me structure the thesis in a meaningful and relevant manner and provided critical comments on earlier versions of this work. Over the years I have had fruitful discussions with Ted Miller that inspired critical thinking and self-criticism. I am especially thankful to Ted for dropping by our lab and sparking up discussions on contemporary ecology and evolution. I am grateful to Bill Montevecchi for introducing me to seabirds as an undergraduate and for discussions on ecology of seabirds and their role as environmental indicators. Dr. Rasul Khan has been an inspiration throughout

my Ph.D. program, providing encouragement, insight and enthusiasm into the study of parasites. I am indebted to him for that. I thank Jeanne Finney-Crawley and Eric Baggs for discussions on parasites and diseases and their roles in population dynamics of parasites.

The fieldwork in this study was conducted with great ease due to a number of people. Greg Robertson of the Canadian Wildlife Service (CWS) facilitated logistics throughout the duration of this study. Dave Fifield was an essential part of Gull Island field camp; data collection and many aspects of this study could not have been done without him. Both Greg Robertson and Dave Fifield had gone far beyond the call of duty to ensure that my logistical and other demands were met. I am indebted to both of them and will cherish the great times in the field. I am indebted to Johanne Dussurealt who collected murres for this study, assisted in fieldwork and data management. I thank Jennifer Lavers for helping in the collection of specimens from the Gannet Islands. I also thank Brianna Newton, Corinne Wilkerson, Sampath Seniveretne, Carla Baker, Stephanie Royston, Keith Lewis, and Rachel Bryant for help with fieldwork and logistics. I thank Tony Power and the staff at Cape St. Mary's Ecological Reserve for their help in taking me to study sites and for pain-stakingly collecting tick specimens for me. I am grateful to Sabina Wilhelm, Pierre Ryan, Scott Gilliland and Paul Harris of CWS for

providing me with murre specimens for this study. I thank Carsten Egevang for sending specimens from Greenland and Tony Gaston and Kyle Elliott for sending specimens from Coats Island. I am indebted to Bill Gin for providing materials for dissection upon request; Bill Brown for providing chemicals for preserving and processing parasites; and Peter Earle for assistance with software related problems.

I am indebted to Peter Rand and Chuck Lubelczyk of the Vector Borne Disease Laboratory of the Maine Medical Center in the United States for facilitating a wonderful collaboration and providing crucial Lyme Disease testing of ticks collected in this study. Funding for this project was also provided by the Atlantic Cooperative Wildlife Ecology Research Network of the CWS. I am especially grateful to Richard D. Elliot, CWS for being such a charming personality and source of constant encouragement and for facilitating and promoting many aspects of my study. Permits for field activity were provided by the Animal Care Services, Memorial University of Newfoundland; the Canadian Wildlife Service (Atlantic Region); and Parks and Natural Areas Division (Newfoundland and Labrador).

I am indebted to my family for enduring years of wildlife and parasites. My brothers, especially Towhid Bin Muzaffar, for providing guidance and encouragement; my parents, particularly my father for being

my biggest fan; and my wife, Sophia J. Shaikh, for conducting field work and being unconditionally supportive of and enthusiastic about all my activities.

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CHAPTER 1. GENERAL INTRODUCTION

The ecology of parasites and disease

Parasites and diseases are important components of ecosystems with long-term effects on fitness, reproductive success and population dynamics of their hosts (Clayton and Moore 1997, Hudson et al. 2002). Globalization and human-induced climatic anomalies have allowed parasites to forge niches by invading into new geographic areas or establishing ranges in unusual localities, giving rise to epidemics and epizootics worldwide (Daszak et al. 2000, Daszak 2005, Wilcox and Colwell 2005, Thomson et al. 2006). Parasites and the diseases they cause have long been regarded as important sources of morbidity and mortality in wildlife (Duffy 1991), yet quantitative studies addressing their impacts are limited (Daszak et al. 2000, Cattadori et al. 2005). This has stimulated ecologists to evaluate the roles of parasites and diseases in ecological systems using quantitative methods, especially over the last three decades (Hudson et al. 2002, Wilcox and Colwell 2005).

Among wildlife, birds have been particularly important in helping bridge the gap between quantitative ecology and parasitology (Loye and Zuk 1991, Clayton and Moore 1997, Cattadori et al. 2005). Over the past two decades, the study of avian parasites has revealed much about the nature of parasites in ecosystems providing insights into the mechanisms of parasite

population dynamics and their influence on host population regulation (van Riper et al. 1986, Hudson et al. 2002, Cattadori et al. 2005).

Determining the role of parasites in modulating life histories of their hosts remains a difficult task in spite of the fact that the basic mechanisms had been conceptualized almost three decades ago (Anderson and May 1978, Anderson and May 1979, Hudson et al. 2002). The few studies that do establish a clear link between parasite- or disease-induced mortality and avian population regulation are long-term studies that often also demonstrate that parasites may operate synergistically with other factors, such as climate change (Cattadori et al. 2005). It is clear however, that parasite and disease dynamics need to be empirically investigated, particularly in the context of ecological processes linking them to their hosts' life cycles. This has been recognized by the scientific community of parasitologists, veterinarians and ecologists, setting the stage for a better understanding of the dynamics and impact of parasites and diseases on hosts (Hudson et al. 2002). These three disciplines, however, operate under very different paradigms, yet each has a role to play in bridging the gaps in our understanding of parasite and disease ecology (Daszak et al. 2000, Horwitz and Wilcox 2005, Wilcox and Colwell 2005, Muzaffar et al. 2006). Although problems associated with fundamental differences in each of these disciplines still persist, interdisciplinary

approaches have gained strength in recent years forming a common platform from which to address questions and challenges in parasite dynamics and disease epidemiology.

Definitions and concepts

The term 'parasite' is difficult to define and every textbook in parasitology starts by examining the difficulties of defining the term (Brooks and McLennan 1993). Here I define parasites as organisms that live in or on other organisms usually doing some damage to the reproductive success of their hosts (Clayton and Moore 1997). Even this definition is incomplete since many parasites have life stages that are completely free-living and the infective stages do not seem to compromise reproductive success. Such definitions also downplay complex ecological and evolutionary interactions that result in host-parasite systems. Moreover, host-parasite interactions may shift from parasitic to mutualistic or vice versa depending on a continuum of context-dependent drives towards either low or high virulence (Ewald and De Leo 2002).

Combes (2001) has defined the parasite-host relationship as an intimate association of parasites (the offender) and hosts (the offended) and the ongoing arms race between the two organisms attempting to overcome

one another's defenses. This is essentially a reformulation of the much older Red Queen Hypothesis relating living organisms (e.g. predators and prey) in the evolutionary arms race (Van Valen 1973). This move from the classical definitions of parasites and parasitism to one in which the interaction is regarded as dynamic, involving behavioral, genotypic and phenotypic adaptive responses in relation to environmental, temporal, spatial and stochastic processes. This aspect of host–parasite interactions is relatively recent and difficult to conceptualize (Brown et al. 2003, Horwitz and Wilcox 2005).

While recognizing this complexity of host-parasite interactions to its fullest, I have still sought refuge in traditional definitions simply due to the ease with which they can be related to different kinds of parasites. Anderson and May (1978) have categorized parasites (somewhat arbitrarily) as *micro* and *macroparasites*, the former encompassing viruses, bacteria and 'protozoans' (a diverse and arbitrary group of generally single-celled organisms with representatives from different kingdoms) and the latter encompassing the more 'traditional' parasites (Platyhelminthes, Acanthocephalans, etc., Dogiel 1964). Throughout this work, I shall refer to macroparasites as parasites. Microparasites are referred to as pathogens.

Objectives of this study

This study examines the linkages and interactions between parasites and diseases with birds. The study has three major objectives:

1. to review our understanding of emerging infectious diseases (Chapter 2) by examining Avian Influenza in North America, Europe and Southeast Asia. I provide the history of avian influenza, the causative agent and the role of birds in the geographic spread of the disease, with especial reference to highly pathogenic avian influenza (HPAI) of H5N1 subtype. I then examine the role of wild birds in the spread of this HPAI.
2. to examine the ecology of Lyme Disease, a tick-borne disease of birds and mammals including humans, in seabird colonies in eastern Canada (Chapter 3), by quantifying aspects of the ecology of the tick *Ixodes uriae* (Chapter 4) and using prevalence data on *Borrelia garinii* (the causative agent) to evaluate the geographic spread of *Borrelia* among seabirds.
3. to examine the ecology of endoparasites of two auk species (Common Murres, *Uria aalge* and Thick-billed Murres, *U. lomvia*), especially the tapeworm genus *Alcataenia* (Chapter 5) with reference to changes in

the marine environment that have led to recent distributional changes of this parasite.

CHAPTER 2. AVIAN INFLUENZA: ECOLOGY, EVOLUTION AND EPIDEMIOLOGY

Emerging infectious diseases

Emerging and re-emerging infectious diseases are important global problems of great concern to human as well as animal health (Daszak et al. 2000, Wilcox and Colwell 2005). The Human Immunodeficiency Virus (HIV) epidemics in Africa, the widespread occurrence of Malaria in Asia, the outbreak of Severe Acute Respiratory Syndrome (SARS) in Southeast Asia are few of the examples of human diseases with large numbers of casualties. Rinderpest outbreak in African ungulates (Rossiter 2001), West Nile Virus in North America (Campbell et al. 2002) and Avian influenza in Eurasia (Olsen et al. 2006) have caused mortality in wildlife and humans alike. The epidemiology of diseases has an important human component, although in many cases wild animals may play a significant role (Daszak et al. 2000). In recent years, human activities have enhanced the chances of disease transmission and persistence. Direct consequences of globalization of the modern marketplace include a frequent movement of humans, trade of products and alterations in the landscape (Wilcox and Colwell 2005). These changes in the context of the biosphere have resulted in spreading of diseases and parasites across the globe (Daszak et al. 2000, Daszak 2005, Wilcox and

Colwell 2005). Whereas local wild bird populations may play a role in the spread of diseases (e.g. West Nile Virus in North America, Campbell et al. 2002), human activities remain the ultimate cause of widespread disease outbreaks (e.g. the arrival of West Nile virus in North America, Campbell et al. 2002; Avian Malaria in the Hawaiian islands, van Riper et al. 1986, Avian Influenza in southeast Asia, Olsen et al. 2006, Muzaffar et al. 2006 to name a few). The widespread occurrence of many infectious diseases has prompted a drive towards understanding ecological processes and mechanisms driving wildlife disease spread (Wilcox and Colwell 2005, Ezenwa et al. 2006).

Here, I review the biology and dispersal of a pathogenic form of avian influenza, an important disease that has swept through modern societies. Although wild birds have been implicated as principal vehicles of spread of avian influenza, the extent to which they do so has been debated. I summarize the data from an extensive literature review to discuss the proximate and ultimate causes of the spread of this disease.

Avian Influenza: the current context

Governments, international bodies, and the public around the world are gravely concerned about the potential impact of the avian influenza viruses on human health and on the global economy (Li et al. 2004; Chen et al.

2005; Ferguson et al. 2005). The recent rapid spread of avian influenza viruses, repeated disease outbreaks caused by highly pathogenic strains in domestic poultry with accompanying economic costs, cases of direct transfer of the virus from birds to humans, and the apparent high death rate among infected humans have combined to make 'avian influenza', 'highly pathogenic' and 'H5N1' household words, and the subject of much preparatory organization by agencies such as The World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Governments have developed surveillance schemes and contingency plans to be able to deal with a pandemic that many claim to be imminent and inevitable (Chen et al. 2005, Fauci 2006).

Migratory birds and waterbirds in general play central roles in this critical issue. It is widely asserted in the press, on websites of governments and international agencies (e.g. The World Health Organization, Food and Agricultural Organization), and even in the scientific literature, that highly pathogenic genotypes of H5N1 virus are spread to domestic poultry through contact with wild birds (Li et al. 2004; Chen et al. 2005). Relatively fewer studies highlight the dearth of evidence linking avian influenza outbreaks to migratory birds (e.g. Olsen et al. 2006, Muzaffar et al. 2006, Gauthier-Clerc et al. 2007). To help in evaluating the role that waterbirds play in the

epidemiology of this disease it is essential to investigate these and other claims critically. At the same time, it is also important to quantify aspects of the epidemiology of avian influenza to fill the gaps in our understanding of the disease.

Influenza Virus Structure and Nomenclature

Influenza viruses belong to the Orthomyxoviridae family of RNA viruses that occur naturally in many species of wild aquatic birds, and are maintained in wild populations (Horimoto and Kawaoka 2001, Earn et al. 2002). Avian influenza viruses infect the gastrointestinal tract in their natural avian host species, but can also infect the respiratory tract and other organ systems. Viral particles are shed in the feces for a time shortly after infection after which viral replication stops due to the immune response of the host. The virus is transmitted very efficiently via bird-to-bird contact and fecal shedding into the water supply (Webster et al. 1992).

The influenza virus has a very small genome with only 8 RNA segments. Six of these code for the proteins HA, NA, NP, PB1, PB2, and PA. The remaining two RNA segments are transcribed to mRNAs and translated in different reading frames to yield two proteins each, M1 and M2, and NS1 and NS2, respectively. Based on variants of the M1 and NP proteins,

influenza viruses are classified into three major 'types': A, B and C (Webster et al. 1992, Murphy and Webster 1996, Earn et al. 2002). Type A influenza virus occurs in a wide range of birds and mammals, is geographically widespread, and is epidemiologically the most important. It is commonly referred to as avian or bird flu. Type B is restricted to and an important cause of illness in humans. Type C is not known to cause illness and very little is known about it. Neither Type B nor Type C have ever been isolated from waterbirds or poultry and are not discussed further.

Two proteins (HA, or hemagglutinin; and NA, or neuraminidase) are arrayed on the envelope of influenza A virions, and interact with receptor sites on the exterior of host cell membranes, and play important roles in gaining the virion access to the interior of cells. The complexity of the molecular interactions on the cell membrane restricts the types of cells that a virion can invade successfully, and usually confines a specific viral genotype to one host species, or at least to specific types of receptor sites. (Earn et al. 2002)

Sixteen forms of HA and nine forms of NA have been described, and their combinations form the basis for the classification of influenza A viruses by 'subtypes'. Hence the H5N1 'subtype' of avian influenza contains the fifth described form of HA and the first described form of NA (HxNy abbreviates

hemagglutinin as 'H' and neuraminidase as 'N'.). Most but not all of the 144 possible pairwise HA – NA combinations have been observed in wild birds. The nomenclature of avian influenza viruses further uses a system that identifies the type (A, B or C), the host species from which it was isolated, the location, the isolate number, and the year. For example, 'A/mallard/Alberta/211/98 (H1N1)' is isolate number 211, a type A, subtype H1N1 virus, from a mallard in Alberta caught in 1998.

Sequence data have revealed much variability within each subtype (Li et al. 2004, WHO 2005). This variability is genetic, and is found in the RNA sequences coding for all of the virus proteins. Antigenic drift (i.e. progressive accumulation of individual mutations resulting in the gradual decline in the intensity of the antibody response) may help explain the need for regular replacement of vaccine strains (e.g. Earn et al. 2002), as details of the structure of the HA and NA molecules change and so reduce the ability of the host's immune system to recognize variant subtypes of the virus. Horimoto and Kawaoka (2001) attribute antigenic drift to point mutations and 'immunological pressure'. Influenza viruses commonly change by antigenic drift which means there are small changes that occur all the time to the HA and NA proteins (Webster et al. 1992, Earn et al. 2002). Type A influenza viruses also can undergo antigenic shifts that are sudden major changes that

can create a new viral subtype through genetic reassortment, the exchange of viral segments when one host is infected by two different viral subtypes. How these processes occur in wild birds is not well known, and clarifying the underlying evolutionary processes would help in understanding the ecology of the virus and its epidemiology (Webster et al. 1992, Murphy and Webster 1996, Earn et al. 2002).

Ecology of Avian Influenza

Most knowledge of avian influenza has come from virology, veterinary science, and human medicine, and most is written from the perspectives of either domestic animal or human health. The available information is extensive and detailed, but little is known about the ecology of avian influenza viruses in natural populations.

All known influenza A virus subtypes have been documented in waterbirds (reviewed by Webster et al. 1992, Murphy and Webster 1996, Ito and Kawaoka 1998, Alexander 2000, Webby and Webster 2001, Fouchier et al. 2005) and many of these form long-term host-virus associations in the wild (Webby and Webster 2001, Webster and Hulse 2004). The virus has also been isolated from a wide range of wildlife: birds of many species, American mink *Mustela vison*, harbor seals *Phoca vitulina*, whales (Cetacea); and domestic

avian and mammalian hosts: pigs *Sus scropha*, chickens *Gallus gallus*, turkeys *Meleagris gallopavo*, ducks (Anatidae), domestic horses *Equus caballus*, humans (Webster et al. 1992, Webby and Webster 2001, Webster et al. 2006). The earliest documentation of wild infections of influenza A viruses dates to the 1960s, with infections in ducks and seabirds (Slemons et al. 1974, Webster et al. 1992, Webby and Webster 2001, Slemons et al. 2003, Laver 2004), but there can be little doubt that wild aquatic birds have a long evolutionary association with the virus. Its widespread occurrence in gulls, shearwaters, other seabirds, shorebirds and ducks has led to the recognition of waterbirds, in general, as the primary natural reservoir of the virus (Hinshaw et al. 1980, Webster et al. 1992, Webby and Webster 2001, Hatchette et al. 2004). Not all subtypes are equally successful in establishing stable associations, because hosts vary in susceptibility and in the efficiency of transmission (Strum-Ramirez et al. 2004).

Studies published on the ecology of avian influenza in wild birds in North America (e.g. Slemons et al. 1974, Hinshaw et al. 1980, Stallknecht and Shane 1988, Webster et al.1992, Stallknecht 1997, Webby and Webster 2003, Krauss et al. 2004, Webster and Hulse 2005) have made attempts to bridge this interdisciplinary gap. Since 1974 thousands of swab samples collected from migratory waterfowl and shorebirds in Alberta and (since 1985) in

Delaware Bay (eastern USA), provide the first and most extensive insight into patterns of occurrence of avian influenza viruses in nature. Influenza A viruses are common in southward migrating waterfowl with 18-60% of juvenile ducks and 4-27% of adult ducks being infected with various influenza subtypes (Hinshaw et al. 1980, Webster et al. 1992). Northward migrating ducks had a much lower prevalence (~0.3%) and diversity of influenza A viruses. Consequently, post-breeding pre-migratory staging areas are thought to be important locations for influenza A transmission (Hinshaw et al. 1980, Ito et al. 1995, Hanson et al. 2002), because of this high prevalence of infection in juvenile ducks, evident from heavy fecal shedding of the virus and increased abundance and densities of a variety of wild bird species co-mingling. The prevalence of Influenza A viruses declines in the course of southward migration, and individuals on southern wintering areas have much lower prevalence of the virus, indicating a loss of viral infection during migration. Influenza A viruses isolated from wintering and resident waterfowl in Texas (southern North America) include greater diversity and rarer subtypes, indicating long-term changes in viral lineages in the wild (e.g. Hanson et al. 2005). Ducks returning northward in spring have much lower viral titers than southward migrants, but in high enough titers to re-establish the virus in their northern breeding grounds (Webster et al. 1992, Ito et al.

1995, Krauss et al. 2004). It is possible that avian influenza viruses could survive the winter and re-infect birds arriving on breeding areas (Webster et al. 1992). Persistence of the virus in the environment (e.g. in water) depends on factors such as pH, temperature, salinity and other physicochemical variables (Stallknecht et al. 1990). Influenza viruses can remain infective outside an avian host for up to 35 days in fecal matter in cold, moist conditions (at 4°C), though less long in warmer conditions (at 20°C). Influenza viruses can be isolated from lake water when waterfowl are present, but once the waterfowl leave for their wintering areas, the viruses can no longer be isolated, suggesting that persistence in the environment is largely dependent on the presence of the hosts.

Certain subtypes seemingly dominate in particular migratory flyways and their prevalence varies from year to year (Hinshaw et al. 1985, Ito et al. 1995, Hanson et al. 2002, Hatchette et al. 2004, Krauss et al. 2004, Hanson et al. 2005). The role of avian species other than waterfowl in perpetuating avian influenza remains unclear (Alexander 2000). Shorebirds (Charadriidae and Scolopacidae) are thought to be important in the dissemination and maintenance of influenza A viruses in the wild (Webster et al. 1992, Webster et al. 2006), although data are very limited. The prevalence of infection in northward migrant shorebirds is higher than in waterfowl (14.2% vs. 0.3%),

but the significance of this is not yet known. Studies of viral ecology in migratory waterbirds in Eurasia indicate similar patterns of viral infection relative to North American studies, with interannual variation in the diversity and seasonality of the viral shedding in relation to migration (Otsuki et al. 1987, Okazaki et al. 2000, Munster et al. 2005). The pattern of occurrence of avian influenza in seabirds is less known, although gulls tend to have higher prevalence of influenza in late summer and early fall (Olsen et al. 2006). These patterns are likely related to gull aggregations in breeding colonies as well as garbage dumps or other feeding areas.

Evolutionary biology of avian influenza

Avian influenza viruses represent a rapidly evolving and diversifying lineage; aquatic birds are indeed the ancestral hosts of avian influenza and shorebirds, ducks and gulls share ancestral genes of several avian influenza subtypes (Webster et al. 1992, Earn et al. 2002, Widjaja et al. 2004). Phylogenetic analyses often do not reveal congruent evolutionary patterns since viral genes that mingle in new hosts may contain diverse reassortants from different host species. For example, in the 1990s a reassortant influenza A (H3N2) virus lineage established itself in USA swine, with genes whose closest and most recent known ancestors were of human, avian and

mammalian origin (Zhou et al. 1999). The viruses that caused the 1957 and 1968 human influenza pandemics were reassortants of human and avian origin, while all the genes in the virus that caused the 1918 pandemic were descended directly from birds (Taubenberger et al. 2005). By more distant ancestry, all influenza genes are of avian origin.

Parallel evolutionary events may be evident in phylogenetic analyses of influenza A viruses. For example, 'swine flu' independently evolved in Eurasia and America. Trees reveal distinct American and Eurasian lineages for several influenza virus A genes. A low pathogenic strain of H5N1 has been detected in healthy wild birds in both Eurasia and North America (CFIA 2005), and is very different from the highly pathogenic Asian strain. These lineages are evolving independently, and while the Eurasian form is highly pathogenic (causing severe disease in chickens, referred to as Highly Pathogenic Avian Influenza, HPAI), the North American form is low pathogenic (not causing any clinical signs of illness in chickens, referred to as Low Pathogenic Avian Influenza, LPAI). The clear separation of the trees is remarkable, because it seems inevitable that there must be some contact on Arctic breeding grounds between migrants of Old and New World origin. Geographical segregation is evident even within the recent phylogeny of H5N1 in China (Chen et al. 2006). Comparison of a large number of samples

from both wild and domestic birds reveals that the current Eurasian H5N1 avian influenza virus originated in China at least a decade ago, and that it has evolved into distinct lineages associated with particular geographic regions.

The mechanisms maintaining the separation (Krauss et al. 2004) are obviously of great current interest with the potential spread of Eurasian H5N1 to America. There is a great deal of speculation in our understanding of the evolution of virulence (Muzaffar et al. 2006). It is clear, however, that the HPAI H5N1 has become endemic in South and Southeast Asia. The increase in pathogenicity can be traced phylogenetically to a progressive accumulation of genes of H5N1 within poultry (Chen et al. 2004, Chen et al. 2006).

It has been predicted that the level of virulence of avian influenza in wild and domestic birds differs greatly (Muzaffar et al. 2006). Wild birds, especially if migratory, must be able to move great distances, and as described above, wild populations have repeated exposure to a great many subtypes of the virus. Consequently, avian influenza viruses are expected to evolve and maintain low virulence in wild birds, as is indeed seen (Webster et al. 1992, Hanson et al. 2005). In contrast, many domestic poultry are housed in high-density, commercially mass-produced situations, and are all similarly immunologically naïve. Moreover, the disease may be borne from farm to

farm by fomites or 'cultural' vectors (Ewald 1994, Ewald and DeLeo 2002) such as garbage trucks, farm workers, or in poultry trade. High pathogenicity thus is likely to be selected for by the conditions prevailing in some commercial poultry production situations, namely dense clusters of immunologically naive hosts, with the potential for 'cultural' vectors such as vehicles, cages and farm workers transporting the virus between such clusters. Indeed, one could hardly imagine a better-designed environment for the evolution of high virulence in a pathogen than the current worldwide network of industrial poultry farms.

HPAI Outbreaks: The Human-Domestic Animal-Wildlife Interface

Three possibilities for the local origin and geographic spread of HPAI can be identified: (1) Wild bird viruses mixing with domestic bird viruses could transfer infections to poultry flocks. The virus could cause asymptomatic or mild disease or it could prove highly lethal. (2) LPAI could evolve into HPAI in domestic poultry in response to one of more of the conditions identified above. The LPAI ancestor virus may be present in the flock, or may have been deposited there by wild birds. Fauci (2006) theorized that HPAI genotypes of the virus are derived from LPAI spread by wild waterbirds, and become highly pathogenic by progressive mutation of the viral genotype while passing from one susceptible host to the next. An infection of an HPAI subtype might then be spread from one farm to another by fomites/environmental conditions/cultural vectors such as inspectors, veterinarians, workers, vehicles, trade in eggs, birds, or feathers, or even by wind or water if the farms are in close proximity. (3) The international trade of wildlife (both legal and illegal) may play important roles in the evolution and spread of HPAI.

Migratory birds and HPAI

It seems less likely that migratory waterbirds are involved in maintaining and spreading HPAI (Chen et al. 2006, Olsen et al. 2006, Gauthier-Clerc et al. 2007). Virulent strains of influenza A have been collected from apparently healthy waterfowl but in most of these cases, a nearby chicken farm with an influenza outbreak had been identified (e.g. Liu et al. 2005, Olsen et al. 2006, Gauthier-Clerc et al. 2007). Recently, a rare occurrence of HPAI in wild birds was documented. In summer 2005, some 1,500 bar-headed geese (*Anser indicus*) and other waterbirds breeding at Qinghai Lake in central China died of an HPAI infection. The strain proved lethal to experimentally infected chickens and mice. In their report, Liu et al. (2005) speculated that the lethal viruses might be emerging from reassortment of genomes in domestic fowl whose LPAI ancestors originated in wild birds overwintering in Southeast Asia. Subsequent work (Chen et al. 2005) showed that the virus was most closely related to a form isolated from poultry in southern China. The high mortality of the bar-headed geese supports the hypothesis that ecological conditions in the wild select against highly pathogenic forms of the virus.

Chen et al. (2006) reported the presence of HPAI H5N1 in two apparently healthy migratory ducks from Poyang Lake in Jiangxi, China.

Isolates from Poyang Lake were also most closely related to the Qinghai Lake isolates, suggesting that the virus has been carried a distance of ~1700 km by migratory birds. The Poyang Lake isolates also retained high pathogenicity in chickens, which may implicate migratory birds in spreading the virus. The isolation of HPAI H5N1 from Mongolia, Siberian Russia, Romania, and Turkey without any clear link to poultry operations have led some to suggest that migratory birds are involved in the spread of the virus. This idea has been vigorously debated in the scientific literature (reviewed by Olsen et al. 2006, Gauthier-Clerc et al. 2007). The role of wild waterfowl in the geographic spread can be evaluated by first determining the susceptibility of wild waterfowl to HPAI H5N1 and their ability to sustain long flights while infected. One recent study (Brown et al. 2006) has shown that some North American waterfowl species infected with Asian HPAI H5N1 strains remained free of clinical symptoms whereas others exhibited clinical symptoms and died. More information is needed on the Eurasian wild waterfowl and their susceptibility to such infections to establish the extent to which they are involved in the spread of HPAI. Additionally, wild waterfowl need to be tested serologically to determine if they have acquired immunity as a result of recent exposure to HPAI.

Even if migratory birds are associated with certain outbreaks, they are unlikely to be major factors spreading the virus through Asia and Europe and into Africa (Chen et al. 2006, Olsen et al. 2006, Gauthier-Clerc et al. 2007). Recently, the discovery of a facility to breed bar-headed geese near Qinghai Lake has further weakened the notion that migratory birds may be important contributors to the spread of the virus (Butler 2006). Chen et al. (2006) conclude that "the establishment of regional virus sublineages suggests that H5N1 virus is perpetuated in poultry largely through the movement of poultry and poultry products rather than by continued reintroduction of viruses by migrating birds". Further work is required to test whether the virus in wild birds originated in domestic birds or vice versa and to clarify how that information could apply to the current spread of the disease across Eurasia. In contrast with wild birds, derivation of HPAI genotypes from LPAI predecessors in poultry has been described several times in the literature. Kawaoka et al. (1987) and Röhm et al. (1996) describe outbreaks of highly pathogenic avian influenza in poultry. The putative LPAI avian ancestors were non-pathogenic to their original wild bird hosts (e.g. tern and swan in Röhm et al. 1996), and while circulating in poultry subsequently acquired the extra amino acids at specific cleavage sites that gave rise to a highly pathogenic variant in poultry.

Poultry and HPAI

As with wild birds, diverse subtypes of influenza A have been reported from the poultry industry and live bird markets (Panigrahy et al. 2002, Webster 2004). Prior to the outbreak of HPAI H5N2 in poultry in several of the United States in 1983 (which caused great economic losses), the virus had been present for a considerable period (as much as 8 years) as an LPAI strain before manifesting as HPAI (CFIA 2004, 2005). In the outbreak of HPAI H7N3 in poultry in British Columbia (February 2004), the virus had been detected a few days earlier as LPAI and had rapidly mutated into the HPAI form. The subsequent 'shift' to HPAI resulted in the depopulation of millions of chickens, turkeys and other domestic poultry to limit the spread of the virus (CFIA 2004, Kermode-Scott 2004). Repeated outbreaks of HPAI H5N1 in Asia during 1997 – present have wreaked havoc in the poultry industries of China, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Korea and Japan. Phylogenetic work reveals that the virus has been present and evolving for at least ten years, first in the LPAI form, and now in the HPAI form.

The Asian context of poultry farms may be significant in the evolution of HPAI H5N1 (Webster 2004). Live-animal markets or wet markets occur

throughout Asia, where a diversity of live domestic and wild geese, chickens, quail, passerine birds, mammals, reptiles and live fish are sold. Poultry are generally kept separated from, but certainly not far from, a wide range of other animals, making these markets ideal places for cross-infection, and the exchange, acquisition and evolution of viral genes (Li et al. 2004, Chen et al. 2004, Webster 2004, Webster et al. 2006). High Pathogenic Avian Influenza H5N1 was first detected in Hong Kong in 1997 and was widespread in poultry markets because of co-housing of a diversity of live animals (Webster et al. 2006). The precursors of this HPAI H5N1 were detected in geese in live poultry markets in Guangdong, China (1996) where they caused a small number of deaths (Webster et al. 2002). This virus however, spread through poultry acquiring gene segments from quail and ducks before becoming a widespread goose virus in the outbreak of 1997 (Webby and Webster 2001). Subsequent to depopulation of all domestic poultry during this outbreak, reassorted subtypes of H5N1 continued to arise from goose and duck reservoirs (Webster et al. 2002, Li et al. 2004). The virus spread to exotic felids, domestic cats, ferrets and mice (Webster et al. 2006). One form, referred to as the Z genotype (Li et al. 2004) became dominant and swept through poultry farms in the region resulting in the culling of millions of domestic birds. Experimental evidence shows that ducks and other poultry may harbor HPAI

H5N1 and can be asymptomatic (Chen et al. 2005, Hulse-Post et al. 2005, Li et al. 2005), suggesting that they are involved in silently amplifying the virus in poultry populations. Clearly, poultry have played and continue to play a central role in the emergence of HPAI H5N1 (Olsen et al. 2006, Gauthier-Clerc et al. 2007).

Local and perhaps even long-distance spread by 'cultural' vectors is implicated in transporting HPAI viruses (Olsen et al. 2006, Chen et al. 2006). The modern day farming industry has vastly improved capabilities of moving large numbers of poultry and poultry products over large geographic areas in very little time. Global trade in poultry is enormous, representing an estimated global consumption of 81.8 million tons in 2006 (FAO 2006). Worldwide, many large industrial operations produce and ship hundreds of thousands of birds per year, and HPAI represents an enormous economic hazard. The movement of poultry and its products typically utilize cultural vectors such as vehicles, implements and workers that spread the virus from farm to farm locally, as in the 2004 LPAI H7N3 outbreak in British Columbia (CFIA 2004). Longer distance spread of the virus is possible in local and international trade. The virus could be carried on crating, on eggs, on feathers, or by birds. The rapid spread of H5N1 across Eurasia can be easily explained by the cultural vector hypothesis.

Wildlife trade and HPAI

The pet trade has become a billion dollar industry and both the legal and illegal movement of birds and other wildlife pose a significant threat with regard to exotic disease spread (van Borm 2005). That exotic diseases can spread rapidly once introduced by the pet trade was illustrated vividly by the entry and spread of West Nile Virus into the Americas (Campbell et al. 2002), which caused human mortality as well as extensive mortality and morbidity in many wild bird species. Avian influenza A viruses have been isolated from cage birds of different kinds in the international pet trade, and subtypes such as H9N2 and highly pathogenic H5N1 have been recorded (Masaji et al. 2001, van Borm et al. 2005).

Caged birds and their ability to amplify the virus have not been studied. It is known that infected birds may remain asymptomatic as was documented in two crested hawk eagles, *Spizaetus nipalensis* smuggled for the falconry trade (van Borm et al. 2005). Cage bird markets, both legal and illegal, potentially assist the spread of HPAI viruses. The isolation of H5N1 from exotic birds in quarantine or confiscated by customs officials in Europe highlight the importance of better surveillance of wildlife trade.

Summary and Conclusions

Understanding the evolution of LPAI to HPAI viruses, as well as the origin and spread of HPAI is of great urgency. Models for HPAI origin and spread most frequently promulgated in the media and official publications appear incomplete, or flawed. These shortcomings in our knowledge of this serious disease could have disastrous consequences for the protection of human health, the global economy, and for domestic poultry operations, in both developed and developing nations, and for populations of wild birds.

Much of the current discussion on the origin of HPAI appears devoid of evolutionary thinking. Often the origin of HPAI genotypes is attributed to the acquisition of 'mutations', while the role of ecological conditions that select for high or low virulence is ignored. Conditions in modern large-scale poultry production seem ideal for the evolution of high virulence, while those faced by free-living migratory birds favor low virulence. Consequently, the global poultry production system with its extensive trade in poultry and poultry products appears the most likely source for the repeated evolution of highly pathogenic strains from LPAI ancestors. HPAI outbreaks seem attributable to this process, and to local and even distant spread of these strains by trade and vectors. It appears unlikely to us that HPAI originates in wild birds, or even that wild birds can spread HPAI very rapidly. Rather, it is

clear that wild birds need protection from these HPAI subtypes. Research on, and surveillance of, disease evolution and transmission in domestic poultry and within the wildlife trade is urgently needed. Measures aimed at improving on-farm biosafety are also essential. In particular, the proper disposal and disinfection of wastes and offal seems paramount in preventing spread of viruses within the poultry industry. The global trade, legal and illegal, in exotic birds and poultry needs careful surveillance and better enforcement of existing laws. Finally, the unprotected disposal from poultry operations of any carcasses, offal and fecal matter to which wild birds might be exposed should be halted.

CHAPTER 3. ECOLOGY OF EMERGING LYME DISEASE IN SEABIRD COLONIES OF THE ATLANTIC COAST OF NORTH AMERICA

Introduction

Lyme Disease and its causative agent

An epidemic of arthritis in Lyme county of Connecticut in the late 1970s associated with a characteristic rash developing from a tick bite led to the description of the etiologic agent of Lyme disease or Lyme borreliosis by Burgdorfer et al. (1982). A spirochete bacterium (Spirochaetes, Spirochaetaceae), *Borrelia burgdorferi*, was subsequently isolated and named from other tick species in both North America and Eurasia (reviewed by Peisman and Gern 2004). Over the two decades following its discovery, variations in *B. burgdorferi* sensu lato (s.l. = in the broad sense) became recognized and at least 12 genospecies (species that include strains that have 70% or more DNA-DNA relatedness under optimal conditions, Christensen et al. 2001) can currently be distinguished: *B. afzelii*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s. = in the strict sense), *B. garinii*, *B. japonica*, *B. lusitania*, *B. sinica*, *B. spielmanii*, *B. tanukii*, *B. turdi*, and *B. valaisiana*

(Kurtenbach et al. 2002a, Peisman and Gern 2004, Richter et al. 2006). The diversity within certain genospecies is large and horizontal gene transfer may occur at certain loci (Dykhuizen and Baranton 2001). It has been proposed that the diversity of *B. burgdorferi* s.l. is maintained by host diversity, rather than tick diversity, and the biochemical responses of the spirochetes to vertebrate host defenses (Kurtenbach et al. 2002a). The transmission cycle involving ticks and their hosts is also intimately dependent on the spirochete's ability to survive in the tick for prolonged periods, before entering the vertebrate hosts.

There is marked host preference among the different genospecies of *B. burgdorferi* s.l. Host- and vector competence (the ability of a host or a vector to maintain non-systemic infections and facilitate transmission), and ultimately, reservoir competence (the ability of a host or a vector to serve as reservoirs of the pathogen) vary significantly between different hosts and vectors (Lane 1991, Peisman and Gern 2004). Broadly, *B. afzelii, B. bissettii, B. japonica* and two serotypes of *B. garinii* are involved in transmission cycles involving rodents and ticks of the genus *Ixodes* (Kurtenbach et al. 2002a, Peisman and Gern 2004). *Borrelia valaisiana, B. turdi* and most serotypes of *B. garinii* are represented in cycles involving birds and *Ixodes* spp. The ecology of Lyme disease, therefore is complex, involving a diversity of spirochetes, a number

of *Ixodes* species and a wide range of mammalian and avian hosts. Only *B. afzelii*, *B. garinii* and *B. burgdorferi s.s.* are capable of causing Lyme Disease in humans and there is variation in the extent of the disease caused by different strains of each genospecies (Kurtenbach et al. 2002a, Lagal et al. 2003, Peisman and Gern 2004).

Many studies have examined the link between *B. burgdorferi* s.s. and its epidemiology in mainland North America in relation to the principal tick vector (*I. scapularis*) and its various mammalian hosts (Deer mice, *Peromyscus maniculatus*, White-tailed Deer, *Odocoileus virginianus*, and humans) (Falco and Fish 1992, Rand et al. 2003, Schulze et al. 2005, Ogden et al. 2005). Limited information exists on bird-tick cycles of Lyme disease (Kurtenbach et al. 2002a, Peisman and Gern 2004), although it is clear that birds form an important component of the cycles involving certain genospecies (Olsen et al. 1993, 1995, Smith et al. 1996, Durden et al. 1997, Rand et al. 1998, Poupon et al. 2006).

Borrelia burgdorferi s.l. in birds

In Europe, *B. garinii* and *B. afzelii* are the most widespread and prevalent of the genospecies present, while *B. burgdorferi* s.s. occurs in western Europe (Peisman and Gern 2004). The overlapping distribution of

many of these genospecies commonly results in mixed infections in ticks of the *I. ricinus* complex (Kurtenbach et al. 2001, Peisman and Gern 2004, Poupon et al. 2006). The bird-tick transmission cycles are typically dominated by *B. valaisiana, B. garinii* and *B. lusitania* with large geographic and seasonal variations in their prevalence (Peisman and Gern 2004, Poupon et al. 2006). Migratory and resident passerines are important hosts for several of these bird-adapted genospecies. Migratory behavior of birds is linked with prevalence of *B. burgdorferi* s.l. and southward migrating birds tend to have higher prevalence of the spirochete compared to northward migrating birds (Poupon et al. 2006).

The dominant genospecies in North America is *B. burgdorferi* s.s. and although it primarily circulates in ticks and mammals (Rand et al. 2003, Peisman and Gern 2004), the spirochete is also abundant in a range of passerines (Anderson et al. 1986, Weisbrod and Johnson 1989, Magnarelli et al. 1992, McLean et al. 1993, Smith et al. 1996, Durden et al. 1997). The few studies that have examined the host competence of passerine birds suggest that there is considerable variation in competence among different host species (Lane 1991). The prevalence of *B. burgdorferi* s.s. and the ability of passerines to disperse infected larval and nymphal *I. scapularis* ticks suggest that migratory passerines serve as reservoirs and are involved in the

movement and expansion of Lyme disease spirochetes in North America (Lane 1991, Smith et al. 1996, Durden et al. 1997, Rand et al. 1998).

Borrelia garinii in seabirds

Compared to other birds, seabirds are extreme in their life history strategies (e.g. Gaston and Jones 1998, Coulson 2002, Schreiber and Burger 2002). They are long-lived, ranging from 5-25 years, with some species living for more than 50 years. During their life cycle, they usually spend relatively small periods of time on land. They generally select coastal cliffs or offshore islands without mammalian predators to breed over periods of 2-6 months. After chicks are reared, they leave the land mass and live a pelagic existence until the next breeding season. Seabird colonies in tropical latitudes may be infested by many species of soft ticks (e.g. *Ornithodoros capensis*) as well as hard ticks (e.g. *Ixodes* spp.) (Duffy 1991). In northern temperate to arctic seabird colonies, *I. uriae* is usually the only tick species (Clifford 1979, Eveleigh and Threlfall 1974). This species is extremely widespread and also occurs in seabird colonies in the southern hemisphere (Zumpt 1952).

Olsen et al. (1993) documented the presence of *B. garinii* from *I. uriae* ticks feeding on Razorbills (*Alca torda*) on the island of Bonden, Sweden (Fig.3.1). Additionally, they found infections from skin biopsies from the

Razorbill. This was the first evidence of a Lyme disease cycle involving seabirds and their ticks. Since the island was rodent-free, this also showed that seabirds could serve as competent reservoir hosts without the involvement of mammalian reservoirs. Subsequently, *I. uriae* ticks feeding on Atlantic Puffins (*Fratercula arctica*) (Faeroe Islands), Black Guillemots (*Cepphus grylle*)(Iceland) and Fork-tailed Storm Petrels (*Oceanodroma furcata*)(Alaska) tested positive for *B. garinii* (Olsen et al. 1995, Gylfe et al. 1999). The presence of *B. garinii* in the southern hemisphere in King penguins (*Aptenodytes patagonicus*) and Black-browed Albatrosses (*Diomedea melanophris*) from Campbell Island off New Zealand and the Falkland Islands indicated their widespread occurrence in both the hemispheres. Five of the nine colonies sampled were free of rodents or had small rodent populations with no evidence of *B. garinii* or tick infestations (Olsen et al. 1995), providing further support for transmission cycles involving primarily seabirds and their ticks (Olsen et al. 1993, 1995). The presence of identical *B. garinii* from ticks on either hemisphere led to the hypothesis that there is trans-hemispheric exchange of the spirochetes (Olsen et al. 1995). The inability of ticks to stay on seabirds long enough (5-10 days on Black-legged Kittiwakes, *Rissa tridactyla*, Finney et al. 1999) to sustain a long migratory flight limits the possibility of the trans-hemispheric movement of ticks as a means of spreading *Borrelia*

(Gylfe et al. 2001). However, seabirds might carry ticks over short distances. For example, newly fledged Kittiwakes prospecting for nesting sites may visit colonies near their natal colonies and this may serve as a means for the exchange of ticks between colonies (Danchin 1992).

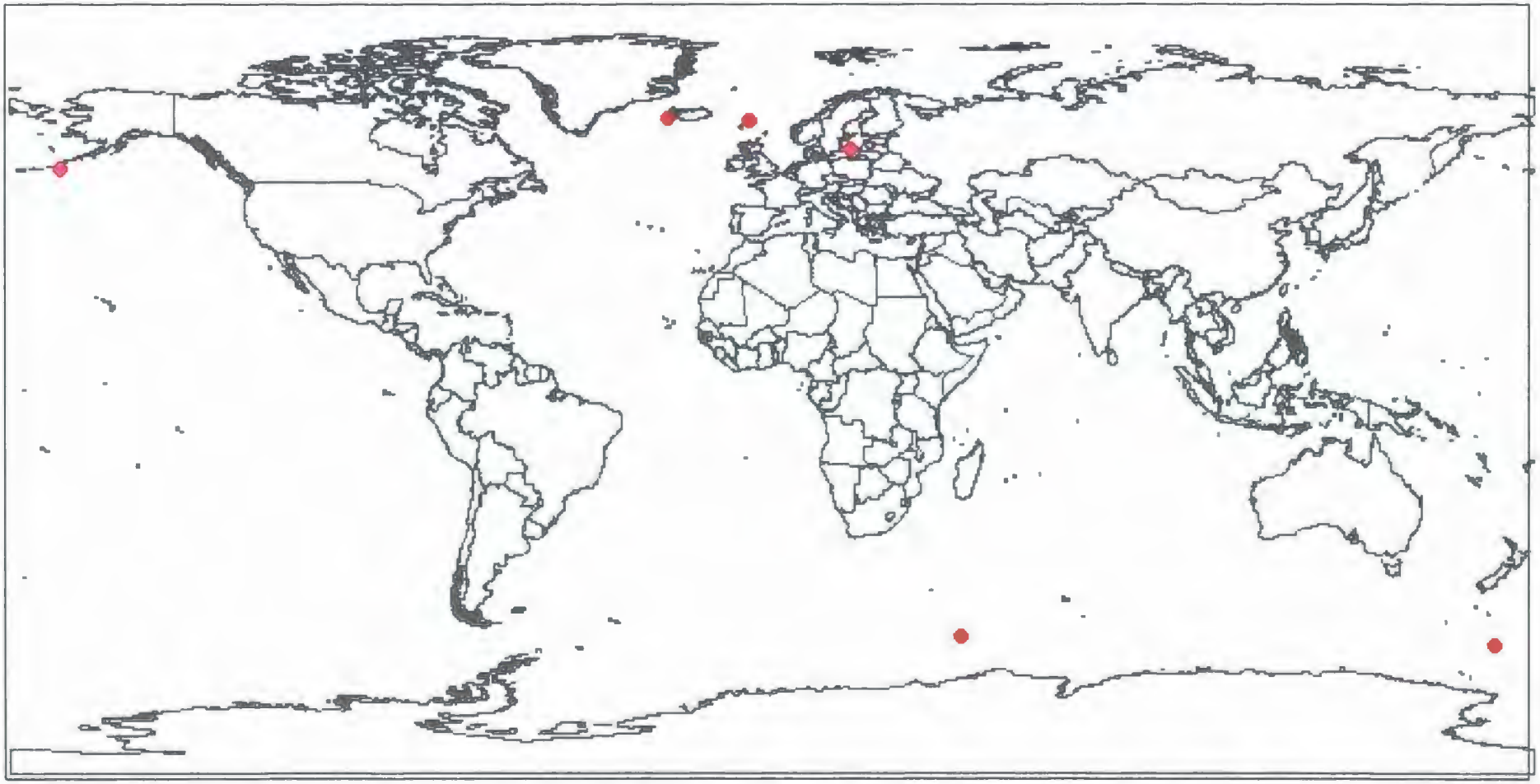

Fig. 3.1. Seabird colonies around the world that have been recorded to have *Ixodes uriae* infected with *Borrelia garinii*. 1) Egg Island, Alaska; 2) Flatey Island, Iceland; 3) Nolsoy, Faeroe Islands; 4) Bonden Island, Sweden; 5) Crozet Islands; and 6) Campbell Island, New Zealand. (from Olsen et al. 1995).

The following predictions can be made about *B. garinii* in seabird-tick transmission cycles resulting in the dispersal or range expansion of the spirochete:

1. *Ixodes uriae* ticks are important in the maintenance of *B. garinii* in seabirds due to the ability of the spirochete infection to be retained during the molt from one stage to the next stage (e.g. larva, nymph or adult female to nymph, adult, or egg). This could have spillover effects into adjoining colonies if fledging seabirds, such as Black-legged kittiwakes, carry infected ticks from their natal colonies to nearby colonies.
2. *Borrelia garinii* can be maintained as systemic infections in seabirds of some species and this could allow long distance movement of the spirochete, but would result in infection of distant colonies only if the infection is of a prolonged duration and the destination colony has existing tick populations.
3. Infected ticks and seabirds could be together involved in the local spread and maintenance of *B. garinii* between colonies within a range of 10s of km. This is contingent upon the seabird species composition and the extent of movements of seabirds between colonies.

4. Once established in a new colony, *B. garinii* could be maintained in ticks as well as in seabirds.

Many important seabird colonies, sustaining globally significant populations occur in the Northwest Atlantic (Lock et al. 1994, Gaston and Jones 1998). Since dispersal and wintering movements of immature and adult seabirds from the North Atlantic (Lock et al. 1994, Gaston and Jones 1998, Huettmann and Diamond 2000) overlap with some of the seabird colonies with recorded *B. garinii* infestations (e.g., Olsen et al. 1995, Bunikis et al. 1996), I hypothesized that infections of *B. garinii* were present in seabirds from some of the Northwest Atlantic seabird colonies. Additionally, since *B. garinii* is widespread and identical strains occur in both hemispheres, I reviewed the evidence on the phylogeny and ecology of *B. garinii* strains from seabirds to attempt an explanation of the current distribution of this spirochete among seabirds.

The objectives of this study, therefore, were

1) to determine the presence of *B. garinii* in selected seabird colonies of Newfoundland and Labrador;

2) to assess temporal variations in the prevalence of *B. garinii* in selected seabird species;

3) to review the potential of seabirds and the associated tick species *I. uriae* in the dispersal of *B. garinii* over short and long distances.

Materials and Methods

Study area

Ticks were collected alive from the Gannet Islands Ecological Reserve, Labrador, in 2005 and 2006; Cape St. Mary's Ecological Reserve in 2006; and Gull Island, (Witless Bay Ecological Reserve) Newfoundland in 2004, 2005 and 2006.

The Gannet Islands Ecological Reserve consists of a group of small islands about 29 km off the coast of southern Labrador (54°00'N, 56°30'W). A cluster of 6 islands is referred to individually as Gannet Clusters 1 through 6 (GC1-6). Five islands (GC1-5) are located within 500m of one another, with GC6, the largest of the cluster located 1.5 km west of the GC1. In addition to the cluster there are Outer Gannet (54° 00′ N, 56° 32′ W) lying 5 km to the north of GC2 and two small outlying rocks, called East Gannet Rock and West Gannet Rock, which lie 4 km southeast of the Gannet Clusters.

The Gannet Islands are situated 29 km northeast of Packs Harbor on

the south Labrador coast. The islands are low lying and are mostly covered by dwarf heath shrub vegetation. Highest seabird densities occur during the summer breeding season on GC1-4 and Outer Gannet. The Gannet Islands collectively host over 39,300 breeding pairs of Atlantic Puffins (*Fratercula arctica*), 10,000 b.p. of Razorbills, over 1270 b.p. of Thick-billed Murres and over 47,000 b.p. of Common Murres.

Cape St. Mary's Ecological Reserve is one of six ecological reserves of Newfoundland and Labrador. It is located about 200 km southwest of St. John's on the southwestern tip of the Avalon Peninsula (46° 50′ N, 54° 12′ W). About 24,000 Northern Gannets (*Morus bassanus*), 20,000 Black-legged Kittiwakes, 20,000 Common Murres, and 2,000 Thick-billed murres live within the reserve during the breeding season.

Gull Island (47° 15′ N, 52° 46′ W) located in southeastern Newfoundland, Canada (see Fig. 4.3). Gull Island is one of four islands in the Witless Bay Ecological Reserve and is about 5 km southeast of the town of Witless Bay (Robertson et al. 2004). Gull Island is 1.6 X 0.8 km in size and is forested, with balsam fir and black spruce being the dominant tree species. The periphery of the island is either grassy or rocky slopes. Narrow ledges and vertical cliffs surround most of the island, with particularly steep cliffs (maximum 69 m) occurring along the northeastern edge of the island. The

southern end has a lower elevation and has three coves and a rocky projection on the southwestern end. Gull Island hosts diverse seabird breeding colonies including Leach's storm petrels (*Oceanodroma leucorhoa*) in most of the forested and forest-edge areas, with most recent population estimate being 350,000 breeding pairs (Stenhouse et al. 2001), about 1600 pairs of Common Murres (restricted to cliff ledges) and 4300 pairs of Black-legged Kittiwakes (Robertson et al. 2004), more than 2600 breeding pairs of Herring Gulls (*Larus argentatus*), along with 88 pairs of Great Black-backed Gulls (*L. marinus*) (Robertson et al. 2001). Gull Island has the largest North American colony of Atlantic Puffins, estimated at about 140,000 breeding pairs (Robertson et al. 2004) occurring on the gently sloping grassy habitat. Small numbers of Razorbills (about 300 pairs), Northern Fulmars (*Fulmarus glacialis*) (about 6 pairs), and Northern Ravens (*Corvus corax*)(several pairs) also occur on the island.

Methods

A total of over 1500 ticks were shipped alive to the Vector Borne Disease Laboratory, Maine Medical Center, U.S.A. during the years 2005 and 2006. The following analyses were conducted by the Maine Medical Center and the data was provided to me. The analytical procedures are briefly

described here. A subset of ticks was dissected and midguts were screened for the presence of spirochetes by fluorescent microscopy using a polyclonal anti-borrelial antibody (Donahue et al. 1987).

DNA was extracted from positive ticks using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia CA). DNA amplification was performed in a designated room using genus-specific primers that include the partial sequence of rrs-rrla intergenic spacer region as described by Bunikis et al. (2004) with use of negative controls. Amplification products were visualized on a 1% agarose gel containing 0.5 ug/ml ethidium bromide. At a second laboratory, ticks positive by fluorescent antibody screen were prepared as above for DNA extraction and Polymerase Chain Reaction (PCR) technique was performed using primers directed at the 16s ribosomal DNA. Sequences of amplicons obtained at both laboratories were confirmed to be *B. garinii* by comparison with known sequences in the Genbank database.

Results

A total of 181 ticks collected from all of the three study sites were tested for *B. garinii* of which a total of 23 ticks (nymphs and adult females) from Gull Island and the Gannet Islands tested positive (Table 3.1), constituting the first record of this spirochete from any Northwest Atlantic

colony (Smith et al. 2006). Specimens sent from the Gannet Islands in 2005 and Gull Island in 2004 could not be tested since they were dead on arrival at the Maine Medical Center. Prevalence of *B. garinii* differed both between seabird species and years (Table 3.1). Higher prevalence was generally observed in female ticks (32.0%) than in nymphs (8.0%) in 2005, although the difference was not significant (Fisher's Exact Test, P=0.074). All of the nymphs tested in 2006 were negative. In general, ticks collected from Herring Gull chicks, Atlantic Puffin adults and chicks showed the greatest prevalence of *B. garinii* (16.7-37.5% in 2005 and 0-28.6% in 2006). The lowest prevalence of the spirochete was recorded from ticks collected from soil or litter samples (5.6-16.7% in 2005, 0-12.5% in 2006). Overall prevalence (prevalence of infection among all ticks collected from all sources) was significantly higher in 2005 (20.4%) than in 2006 (7.4%) in Gull Island (Fisher's Exact Test, P=0.028). Preliminary comparisons of small sections of the genome of the *B. garinii* isolates were similar to strains collected from the Faeroe Islands, Slovenia and West Siberia.

Table 3.1. Prevalence *of Borrelia garinii* among *Ixodes uriae* ticks tested from different localities in the northwestern North Atlantic.

Year	Locality	Source	Life Stage	Numbers tested	Number infected	Prevalence
2005	Gull Island	Atlantic puffin	Nymph	6	1	16.7
			Female	11	4	36.4
		Herring Gull (chick)	Female	8	3	37.5
		Soil (puffin slope)	Nymph	18	1	5.6
			Female	6	1	16.7
		Pooled (all sources)	Nymph	24	2	8.3
			Female	25	8	32.0
			Total	49	10	20.4
2006	Gull Island	Atlantic puffin	Larva	2	0	0.0
			Nymph	3	0	0.0
			Female	29	3	10.3
		Atlantic puffin (chick)	Female	7	2	28.6
		Kittiwake (chick)	Female	6	0	0.0
		Soil (puffin slope)	Larva	1	0	0.0
			Nymph	34	0	0.0
			Female	4	0	0.0
	Renews	Common Murre	Female	11	2	18.2
		Pooled (all sources)	Nymph	37	0	0.0
			Female	57	7	12.3
			Total	94	7	7.4
	Gannet Islands	Common Murre	Female	14	2	14.3
		Razorbill (chick)	Female	12	3	25.0
		Soil (puffin slope)	Nymph	1	0	0.0
			Female	8	1	12.5
		Pooled (all sources)	Nymph	1	0	0.0
			Female	34	6	17.6
			Total	132	13	9.8
			TOTAL	181	23	12.7

Table 3.2. *Borrelia garinii* strains isolated from *Ixodes uriae* collected from various seabird colonies around the world.

Year	Locality	Ticks tested	+ve Ticks	Collected from	Study
1995	Faeroe islands	120	38	Atlantic Puffins	Olsen et al. 1995
1995	Flatey Is, Iceland	60	29	Black Guillemots	Olsen et al. 1995
1995	Bonden Is combined	67	180	Razorbills and Common Murres	Olsen et al. 1995
1995	Egg Is and St.Lazaria Is, AK	24	11	Fork-Tailed Storm Petrel	Olsen et al. 1995
1995	Campell Is, NZ	41	3	Black-browed albatross	Olsen et al. 1995
1995	Crozet Is, Antarctica	46	33	King Penguins	Olsen et al. 1995
1996	Malgundret, Banden Island			Atlantic Puffins	Bunikis et al. 1996
2005	Gull Is, NL	59	18	Atlantic Puffins, and flagged from puffin habitat	from Smith et al. 2006 and present study
2005	Gannet Islands	35	5	removed from Razorbills, Common Murres and collected from puffin habitat	present study
2006	Gull Is	56	2	Common Murres	present study
2006	Gull Is	50	5	Atlantic puffins (adults and chicks)	present study

Discussion

This study constitutes the first record of *B. garinii* in the Northwest Atlantic (Smith et al. 2006) and the presence of this spirochete in the Gannet Islands and Gull Island is consistent with previous predictions suggesting a more widespread occurrence of this spirochete in the greater North Atlantic ecosystem (Bunikis et al. 1996, Gylfe et al. 1999). The extent of occurrence and the seabird species involved in the maintenance of the spirochete need to be determined. This study showed that prevalence of *B. garinii* varied between years and among seabird species. Atlantic Puffins had consistently high prevalence in both years, although Herring Gull chicks yielded the highest prevalence in 2005. The ticks collected opportunistically from other seabird species did not reveal any clear trend in prevalence of *B. garinii*. A more systematic screening of different seabird species is in order and this could elucidate the species preferences of *B. garinii* towards their seabird hosts. Additionally, a careful study of the role of each stage of the tick (larvae, nymphs and adult females) is needed.

Phylogeny and distribution of *B. garinii* in seabirds

An examination of the phylogeny of *B. burgdorferi* s.l. and specifically, *B. garinii* is needed to hypothesize on the widespread distribution patterns of the spirochete from seabirds. The phylogeny of *B. burgdorferi* s.l. is very complex and depending on which segments of the genome are considered for phylogenetic studies, a variety of relationships can be inferred between the known genospecies (Farlow et al. 2002, Lagal et al. 2003, Richter et al. 2006). Broadly speaking, *B. garinii* clusters with *B. afzelii*, *B. valaisiana*, *B. turdi* and *B. lusitania* in phylogenetic trees constructed using a large proportion of the genome (Gylfe et al. 2001). These phylogenetic trees are by no means straightforward and are complicated by genetic exchange between *Borrelia* strains co-infecting a single host and exchanging genetic material (Dykhuizen and Baranton 2001). There is great diversity even within *B. garinii* strains and 11 groups are recognized based on variations in outer surface proteins controlled by the ospC gene (designated G1-G11, Lagal et al. 2003). Figure 3.2 shows a simplified phylogenetic tree redrawn from Lagal et al. (2003) illustrating the different *B. garinii* groupings in relation to *B. afzelii* and *B. burgdorferi* s.s. Two isolates from the *I. uriae* from Nolsoy, Faeroe Islands, used in their study clustered within the G1 group of strains and G1, G2 and G4 strains all clustered together. The other strains within these clusters included

isolates from Slovenia, Austria, Germany and Switzerland suggesting a common ancestry of these central European strains and the strains from seabird cycles (Livey et al. 1995, Wilske et al. 1995, Gylfe et al. 1999, Lagal et al. 2003). There is not a great deal of diversity within the strains of *B. garinii* from seabirds (Olsen et al. 1995, Bunikis et al. 1996, Gylfe et al. 1999), and further phylogenetic analyses are likely to cluster them with G1 strains.

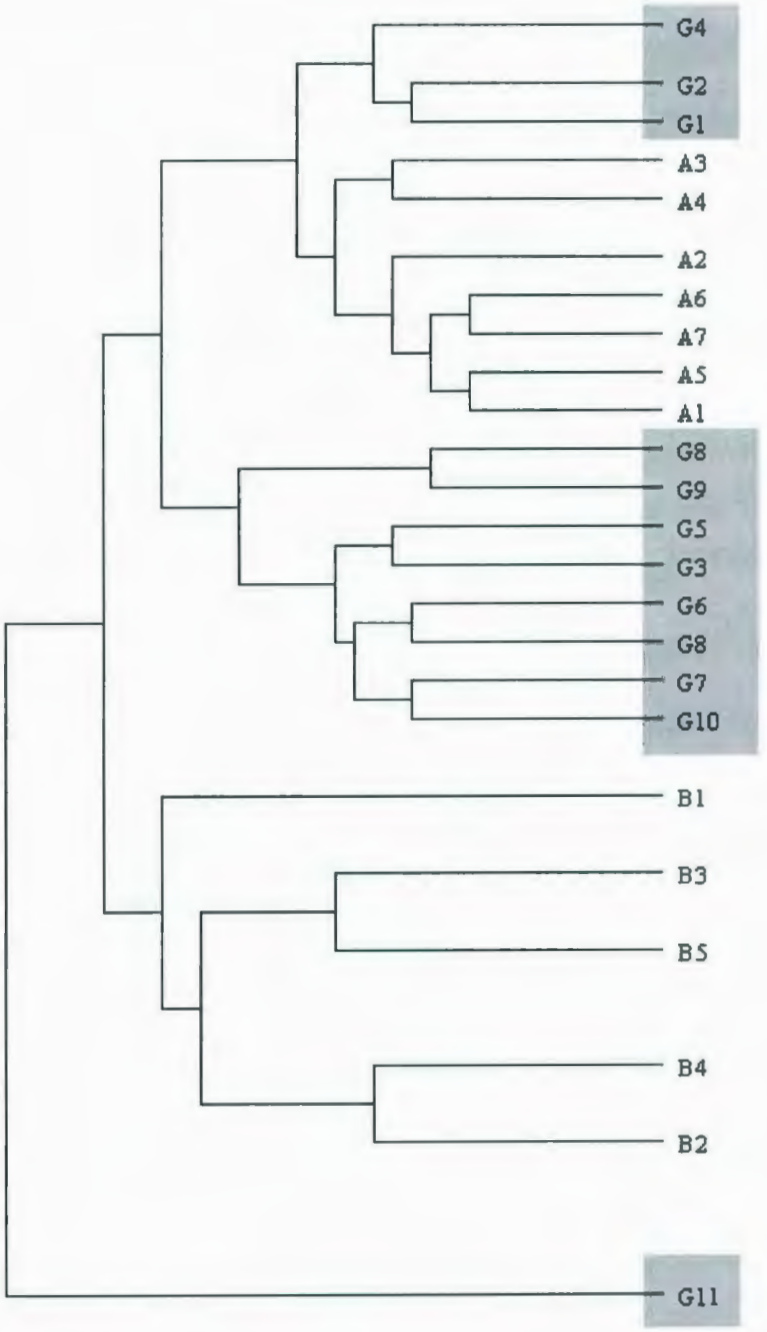

Fig. 3.2. Simplified phylogenetic tree portraying the relationship between different *Borrelia garinii* groups (G1-G11), *B. afzelii* groups (A1-A7) and *B. burgdorferi* s.s. groups (B1-B5). The G1 group contained two *B. garinii* from seabird colonies in Nolsoy, Faeroe Islands (from Lagal et al. 2003).

The first recorded incidence of *B. garinii* in seabirds in the Bonden Island (Olsen et al. 1993) and subsequently in Malgrundet in the Bothnian Gulf off the Baltic Sea could therefore have originated in mainland Europe (Bunikis et al. 1996) (Fig. 3.3, Table 3.2). The Bothnian Gulf ecosystem hosts a number of seabird colonies that are close to the mainland (Bonden is about 12 km from the mainland), and have overlapping populations of *I. uriae* and *I. ricinus* (Clifford 1979, Bunikis et al. 1996). Coastal sites within the Bothnian Gulf off Sweden and Finland as well as Norway, Denmark, Germany and the British Isles lining the North Sea have similar overlapping distributions of these two tick species (Mehl and Traavik 1983, Jaenson et al. 1994). This presents the unique opportunity for *B. garinii* strains from passerines to come in close proximity with *I. uriae*, the vector of *B. garinii* strains in seabirds (Jaenson et al. 1994, Bunikis et al. 1996). Although the two tick species have different ecological niches, their overlapping distributions sometimes make them co-occur in similar habitats (Jaenson et al. 1994). Similar strains of *B. garinii* have been collected from both of these tick species, supporting this idea (Gylfe et al. 1999). This also suggests that strains of *B. garinii* in the Bothnian Gulf and in the North Atlantic colonies represent a northwestward range expansion of the mainland strains of *B. garinii* (Bunikis et al. 1996, Fig. 3.3). Once *B. garinii* had adapted to the seabird transmission cycles involving

I. uriae, it could have then established in seabird colonies along the Northeast Atlantic colonies through dispersal movements of infected birds between colonies. This could also be facilitated by dispersive movements of *I. uriae* ticks on prospecting fledglings of seabirds such as Black-legged Kittiwakes (Danchin 1992, Boulinier et al. 2001). Subsequently, movements over greater distances could have resulted in the colonization of *B. garinii* in the Faeroes and colonies around Iceland reported by Gylfe et al. (1999). Thick-billed Murres banded in Spitsbergen, for instance, have been recovered from southwest Greenland and Newfoundland (Gaston and Hipfner 2000). Similarly, Razorbill chicks banded in a colony in Scotland have been found nesting in the Gannet Islands, Labrador. With new pockets of endemicity in the eastern North Atlantic, long dispersal movements of seabirds could then have facilitated the spread of *B. garinii* to colonies in the Northwest Atlantic.

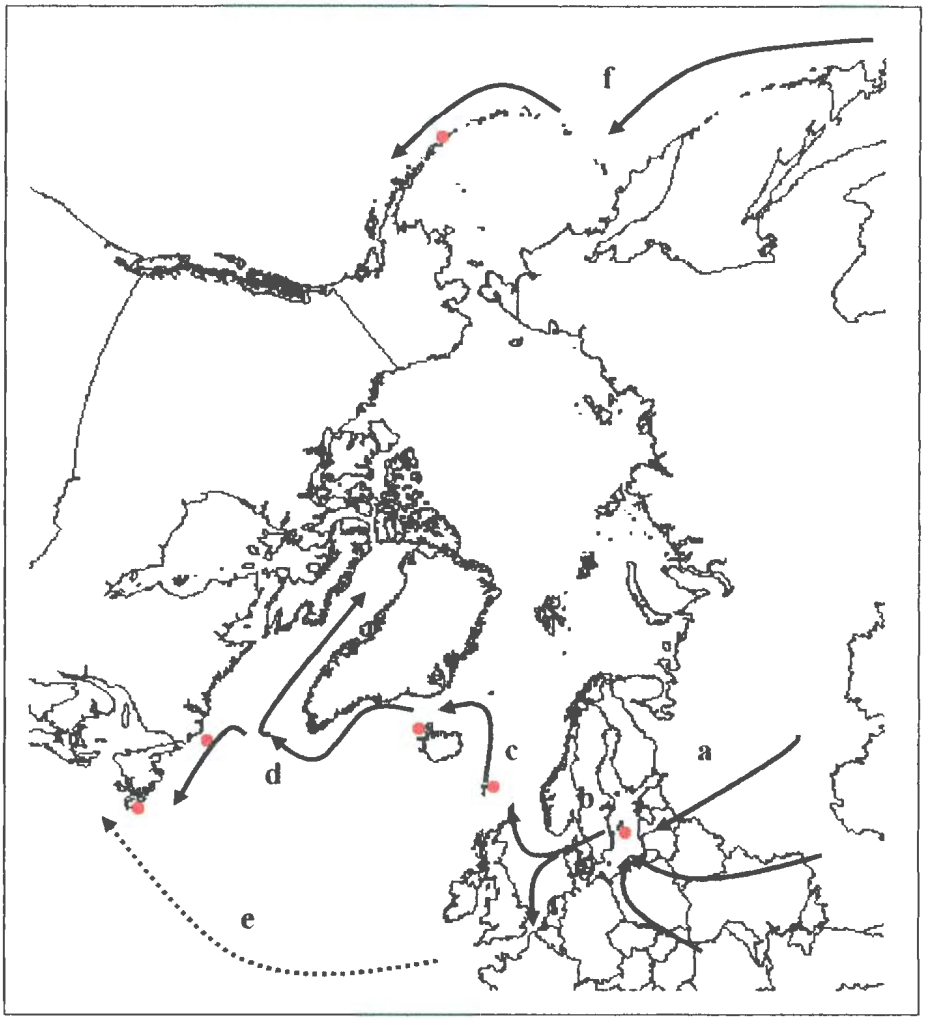

Fig. 3.3. Hypothesized movement of *Borrelia garinii* from Europe into seabird colonies in the North Atlantic. a) Co-occurrence of *Ixodes ricinus* and *I. uriae* on seabird colonies in the Bothnian Gulf and nearby areas. Movement of *B. garinii* from terrestrial to seabird cycle; b) establishment of B. *garinii* in seabird colonies along the Northeast Atlantic. c) Spread of *B. garinii* from endemic focus in Northeast Atlantic to Faeroe Islands and Iceland; d) movement of *B. garinii* from Iceland to colonies off the coast of Greenland and Northwest Atlantic. e) low level movement of *B. garinii* infections with birds moving across the Atlantic; f) Hypothetical route of infection of Alaskan islands from eastern Russia.

Distribution and Movement of *B. garinii*

Smith et al. (2006) collected ticks from six sites in the Northwest Atlantic: Machias Seal Island, Matinicus Rock, Petit Manan Island and Seal Island from Maine, USA; and Gannet Islands, Labrador and Gull Island, Newfoundland in Canada. None of these sites had yielded any evidence of *Borrelia* infections until 2005 (Smith et al. 2006) and 2006 from the Gannet Islands and Gull Island. Previous studies of ticks from a variety of seabirds in the northwest Atlantic colonies, including the Gannet Islands, had failed to find any evidence of *B. garinii* (Gylfe et al. 1999). The absence of any evidence of the spirochete could reflect either i) the absence of *B. garinii* until recently (i.e., recent emergence); ii) the periodic appearance of *B. garinii* depending on host-vector-parasite dynamics; or iii) the scattered, discontinuous sampling associated with small sample sizes. Tick specimens that have been collected and tested for Lyme Disease from colonies around Newfoundland (Cape St. Mary's and Gull Island) have never yielded *B. garinii* (Bennett 2005). Bennett (2005) collected 91 specimens of *I. uriae* in 2003-2004 from Gull Island, Newfoundland but these did not yield any evidence of the spirochete. *Borrelia garinii* is very distantly related to the North American strains of *B. burgdorferi* s.l. (Lagal et al. 2003). The finding of *B. garinii* in this study could therefore represent a movement of *B. garinii* from other areas of known endemicity in

the North Atlantic again supporting a central European origin (Bunikis et al. 1996, Lagal et al. 2003). Whether the movement has occurred recently cannot be ascertained without further detailed phylogenetic studies of the *B. garinii* strains from seabird cycles in the North Atlantic.

Identical *B. garinii* isolates from Campbell Island, New Zealand, and Egg Island, Alaska as well as from Crozet Islands, Antarctica and Nolsoy, Faeroe Islands (Olsen et al. 1995) pose another important problem in the distribution of the spirochete. Ticks falling off the host (during the migration) need to be near suitable habitat (a seabird colony or a suitable land mass) to be able to survive and thereby maintain the *B. garinii* cycle. This is widespread in passerines in Europe and North America (Jaenson et al. 1994, Durden et al. 1997, Rand et al. 1998). Seabirds, however, spend most of their time at sea and biting opportunities for ticks are limited to the breeding seasons (Eveleigh and Threlfall 1974), when their hosts are not undertaking long-distance migrations (Cramp et al. 1985, Gaston and Jones 1998). Dispersive and migratory movements occur over open sea making the infected-tick borne range expansion of *B. garinii* an unlikely means of trans-hemispheric exchange (Gylfe et al. 2001). This is also supported by phylogenetic studies of *I. uriae,* which show that the species likely originated in Australia and then spread to the two hemispheres with seabirds (Zumpt

1952, Gylfe et al. 2001). But the North American clade of *I. uriae* is monophyletic, suggesting that trans-hemispheric exchange of the ticks no longer occurs (Gylfe et al. 2001). Distribution and range expansion of *B. garinii*, if dependent solely on ticks, could therefore take place only at small spatial scales (within 10s of km) during the breeding season. *Ixodes uriae* are capable of dispersing to nearby colonies on fledgling Black-legged kittiwakes and this is regarded as a means of colonization of new areas by the tick (Danchin 1992, Boulinier and Danchin 1996). Studies of short-distance dispersal and migratory movements of kittiwakes and gulls is urgently needed in assessing their roles in *B. garinii* distribution. Gulls may be of particular importance since this study showed high prevalence of the spirochete in Herring Gull chicks that spend their winters in coastal and even inland areas allowing tick dispersal to the mainland from offshore islands.

Alternatively, if systemic infections in seabirds are regarded as an important method of range expansion and long-distance dispersal of *B. garinii*, then the scale over which dispersal can occur could be much larger. Inducing migratory restlessness in passerine birds, such as the Redwing Thrush (*Turdus iliacus*), also elicits physiological stress that in turn suppresses the immune system (Apanius 1998). Redwing Thrushes exhibiting stress can reactivate existing dormant infections of *B. burgdorferi* s.l. and this has been

suggested as a mechanism facilitating long-distance dispersal of the spirochete (Gylfe et al. 2000). Whereas such reactivation mechanisms are possible in seabirds, there is no information on the ability of seabirds to maintain systemic infections of *B. garinii* long enough to sustain a trans-hemispheric flight. For instance, reservoir competence of the seabirds found with *B. garinii* infections is not known (Olsen et al. 1995). Experimentally infected pheasants can remain infective 2 weeks after being infected (Kurtenbach et al. 2002b), which provides ample opportunity for amplification if high tick densities are present. Similarly mechanisms of reactivation in passerines provide opportunities for ticks along the migratory pathways to propagate transmission cycles (Gylfe et al. 2000).

The movement patterns of long distance migrants are also important in our understanding of *Borrelia* dispersal. The migratory routes taken by Sooty Shearwaters (*Puffinus griseus*) form a broad figure-eight shaped pathway spanning across the North and South Pacific (Spear and Ainley 1999). This remarkable migratory route is also consistent with the hypothesized trans-hemispheric spread and maintenance of *B. garinii* strains between the Alaskan islands and Campbell Island in the Pacific. However, Sooty Shearwaters generally do not spend any time on land during their presence in the northern hemisphere, limiting biting opportunities for ticks and chances of

infection with *B. garinii*. Further studies are required to assess this and other seabird species with respect to competence and dispersal capability of *B. garinii* strains (Olsen et al. 1995, Gylfe et al. 1999). The presence of identical strains of *B. garinii* in the Faeroe Islands and Crozet Islands also requires closer examination with regards to seabird species involved, prevalence of *B. garinii* infections in these species and the duration of their infectivity. To date, none of the suspected seabird species (Gylfe et al. 1999) have tested positive for *B. garinii* and their role in the maintenance of this spirochete remains unknown. Long-term studies over large geographic scales involving seabirds from both hemispheres and their ticks are needed to answer this ecological question with any certainty.

An alternate hypothesis

Oceanic islands throughout the world have suffered from deliberate and accidental introductions of exotic animals (reviewed by Courchamp et al. 2003). The role of these introductions in causing population crashes and species extinctions through competition, predation, habitat alteration or diseases is well documented (Courchamp et al. 2003). Introduction of three rat species in remote islands on both hemispheres has resulted in population level changes in many seabird species. Many of these introductions took place

in the 1700s and 1800s when ships traveling along oceanic routes landed on islands. Rat populations had established in these islands. Rodents are competent hosts for *B. garinii* in terrestrial cycles in Europe (Peisman and Gern 2004). The rats (*Rattus* sp.) of Campbell Island, for instance, have only recently been eradicated (Courchamp et al. 2003). Similarly, several of the Aleutian Islands still have large populations of rats within seabird colonies (Major et al. 2007). Iceland and many of their adjoining islands have populations of other rodents (*Apodemus* sp.).

Once on these islands, *B. garinii* strains co-adapted to both seabirds and rodents may have established themselves in transmission cycles. Subsequent dispersal of seabirds to surrounding colonies could then have aided in the movement and dispersal of the spirochete to different islands. The role of introduced or exotic mammals on islands in maintaining *B. garinii* needs to be examined in detail. Passive study of Deer Mice on the Gannet Islands was unable to find any evidence to indicate that ticks fed on these rodents (this study). These aspects of tick feeding preferences need to be ascertained. Rodents on islands also need to be tested for antibodies to *Borrelia* to help assess their exposure to the spirochetes. This would help establish if rodents had played a part in the expansion of *B. garinii* to seabirds.

Smith et al. (2006) have also speculated about the potential of North American cycles involving *I. scapularis* and mammals coming in close proximity with *I. uriae* and seabirds in such areas of co-occurrence of the two tick species. Although the Cape St. Mary's seabird colony is on mainland Newfoundland, it has populations of Common Murres and *I. uriae* ticks. Cape St. Mary's is not far from Gull Island (in the order of 200 km) and short distance movements of seabirds could facilitate arrival of *B. garinii* into this colony. Domestic sheep within the seabird nesting areas and the abundance of small mammals (Meadow voles, *Microtus pennsylvanicus*) in Cape St. Mary's could also facilitate a transfer of *B. garinii* from seabirds to terrestrial transmission cycles involving mammals and *I. uriae*. Although purely speculative, this aspect of the ecology of *B. garinii* in seabirds could be of human health concern, since tourists visiting the colony are often seen returning to the visitor center with ticks.

Summary and Conclusions

Borrelia garinii has been circulating in seabird colonies in the North Atlantic since at least the early nineties. The likely source of infections is from areas of endemicity in the Bothnian Gulf and the Northeast Atlantic seabird colonies, where seabirds, songbirds and two different tick species come in

close proximity to each other. The likely source of the Alaskan *B. garinii* strains is eastern Russia. Phylogenetic studies suggest a gradual movement of the European strains into seabird colonies in the Northeast Atlantic and Pacific. Dispersive movements of seabirds may have driven the subsequent spread of *B. garinii* to the greater North Atlantic and then to the Northwest Atlantic colonies. Less is known about *B. garinii* in the North Pacific, but a similar process may be involved. Long-distance movement of *B. garinii* is reflected in the presence of identical strains in two hemispheres. Further genetic studies are required to provide a better picture of this range expansion of the seabird strains of *B. garinii*. This should also be complimented with studies in the ecology of the vector and the various seabird hosts across large geographic areas to ascertain reservoir competence of hosts to this spirochete. At present, there is evidence to suggest that Atlantic Puffins are suitable reservoirs, and other seabirds may be involved in the amplification and trans-hemispheric spread of the spirochete. Additionally, the role of mammals such as rodents and lagomorphs (rabbits), on oceanic islands in the introduction and persistence of *Borrelia garinii* in marine ecosystems needs to be determined.

CHAPTER 4. ACTIVITY PERIODS AND QUESTING BEHAVIOR OF THE SEABIRD TICK *IXODES URIAE* (ACARI: IXODIDAE) ON GULL ISLAND, NEWFOUNDLAND

Introduction

The widespread occurrence of *Borrelia burgdorferi* s.l. in terrestrial and marine ecosystems is intimately associated with the distribution and ecology of its tick vectors (Kurtenbach et al. 2002a, Peisman and Gern 2004, Chapter 3). Whereas a great deal of work has been done on the ecology of mainland tick species (reviewed by Gray 1998), relatively little work exists on the ecology of seabird ticks that are ubiquitous parasites of seabirds in most breeding colonies (Clifford 1979). A handful of studies have documented costs of tick parasitism such as retarded chick-growth rates, nest desertion and reproductive failure in a few seabird species (Duffy 1991, Morbey 1996, Gauthier-Clerc et al. 1998, 1999, Bergstrom et al. 1999, Mangin et al. 2003). Others, however, were unable to find any relationship between tick infestations and reproductive success or survival (e.g., Barton 1996, Gauthier-Clerc et al. 2003).

Ixodes uriae (Acari: Ixodidae) is perhaps the most widespread seabird tick, with a circumpolar distribution on both hemispheres, occurring in temperate and sub-polar lattitudes (Clifford 1979, Fig. 4.1). The length of its life cycle ranges from as short as two years in the southern hemisphere (Murray and Vestjens 1967, Frenot et al. 2001) to as long as 8 years in the northern hemisphere (Arthur 1962, Balashov 1972, Eveleigh and Threlfall 1974, Steele et al. 1990, Barton et al. 1996). The species is a generalist, feeding on more than 50 seabird species, apparently having a preference for Common Murres in the northern hemisphere (Eveleigh and Threlfall 1974). A similarly high prevalence on Black-legged Kittiwakes, that are ecologically and behaviorally different from murres, illustrates the flexibility of *I. uriae* in host preference and a dependence on host availability (e.g. Mehl and Traavik 1983, Danchin 1992, Barton 1996, McCoy and Tirard 2002). *Ixodes uriae* may even 'specialize' on kittiwakes within a colony, giving rise to genetically distinct local races (McCoy et al. 1999, 2003, 2005). The prevalence and abundance of *I. uriae* on seabirds varies with species and geographic locality, and interest has risen in recent years due to its involvement in the maintenance of *B. garinii*, 1 of the 3 genospecies of spirochete recognized as the causative agents of Lyme disease (Olsen et al. 1993, 1995, Muzaffar and Jones 2004, Smith et al. 2006). Several viruses (such as orbiviruses, flaviviruses, and bunyaviruses)

have been isolated from *I. uriae* (Main et al. 1973, Oprandy et al. 1988, reviewed by Muzaffar and Jones 2004) and the ticks play an important role in the persistence of these microbes in nature (Oprandy et al. 1988).

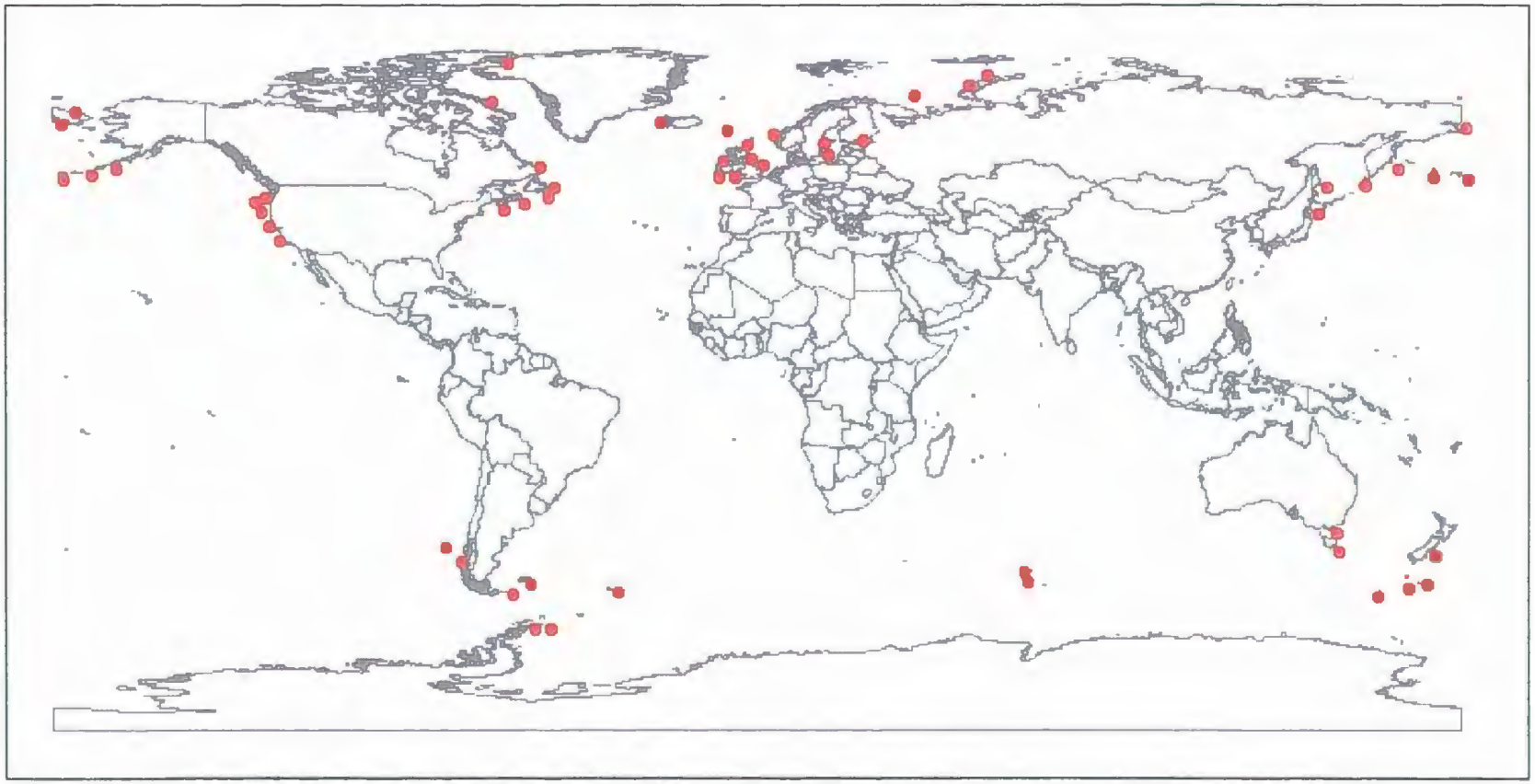

Fig. 4.1. Distribution of *Ixodes uriae* from known colonies around the world (from Muray and Vestjens 1967, Eveleigh and Threlfall 1974, Clifford 1979, Muzaffar and Jones 2004).

Life Cycle of *Ixodes uriae*

Ixodes uriae have three feeding life stages (three-host ticks), namely the larva, the nymph and the sexually dimorphic adult stage (Eveleigh and Threlfall 1974, Fig. 4.2). There is a great deal of variation in the feeding preferences and timing of activity of the larvae, nymphs and adults of *I. uriae* (Murray and Vestjens 1967, Eveleigh and Threlfall 1974, Steele et al. 1990, Barton et al. 1996, Frenot et al. 2001). Each stage requires a blood meal from a suitable host, which it acquires over a period of 6-12 days, in order to transform into the subsequent stage (Murray and Vestjens 1967, Eveleigh and Threlfall 1974, Finney et al. 1999). Adult males do not feed, but the females require a blood meal to successfully lay eggs.

In the northern temperate latitudes, each life stage is believed to feed only once a year, since the short duration of summer and availability of seabird hosts restrict the tick activity periods to about two months and the slow development rates prevent molting into the subsequent stages within the same season. Contrastingly, the shorter length of the life cycle in the southern hemisphere is driven by the fact that temperatures are suitable in some colonies to allow development of ticks to subsequent stages within the same season and hosts are present for up to 7 months (Murray and Vestjens 1967, Frenot et al. 2001). Therefore, seasonal variation in the activity periods

of different life stages and the availability of suitable seabird hosts together determine the successful completion of the life cycle.

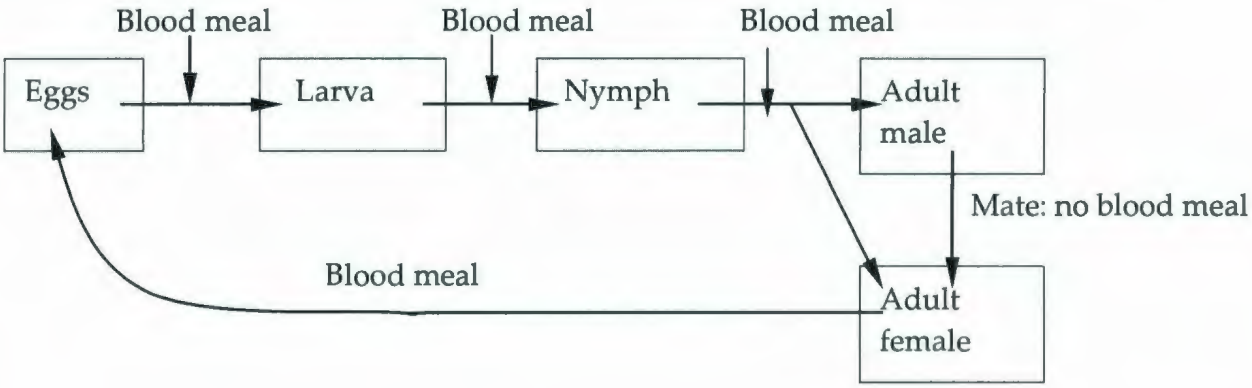

Fig. 4.2. Life cycle of *Ixodes uriae*, a three-host tick.

Questing behavior (the active search by ticks for a host) of ixodid ticks is crucial to locating a host and acquiring a blood meal. Since *I. uriae* is regarded as a nidicolous species, living in cracks in the rocks near nesting areas or in and around burrows of seabirds, questing behavior has not been quantified in most previous studies (e.g., Eveleigh and Threlfall 1974, Steele et al. 1990, Barton 1996, Barton et al. 1996) although it has been observed and reported in some studies (e.g. Mehl and Traavik 1983). Questing behavior of different tick stages is integral to understanding and modeling population dynamics of ticks. Questing behavior has been quantified in many non-nidicolous species (Rand et al. 2003, Ogden et al. 2005), but relatively few

nidicolous species (Sonenshine 1991). Comparisons of on-host and off-host abundance of ticks are also paramount to determining the risk of tick-borne diseases (LoGiudice et al. 2003, Ogden et al. 2005).

To this end, I initiated a study of *I. uriae* behavior at a major seabird colony in Newfoundland. The primary objectives of this study were to: a) quantify seasonal pattern of questing behavior of nymphs and adults; b) quantify the infestation levels of nymphs and adults on readily available seabird hosts; and c) assess the relative roles of different seabird species and ages (adults versus chicks) in hosting different life stages of the tick.

Materials and Methods

Study area

This study was conducted at Gull Island (47°15'N, 52°46'W) located in southeastern Newfoundland, Canada (Fig. 4.3). Gull Island is one of four islands in the Witless Bay Ecological Reserve and is about 5 km southeast of the town of Witless Bay (Robertson et al. 2004). Gull Island is 1.6 X 0.8 km in size and is forested, with balsam fir and black spruce being the dominant tree species. The periphery of the island is either grassy or rocky slopes. Narrow ledges and vertical cliffs surround most of the island, with particularly steep cliffs (maximum 69 m) occurring along the northeastern edge of the island.

The southern end has a lower elevation and has three coves and a rocky projection on the southwestern end. Gull Island hosts the largest North American colony of Atlantic Puffins, estimated at about 140,000 breeding pairs (Robertson et al. 2004) occurring on the gently sloping grassy habitat (See Chapter 3 for other seabird species in Gull Island).

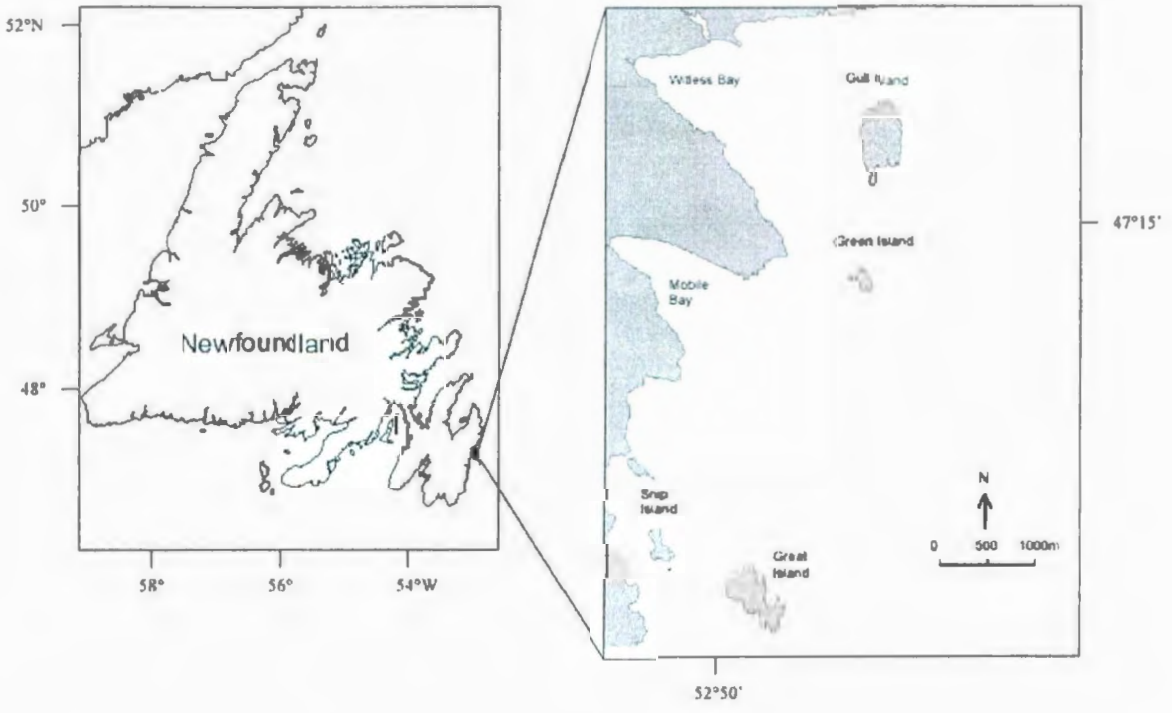

Fig. 4.3. The location of Gull Island, Witless Bay Ecological Reserve.

Bird species and quantification of on-host ticks

I inspected a total of 98 adult Atlantic Puffins, 98 Atlantic Puffin chicks and 145 Herring Gull chicks for ticks during the breeding seasons of 2004 and 2005. Adult puffins were captured mostly using a large dip-net held in the flight path of birds. Chicks of both species were collected by hand from their breeding sites. Each bird was held briefly in a cotton bag, weighed and measured and then searched for ticks using palpation (Danchin 1992), whereby the entire body surface is gently felt for the presence of biting ticks. This method was originally found to be very useful in assessing tick loads on Black-legged Kittiwake chicks (Danchin 1992), and was deemed suitable for the assessment of Atlantic Puffin chicks. I recognize that the method may underestimate the number of ticks in adult birds, and that this measure would only provide an index of abundance rather than a total count of ticks (Choe and Kim 1987).

Initially, palpation of adult Atlantic Puffins showed that ticks could still be collected using a pair of forceps and gently pulling at the base of the hypostome of the feeding tick. Dislodged ticks that inadvertently detached and remained in the bird bag were also retained. Ticks were visually categorized as either nymph or adult female, based on size. Larvae, that are

significantly smaller than nymphs, were not included in the analysis due to the difficulty of detecting most individuals from the body of live birds.

Quantification of questing tick abundance and behavior

Questing ticks were collected using a $1m^2$ white flannel flag (adapted from Falco and Fish 1992) that was dragged along the puffin slopes between 10:00 AM-1:00 PM between 1-8 times every two weeks during the months of May, June, July and August. Hereafter, I refer to these samples as flag samples. I attempted to collect at least three flag samples per week to ensure that the peaks in questing activity were reflected in our samples (Daniels et al. 2000). I was unable to collect flag samples at this rate for the entire sampling period due to periodic bad weather but I attempted to sample at least once a week during these periods. I believe that this did not significantly change my estimates of questing activity since most of the bad weather occurred early or late in the season when tick activity was at a minimum. Nymphs and adult females were counted and removed from the flag using forceps into vials for identification. The flag was dragged between 30-120 minutes, with an interval of 30 seconds between tick checks. The frequency of tick occurrence was indexed quantitatively as the number of nymphs and adult females captured per hour of dragging (Falco and Fish 1992, Rand et al. 2003). The relative

seasonal abundance of questing ticks was then presented as mean number of ticks captured per hour.

Statistical Analyses

I used the software Quantitative Parasitology 2.0, specifically developed to account for aggregated parasite distributions and allow distribution-free statistical tests to compare parasite loads on hosts (Reiczigel and Rózsa 2001). I quantified mean intensity (mean number of ticks per infected host), prevalence (proportion of hosts that were infected) and median intensity (most commonly occurring number of ticks per infected host) since no single measure of parasite 'load' is appropriate and a combination is recommended (Rózsa et al. 2000). Confidence intervals (at 95% confidence level) for mean intensities were computed using bootstrap techniques with 2000 replications (Rózsa et al. 2000). Exact Confidence intervals (at 95% confidence level) were calculated for prevalence using the Clopper-Pearson method (Rózsa et al. 2000). Confidence intervals for median intensities were also calculated, with the exact confidence level being reported, rather than the desired level, due to the discrete nature of the data (Rózsa et al. 2000). Mean intensities of different hosts were compared using Bootstrap t-tests, p-values being generated from 2000 replications (Rózsa et al.

2000). Prevalence of ticks on different hosts were compared using Fisher's Exact Test, with the exact p-value reported whenever possible. Median intensities were compared using Mood's Median Test. An α level of 0.05 was used to determine significance in each case. Spearman's Rank Correlation (2-tailed) was used to examine associations between on-host and questing ticks.

Results

Questing activity

The questing of ticks in search of suitable hosts varied between the two years of our study (Fig 4.4). First activity of tick nymphs was noted on June 19 in 2004 compared to May 18 in 2005 (Figs. 4.4 a, 4.4 c). Nymph activity reached a peak (79 and 110 individuals/hr in 2004 and 2005 respectively) at around mid-July in both years, although peaks of smaller magnitude occurred before and after the highest peak in 2004. Female ticks quested later in the season, compared to nymphs, starting at around late June and early July (Figs. 4.4 b, 4.4 d). Questing females did not reach the massive peaks observed in nymphs, with 12 and 21 individuals/hr captured in 2004 and 2005 respectively. Questing of both nymphs and females ended by early August in both years.

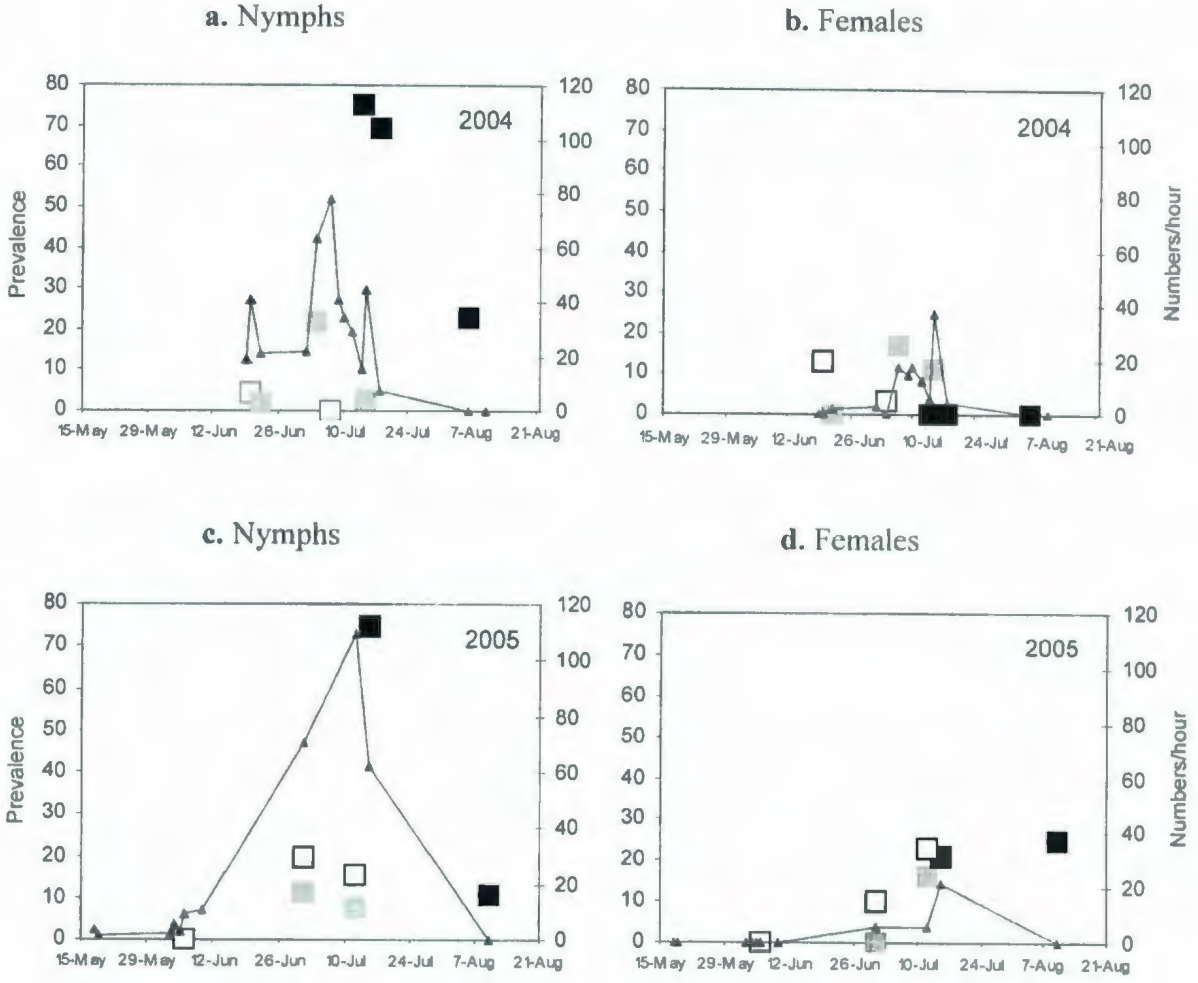

Fig. 4.4. Variation of questing activity (solid line) in relation to prevalence of nymphs and adult females on adult Atlantic Puffins (open squares), Atlantic Puffin chicks (black squares) and Herring Gull chicks (gray squares).

On-host ticks and questing

Nymphs and females feeding on hosts varied with time of year and between species (Fig. 4.4). Mean Intensity, Median Intensity and Prevalence

of nymphs and female ticks, pooled over the entire field season showed distinct patterns across host species and host age (Table 4.1). Mean Intensity and Median Intensity of nymphs and female ticks did not vary statistically in any of the three host-types ($p<<0.05$ in all cases of Bootstrap t-tests and Mood's Medial tests, respectively; Table 4.1). Nymphs were seen to feed opportunistically on adult puffins and Herring Gull chicks during the early part of July (in both years) although this did not necessarily relate to tick questing (e.g. Fig 4.4 c). The prevalence of nymphs did not vary significantly on Herring Gull chicks between the two years (Fisher's Exact Test, 12.6% in 2004 versus 8.6% in 2005, $p=0.591$; Figs. 4.4a, 4.4 c, Table 4.1). Nymph prevalence on Herring Gull chicks was significantly correlated with questing activity (Spearman's Rank Correlation, n=8, $p<0.01$ in 2004; n=7, $p<0.01$ in 2005). The prevalence of female ticks on Herring Gull Chicks was also low, but was not significantly correlated with questing activity ($p>>0.05$ in both years).

Table 4.1. Variation in the prevalence, Mean Intensity and Median Intensity of *Ixodes uriae* nymphs and adult females on adult Atlantic Puffins, Atlantic Puffin chicks and Herring Gull chicks. **indicate confidence intervals that could not be computed due to low numbers of ticks. Values in parentheses indicate the exact confidence, in percentage, of the confidence interval of Median Intensity.

	2004			2005		
Nymphs	Puffin (adult)	Puffin (chick)	Gull (chick)	Puffin (adult)	Puffin (chick)	Gull (chick)
N	55	42	87	43	56	58
Prevalence (%)	1.8[a]	57.1[b]	12.6[c]	23.3	30.4	8.6[c]
C.I.	0.09-9.72	40.96-72.28	6.48-21.50	11.75-38.64	18.77-44.1	2.85-18.99
Mean Intensity (#s/infected host)	1	3	3.91	2.1	2.41	1.8
C.I.	**	2.21-4.29	1.91-7.64	1.3-3.10	1.47-3.94	1.00-3.00
Median Intensity (#s/infected host)	1	2	2	1.5	2	1
C.I.	**	1-4 (99.7%)	1-6 (96.7%)	1-4 (98.8%)	1-2 (99.3%)	1-4 (93.8%)
Females						
N	55	42	87	43	56	58
Prevalence	7.3[a]	0	13.4	34.9	8.9	15.5
(%)C.I.	2.01-17.59	0	7.33-22.86	21-50.93	2.96-19.62	7.34-27.43
Mean Intensity (#s/infected host)	2.25	0	1.42	1.8	1.4	1.78
C.I.	1-2.75	0	1.08-1.83	1.27-2.80	1.00-1.60	1.22-2.89
Median Intensity (#s/infected host)	2.5	0	1	1	1	1
C.I.	**	0	1-2 (99.7%)	1-2(98.2%)	1-2 (93.8%)	1-2 (97.9%)

a. Significantly different from adult puffins in 2005.
b. Significantly different from adult puffins in same year
c. Significantly different from puffin chicks in same year

The prevalence of nymphs on adult puffins was significantly lower in 2004 (Fisher's Exact Test, 1.8% in 2004 versus 23.3% in 2005, p=0.001; Figs. 4.4a, 4.4 c) whereas mean intensity and median intensity did not vary (Table 4.1). Adult puffins had higher prevalence of female ticks that nymphs in both years but prevalence was significantly correlated with questing activity only in 2005 (Figs. 4.4 b, 4.4 d, Spearman's Rank Correlation, n=7, p<0.01). Similarly, increased abundance of questing nymphs was related to a significantly higher prevalence of nymphs on adult puffins in 2005 (Spearman's Rank Correlation, n=7, p<0.01), but not in 2004 (Table 4.1, Figs. 4.4a, 4.4 c, Spearman's Rank Correlation, n=8, p>>0.05).

Puffin chicks showed the highest prevalence of nymphs in both years and this was well synchronized with the peak questing activity of nymphs (Figs. 4.4 a, 4.4 c, Table 4.1, Spearman's Rank Correlation, n=8, p<0.01 in 2004; n=7, p<0.01 in 2005). The prevalence of nymphs on puffin chicks did not vary between the years (Fisher's Exact test, 57.1% in 2004 versus 30.4 in 2005; p<0.05; Table 4.1). Female ticks were not found on puffin chicks in 2004 and occurred in low prevalence (compared to nymphs) in 2005, although this was not correlated with questing (Spearman's Rank Correlation, n=7, p>>0.05). Prevalence of nymphs over the entire season was significantly higher in

puffin chicks compared to adults in 2004 and Herring Gull chicks in both years (Table 4.1).

Discussion

General host-preference

The preferred host of *I. uriae* is considered to be the Common Murre, although this tick species thrives on colonies of seabirds even when murres are absent (Eveleigh and Threlfall 1974, Morbey 1996, Muzaffar and Jones 2004). Gull Island harbors substantially larger populations of both breeding Atlantic Puffins and Herring Gulls compared to Common Murres, yet ticks were extremely abundant on grassy slopes occupied by breeding puffins and gulls, and both these seabird species were utilized extensively as hosts.

Questing activity

Questing or host-seeking activity is an important aspect of tick biology and is crucial to the understanding of the epidemiology of tick-borne diseases (Rand et al. 2003, Ogden et al. 2005). Olfactory, thermal, tactile and visual cues may all serve as stimuli for ticks to assist in seeking out and feeding on suitable hosts (Sonenshine 1991). Questing strategies are broadly categorized as either 'active' or 'passive' and many tick species may use a combination of

both strategies to find hosts (Balashov 1972, Sonenshine 1991). Some non-nidicolous tick species climb onto vegetation and wait to grab onto passing hosts. Other species use the hunter technique whereby they actively crawl and run towards their hosts. Nidicolous species use cues that are similar to non-nidicolous species, although the behavior and the extent to which they respond to such cues (or their combinations) vary among species (Sonenshine 1991). The exact questing strategy varies between species and in many cases between the different stages within a species (Sonenshine 1991). Ticks that actively quest for hosts go through periods of questing followed by inactivity (quiescence) which vary on a daily as well as a seasonal basis (Burkot et al. 2001, Bown et al. 2003, Perret et al. 2003).

Ixodes uriae is a nidicolous species that lives in the proximity of nesting areas, cracks in rocky cliffs or burrows of seabirds (Sonenshine 1991). Collection of *I. uriae* from soil samples shows that there is a great deal of variability in the timing and duration of the abundance of different tick stages across geographic localities (Eveleigh and Threlfall 1974, 1975, Mehl and Traavik 1983, Barton 1996). Eveleigh and Threlfall (1974) established the life cycle of *I. uriae* and quantified the abundance of larvae, nymphs and adults in soil samples from Gull Island, Newfoundland. They measured the increase in the abundance of different tick stages from soil samples and equated this to

'activity', although this did not differentiate between questing and inactive ticks in soil samples. Patterns of abundance were characterized by a marked peak of larvae and nymphs in early July, whereas adult males and females were generally lower in abundance relative to nymphs and larvae, and were most abundant late in the season (August) (Eveleigh and Threlfall 1974). Barton (1996) reported earlier activity of *I. uriae* in the Isle of May (Scotland). He collected soil samples on a monthly basis over two years and used the proportion of engorged ticks in a sample as an indication of activity. Ticks of all stages were found to be most active in the months of May and June.

My observed seasonal patterns of abundance of questing nymphs and adult females were similar to the patterns of abundance observed by Eveleigh and Threlfall (1974). In both years, nymph questing activity peaked around mid-July, although the magnitude of the peaks was different in each year. Additionally, in 2004, there were peaks earlier and later in the season that were of lower magnitude. Such changes in tick activity patterns were likely weather driven, with less ticks being active in wet or cold conditions (personal observation; Benoit et al. 2006). In non-nidicolous species, the influence of environmental factors on feeding and host-seeking activities has been determined in many tick species. Harlan and Foster (1990) showed that questing activity (determined by drag sampling) of *Dermacentor variabilis* was

significantly affected by ambient temperature, and to a lesser extent, relative humidity and vapor pressure. The variation in questing activity patterns in *I. scapularis* and *Amblyomma americanum* were related to temperature and relative humidity, the former species being most active under conditions of low temperature and high relative humidity whereas the reverse was true for the latter species (Schulze et al. 2001). Species such as *I. persulcatus* and *I. ricinus* ticks were more active under conditions of optimal temperature and relative humidity ranges characteristic of each species (Balashov 1972). In nidicolous species such as *Ixodes trianguliceps,* temperature and other environmental factors influence the development of stages rather than questing (Randolph 1975).

Ixodes uriae do not quest by climbing on to vegetation like many other *Ixodes* species (Balashov 1972, Eveleigh and Threlfall 1974), especially since many seabird colonies have limited vegetation to permit such a strategy (Barton 1996, Eveleigh and Threlfall 1974, Frenot et al. 2001). Visual cues could be a factor since the puffin slopes were typically covered in puffins in some days and Herring Gulls had considerable activity on and around their nest sites. Most hard ticks, including members of the eyeless genus *Ixodes,* have photoreceptors that aid in responding to shades of light (Sonenshine 1991, Fourie et al. 1993). *Ixodes rubicundus* respond to variations in shadowing

more than to carbon dioxide or host odors (Fourie et al. 1993). *Ixodes ricinus* use the onset of darkness as a cue to initiate questing, but other olfactory cues (such as carbon dioxide gradients) are more important in host detection (Balashov 1972, Perret et al. 2003). The relative importance of different cues to help detect and seek out potential hosts remains unclear in *I. uriae* and laboratory experiments are required to illuminate these behavioral attributes.

On host ticks

Ixodes uriae ticks exhibited differential host-preference, with nymphs feeding preferentially on puffin chicks and occasionally on puffin adults and Herring Gull chicks. Female *I. uriae*, however rarely fed on puffin chicks but were more prevalent on adult puffins, whereas there was no preference of nymphs or female ticks for Herring Gull chicks.

The prevalence of nymphs and female ticks on adult puffins was correlated with questing only in 2005, the year that had a greater amount of tick questing activity compared to 2004. The prevalence of nymphs on adult puffins, however, was still low in 2005, suggesting that adult puffins are important hosts for nymphs only when tick populations are larger. The prevalence of female ticks was higher on adult puffins in 2005, although the flag samples did not indicate any real increase in their questing activity in

that year. Additionally, female ticks were higher in prevalence in the early part of the season (mid-June to early-July), when questing activity was just starting to increase (Figs. 4.4 b, 4.4 d). Female ticks could therefore have been preferentially feeding on adult puffins during the early part of the season, when they were still incubating their eggs. Since there was no clear relationship between questing activity of female ticks and prevalence on adult puffins (significant in 2004 but not 2005), it would seem likely that the feeding was taking place without aggressive questing (i.e., ticks passively waiting in burrows). Herring Gull chicks were present on puffin slopes during the incubation period of puffins and are known to hide (partially or fully) in puffin burrows when threatened. The prevalence of female ticks on Herring Gull chicks followed a similar pattern of higher prevalence during this time of the season, further supporting the contention that female ticks could be passively waiting for hosts in puffin burrows or other suitable places.

Nymphs were found on puffin chicks soon after they had hatched and the prevalence exceeded 70% in both years in early- to mid-July. The on-host abundance of nymphs on puffin chicks reflected the questing activity determined by the flag samples. Prevalence of nymphs on puffin chicks declined as activity of nymphs declined. This suggests a preference of

nymphs for puffin chicks. Eveleigh and Threlfall (1975) stated that puffin chicks were not important in the life cycle of *I. uriae* since very few were found on these chicks. This contention was made based on 15 individuals searched for ticks. Puffins are highly variable in the timing of hatching and we suggest that the puffin chicks are important particularly when both nymphs and puffin chick abundance were synchronized. Large prevalence of nymphs on puffin chicks indicated that other cues (such as olfactory) were likely responsible for helping ticks detect, approach and attach to these hosts in their natal burrows. The correlation between questing activity and prevalence on puffin chicks also suggested that the nymphs were using a combination of passive and aggressive questing.

Ecology of *Ixodes uriae* and host preference

Ixodes uriae occurs in a diversity of moist, coastal habitats of seabird colonies ranging from grassy tussocks and burrows, well-defined nests, vertical cliffs and sloping rocky substrates without vegetation (Eveleigh and Threlfall 1974, Steele et al. 1990, Danchin 1992, Barton et al. 1996, Frenot et al. 2001). The variation in activity patterns of this tick is a reflection of the prevailing environmental factors, diversity of habitats and abundance of hosts. Experimental studies on *I. uriae* collected from seabird colonies at the

Humble and Anvers Islands off the Antarctic Peninsula demonstrate that the species is hydrophilic with water loss constituting one of the most important factors influencing survival of eggs as well as the different mobile stages (Benoit et al. 2006). *Ixodes uriae* ticks reduce water loss by seeking out moist, cool habitats. The resulting aggregation of large numbers of ticks (e.g. 1000s of ticks in rock cracks, Barton et al. 1996, Benoit et al. 2006) helps to reduce water loss significantly since individuals absorb water from the enhanced humidity around such aggregations (Benoit et al. 2006). *Ixodes uriae* is incapable of imbibing water with their mouthparts and is therefore probably dependent on absorption of water from humid areas around water droplets as seen in *I. ricinus* (Kahl and Alidousti 1997). Control of water loss through these and other methods is common in many ticks including some within the genus *Ixodes* (Sonenshine 1991, Benoit et al. 2006). Additionally, cold tolerance in *I. uriae* aids in water retention allowing them to be widely distributed in cooler latitudes (Lee and Baust 1987, Sonenshine 1991).

Ixodes uriae ticks have temperature-dependent development periods that in turn determine the abundance of different stages at different times of the year (estimated by Murray and Vestjens 1967, Eveleigh and Threlfall 1974, Barton et al. 1996). Different tick stages ready to feed, however, need to seek out and feed on suitable hosts. Absence of hosts during some parts of the year

prevents further development since the blood meal is necessary in this species (Eveleigh and Threlfall 1974). The abundance of hosts then becomes the determinant of the length of the life cycle (Eveleigh and Threlfall 1974, Barton et al. 1996, Frenot et al. 2001, Benoit et al. 2006). Hosts, such as different penguin species, may be present for up to 7 months in some Antarctic colonies, making tick feeding possible thereby allowing two stages to develop within one season (Murray and Vestjens 1967, Frenot et al. 2001). This shortens the life cycle to 2 years. Additionally, tick activity patterns from spatially separate colonies on Possession Island within the Crozet Islands show different activity patterns based on the abundance patterns of the hosts (Frenot et al. 2001). Ticks from King Penguin colonies have a narrower range of activity periods since the species is present at the colony for shorter durations of time compared to Macaroni penguins (Frenot et al. 2001).

Clearly, a great deal of variation exists in the ecology and behavior of *I. uriae* throughout its range. The role of ticks in various aspects of host ecology and in the maintenance of diseases in seabird colonies remains unknown. Our study shows that questing behavior varies between different stages of *I. uriae*, thereby making different host species susceptible to infestations during different times of the breeding season. Additionally, different stages feed preferentially on different hosts. This particular aspect is of great ecological

and epidemiological significance since the nymphs of other *Ixodes* species (e.g. *I. scapularis*) are very important as reservoirs of *B. burgdorferi* s.l., and feeding behavior of nymphs are critical in the perpetuation of this pathogen. Preliminary evidence of the presence of *B. garinii* has been documented from ticks collected from puffins in Gull Island (Smith et al. 2006) and the Gannet Islands (Smith et al. 2006, Chapter 3). Therefore we urgently need more information on the behavior, ecology and host-preference of different life stages of *I. uriae* to better evaluate and understand the impact of a seemingly emerging disease in the seabird colonies of the Northwest Atlantic.

CHAPTER 5. ENDOPARASITES OF MURRES: MARKERS OF CHANGE IN THE MARINE ENVIRONMENT

Introduction

Seabirds are prominent components of the North Atlantic marine environment representing tens of millions of breeding pairs and constituting a large portion of the total biomass of this system (e.g., Barrett et al. 2006). Such large numbers of seabirds consume enormous amounts of invertebrates and fish, totaling to a biomass of millions of tons annually (Gaston and Jones 1998, Barrett et al. 2006). Dispersal and migratory movements after the breeding season vary among and within species (Gaston and Jones 1998, Huettmann and Diamond 2000) resulting in the exploitation of different prey taxa over the course of a year (Gaston and Jones 1998). Variation in the diet of seabirds also exposes them to a suite of parasites occurring across their geographic ranges (Muzaffar and Jones 2004, Hoberg 1996, Hoberg 2005). However, limited information exists on the impact, abundance, prevalence and distribution of parasites of seabirds (Threlfall 1971, Hoberg 1984a, 1984b, 1986, Muzaffar and Jones 2004). Nevertheless, more than 700 species of helminth parasites (primarily gastrointestinal) are known to occur in over 165 seabird hosts (Hoberg 1996, Hoberg 2005). Very few studies have assessed the

zoogeography of this diverse array of parasite species of seabirds (e.g. Threlfall 1968, 1971, Hoberg 1984a) and even fewer studies have explored the impact of parasites (Fagerholm 1996, Galaktionov 1996) on their seabird host individuals and populations. Muzaffar and Jones (2004) reviewed the known diseases and parasites of the auks (Alcidae) and of the 57 helminth taxa recorded, many species were potentially detrimental to their hosts' health and possibly survival.

Distribution of endoparasites of murres

Parasites (especially endoparasites) can serve as elegant biomarkers of ecological interactions at different trophic levels (MacKenzie 2005, Hayward 2005) although their roles in this context have not been explored in seabirds (Hoberg 1996, 2005). Threlfall (1971) conducted a detailed study (between 1966-1969) of endoparasites of alcids in the Northwest Atlantic, with specimens collected primarily from colonies in eastern Newfoundland and from Greenland. He found an abundance of tapeworms in the genus *Tetrabothrius* in many alcids, but particularly murres. The other genus of tapeworm found in alcids is *Alcataenia*, and these occurred in relatively lower prevalence. Hoberg (1984a) conducted a similar, but more comprehensive, study on the zoogeography of endoparasites of alcids on the North Pacific

Basin. Zoogeographic studies of the *Alcataenia* have revealed interesting patterns of dispersal and colonization in its seabird hosts (Hoberg 1986). *Alcataenia* is primarily a parasite of the auks (at least 8 species) and to a lesser extent, gulls (Laridae; at least 2 species) and host-specificity in many of these tapeworm species makes them suitable candidates for analyzing patterns of zoogeography and host ecology (Hoberg 1984a). Additionally, *Alcataenia* utilizes Euphausiid shrimps (e.g. *Thysanoessa inermis*) as an intermediate host (Shimazu 1975, Fig. 5.1), thereby providing information on its seabird hosts' feeding patterns.

Galaktionov (1994) showed that seabirds (gulls and auks) in the Barents Sea ecosystem, that had undergone long-term changes in their diet, had drastically different endoparasite fauna. These shifts in diet were linked with long-term changes in the Barents Sea ecosystem. Divergent endoparasite fauna can therefore be reflective of long-term changes in the greater marine ecosystem. Acquisition of parasite fauna that use intermediate hosts would require an overlap between the distribution of both infected/infective intermediate hosts (euphausiids) and definitive hosts (Hoberg 1996, Hoberg 2005). Such overlap in distributions of hosts and parasites is maximized during the breeding season around colonies, where large numbers of seabird hosts undergo foraging trips within certain areas providing opportunities for

transmission of parasites to both a variety of potential intermediate and definitive hosts. The juxtaposition of parasite, intermediate host and definitive host is far less regular outside the breeding season when seabirds have a pelagic existence over much larger geographic areas, suggesting that less transmission of endoparasites occurs outside the breeding season.

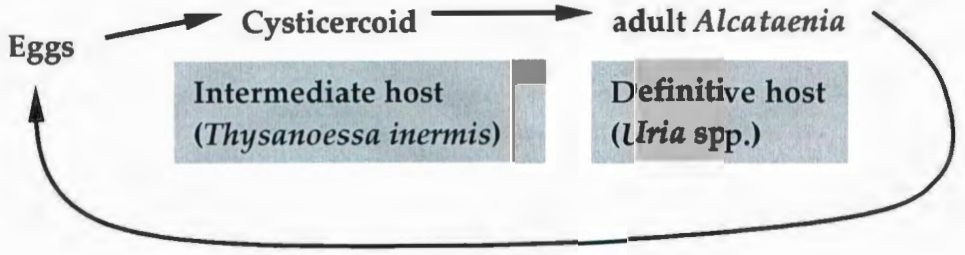

Fig. 5.1. Life cycle of *Alcataenia* tapeworms.

Distribution and ecology of murres

Murres (*Uria* spp.) are among the most numerous seabirds in the northern hemisphere (Tuck 1961, Nettleship and Birkhead 1985, Gaston and Jones 1998, Ainley et al. 2002, Gaston and Hipfner 2000). Global populations of Common Murres number between 13 and 21 million breeding pairs (b.p.) (Ainley et al. 2002) whereas Thick-billed Murres (*U. lomvia*) range between 15 and 20 million b.p. (Gaston and Jones 1998). In the Pacific colonies, there is extensive overlap between the two species, although this is not the case at Atlantic colonies, where Thick-billed Murres tend to be more northern in their distribution compared to Common Murres (Fig. 5.2). Common Murres occur in extremely dense colonies, with 20-70 individuals m^{-2} breeding on broad, flat, rocky outcrops on headlands and offshore islands (Cramp et al. 1985, Nettleship and Birkhead 1985, Gaston and Jones 1998). In contrast, Thick-billed Murres nest in dense clumps on nesting sites that are typically located on narrow ledges (as little as 10 cm wide) along steep cliffs directly adjacent to the sea (Gaston and Nettleship 1981, Nettleship and Birkhead 1985). Densities vary between 1 and 37 pairs m^{-2} (Cramp et al. 1985).

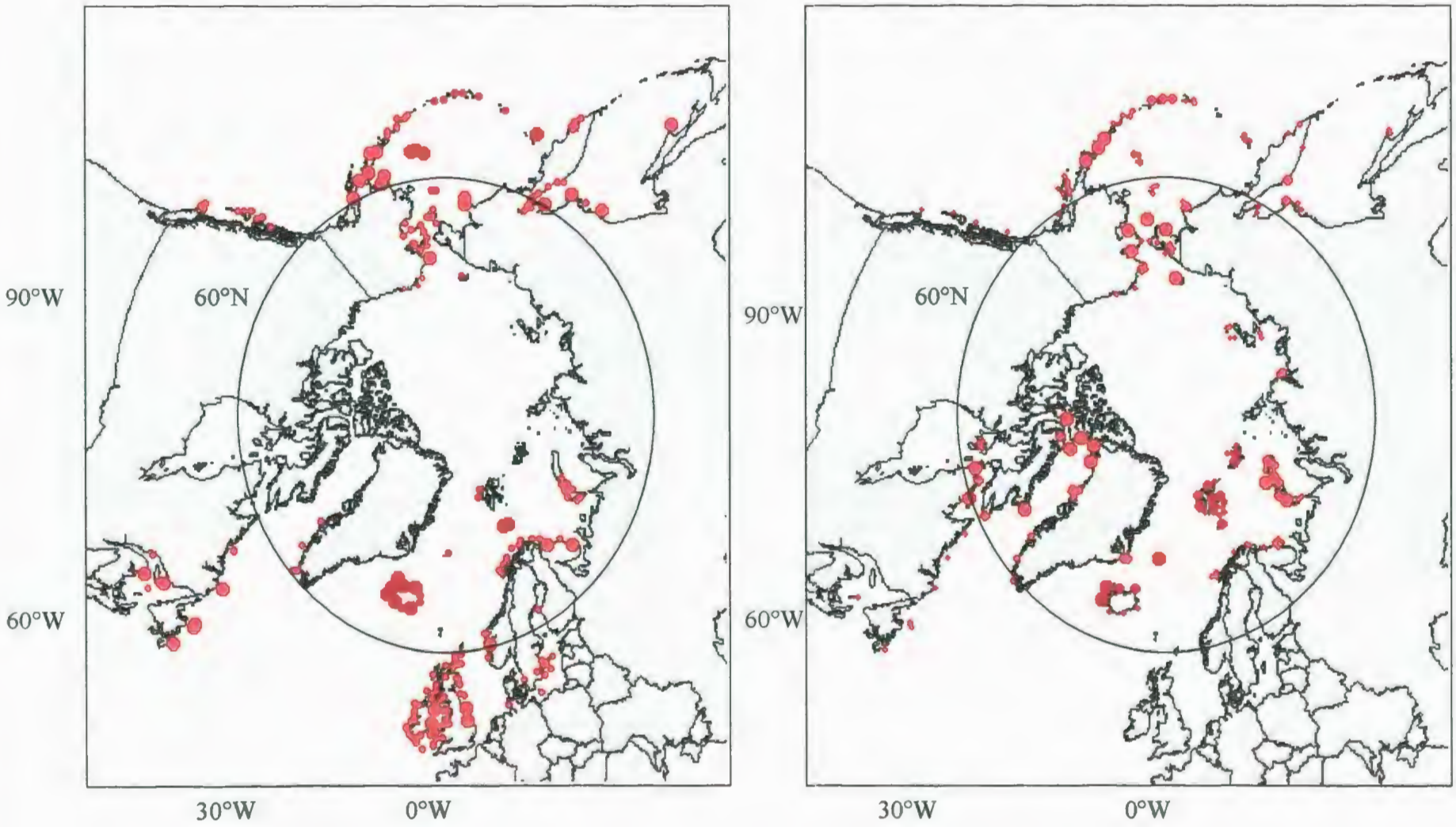

Fig. 5.2. Breeding distribution of a) Common Murres and b) Thick-billed Murres (the diameters of the circles indicate greater densities). Redrawn from CAFF (2004), Ainley et al. (2002), and Gaston and Hipfner (2000), Outline map from ESRI 2004, QEII Library, Memorial University of Newfoundland)

Murres breeding in higher latitudes, especially Thick-billed Murres, are significantly influenced by sea ice that determines their summer distribution, timing of breeding and even breeding success or failure (Gaston and Jones 1998, Gaston and Hipfner 2000, Ainley et al. 2002). Patterns of migration are generally complex and vary between Pacific and Atlantic populations. Common Murres in the Bering Sea are pushed towards the Aleutians in large numbers by expanding sea ice. Movements of more southern populations in the Pacific are relatively less extensive, with southward migration closely following the coastline and limited off shore movements. In the northwest Atlantic, movements of Common Murres are away from coastal areas across the relatively broad outer continental shelves. Birds move further south of Labrador waters as sea ice expands, reaching the Scotian shelf and the Bay of Fundy by December. Thick-billed Murres are markedly different in their migratory and dispersal movements (Huettmann and Diamond 2000, Gaston and Hipfner 2000). In the Pacific, this species tends to remain within the breeding range although they are displaced in Arctic Alaska by sea ice. Birds that breed in the eastern Canadian Arctic move along the Greenland coast to southwest Greenland. Large congregations of Thick-billed murres subsequent to breeding occur along the Labrador shelf.

First and second year individuals arrive in Newfoundland waters by November, while older individuals arrive later.

Feeding ecology of murres

Murres feed on a variety of invertebrate and fish species (Bradstreet and Brown 1985, Elliot et al. 1990, Gaston and Jones 1998, Rowe et al. 2000, Gaston and Hipfner 2000, Ainley et al. 2002). Common Murres have a greater dominance of fish in their diets with additional euphausiids, amphipods and polychaetes. Contrastingly, Thick-billed Murres have a far greater dominance of euphausiids, with fish and amphipods constituting a smaller proportion of their diets. There is a great deal of regional variation in the kinds of prey taken depending on the geographic distribution of prey in relation to the colony (Bradstreet and Brown 1985). Chicks of either species are fed on fish, particularly species such as Capelin in the northwest Atlantic.

Changes in the environment have been reflected in changes in the distribution and abundance of several important forage fish as well as invertebrate species in the Atlantic (Carscadden et al. 2001, Barnard et al. 2004). This, in turn, has been reflected in the fish species brought in for chicks in the colonies in the northwest Atlantic. Common and Thick-billed Murres in the Gannet Islands, Labrador, for instance have undergone a massive shift

from Capelin (*Mallotus villosus*) in the 1980s to Sandlance (*Ammodytes americanus*) and Daubed Shanny (*Leptoclinus maculatus*) in the late 1990s and into the 2000s (Bryant et al. 1999). Similar changes in food of chicks have been recorded in many colonies in the Northwest and Northeast Atlantic (e.g. Daveron and Montevechhi 2003, Wanless et al. 2005, Barrett et al. 2006). The general purpose of this study was to examine whether changes in the diversity of murre parasites have corresponded to changes in murre diet and changes in the Northwest Atlantic marine ecosystem over the last few decades.

Objectives

To assess such changes in the Northwest Atlantic, I quantified

1. the endoparasite fauna of Common and Thick-billed Murres from the Northwest Atlantic between 1966-1969 and 2005-2006. One detailed historical study of auk parasites (Threlfall 1971) in this region offers an opportunity to compare the current murre endoparasite loads and species composition to previous levels;
2. the prevalence and abundance of the tapeworms, particularly the genus *Alcataenia*, in Common and Thick-billed Murres. Given the large number of changes in the feeding of a range of seabird species in the

Northwest Atlantic (e.g. Bryant et al. 1999, Rowe et al. 2000, Daveron and Montevecchi 2003, Lavers 2007), I expected to find major changes in the prevalence and abundance of certain endoparasite taxa between the 1966-1969 and 2005-2006 periods. Specifically, I expected changes in the composition of *Alcataenia* species.

3. the diet of both murre species, from representative locations, to help explain the trends in the species composition, distribution and abundance of endoparasites.

Materials and Methods

Parasite collection methods and study area

Thick-billed and Common Murres specimens were collected from several different sites (Table 5.1, Fig. 5.3). I examined 15 Common Murres and 11 Thick-billed Murres collected by hunters during the Newfoundland Murre hunt in the 2005-2006 hunting seasons. Most of the wintering murres were collected either from Harbor Breton (45° 28'N, 55° 47'W) or St. Mary's Bay (46° 55'N, 53° 34'W) along the southern side of the Avalon Peninsula. To determine the infection rates of parasites from murres in colonies, I collected 15 Thick-billed Murres and 15 Common Murres from the Gannet Islands, Labrador (see Chapter 3 for a description of the Gannet Islands) in the

breeding season of 2006. I also collected 13 Common Murres from a by catch in the Renews area, Newfoundland in July 2005. These birds were most likely breeding birds from the Witless Bay Ecological Reserve (see Chapter 3 for a description of Gull Island, Witless Bay Ecological Reserve) that is 30 km north of Renews.

Sixteen Thick-billed Murres were collected, frozen and shipped from Coats Island in 2006. Coats Island lies at the northern end of Hudson Bay (62° 17′ N, 83° 00′ W) in the Kivalliq Region of Nunavut and has an area of 5,600 km^2 and is about 130 km long (Gaston and Ouellet 1997). The maximum elevation above sea level is about 185 m. Coats Island hosts about 33,000 b.p. of Thick-billed Murres in addition to a variety of other wildlife (Gaston and Ouellet 1997).

An additional 15 Thick-billed Murres were collected from the Nuuk region of western Greenland (64° 00′ N, 52° 00′ W) by hunters soon after the breeding season in 2006, frozen and shipped to me. All collections were made under permits issued by the Canadian Wildlife Service, Atlantic Region; Parks and Natural Areas, Newfoundland and Labrador; and Animal Care Services, Memorial University of Newfoundland.

All murre specimens were frozen after collection and retained for future examination. Each specimen was aged based on body plumage to

either adult or juvenile. Standard measurements such as wing cord, total tarsus, tarsus bone, total mass, and head measurements were recorded. Later, the gastrointestinal tract, liver, gall bladder, lungs, heart and kidneys were removed for examination for endoparasites. The sex of the individual was also determined during the dissection. Each of the organs was separately examined under a dissecting microscope after washing in clean, cold water over a sieve with 0.25 mm mesh size. Endoparasites were preserved in 70% ethanol. Identifications were done following Yamaguti (1959a, 1959b), Hoberg (1984a, 1984b), Barus et al. (1978) and Nagasawa et al. (1998a, 1998b). Specimens were also submitted to E.P. Hoberg at the U.S. National Parasite Collection for confirmation and cataloguing (see Appendix I).

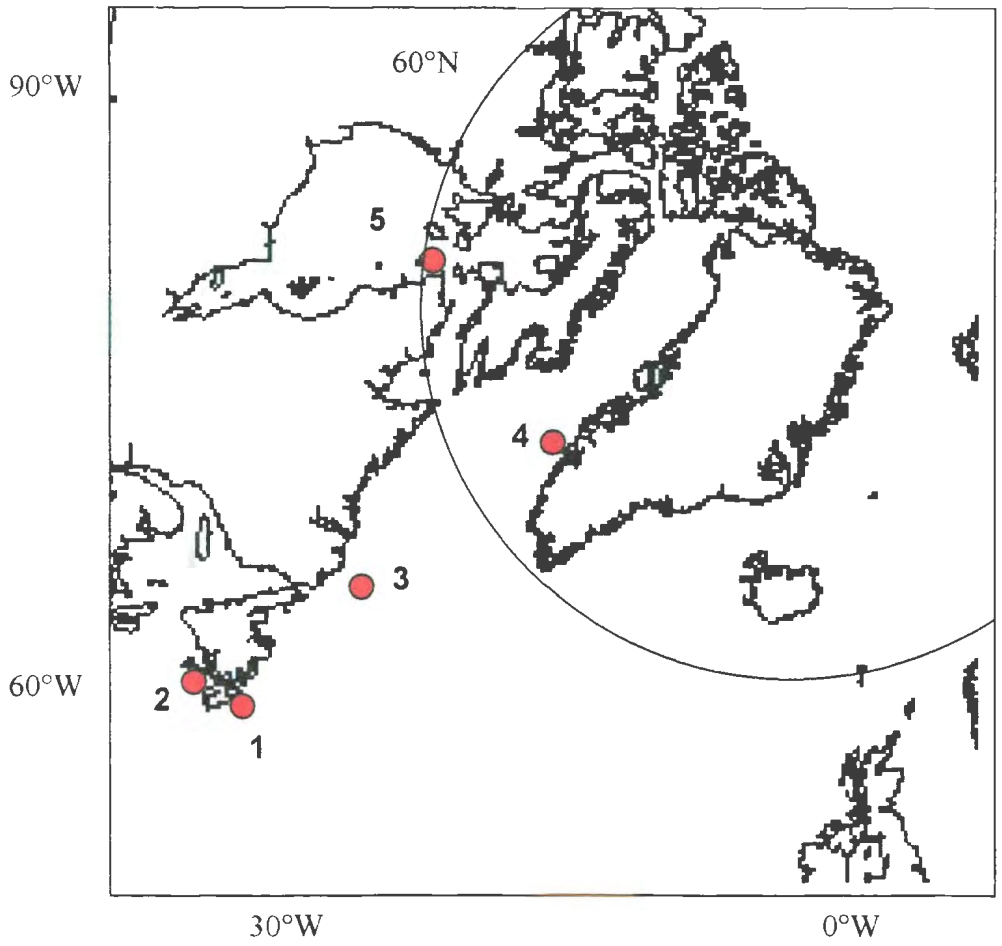

Fig. 5.3. The sites of murre collections: 1) Witless Bay Ecological Reserve (Newfoundland), 2) wintering murres from various locations off Newfoundland, 3) Gannet Islands, Labrador, 4) off shore from Nuuk, Greenland, 5) Coats Island, Nunavut.

The proventriculus, gizzard and intestines (referred to henceforth as intestines) of many of the murres had remnants of food and this was quantified. Since relative abundance of food items may be difficult to quantify and interpret due to varying digestion rates, I calculated the number of intestines containing certain kinds of food (Elliot et al. 1990, Rowe et al. 2000). The otoliths of fish were retained to identify them to species (when possible) following the Campana (2004). Euphausiids, Amphipods and squids were sorted and enumerated, but not identified further.

Table 5.1. Murres collected and their localities.

	Thick-billed Murre	Common Murre
Coats Island, Nunavut	16	0
Gannet Islands, Labrador	15	15
Witless Bay, Newfoundland	0	13
Nuuk, Greenland	15	0
Newfoundland (winter)	11	15
Total	**57**	**43**

Quantification and statistical analyses

I used the software Quantitative Parasitology 2.0, specifically developed to account for aggregated parasite distributions and allow distribution-free statistical tests to compare parasite loads (Reiczigel and Rózsa, 2001). I quantified mean intensity (mean number of parasites per infected host) and prevalence (proportion of hosts that were infected) since

no single measure of parasite 'load' is appropriate and a combination is recommended (Rózsa et al., 2000). Confidence intervals (at 95% confidence level) for mean intensities were computed using bootstrap techniques with 2000 replications (Rózsa et al., 2000). Exact Confidence intervals (at 95% confidence level) were calculated for prevalence using the Clopper-Pearson method (Rózsa et al., 2000). Confidence intervals for median intensities were also calculated, with the exact confidence level being reported, rather than the desired level, due to the discrete nature of the data (Rózsa et al., 2000). Mean intensities in different hosts were compared using Bootstrap t-tests, p-values being generated from 2000 replications (Rózsa et al., 2000). Prevalence of parasites among murre species was compared using Fisher's Exact Test, with the exact p-value reported whenever possible. An α level of 0.05 was used to determine significance in each case.

The proportion of intestines with certain kinds of food was used to compare the diets of the two murre species. Dietary differences among different regions were also compared using a binomial test comparing the proportion of stomachs with a certain type of food item between specimens from two regions. Tests were performed in Minitab 14.1. If sample sizes were too low and the p-value was significant but between 0.01 and 0.05, then the Fisher's Exact test in Quantitative Parasitology 2.0 was used.

Results

Comparison of parasite assemblages between current and past studies

A total of 623 endoparasites representing Digenea (Trematoda, flatworms), Eucestoda (tapeworms), Nematoda (roundworms) and Acanthocephala (spiny-headed worms) were collected from the two murre species (Tables 5.2 and 5.3). The bulk of the specimens (>85%) was represented by tapeworms. Comparison of tapeworm species composition and abundance, however, differed between the two studies with an almost complete absence of the genus *Tetrabothrius* in the current study and a much higher prevalence and abundance of species of *Alcataenia* (Tables 5.2 and 5.3). The most notable among the *Alcataenia* was the presence of *A. longicervica*, particularly in Thick-billed Murres. This species is endemic to the North Pacific basin (Hoberg 1984a) and my study constitutes the first record of this species in the North Atlantic. *Alcataenia meinertzhageni* were generally rare, consistent with previous studies, while *A. armillaris* had variable abundance, with lower prevalence and intensity in Thick-billed murres compared to Threlfall (1971) and total absence in Common Murres. The prevalence and abundance of the nematode *Stegophorus stellae-polaris* was higher in both murre species (but particularly in Thick-billed Murres) compared to Threlfall

(1971). Previously, several other species including *S. stellae-polaris* were found from both murre species, but in much lower prevalence and intensity. The only other species of nematode recorded in this study was *Contracaecum spiculigerum*, single specimens of which were collected from both murre species.

Table 5.2. Comparison of parasite community structure in Common Murres from 1966-1969 (Threlfall 1971) and 2005-2006 (present study). (p=prevalence, MI=mean intensity, R=Range).

	1966-1969[1]			2005-2006[2]		
	P	MI	R	P	MI	R
Digenea						
Ornithobilharzia lari	<1	1	1			
Cryptocotyle lingua	<1	1	1			
Renicola sp.				2.3	5	5
Eucestoda						
Tetrabothrius cylindraceus	1	5	1-25			
Tetrabothrius erostris	<1	2	2			
Tetrabothrius jagerskioldi	7	2	1-19			
Tetrabothrius sp.	1	1	1-3			
Alcataenia armillaris	15	3	1-27			
Alcataenia camplyacantha	1	2	1-5			
Alcataenia longicervica				4.7	4.5	1-8
Alcataenia meinertzhageni	2	2	1-3			
Alcataenia micracantha	<1	2	1-4			
Alcataenia sp.	1	1	1-2	11.6	7.4	1-14
Unidentified tapeworms				2.3	5	5
Nematoda						
Eustrongylides mergorum	<1	1	1			
Contracaecum spiculigerum	3	1	1-2	2.3	1	1
Contracaecum sp.	<1	1	1			
Cosmocephalus obvelatus	<1	1	1			
Stegophorus stellae-polaris				9.3	1	1
Anisakis sp.	<1	1	1			
Unidentified nematodes				11.6	4.2	1-7
Acanthocephala						
Unidentified acanthocephala				2.3	1	1

1. Threlfall 1971.
2. N= 43

Table 5.3. Comparison of parasite community structure in Thick-billed Murres from 1966-1969 (Threlfall 1971) and 2005-2006 (present study). (p=prevalence, MI=mean intensity, R=Range).

Parasite taxa	1966-1969[1]			2005-2006[2]		
	P	MI	R	P	MI	R
Digenea						
Cryptocotyle lingua	2	1	1			
Eucestoda						
Tetrabothrius jagerskioldi	8	2	1-4			
Tetrabothrius sp.				1.8	1	1
Alcataenia armillaris	26	5	1-12	17.5	6.3	1-21
Alcataenia longicervica				26.3	14.6	1-71
Alcataenia meinertzhageni				3.5	2.5	2-3
Alcataenia sp.	8	1	1	22.8	2.5	1-30
Nematoda				8.8	3.2	1-8
Eustrongylides mergorum	2	1	1			
Contracaecum spiculigerum	10	2	1-4	1.8	1	1
Stegophorus stellae-polaris	5	3	1-5	28.1	2.8	1-7
Acanthocephala						
Unidentified acanthocephala				1.8	1	1

1. Threlfall 1971.
2. N= 57

Prevalence and intensity of *Alcataenia longicervica*

The North Pacific tapeworm species *A. longicervica* occurred at Coats Island, Witless Bay and southwest Greenland (Table 5.4) from both murre species in the present sample. *Alcataenia longicervica* was the only tapeworm species in Common Murres and its prevalence and mean intensity were low (Table 5.4) in specimens collected from Witless Bay, Newfoundland, and wintering birds off the coast of Newfoundland. No *Alcataenia* specimens were found in Common Murres from the Gannet Islands. In contrast, Thick-billed Murres had greater prevalence and mean intensity of *A. armillaris* and *A. longicervica,* although *A. meinertzhageni* was relatively rare. The greatest prevalence and intensity of *A. longicervica* were recorded from Thick-billed Murres from Nuuk, Greenland followed by those from wintering birds in Newfoundland.

Table 5.4. Variation in the abundance of three *Alcataenia* species in two murre species in relation to locality. Dashes indicate sites where collections of a host species were not made. (P=Prevalence, MI=Mean intensity, R=Range; CI=Coats Island, Nunavut; GI=Gannet Islands, Labrador; GR=Nuuk, Greenland; NL-w=Newfoundland-winter; WB=Witless Bay).

	A. armillaris			*A. longicervica*			*A. meinertzhageni*		
	P	MI	R	P	MI	R	P	MI	R
Common Murre (N=43)									
CI	-	-	-	-	-	-	-	-	-
GI	0	0	0	0	0	0	0	0	0
WB	0	0	0	7.7	1	1	0	0	0
GR	-	-	-	-	-	-	-	-	-
NL-w	0	0	0	6.7	8	8	0	0	0
Thick-billed Murre (N=57)									
CI	6.3	1	1	6.3	1	1	6.3	2	2
GI	0	0	0	0	0	0	0	0	0
WB	-	-	-	-	-	-	-	-	-
GR	46.7	4.3	1-21	53.3	27.3	5-71	6.7	3	3
NL-w	18.2	10	10	54.5	13	3-39	0	0	0

Diet of Murres

When both murres were considered, the diets were typical of each species, with the Common Murres having more stomachs with fish and the Thick-billed Murres species having more crustaceans such as euphausiids and amphipods (Table 5.5). Among the Common Murres, sandlance and capelin were the dominant food items found in the majority of stomachs. Contrastingly, euphausiids, followed by amphipods were the most common items in Thick-billed Murre stomachs. When murres were compared by region (Table 5.6), Common Murres still had a greater proportion of stomachs with fish compared to Thick-billed Murres. The notable exception was the Gannet Islands, in which a large proportion of stomachs of Common Murres had crustacean (including euphausiid) remains. Regional differences in Common Murre stomach contents were not statistically significant. Thick-billed Murres from the Gannet Islands, on the other hand, had a much larger proportion of stomachs with fish and squid compared to euphausiids. Proportions of stomachs with fish were significantly higher compared to wintering birds from Newfoundland (n=26, $Z=2.75$, $p=0.003$) but not to birds from Greenland (n=30, $Z=1.35$, $p=0.088$). Proportions of Thick-billed Murre stomachs with euphausiids in the Gannet Islands were significantly lower

than those from wintering Newfoundland birds (n=26, Z = 5.96, $p< 0.0001$) as well as from Greenland (n=30, Z = 3.16, $p= 0.001$).

Table 5.5. Comparison of the diets of Thick-billed Murres (N=42) and Common Murres (N= 38) in this study. (Values shown are proportion of intestines containing each food item).

	Thick-billed Murre	Common Murre
Cod species		2.3
Sandlance		39.5
Capelin	7.0	23.3
Daubed Shanny		4.7
Unidentified fish	24.6	48.8
Hyperiid Amphipod	22.8	4.7
Euphausiid	38.6	4.7
unidentified crustacea	14.0	9.3
Squid	19.3	4.7
Mollusc	1.8	

Table 5.6. Comparison of the diets of Thick-billed and Common Murres expressed as proportion of intestines with each food item and sorted by region. (NL=Newfoundland winter; GI=Gannet Islands; WB=Witless Bay; GR=Nuuk, Greenland; CI=Coats Island, Nunavut).

	Common Murre			Thick-billed Murre			
	NL	GI	WB	NL	GI	GR	CI
Sample size (N)	14	11	13	10	10	15	7
Cod species		9.1					
Sandlance		45.5	92.3				
Capelin		36.4	46.2			26.7	
Daubed Shanny		18.2					
Unidentified fish	92.9	63.6	7.7	10.0	60.0	33.3	28.6
Hyperiid Amphipod	7.1	9.1		20.0	20.0	60.0	
Euphausiid		18.2		90.0	10.0	60.0	42.9
Unidentified crustacea	7.1	27.3		10.0		26.7	42.9
Squid		18.2			30.0	40.0	28.6
Mollusc						6.7	

Discussion

Changes in parasite assemblages

Parasite communities in seabirds are significant but virtually ignored components of the ecosystem that can provide information on feeding ecology, distribution patterns, evolution and environmental change in marine ecosystems (Hoberg 1986, Galaktionov 1996, Hoberg 1996, Hoberg 2005). In this study, I recorded drastic changes in the endoparasites assemblages of two murre species in the Northwest Atlantic between the late 1960s and mid 2000s. Compared to Threlfall's (1971) study, one widespread tapeworm genus

Tetrabothrius had been almost completely replaced by *Alcataenia* species. This fact alone indicated fundamental dietary shifts in the murre hosts, since *Tetrabothrius* utilizes a range of different intermediate hosts including cephalopods (squids), bony fish and crustaceans (Hoberg 1984a, Hoberg 1994, Muzaffar and Jones 2004). *Alcataenia,* in contrast, utilizes a more restricted range of intermediate hosts, namely euphausiid shrimps such as *Thysanoessa inermis* (Shimazu 1975, Hoberg 1984a, 1984b). The change in the parasite assemblages could reflect limited abundance (availability) of food species, changes in the distribution of food species or regional factors (limited to colonies) limiting prey choice (Hoberg 1996). At least, my results provide evidence that murre prey has likely undergone a fundamental shift from a mixed collection of fish and invertebrates in the 1960s to predominantly euphausiids during the 2000s.

Global climate change

Changes in the marine environment have been (and continue to be) documented in many studies (Pedersen and Smidt 2001, Beaugrand et al. 2002, Barnard et al. 2004, Doney 2006, Ritcher-Menge et al. 2006, Smetacek and Nicol 2006). These include changes in sea temperature, ocean currents, upwelling patterns, nutrient flux, extent of sea ice (at the poles), and growth

and distribution of phyto- and zooplankton. These changes can in turn influence distribution and feeding ecology of seabirds and other apex predators (Hoberg 1996, ACIA 2005, Hoberg 2005, Palter et al. 2005, Ritcher-Menge et al. 2006) and the community composition, abundance and distribution patterns of their parasites (Hoberg 1996, Marcogliese 2001, Muzaffar and Jones 2004, Hoberg 2005).

The Arctic Ocean has been central to our understanding of long-term climate change and recent papers highlight fundamental ecosystem processes and climate-mediated changes in this extreme environment (Ottersen et al. 2001, Beaugrand et al. 2002, ACIA 2005, Doney 2006, Smetacek and Nicol 2006, Ritcher-Menge et al. 2006). Water circulation in the Arctic provides a basis for our understanding of ecosystem change. Warm waters enter from the North Pacific into the Arctic via the Bering Strait and from the North Atlantic via the Fram Strait and Nordic Seas (ACIA 2005). Two important systems, namely the Beaufort Gyre spanning across the Canadian Basin and the Transpolar Drift, extending from the Siberian coast to the Fram Strait, are important transporters of large volumes of water in and out of the Arctic. Water leaves the Arctic either through the Canadian Archipelago or the Fram Strait. The latter water mass moves along the east Greenland coast, across the southern tip and into the Labrador Sea and Baffin region, where it eventually

encounters water coming in through the Canadian Archipelago. Temperature and salinity of the Arctic fluctuate depending on, among other things, the volumes of water entering through the Atlantic and Pacific, the extent of sea ice, surface water runoff, heat exchange with the atmosphere and direct precipitation.

Global temperature anomalies associated with altered weather patterns have changed these systems in the Arctic (ACIA 2005). There is clear evidence of reduced ice cover over longer periods of time in a year; a more eastward influence of the Beaufort Gyre; a more pronounced Transpolar Drift; and greater encroachment of North Pacific and North Atlantic waters into the Arctic (ACIA 2005). These changes are in turn associated with salinity changes and varying thermohaline, shifts in the Artic-Atlantic and Arctic-Pacific water boundaries and altered coastal currents.

Dietary shifts, prey distributions and parasite loads

The oceanographic variables in the Arctic help determine the plankton community structure, distribution, production and movement (ACIA 2005). Zooplankton in the Arctic, represented by about 260 species, collectively constitute about 50% of the pelagic biomass. Among these, euphausiids (krill) are abundant on the Atlantic side and the Bering Sea, but not in the central

Arctic or the East Siberian, Laptev and Kara Seas (Siegel 2000, ACIA 2005). Copepods and amphipods are also very abundant and form significant components of the mesozooplankton. Changes in the distribution of zooplankton, particularly euphausiids have been noted in the literature (Dalpadado and Skjoldal 1991, 1996, Beaugrand et al. 2002, Barnard et al. 2004, ACIA 2005). Their presence and abundance is dependent upon a 'match' or overlap between phytoplankton abundance and distribution (Dalpadado and Skjoldal 1991, 1996, ACIA 2005). Fish, seabirds and marine mammals depend on the abundance of a range of zooplankton and other fish. Oceanographic changes altering zooplankton abundance patterns, therefore, also alter abundance of these animals in higher trophic levels along large spatial and temporal scales (Dalpadado and Skjoldal 1991, 1996, Carscadden et al. 2001, Carscadden and Vilhjálmsson 2002, ACIA 2005).

Dietary shifts are important ways of measuring such alterations in the marine environment and the diet of seabird species has been studied extensively in this regard (Croxall 1987, Montevecchi 1993, Montevecchi and Myers 1995, 1996, 1997, Bryant et al. 1999, Daveron et al. 2002, Daveron and Montevecchi 2003, Abraham and Sydeman 2004, Barrett et al. 2006). Murres in the Gannet Islands (Bryant et al. 1999), Funk Island (Daveron et al. 2002, Daveron and Myers 2003) and wintering areas (Elliot et al. 1990, Rowe et al.

2000) reflect long-term change in chick provisioning and adult diet, indicative of changes in the prey base. It has been argued that Common and Thick-billed Murres may both feed preferentially on euphausiids during the breeding season in years with lower abundance of fish (Mehlum 2001).

Local variations in feeding behavior in Thick-billed Murres can also influence the kinds of food acquired and breeding success (Falk et al. 2002). I found a greater proportion of stomachs with euphausiids, amphipods and other crustaceans in Common Murres at the Gannet Islands (relative to other sites) although over 60% of the stomachs still had fish. This could be reflective of lower abundance of fish in the foraging areas around the colony. Similar anomalies have been recorded in Razorbills at the Gannet Islands, with a larger proportion of parents bringing in larval fish in 2005, that is atypical of Razorbills (Lavers 2007). Contrastingly, Thick-billed Murres in the Gannet Islands had a smaller proportion of stomachs with euphausiids (10%) or amphipods (20%) and a much larger proportion of stomachs with fish (60%), compared to other sites. This finding was puzzling since generally Thick-billed Murres have euphausiids and other crustaceans as a dominant food item (Gaston and Jones 1998, Gaston and Hipfner 2000, Mehlum 2001, other sites in this study). This could indicate regional processes restricted to the Gannet Islands driving foraging patterns and diet in murres and other

seabirds at this colony. Falk et al. (2002) found that Thick-billed Murres in Coburg Island in the Canadian Arctic spent less time feeding, indicating good food supply for the chicks, compared to those in Hakluyt Island, a colony in southwestern Greenland. The less time spent in feeding, however, meant that they had lower chick growth rates resulting in later fledging dates. Similar processes may be at play in the Gannet Islands colony. The absence of tapeworms in the murres from the Gannet Islands could also be explained by the lower proportion of euphausiids in the diet relative to fish, since the euphausiid *T. inermis* is essential in the transmission of the *Alcataenia* species to the murre hosts. Other sites that had higher prevalence and abundance of *Alcataenia* in Thick-billed Murres also had a larger proportion of stomachs with euphausiids (e.g. Greenland, Newfoundland winter).

Euphausiid distributions and *Alcataenia longicervica*

Of the Cylophyllidean cestodes that parasitize murres, the genus *Alcataenia* is represented by at least ten species of which eight are restricted and specific to the auks and two species occur in gulls (Laridae)(although they may occur incidentally in auks). Hoberg (1986) investigated the historical biogeography of the host-parasite assemblage and concluded that the speciation of *Alcataenia* must have occurred later than the speciation of

their hosts. Distributions of cestode and other helminths are often a product of host-parasite ecological associations, with the interactions between the distributions of intermediate hosts tending to limit cestode distributions (Hoberg 1996). Since intermediate hosts and parasites often overlap during the breeding season of the auk hosts, frequency of transmissions is likely to be high during the breeding season. The intermediate hosts of *Alcataenia* are Euphausiid crustaceans such as *Thysanoessa* species (Shimazu 1975), which also forms an important dietary component of breeding and wintering murres, particularly Thick-billed Murres (Gaston and Noble 1985, Birkhead and Nettleship 1987, Elliot et al. 1990, Rowe et al. 2000, Ainley et al. 2002). *Alcataenia armillaris* Rudolphi 1810 and *A. meinertzhageni* Baer 1956 are restricted to the murres and have been recorded from different locations in the Holarctic (Threlfall 1971, Hoberg 1986, Fig. 5.4). *Alcataenia longicervica* Hoberg 1984 was described from murres in the North Pacific basin and has been regarded as endemic to the region (Hoberg 1986) and extensive surveys of murres from the North Atlantic have failed to find the species (e.g. Threlfall 1971).

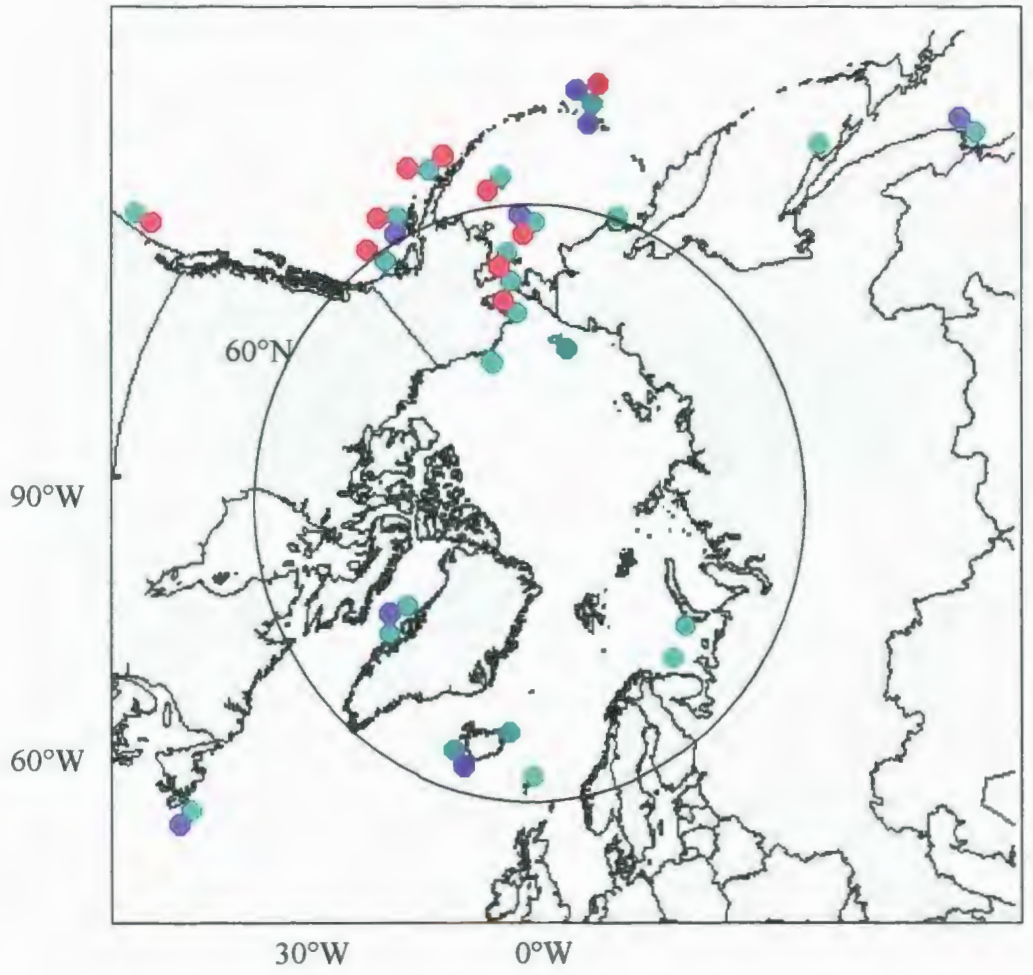

Fig. 5.4. Former distribution of *Alcataenia* species in murres: *A. meinertzhageni* (blue), *A. armillaris* (green) and *A. longicervica* (red). (From Hoberg 1984a, 1986, Threlfall 1971).

Two species of Euphausiids, *Thysanoessa inermis* and *T. rauschii* are abundant in the Bering, Beaufort and Chukchi Seas (Fig. 5.5) (Siegel 2000). Advective movements can strongly influence zooplankton and their distributions (Siegel 2000, Edvardsen et al. 2003). Influenced by such forces and water influx into the Arctic, Beaufort and Chukchi Seas have periodic geneflow from the populations of both *Thysanoessa* species in the Bering Sea (Siegel 2000). Additionally, the sporadic occurrence of the two species in the Laptev and Kara Seas (Drobysheva 1994) indicates that there are mechanisms that facilitate the inflow of genes between North Pacific and North Atlantic stocks of these euphausiids (Siegel 2000). Infected populations of *Thysanoessa* moving into the Beaufort Sea and beyond could expose breeding Thick-billed Murres of the eastern Canadian Arctic to *A. longicervica* infections originating in the North Pacific. Infected Thick-billed Murres could have then introduced infections into populations of *Thysanoessa* species in the Labrador Sea and subsequently to Newfoundland waters, thereby exposing Common Murres (distributed further south) (Fig. 5.5).

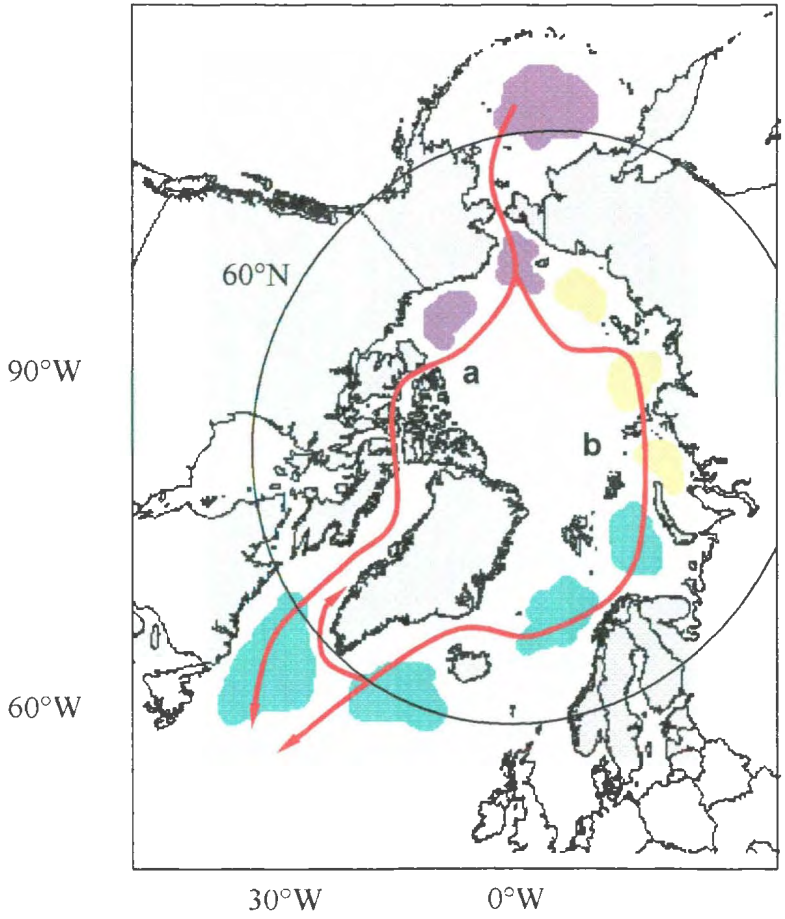

Fig. 5.5. Distribution of populations of *Thysanoessa* species in the Bering, Chuckchi and Beaufort Seas (Purple); Labrador, Norwegian and Barents Seas (Green); and periodic distributions in the East Siberian, Laptev and Kara Seas (Beige). The red arrows show possible paths taken by infected *Thysanoessa* from the North Pacific into the Northwest Atlantic. a) Movement of *Thysanoessa* infected with *A. longicervica* from the Bering Sea along Beaufort Sea and into the Canadian Arctic. b) Movement along the East Siberian Sea via the Barents Sea into the Northwest Atlantic.

Alternatively, movement of *T. inermis* infected with *A. longicervica* could have occurred along the East Siberian and Laptev Seas influenced by the pronounced Beaufort Gyre and Transpolar Drift (Ottersen et al. 2001) resulting in infected Thick-billed Murre colonies along Novaya Zemalaya, Svalbard, Iceland and northeastern Greenland (Fig 5.5). Establishment of areas of endemism of the tapeworm in these colonies could then facilitate movement along the eastern Greenland coast and into the Labrador Sea, driven by mixing of infected *Thysanoessa* populations along these current systems. My study strongly supports this latter route as the primary mode range expansion of *A. longicervica* into the Northwest Atlantic. Colonies along the former route in my study (Coats Island, Gannet Islands, and Witless Bay) all revealed low or no infections in Common and Thick-billed Murres. In contrast, specimens collected from the Nuuk region of Greenland showed very high prevalence and abundance of *A. longicervica* in Thick-billed Murres. Similarly, wintering Thick-billed Murres from Newfoundland also revealed high prevalence and abundance of this tapeworm. It is widely believed that a significant portion of wintering murres in Newfoundland originates in Greenland (Gaston and Jones 1998). The prevalence and abundance of the tapeworm in specimens from either sample did not vary significantly suggesting strongly a Greenland origin of these birds.

Summary and Conclusions

Ecology and evolution of endoparasites in the marine environment are strongly linked with environmental factors and host associations. In this study, I investigated this by evaluating the endoparasite fauna of murres from several localities in the Northwest Atlantic. Assemblages of parasites differed from those collected in the same region in earlier studies. Tapeworms of the genus *Alcataenia* were dominant among the tapeworms, especially in the Thick-billed Murres. I recorded the first evidence of a North Pacific species, *A. longicervica* in the North Atlantic. Patterns of prevalence and intensity of this tapeworm in my study suggested that two possible routes might have been used in the range expansion of the species. I provide evidence to suggest that a greater movement of this tapeworm likely occurred in the route involving overlapping distributions of *Thysanoessa* species in the Chukchi Sea and the East Siberian and Laptev Seas. Climatic variations and associated anomalies in the Arctic Ocean most likely facilitated this range expansion. My data also supports the widely held belief that a considerable portion of the wintering Newfoundland murres is from Greenland. The role of parasites in evaluating climate-related change in the greater marine ecosystem is emphasized. Limited information is available on parasites in the marine environment in general and their utility in understanding marine

ecosystem dynamics has been neglected. With rapidly changing dynamics of the world's climate and its associated impacts on terrestrial and aquatic ecosystems, there is an urgent need to incorporate parasites in ecological studies in general and marine ecological studies in particular.

CHAPTER 6. PARASITES AND DISEASES IN A CHANGING WORLD

Parasites and diseases, their ecology, evolution and distribution patterns have come to the forefront in our recognition of a changing world (Cohen 2000, Hudson et al. 2002, Lafferty and Kuris 2005, Wilcox and Colwell 2005). This is primarily due to widespread emergence and re-emergence of diseases of human concern. Congruent to this has been an emerging paradigm suggesting a link between environmental stress and *increased* risk of disease emergence (Cohen 2000, Wilcox and Colwell 2005, Lafferty and Kuris 2005). Whereas there are clear relationships between anthropogenic environmental stresses (such as habitat alteration, biodiversity loss, pollution, climate change, exotic species) and diseases and parasites, the relationships are not necessarily linear, straightforward or invariably capable of causing increases in parasites and diseases (Lafferty and Kuris 2005).

The diverse and complex life histories of parasites and their hosts encourage the abundance of certain parasites under stressful conditions while eliminating others. Changes in foraging habits resulting in the altered feeding ecology of gull species may result in changes in the prevalence of digenean trematodes (Bustnes and Galaktionov 1999). Gulls feeding more frequently on

offal, feed less frequently on coastal snails that are intermediate hosts to several digenean species, thereby gradually diminishing and eventually eliminating the final link (entry into the seabird definitive host) of the parasite's life cycle. This in turn could eliminate the parasite locally (Bustnes and Galaktionov 1999). Habitat alterations could influence the spread of vectors of disease thereby increasing the prevalence of diseases. A well-known example of this is the rapid spread of Lyme disease in continental North America. Reforestation of formerly deforested areas coupled with unchecked increases in White-tailed Deer, *Odocoileus virginianus*, in the absence of its native predators (wolves, *Canis lupus*, and other predators) and a substantially reduced hunting pressure, have resulted in the unprecedented range expansion of the Deer Tick (*I. scapularis*) throughout the United States and southern Canada. The increased interactions of humans with nature have resulted in greater exposure of ticks to humans resulting in increasing cases of Lyme Disease in North America. Climate change models, predict that increasing temperatures are likely to further increase distributions of Lyme Disease in North America (Ogden et al. 2005).

In my study using avian parasites and diseases, I have attempted to illuminate several points:

a) Parasites and diseases are integral components of ecosystems and their

study should be approached from a holistic, ecological viewpoint, rather than just the medical viewpoint. The study of endoparasites of auks illustrates the complexity of tapeworms and their associations with their seabird hosts. This also reflects interactions at different spatial and temporal scales, with multiple factors contributing to observed distribution patterns. Studying such complex associations constitute an important challenge to the ecological study of parasites.

b) Anthropogenic perturbations strongly influence parasite communities and disease transmission and assessing this aspect of parasite ecology is critical. The presence of *Borrelia garinii* in seabird colonies could have been aided by the increase in prevalence of this spirochete in human dominated landscapes, coupled with bird movements and introductions of mammals on remote seabird colonies. The presence of *B. garinii* in seabird colonies close to the coast could lead to its establishment in mainland colonies such as Cape St. Mary's, Newfoundland. Visitors on the reserve often return from tours close to the seabird colonies with ticks on their clothing and therefore, the human concern of this seabird-tick-*Borrelia* cycle needs to be better studied and understood.

c) Profound anthropogenic changes may influence parasite distributions and ecology. The range expansion of *Alcataenia longicervica* from the North Pacific

to the North Atlantic could be an indirect result of human induced climate change in the Arctic, inducing changes in oceanic currents, host distribution patterns and feeding behavior.

d) The current world is far from natural and human movements and trade provided unprecedented opportunities for parasites to spread, evolve and thrive over wider geographic areas. The avian influenza outbreaks in Asian poultry and their spread into various countries using cultural vectors highlight this new socio-ecological landscape that facilitates parasite and disease spread.

Recognition alone of these various factors influencing parasite and disease dynamics in a profoundly different world will be of no use. We urgently need to determine how parasites behave, spread and evolve in these novel circumstances through carefully designed ecological studies addressing key questions in epidemiology and ecology of diseases. Only through these studies will we be able to better understand and predict the future dynamics of diseases and better assess threats associated with emerging diseases.

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Appendix I: Voucher specimens of endoparasites submitted to the United States National Parasite Collection[1, 2]

Parasite: ALCATAENIA LONGICERVICA. Class: CESTODA
Host: URIA LOMVIA. Body Location: SM INTESTINE (ant 3rd)
Locality: NORTH AMERICA, Canada,NL,Harbor Breton. Identifier: MUZAFFAR, S B & HOBERG, E P 03 OCT 2005. Collector: ROBERTSON, G 02 FEB 2005.
Accession No.: 097386.00. Type: VOUCHERS. Storage No. 207A-24/25
Comments: 2 slides, vouchers (scolices, few proglottids). GRobertson-SBM-TBMU-007-1 & 2 (Candian Wildfile Service). Semichon's acetocarmine/Canada balsam. Pub Marine Ornithology: in press.

Parasite: ALCATAENIA LONGICERVICA. Class: CESTODA
Host: URIA LOMVIA. Body Location: SM INTESTINE (ant 3rd)
Locality: NORTH AMERICA, Canada,NL,Harbor Breton. Identifier: MUZAFFAR, S B & HOBERG, E P 03 OCT 2005. Collector: ROBERTSON, G 02 FEB 2005.
Accession No.: 097387.00. Type: VOUCHERS. Storage No. 209A-11/13
Comments: 3 slides, vouchers (scolices, few proglottids). GRobertson-SBM-TBMU-009-1, 2 (scloex imbedded in gut wall) & 4 (Candian Wildfile Service). Semichon's aceto-carmine/Canada balsam. Pub Marine Ornithology: in press.

Parasite: ALCATAENIA LONGICERVICA. Class: CESTODA
Host: URIA AALGE. Body Location: SM INTESTINE (ant 3rd)
Locality: NORTH AMERICA, Canada,NL,St. Mary's Bay. Identifier: MUZAFFAR, S B & HOBERG, E P 03 OCT 2005. Collector: DUSSUREAULT, J 08 FEB 2005.
Accession No.: 097388.00. Type: VOUCHERS. Storage No. 209A-14/18
Comments: 5 slides, vouchers (5 specimens, many proglottids). GRobertson-SBM-COMU-004-1 (2 slides), 2, 3 , & 4 (Candian Wildfile Service). Semichon's acetocarmine/Canada balsam. Pub Marine Ornithology: in press.

1. **United States National Parasite Collection**
   Animal Parasitic Disease Laboratory
   USDA, Agricultural Research Service
   BARC East No. 1180, 10300 Baltimore Avenue
   Beltsville, Maryland 20705

2. **Generated by the online searchable database of the USNPC: http://www.anri.barc.usda.gov/bnpcu/parasrch.asp**