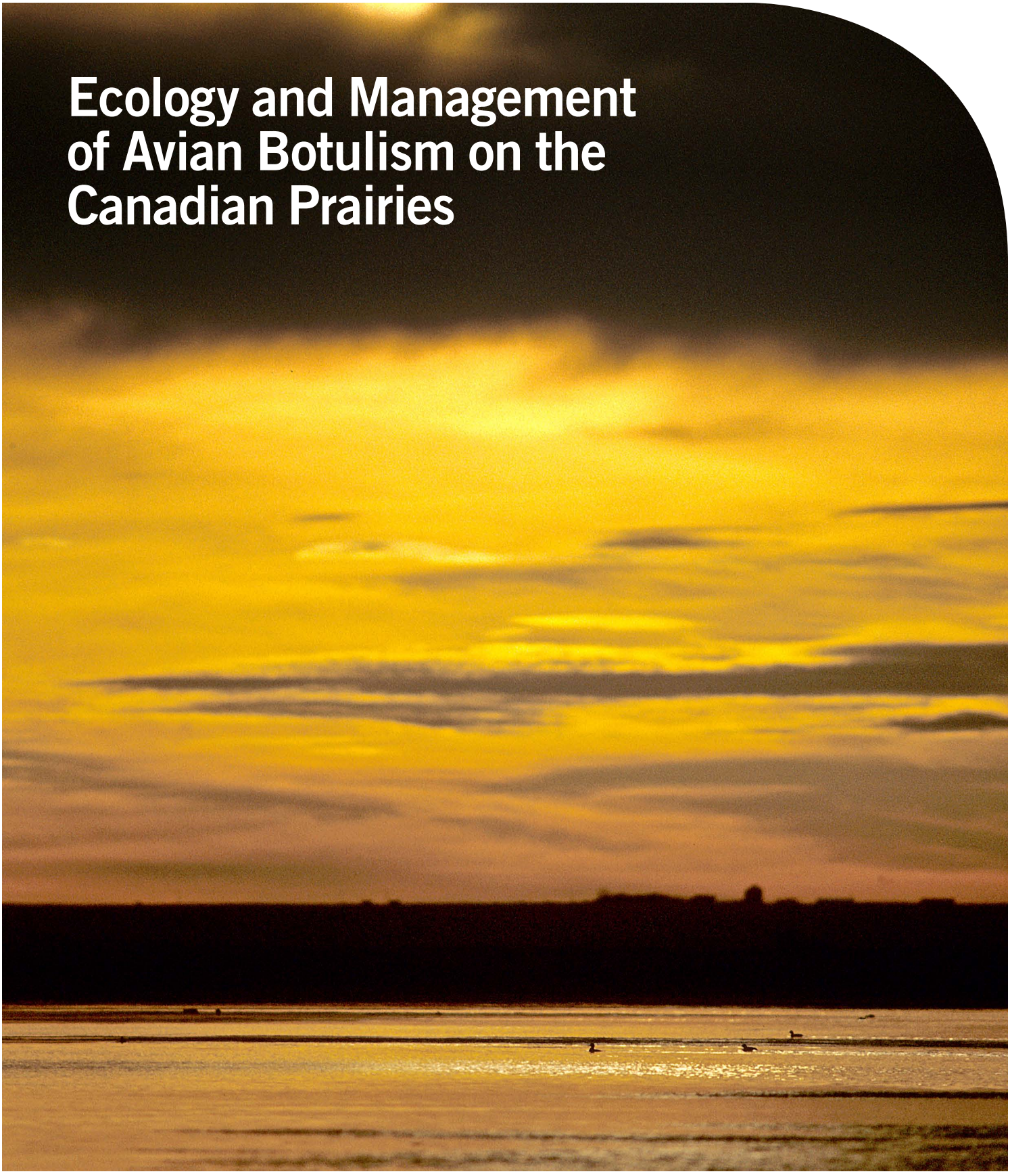
Ecology and Management of Avian Botulism on the Canadian Prairies



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ECOLOGY AND MANAGEMENT OF AVIAN BOTULISM ON THE CANADIAN PRAIRIES

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EXECUTIVE SUMMARY

Avian botulism (*Clostridium botulinum*, Type C; hereafter 'botulism') has occurred on the Prairies and elsewhere for centuries. Botulism affects varying numbers of birds, often in multiple locations, at some time virtually every year. After the formation of the Prairie Habitat Joint Venture (PHJV) in 1988, and as early as 1994, it was becoming increasingly apparent that very large numbers of ducks and shorebirds were dying annually during botulism outbreaks at several large wetlands on the Canadian Prairies. The magnitude of these losses created considerable consternation in the North American waterfowl and wildlife management community.

At the time, wetland surveillance and carcass removal (or 'clean-up') were used to try to limit the severity of these outbreaks, but the efficacy of this approach was unknown. In 1997, the PHJV Advisory Board responded by creating an Avian Botulism Working Group to identify critical research needs and management objectives. From 1998 to 2001, this group coordinated a Prairie-wide investigation to determine whether carcass removal could reduce waterfowl losses and whether late-summer survival of mallards (*Anas platyrhynchos*) was reduced during botulism outbreaks. The group also sought to learn more about the basic ecology of avian botulism, with the goal of providing clues about other management options.

Botulism Ecology

Initial investigations of botulism ecology focused on whether and why abiotic and biotic factors could trigger and perpetuate botulism outbreaks. The source of substrate for initial proliferation and toxigenesis of botulism prior to outbreaks was unknown.

One study investigated the factors involved in the initiation of avian botulism outbreaks, by focussing on the role of Franklin's gull (*Larus pipixcan*) mortality as a source of initial substrate for *C. botulinum*. Hatch-year Franklin's gull carcasses were the predominant source of toxin-laden maggots found prior to outbreaks of avian botulism in waterfowl. Peak carcass density of gulls occurred one to two weeks prior to the onset of botulism outbreaks in waterfowl.

Gull carcasses were 22.7 times more likely to become maggot-laden at ambient temperatures ≥20 C than were carcasses exposed to lower temperatures. High *C. botulinum* toxicity of maggots produced in gull (or eared grebes [*Podiceps nigricollis*]) chick carcasses coincided with high densities of susceptible birds. Hence, mortality of gulls or other birds may be a major initiating factor to trigger some botulism outbreaks.

In controlled studies of mallards collected from a range of wetlands, abundance of botulism spores and the potency of toxins produced from spores in birds varied considerably among study wetlands. The proportion of carcasses producing type C *C. botulinum* toxin under controlled conditions in mallard carcasses varied from 0 to 100% across lakes. The prevalence of toxin production on non-botulism lakes ranged from 0 to 38% (median = 17%), whereas lakes with a history of botulism had prevalences ranging from 15 to 100% (median = 71%).

The model of botulism ecology, developed by the working group in 1998, should continue to be used as a basis for management and research, and should be revised as new data become available. However, new quantitative evaluations should also be undertaken to determine the relative importance of different risk factors that could contribute to the initiation and perpetuation of botulism outbreaks.

Carcass Removal

Removing carcasses to prevent propagation of botulism outbreaks is a long-standing approach to managing avian botulism. However, to our knowledge, its effectiveness under field conditions had not been investigated prior to this study.

We demonstrated that carcass clean-up operations conducted during botulism outbreaks removed <50% of carcasses on small wetlands, at best, whereas <10% of carcasses were found on larger, heavily-vegetated wetlands. Most marked carcasses did not sink and were not removed by scavengers for >12 days, and therefore should have been available for detection by clean-up crews. Thus, the main factor causing low carcass recovery was the inability of clean-up crews to discover carcasses in dense vegetation.

Mallard Survival

Using radio-marked mallards, we determined probable cause(s) of mallard mortality, comparing survival among wetlands with carcass removal to wetlands where carcasses were not collected, and evaluated how survival was related to lake-wide estimates of carcass density. Mallard mortality risk was related to carcass density, such that birds exposed to high carcass densities (especially high densities of maggot-laden carcasses) had high mortality risk, consistent with the assumption that reduction of carcass densities to low levels should enhance survival of healthy birds. However, carcass removal did not consistently enhance survival of radio-marked, moulting mallards, even on small wetlands with intense surveillance.

Although catastrophic losses of waterfowl to botulism are well documented, little is known about the impact of the disease at the population level. We used band-recovery data from mallards trapped at nine major outbreak sites in Prairie Canada, to test the hypothesis that individuals exposed to outbreak levels of botulism during the post-breeding season suffer reduced late-summer survival as a consequence. Direct recovery analyses revealed that late-summer survival of mallards from Saskatchewan and Manitoba was reduced substantially (14-44% depending on year and location) when birds were associated with botulism outbreaks. In contrast, results from Alberta varied but did not show reduced direct recovery rates for reasons that remain unclear. On botulism-prone wetlands, direct recovery rates of banded mallards were not higher on clean-up versus non-clean-up lakes. Thus, there was no compelling evidence that clean-up was effective in reducing botulism losses.

Studies are needed to determine the extent to which results for mallards can be generalized to other avian species, particularly those of potential management concern (e.g., northern pintails

[*Anas acuta*]). Impacts of botulism on population growth rates may be evaluated most effectively within a population modeling framework. The effects of botulism on continental populations will depend not only on the mortality rates of exposed individuals, but also on the proportion of individuals in the population that are routinely exposed, and acquiring information on the size of the population at risk will be essential.

In addition, further work is needed to determine how spore and toxin production and potency interact to affect survival, and whether these factors may be managed to reduce occurrence or severity of botulism outbreaks.

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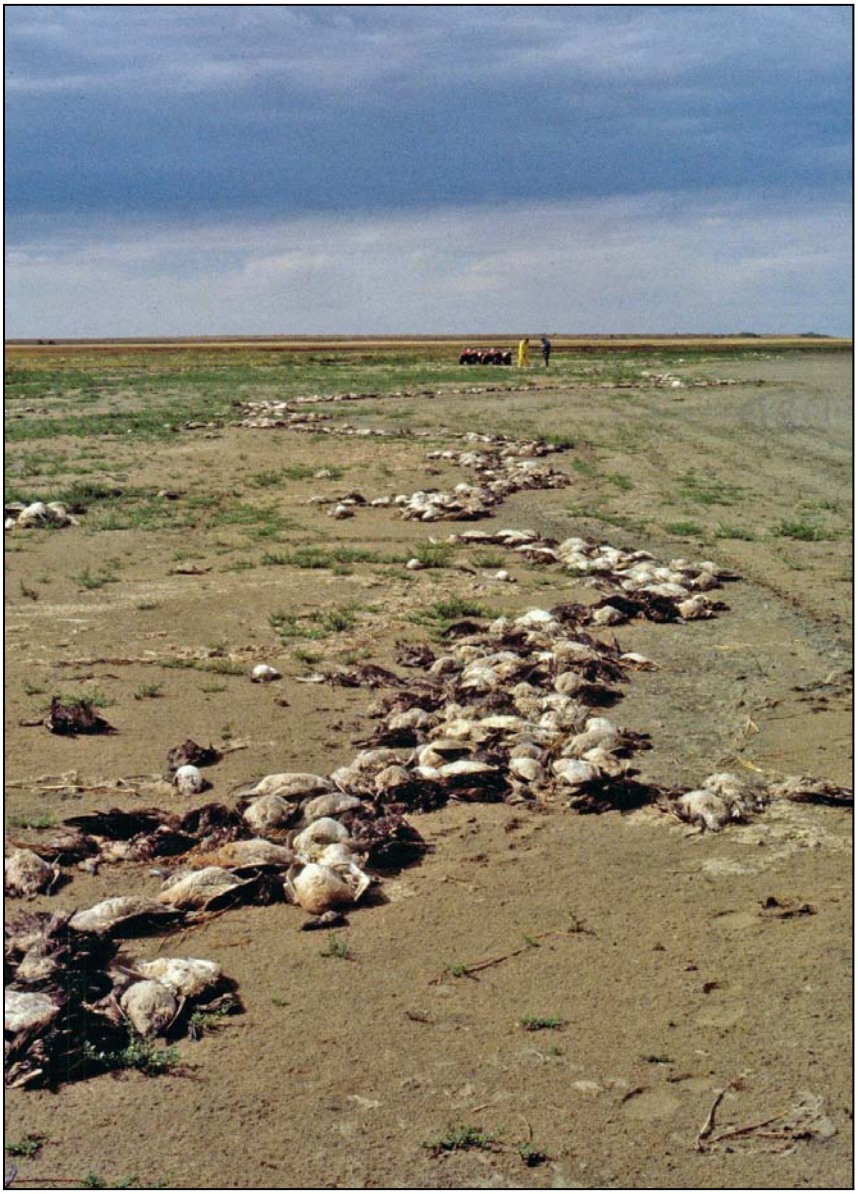
GENERAL INTRODUCTION

Type C *Clostridium botulinum* is a naturally occurring bacterium, which produces the neurotoxins that cause the paralytic disease known as botulism. Botulism results in significant mortality in North American birds (Hunter et al. 1970; Smith 1976; Samuel 1992) and has resulted in die-offs (see below) of hundreds of thousands of waterbirds on a single wetland, in a single season (Rocke and Bollinger 2007).



There is evidence that botulism has occurred in North America for almost 100 years, and that it recurs every year. Botulism has been reported on several lakes in Prairie Canada (Figure 1; Table 1; see Appendix 1 regarding Alberta), including significant outbreaks in the 1990s. These outbreaks raised serious concerns because avian breeding success and mortality in this region has significant impacts on waterfowl and other migratory birds at a continental scale.

The outbreaks of the 1990s raised questions, not only about the impact of botulism on waterfowl populations, but also about the potential impacts of the disease on programs designed to conserve waterfowl and their habitats. The public began to question organizational commitment to waterfowl management within Canada if conservation agencies could allow such mortality to occur. At the same time, organizations involved in botulism management started to question the efficacy of traditional management techniques, and expressed concern over the apparent lack of options for dealing with disease outbreaks.

Research from the 1970s supported the idea that botulism outbreaks were perpetuated by the proliferation of *C. botulinum* toxin in decomposing bird carcasses, and the subsequent consumption of toxic maggots from these carcasses by healthy birds (Hunter 1970). Therefore, the management response in Canada was to continually survey botulism-prone wetlands and to remove dead birds (see below). By reducing the availability of toxin-laden maggots, this technique (hereafter carcass collection, pickup, removal, or clean-up) was expected to break the carcass-maggot botulism cycle (Hunter 1970) and thus increase waterfowl survival. Yet, despite the significant cost of carcass collection, which was roughly C$1 million in 1997, its efficacy was never tested under field conditions in the 60 years since it was first employed.

With concerns about botulism growing, questions relating to its ecology, its impact on bird populations, and how it should be managed remained unanswered. At the time of the 1990s outbreaks, studies of botulism had been fragmented and short-term, and usually occurred at a local level. Left with an incomplete understanding of the factors that precipitated botulism outbreaks and an inability to predict outbreaks, managers continued to carry out carcass removal while calling for more research to inform future management decisions.

PHJV AVIAN BOTULISM WORKING GROUP

Agencies involved in waterfowl and avian habitat management in Prairie Canada are drawn together under a partnership called the Prairie Habitat Joint Venture (PHJV). In support of the goals of the North American Waterfowl Management Plan (NAWMP), the PHJV mission is to achieve healthy and diverse populations of waterfowl and other birds through conservation programs and actions. To address concerns raised during 1990s botulism outbreaks, the PHJV formed the Avian Botulism Working Group in 1997. This working group brought together biologists experienced with botulism, including provincial agencies and Ducks Unlimited Canada (DUC), as well as scientists from Environment Canada, DUC's Institute for Wetland and Waterfowl Research, the National Wildlife Health Centre (U.S. Geological Survey), the U.S. Fish and Wildlife Service, Utah State University, and the Canadian Cooperative Wildlife Health Centre (CCWHC).

Initially, the working group reviewed the botulism situation in Prairie Canada and agreed that comprehensive studies were required to address the following three critical information gaps:

1. The impact of botulism on:
   - local populations on individual lakes
   - continental populations
   - populations of species of conservation concern
2. The effectiveness of carcass pickup/recovery/clean-up operations
3. Methods for assessing risks of botulism on a wetland

SUMMARY OF PREVIOUS REPORTS

In 1998, the Avian Botulism Working Group presented the PHJV Advisory Board with a report, which outlined their initial findings and made recommendations to guide further action and investigation. The group also reported on their progress and plans in a 1999 document entitled *A Summary and Pre-Proposal for Adaptive Resource Management of Avian Botulism on the Canadian Prairies*. Both reports are summarized below. Because the information presented in these summaries is taken directly from the original documents, it reflects the understanding of botulism prior to 1999. Subsequent sections will outline how this knowledge has since expanded, and will make recommendations as to how these insights can guide further action.

1998 report

General information about botulism

C. botulinum is a naturally occurring bacterium with worldwide distribution. It produces neurotoxins, which are among the most poisonous substances in the world and cause the paralytic disease known as botulism. These neurotoxins can be divided into seven toxinotypes (A to G) based on their antigenicity. This discussion deals only with type C because botulism in wild birds, with a few exceptions in fish-eating birds, is caused by that toxinotype.

Type C *C. botulinum* exists as spores, or as vegetative (growing) cells that may produce toxin. Spores of this bacterium are very widespread in soils, especially in wetlands (Dobbs 1992), and sites of former botulism outbreaks can be heavily contaminated with them (Wobeser et al. 1987). The spores are extremely resistant and can persist in wetlands that are dry for years (Wobeser et al. 1987). However, the factors that influence spore density in soil are poorly understood.

Spores only begin vegetative growth under specific circumstances, including: anaerobic (free of oxygen) conditions; temperatures of 10 C or higher; a pH between 5 and 9; and a nutrient substrate with a high protein content, such as the carcass of a vertebrate or invertebrate animal

(Coburn and Quortrup 1938; Bell et al. 1955). In the absence of these conditions, a reversion to the spore form occurs. Vegetative growth can also be inhibited by other factors that are not well understood. How these limiting factors might apply to type C *C. botulinum* growth in different substrates, such as within carcasses, is also largely unknown.

Botulism in wetlands and waterfowl

Type C *C. botulinum* spores are readily available in wetlands, and a wide range of wildlife species ingest them continuously. Spores have been detected in the liver of birds not affected with botulism (Gunderson 1933), as well as in the intestines of fish (Itoh et al. 1978) and within the liver and intestines of healthy mallards (*Anas platyrhynchos*) (Reed and Rocke 1992). The frequency of occurrence, as well as the density and residency time of spores in different animals, have not been established; nor has the relationship between the prevalence of spores in the environment and the prevalence of spores in animals.

When an animal with spores in its gut or tissue dies, its carcass becomes anaerobic and its tissues are invaded by *C. botulinum* spores, which then begin vegetative growth and toxin production if conditions are favourable (Smith and Turner 1987). This process of toxin development has been reproduced in various insects, birds, and mammals (Hunter 1970; Notermans et al. 1980; Reed and Rocke 1992). Vertebrate carcasses tend to support particularly high levels of *C. botulinum* toxin production; mostly likely because they provide a large amount of suitable substrate, a self-contained microenvironment, and high temperatures that are optimal for growth and toxin production (Duncan and Jensen 1976; Wobeser and Galmut 1984). The amount of toxin produced in different types of animal carcasses may vary (Smith and Turner 1989), but this has not been examined carefully.

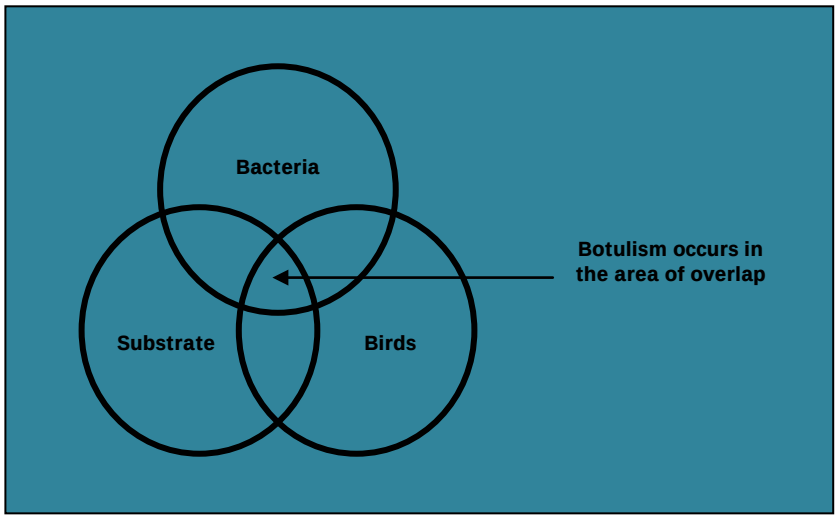
When the type C neurotoxin is ingested by a bird, it is absorbed into their blood and binds to specific receptors on their motor neurons. The toxins then block the transmission of nerve impulses involved in muscle activation, which results in the flaccid paralysis seen in birds affected by botulism (Schiavo et al. 1995) (see left). Ingestion of pre-formed toxin is the major route of botulism intoxication in all wildlife species, including wild

birds. There is no evidence that birds become intoxicated through drinking water, possibly because toxins do not reach high enough concentrations in water to cause poisoning. The most likely source of toxin is toxin-bearing invertebrates, and bird species differ markedly in the probability of ingesting these due to differences in feeding behaviour. All birds, with the possible exception of vultures (Kalmbach 1939; Cohen et al. 1969), are susceptible to ingested type C neurotoxin, but there is very little information on interspecific differences in susceptibility.

In California, Rocke et al. (1999) determined that certain wetland environmental conditions were associated with botulism outbreaks. They found an association, in time, between the occurrence of outbreaks and: increasing sediment and water temperature; increasing abundance or biomass of invertebrates; and decreasing turbidity. Wetlands with botulism outbreaks also had lower redox potential than non-outbreak wetlands. In later studies, which were conducted on a larger geographic scale, outbreaks were more likely when water pH was in the 7.5-8.5 range, redox potential was negative, and temperature exceeded 20 C, although the relationship among these variables was complex (Rocke and Samuel 1999). How these physiochemical factors might relate to bacterial abundance, growth and toxin production, as well as the availability of toxin to birds, is unknown.

Models of botulism ecology

The most basic conceptual model of avian botulism has three components: i) the bacterium, ii) the substrate within which the bacterium grows and produces toxin, and iii) a population of susceptible birds. Each of these three components is influenced by many other environmental factors, and botulism only occurs when all three components are present and environmental conditions are favourable. For example, toxin may be produced if toxin-producing bacteria and substrate are present, but the disease won't occur if birds are absent. Similarly, if the bacterium and birds are present but substrate is not available for bacterial growth, no toxin will be formed. In a simple schematic model, the risk of botulism is dependent upon the overlap among all three components (see right).



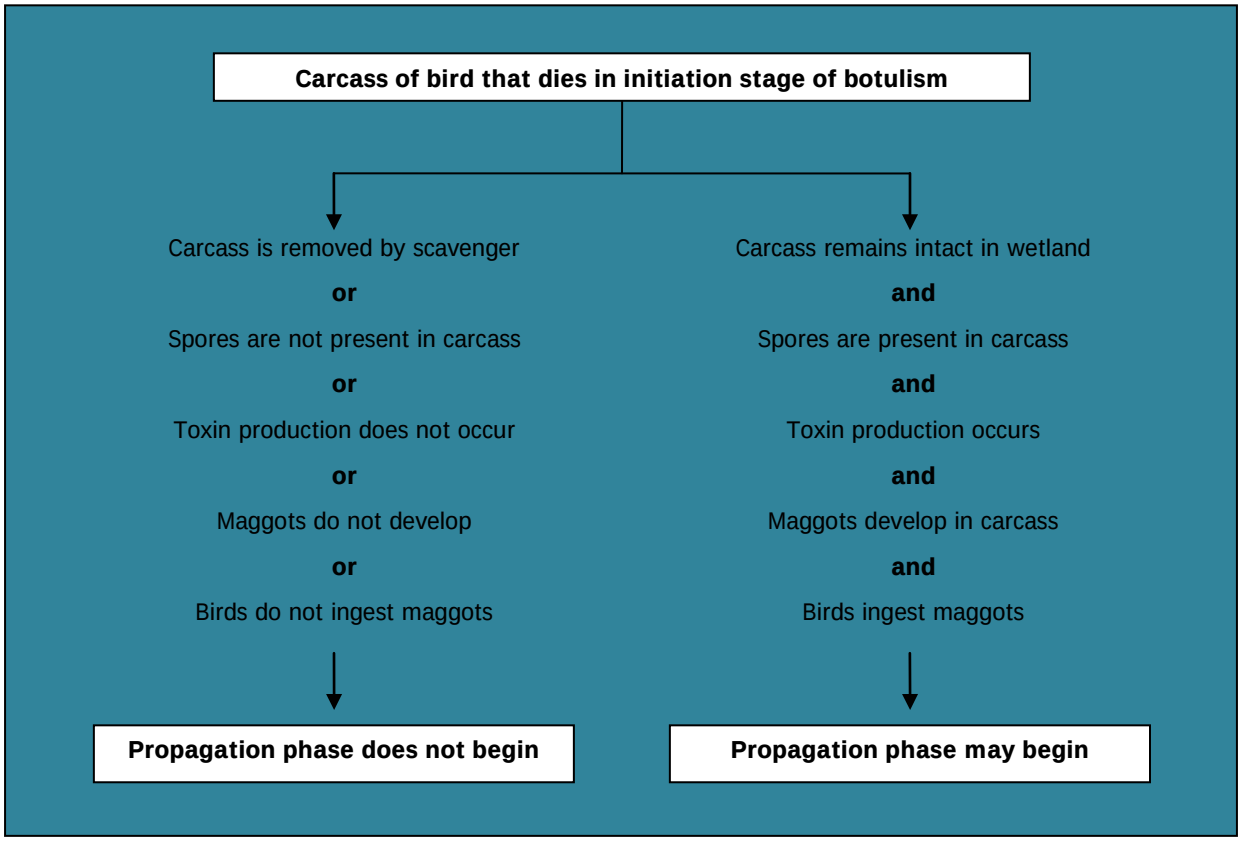
There are two distinct phases in the ecology of botulism: an initiation phase and a propagation phase. The basic bacteria-substrate-birds model (see above) applies to both phases, but they differ in the type of substrate involved. In the initiation phase, any protein-rich organic matter

could potentially serve as substrate for bacterial growth. The actual source of toxin (i.e., the substrate involved) that initiates mortality in wild birds is almost never known, but three potential substrates have been identified: invertebrate carcasses, vertebrate carcasses, and non-carcass proteinaceous material. In the propagation phase, carcasses of birds that have succumbed to botulism are the substrate within which toxin production occurs. Although waterbirds, other than gulls, do not feed directly on carcasses, they can contract the toxin by eating toxin-bearing invertebrates such as maggots. Once intoxicated, they will then succumb to botulism and their carcasses will, in turn, produce more toxin-bearing maggots. Through this carcass-maggot botulism cycle, the potential for expansion of botulism from a single carcass is immense.

Because the three components (bacteria, substrate, and birds) of the botulism model are normally present in most wetlands, it is likely that the initiation phase of botulism occurs commonly and repeatedly in wetlands. When conditions are favourable and a large amount of toxin is produced, many birds may die. This could explain why some botulism outbreaks begin after some environmental event, such as a hail storm that kills many birds, a fish kill, or other events that provide suitable conditions for bacterial growth and toxin formation.

The carcass of a bird that dies in the initiation phase may or may not become substrate for toxin production. For this to occur, the carcass must contain spores and must remain intact long enough for toxin and maggots to develop. Factors that influence this step include the rate at which toxin and maggots develop in the carcass, and the rapidity and thoroughness with which

carcasses are removed by larger scavengers. When temperatures are high and flies are abundant, carcasses may contain toxin-laden maggots within two to three days. Under cool conditions, carcasses may remain free of maggots for weeks (Wobeser and Galmut 1984). If scavengers are abundant, and carcasses are relatively few in number, most may be removed before toxin develops. However, if there are many carcasses in an area, scavengers may be saturated so that most carcasses persist until maggots are present (Cliplef and Wobeser 1993). When a bird succumbs to botulism, its carcass can be involved in one of two pathways (see below).



1999 report

Avian botulism losses and management

Avian botulism continued to be a problem on the Canadian Prairies, with total waterfowl losses estimated to be in excess of one million birds in 1998. Although these losses were comparable to those of 1997, the pattern of mortality was different in 1998. In 1997, as in the previous three years, the greatest losses were observed on three large terminal basins in the southern Canadian Prairie: Pakowki Lake, Alberta (45,048 *collected*); Old Wives Lake, Saskatchewan (1,000,000 *estimated dead*); and Whitewater Lake, Manitoba (49,000 *collected*). In contrast, avian botulism mortality was relatively low on these three lakes in 1998, but was recorded on approximately 23

additional lakes and marshes. Although this was the first time botulism had been detected on some lakes, botulism had not been reported on others for the previous 20-40 years. The outbreaks of 1998 were also unusual in that two of the largest die-offs were in the boreal forest ecoregion of northern Alberta, and diving ducks comprised a significant proportion of the carcasses collected in one of these die-offs. In total, approximately 210,000 carcasses were collected during clean-up operations across the Canadian Prairie in 1998, and direct costs of this clean-up were conservatively estimated at C$950,000.

Regional investigations

In response to high botulism losses, the PHJV funded a regional coordinator and investigation unit to conduct studies recommended by the Avian Botulism Working Group, on Pakowki, Old Wives, and Whitewater lakes June-August 1998 (see below). From these investigations, the working group learned that carcass counts from removal efforts during botulism outbreaks underestimated total mortality by a factor of four to ten, which was significantly greater than the correction factor of two that had been proposed in the past. Using a correction factor of five, and records indicating that over 200,000 carcasses were retrieved during botulism outbreaks in 1998, it was estimated that at least one million birds died of botulism on the Canadian Prairies that year. Low clean-up efficiencies meant that a large number of carcasses remained on the marsh to perpetuate the carcass-maggot botulism cycle. This was confirmed by carcass density maps, which revealed areas of marshes with densities of 20-50 carcasses/ha during clean-up operations.

Potential risk factors for botulism outbreaks were also identified at the three lakes in 1998. First, high carcass densities were associated with Franklin's gull (*Larus pipixcan*) colonies. Second, a greater proportion (~90%) of maggot-laden carcasses contained type C toxin on all three lakes than on outbreak sites (~40%) in California (Reed and Rocke 1992), implying a high risk of exposure to toxin at these Canadian lakes.

Priority areas

In order to move forward, the working group proposed to focus on three main priority areas and associated objectives:

1. Effectiveness of management
   At the time of the 1999 report, it was evident that methods of surveillance and carcass removal were not effective on large lakes. Therefore, the group proposed to determine whether carcass removal can be made more effective and, if so, how this could be achieved. They also proposed to determine whether birds are saved as a direct result of management, and planned to address these questions in the following three inter-related ways:
   a) Vary the level of management effort on three to four wetlands, and determine the effort required to reduce densities of toxin-producing carcasses to levels where survival of birds is increased (see b, below).
   b) Directly measure survival of radio-marked birds (pintails, if possible) on wetlands subjected to different levels of management effort (see a, above). This could also indicate the level of effort required to increase survival of birds.
   c) Over time (e.g., three years), specify a level of effort required to reduce carcass density and bird mortality, and then measure carcasses and survival of birds in response to management effort, comparing predicted against measured responses.

2. Effects on populations
   On the scale of individual wetlands, it is possible to estimate whether mortality is reduced as a result of management (see 1, above). However, if subsequent fall or breeding population sizes are not reduced because of botulism mortality, then outbreak management is not necessary because these efforts would not increase the number of birds significantly. While the group felt this issue couldn't be ignored, they also cautioned that it wouldn't be easily addressed. In 1998, they began to tackle the issue by banding and vaccinating ducks on two lakes (one with clean-up and the other without). However, they encountered problems associated with timing of banding relative to disease outbreak, as well as uncertainty surrounding the efficacy of the vaccine employed. The group planned to address these problems in three ways:
   a) Determine whether the vaccine increases survival in the field using radio-marked ducks and controls and, if not, assess ways of making the vaccine work.
   b) Continue banding (and vaccination if the vaccine is effective).
   c) Explore the possibility of modelling effects of botulism on pintail population dynamics once better estimates of survival rates are acquired.

3. Evaluating other management options
   To ensure that newly proposed management efforts weren't misdirected, the group required a better understanding of additional factors. First, the group needed to know more about the key components of the ecology of botulism, such as other sources of bird mortality and alternate botulism vectors. Second, the group needed to know how many birds die for reasons other than maggot ingestion during an outbreak. This could pose a

challenge because management assumed that maggots are the source of toxin, and was therefore geared to eliminating maggot-laden carcasses. To address these two issues, a series of small-scale studies were proposed to evaluate factors that might contribute to the occurrence and magnitude of an outbreak but are not directly associated with the carcass-maggot cycle, such as: nutrients, blue-green algae, weather, and other animals (e.g., gulls).

ORGANIZATION OF THIS REPORT

The remainder of this report summarizes the studies conducted in association with the PHJV Avian Botulism Working Group since 1999. These findings, coupled with the background information outlined above, provide a framework for future efforts to understand this disease and manage its impacts on waterfowl populations.

Carcass collection has been the only active management technique used in Western Canada for botulism, yet its efficacy has not been assessed under field conditions. Investigations of the efficacy of carcass collection are presented in Part I of this report

In their 1998 report, the working group created a working model of botulism ecology. Information required to revise this model, as well as factors that influence the progression of botulism, have since been identified and studied. The results of these studies are discussed in Part II and Part III of this report. The working group also explored the potential role of blue-green algae in causing initial mortality events that could act as a catalyst for botulism outbreaks, and presents results of this study in Appendix 2.

Population impacts of botulism must be considered at local, regional, and continental levels. The effect of this disease on species of conservation concern, such as the northern pintails and shorebirds, requires special attention. Fundamental to assessing population effects and the efficacy of botulism management techniques is the need to determine the mortality rate (the number of birds that die of botulism / number of birds in the population at risk) for different species and groups. Three separate studies were undertaken to assess the population impacts of botulism on mallards, and these are presented and Parts II, III, IV, and VI of this report.

Key findings of all studies are discussed in the Main Conclusions and Recommendations section at the end of this report. This last section presents results in an adaptive management framework, to guide future efforts to advance our understanding of the ecology of botulism and its impacts on avian populations, as well as potential management strategies.

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Figure 1. Map indicating lakes where botulism has been reported in Prairie Canada to date. Names and coordinates of individual lakes are provided in Table 1.

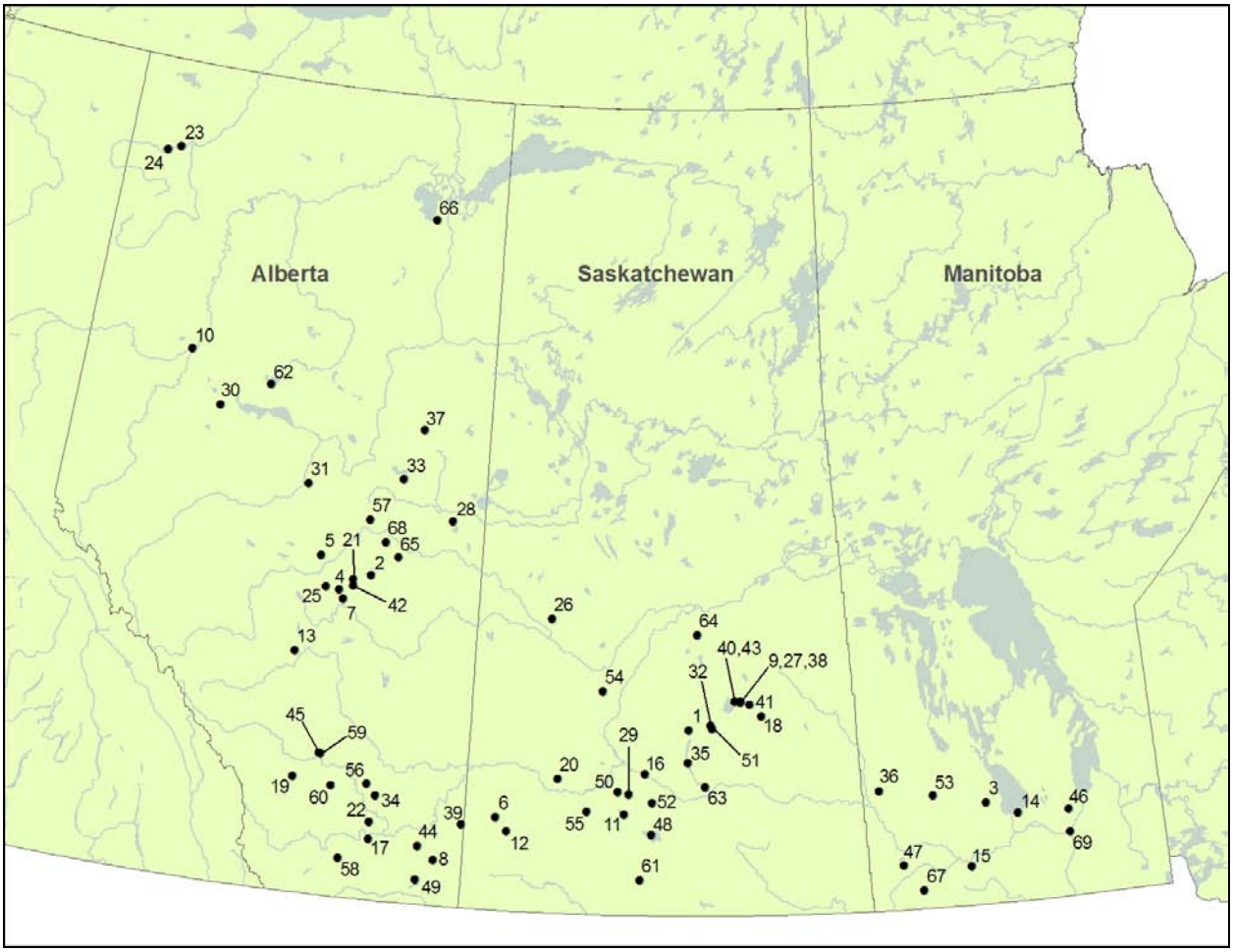

Table 1. Names and locations of lakes where botulism has been reported in Prairie Canada to date. Lake numbers correspond to the numbers in Figure 1.

Lake #	Location	Province*	Latitude	Longitude
1	Axe Lake	SK	51.53333	-105.21667
2	Beaverhill Lake	AB	53.41667	-112.50000
3	Big Grass Marsh	MB	50.38194	-98.85528
4	Big Hay Lake	AB	53.17000	-113.20000
5	Big Lake	AB	53.61667	-113.70000
6	Bigstick Lake	SK	50.26666	-109.33333
7	Bittern Lake	AB	53.05000	-113.08333
8	Buffalo Lake	AB	49.61667	-110.58333
9	Campbell Project, SE Little Quill Lake	SK	51.92000	-104.08000
10	Cardinal Lake	AB	56.14000	-117.44000
11	Chaplin Lake	SK	50.36670	-106.60000
12	Crane Lake	SK	50.08000	-109.08000
13	Cygnet Lake	AB	52.28000	-114.02000
14	Delta Marsh	MB	50.19861	-98.20473
15	Dowd Slough	MB	49.53000	-99.27000
16	Eyebrow Lake	SK	50.93333	-106.14999
17	Fincastle Lake	AB	49.83333	-111.98333
18	Foam Lake	SK	51.71667	-103.61667
19	Frank Lake	AB	50.57000	-113.72000
20	Galloway Bay	SK	50.83333	-108.03335
21	Gambling Lake	AB	53.33333	-112.90000
22	Grantham	AB	50.06667	-112.00000
23	Hay Lake	AB	58.82560	-118.66850
24	Hay Zama Lakes	AB	58.75000	-119.00001
25	Hay Zama Lakes II	AB	53.20000	-113.50000
26	Jackfish Marsh	SK	53.01370	-108.31931
27	Jesmer Marsh, NE Little Quill Lake	SK	51.92000	-104.08000
28	Jessie Lake	AB	54.25380	-110.73770
29	Kettlehut Lake	SK	50.65000	-106.50000
30	Kimiwan	AB	55.45000	-116.55000
31	Kings Lake	AB	54.56667	-114.20001
32	Kutawagan Lake	SK	51.60000	-104.73333
33	Lac La Biche	AB	54.77000	-111.97000
34	Lake Newell	AB	50.43000	-111.92000
35	Last Mountain Lake	SK	51.08333	-105.23333
36	Lidcliffe Marsh	MB	50.62917	-101.13333
37	Little Fish Lake	AB	55.46000	-111.59000

Lake #	Location	Province*	Latitude	Longitude
38	Little Quill Lake	SK	51.91667	-104.08333
39	Many Island Lake	AB	50.13333	-110.05000
40	Middle Quill Lake	SK	51.92000	-104.20000
41	Milligan Creek, E of Little Quill	SK	51.88000	-103.87000
42	Miquelon Lake	AB	53.25000	-112.88000
43	Mud Lake	SK	51.92000	-104.20000
44	Murray Lakes	AB	49.80000	-110.93000
45	Namaka Lake	AB	50.93000	-113.22000
46	Oak Hammock Marsh	MB	50.18750	-97.12500
47	Oak/Plum Lakes	MB	49.60000	-100.68332
48	Old Wives Lake	SK	50.10000	-106.00000
49	Pakowki Lake	AB	49.33333	-110.91667
50	Paysen Lake	SK	50.68000	-106.73000
51	Pel Lake	SK	51.55000	-104.70000
52	Pelican Lake	SK	50.53333	-106.00000
53	Proven Lake	MB	50.53333	-99.98333
54	Rice Lake	SK	52.05000	-107.11667
55	Rush Lake	SK	50.40420	-107.40170
56	San Francisco Lake	AB	50.58333	-112.13332
57	Smoky Lake	AB	54.16667	-112.66667
58	Stirling Lake	AB	49.53000	-112.58000
59	Stobart Lake	AB	50.92000	-113.18000
60	Town of Milo	AB	50.50050	-112.88380
61	Twelve Mile Lake	SK	49.48333	-106.23333
62	Utikuma Lake	AB	55.83333	-115.41667
63	Valeport	SK	50.75000	-104.86667
64	Waterhen Marsh	SK	52.83333	-105.03332
65	Watt Lake	AB	53.70000	-111.93000
66	Welstead Lake	AB	58.33000	-111.78000
67	Whitewater Lake	MB	49.25000	-100.30000
68	Whitford Lake	AB	53.88333	-112.25000
69	Winnipeg	MB	49.88000	-97.15000

* SK = Saskatchewan; AB = Alberta; MB = Manitoba.

PART I

EFFICACY OF CARCASS CLEAN-UP DURING AVIAN BOTULISM OUTBREAKS

TRENT K. BOLLINGER, DANIEL D. EVELSIZER AND MARNIE ZIMMER

ABSTRACT

Carcass clean-up is a common management response to waterfowl die-offs caused by *Clostridium botulinum,* Type C (hereafter, botulism). We evaluated the effectiveness of carcass clean-up in reducing carcass densities on seven wetlands in Prairie Canada, under typical to enhanced botulism clean-up efforts. The wetlands varied from 250-7,850 ha in area, and had varying density of vegetation. During clean-up operations, we regularly estimated carcass densities remaining on lakes using line transect surveys. Fresh carcasses were marked with leg bands and the proportion of carcasses retrieved during clean-up operations was estimated for each lake. The proportion of carcasses retrieved varied from 7-42% and, as predicted, higher values were associated with smaller wetlands that had greater clean-up effort per ha. Clean-up on large, heavily vegetated wetlands was ineffective, resulting in removal of only 7% of carcasses and leaving 20-40 carcasses/ha over extensive areas. Costs for clean-up varied from C$11-144 / ha (estimated in 2000), with higher costs associated with clean-up on smaller lakes, where the highest proportions of carcasses retrieved were under 50%. Extrapolating labour estimates for clean-up on small wetlands to project requirements for the largest wetlands, we estimated that 47 airboats would need to be deployed, for eight hours a day, every day of the summer to achieve levels of carcass removal similar to those obtained on smaller wetlands. Carcass clean-up of botulism outbreaks is costly and time consuming, and recovers <50% of carcasses at best. Clean-up on large, heavily vegetated wetlands is ineffective and impractical.

INTRODUCTION

In Type C *Clostridium botulinum* (botulism), carcasses act as a source of toxin for susceptible birds. Decaying carcasses that contain type C botulism spores provide a highly suitable substrate for vegetative growth of this anaerobic bacterium and provide conditions for production of *C. botulinum* toxin. Larvae of necrophagous invertebrates, in particular blowfly (*Calliphora spp.*) maggots, feed on decaying, toxin-laden carcasses and then pass the toxin to other birds that use the larvae for food. This process of propagation of type C botulism intoxication can lead to die-offs of hundreds of thousands of waterbirds on a single wetland, in a single season (Rocke and Bollinger 2007).

Although removing carcasses to prevent propagation of botulism outbreaks is a logical and long-standing approach to managing avian botulism, its effectiveness under field conditions has not been evaluated. Reed and Rocke (1992) reported that the daily relative risk of sentinel mallards (*Anas platyrhynchos*) contracting botulism was 4.5 times lower when their 0.8 ha enclosures contained zero carcasses compared to when they contained 12.5 carcasses/ha. High carcass density also produced high mortality risk in free-ranging mallards (Evelsizer et al. 2010a). Unfortunately, a carcass density of zero cannot be obtained under field conditions because of the difficulty of finding carcasses in the heavily vegetated wetlands where botulism occurs. For instance, Stutzenbaker et al. (1986) reported that only 6% of avian carcasses were found by eight searchers walking through a 40.5 ha marsh in southeast Texas. In that study, none of the marked carcasses placed in locations obscured by overheard cover were found, whereas 12% of carcasses placed on top of cover were detected. Cliplef and Wobeser (1993) reported that only 32% of marked carcasses were recovered in routine carcass collection activities using an airboat and two-person crew on 850 ha Eyebrow Lake in south central Saskatchewan. This estimate was an average obtained from two visits, with searches lasting approximately six hours each.

During the mid to late 1990s, large outbreaks of avian botulism occurred on wetlands in Prairie Canada. Intensive clean-up (or 'removal') operations were undertaken to reduce losses of waterfowl on many of these wetlands. Daily clean-up of individual wetlands, over a period of weeks to months, resulted in the removal of 10,000-120,000 avian carcasses per lake, per year. Because of the intensity of the clean-up effort, it was generally believed that over half of the carcasses on each wetland were being removed and, more importantly, that this had a beneficial effect in reducing losses to avian botulism.

Our objectives were to determine: 1) efficacy of carcass removal effort and, more specifically, the proportion of carcasses removed; 2) carcass density remaining after clean-up; 3) relative costs of the clean-up effort; and 4) clean-up effort required to substantially reduce carcass densities.

METHODS

Evaluation of Routine Clean-up

The efficacy of carcass clean-up was evaluated during routine clean-up operations of botulism outbreaks on seven lakes in Prairie Canada, from 1998-2001. One lake was evaluated in two consecutive years, for a total of eight different estimates of the efficiency of carcass removal. The lakes ranged from 250-7,850 ha in area (Table 1). These wetlands had experienced repeated occurrences of avian botulism over the previous several decades, and outbreaks had typically been managed by carcass removal. The lakes were typical of Prairie wetlands, with varying water levels and varying amounts of emergent vegetation (Figure 1), including *Scirpus spp.*, *Typha spp.*, *Carex spp.*, grasses, and the submergent plant, *Potamogeton spp*.

Clean-up activities

Clean-up activities were typical of the type of carcass clean-up operations that had been undertaken on wetlands in Western Canada. Airboats, with a driver and one or two crew members, were used to systematically search and collect carcasses, usually beginning in mid to late June. Some wetland shorelines were also searched on foot or with all-terrain vehicles. All observed carcasses were collected, as were sick birds, which were humanely killed. Carcasses where buried in a pit after treating with lime (see right). In 1998 and 1999, lakes were initially searched every three to seven days by a single airboat. When present, carcasses were collected during this monitoring period but an intensive clean-up was not initiated until numbers of carcasses appeared to be increasing, usually in mid to late July. During intensive clean-up, several airboats and crews

visited the site daily. This strategy had been used commonly in past botulism management programs. In 2000 and 2001, daily intensive clean-up programs were initiated earlier in July, before carcass densities had increased. All areas of the lakes were searched, but areas with higher carcass density were searched more intensively. These areas were identified by clean-up crews, and the research crews that performed transect surveys and marked carcasses also informed clean-up coordinators of areas where carcass density was high. Lakes were typically divided into sectors, to assist in clean-up efforts (Figure 2). Carcasses were checked for U.S. Fish and Wildlife leg bands and poultry bands that had been placed on them to monitor clean-up efficiency.

Surveys and marked carcasses

While clean-up procedures were underway, an airboat with a two-person crew performed surveys to estimate carcass densities remaining on the wetland and to map vegetation. Surveys took place every two weeks in 1998 and were run approximately every week in the remaining years. Transects were run in a north-south or east-west direction, depending on the orientation of the lake (Figure 3). The first transect position was randomly determined and all other transect lines were placed at 50-500 m intervals depending on the size of the lake and the expected carcass density. Transects were run from shoreline to shoreline, as far as the airboat could travel, at consistent intervals from the position of the first transect. Hand-held Global Positioning System (GPS) units (Garmin Inc.™) were used for guidance. One observer (and occasionally two) stood at the front of the boat, counting all carcasses and estimating the perpendicular distance from the carcass to the centre of the transect (i.e., the centre of the boat). In 1998, these observations were truncated at 2.5 m; however there was no truncation in subsequent years. UTM coordinates of each carcass were recorded.

For all carcasses within 2.5 m of the transect line, species, age, sex and state of decomposition (SOD) was recorded. At Pakowki Lake in 1998, carcass searches were also performed on transects placed randomly around the perimeter of the lake (shoreline transect), to account for the accumulation of carcasses in these locations and to mimic clean-up strategies (Figure 3). SOD was evaluated and recorded according to six categories: SOD 1, sick bird; SOD 2, freshly dead with no evidence of decomposition (see left); SOD 3, autolyzed carcass without evidence of maggots; SOD 4, early maggot development with little or no penetration of the carcass; SOD 5, carcass completely inundated with maggots; and SOD 6, carcass consisted of skin and skeleton with few, if any, maggots. A numbered aluminum poultry leg band (National Band and Tag Company, Newport, Kansas) was placed on the tarsometatarsal bone of the majority of relatively fresh carcasses (SOD categories 1-3) along the 5 m shoreline transect. In some cases, carcasses found off transect were also marked to increase the number of

marked carcasses potentially available to be retrieved by search crews. All carcasses were returned to the marsh, to approximately the same location where they were found.

The UTM coordinates of transition zones between the various vegetation density zones were recorded along the length of each transect. Vegetation zones were categorized as: open water; light vegetation (>10 m visibility); medium vegetation (2.5-10 m visibility); or heavy vegetation (<2.5 m visibility).

The program DISTANCE 4.1 was used to derive detection probability functions and to estimate densities of carcasses that remained on the wetland during carcass clean-up efforts (Thomas et al. 2006). Carcass location information was plotted on GIS layers within ArcView (ESRI, Inc.) and isoclines of carcass density were derived using Spatial Analyst (ESRI, Inc.).

To ensure that carcasses were not being lost due to scavenging, and to monitor rates of decomposition, radio transmitters (prone and suture and intra-abdominal types) were firmly attached to freshly dead carcasses. Radio-marked carcasses were left on wetlands during clean-up operations, as well as on four other wetlands without clean-up. These carcasses were monitored daily and their rates of decomposition and fates were recorded; however, clean-up crews were instructed to collect these radio-marked carcasses if found.

Intensive search trial

To determine the efficacy of an intensive search in a small, predefined area of heavily vegetated wetland, we measured pickup efficiency within a 500 x 500 m square study area (25 ha) established in dense emergent vegetation on Old Wives Lake on 18 August 1997, during an avian botulism epizootic. The perimeter of the area was marked every 100 m with 3 m wooden poles and flagging tape. Carcass density prior to the clean-up was estimated by counting all carcasses on five 500 x 4 m strip transects that were spaced 100 m apart (i.e., on 40% of total area). The first transect ran along one edge of the study area. Strip transects were searched using an airboat and a three-person crew. Coloured plastic, spiral poultry leg bands (Kuhl Corporation, Flemington, NJ) were placed over the tarsometatarsus bone (or occasionally the distal tibiotarsal bone) of 59 of the least decomposed carcasses. These marked carcasses were deployed from a moving airboat, in an arbitrary fashion, within the study area. When the survey was complete and the marked carcasses were distributed, the study area was searched by an experienced clean-up crew consisting of two ‘pickers’ and an airboat driver (see above). They

searched the area thoroughly, collecting all carcasses encountered and recording all marked carcasses, until they felt the area was completely covered.

The trial was repeated on 28 August using the same study area. Procedures were identical to those used 10 days previously, except different coloured leg bands were used to mark the carcasses. Also, an additional six carcasses were collected and banded from outside of the study area, to bring the total number of marked birds distributed within the study area to 30.

RESULTS

Evaluation of routine clean-up

The surface area covered by open water, light, medium, and heavy vegetation on each of the research lakes is shown in Figure 1. The area of the lakes and the area of vegetation are presented in Table 2. The proportion of marked carcasses found by clean-up crews during botulism outbreaks on the eight research lakes ranged from 7-45% (Table 2). Only 7% of marked carcasses were found on Whitewater Lake (7,850 ha), whereas 28-45% of marked carcasses were found on lakes that were 250-550 ha in area. Kimiwan and Pakowki lakes, which were also large lakes, had pickup rates of 32 and 28%, respectively. However, the proportion of these lakes that was heavily vegetated, which is where most of the carcasses were found and where most of the clean-up effort was concentrated, was much smaller than the total area of the lake (Figure 3). The average cost per ha on the four small lakes was ~C$123 (Table 1). On Whitewater Lake, the cost per ha was ~C$11 in 1998 and the clean-up rate remained at 7% in 1999, despite an increased clean-up effort and a 40% increase in costs.

The fates of radio-marked carcasses on three lakes with clean-up and four lakes without clean-up are presented in Table 3. Scavengers removed 7% and 5% of marked carcasses on Paysen Lake in 2000 and 2001, respectively. Although scavengers removed one of the two radio-marked carcasses on Chaplin Lake, scavenging rate could not be determined at that site because of low sample size. No radio-marked carcasses were removed by scavengers on the remaining four lakes. Overall, <2% of radio-marked carcasses were removed by scavengers during the periods of observation. Avian scavengers were likely involved at Paysen Lake, based on observations that flesh and viscera were removed with minimal disturbance of the carcass, and bite wounds were not evident. The radio-marked carcass at Chaplin Lake could not be found, which may indicate removal by a terrestrial predator, although this could not be confirmed.

Rates of maggot development and decay of radio-marked carcasses are shown in Table 4. Carcass density remaining on the lakes, determined by mark-recovery of carcasses and estimated based on strip transects in 1998 and by line transects in subsequent years, are presented in Table 5. Although mean carcass density over the summer ranged from 1.2-6.2 carcasses/ha at Whitewater Lake, density varied dramatically within the lake over short periods of time, as indicated by carcass density plots generated from line transect data collected at ~5-7 day intervals (e.g., compare Figures 4 and 5).

Intensive search study

On 18 August, 132 dead birds were counted on transects within the 25 ha study area on Old Wives Lake, resulting in an estimate of 3,300 carcasses (132 carcasses/ha) for the entire study area. Of the 59 marked carcasses placed arbitrarily within the study plot, 36 (61%) were collected during the subsequent clean-up of the entire area. A total of 2,477 carcasses was collected by the clean-up crew over 13 hours, involving 5.5 hrs on 18 August and 7.5 hrs on 19 August. Based on a carcass pickup rate of 61% and 2,477 carcasses retrieved, the total number of carcasses on this study area was estimated at 4,060 (700 higher than the estimate derived from the transects). If we use the lower estimate of 3,300 carcasses and assume that 39% of carcasses were missed, then an estimated 1,287 carcasses (51 carcasses/ha) remained on the study area.

On 28 August, 600 new carcasses were estimated to be present on the study area, based on transect counts. Using an airboat, a three-person crew collected a total of 604 birds over approximately six hours and found 10 of 30 marked carcasses (33.3%). They also found two carcasses that had been marked 10 days previously. Using a correction factor of three, the number of carcasses in the study plot was estimated to be 1,812, or 72.5 carcasses/ha. This total should reflect the number of carcasses left after the first clean-up nine days earlier (approximately 1,300 carcasses) and an additional 600 new carcasses, based on the estimate derived from transects. This value of 1,900 is very close to the estimate of approximately 1,800, which was derived from the number of carcasses collected during the clean-up multiplied by the correction factor of three. Many carcasses left on the study area over the previous nine days would be lost due to complete decomposition. Based on the observation that only one in three carcasses was found in this second trial, approximately 1,200 carcasses (2 x 604) remained on the 25 ha study plot. This equates to a density of 48 carcasses/ha, which is very similar to that observed in the first trial (51 carcasses/ha), when initial carcass densities were much higher, as was the proportion of carcasses found (61%).

CONCLUSIONS

Although carcass clean-up operations during this study were typical of past responses to avian botulism outbreaks, the level of effort applied was much greater than in the past in most cases. Yet, less than half of the birds dying of botulism were actually retrieved. This was likely due to poor visibility of carcasses within stands of emergent vegetation and the high cost of clean-up. Wetlands with approximately 200-600 ha of emergent vegetation had carcass pickup efficiencies of 25-45%. The highest recovery (45%) of marked carcasses occurred on Paysen Lake, a 445 ha wetland with very intensive clean-up efforts of 1.04 boat hrs/ha over the duration of the outbreak, at a cost of ~C$117.00/ha. In spite of these efforts, an average of 1.3 carcasses/ha remained on the wetland over the summer; focally, these densities would have been much higher. These results are comparable to those of Cliplef and Wobeser (1993), who reported that 32% of marked carcasses were found during routine botulism clean-up operations using an airboat. In their study, marked carcasses were placed on the wetland one day prior to clean-up and the experiment was repeated twice without the opportunity for repeated searches of the area over

time. Our experiment differed in that we marked carcasses at regular intervals throughout the summer and monitored the proportion of marked carcasses found over the course of the entire clean-up. In spite of the perception that the clean-up efforts were more intense due to continuous searches of the lake, the proportion of carcasses found was similar to previous reports.

Intensive clean-up of well defined areas has the potential to improve pickup rates. Although we achieved a pickup rate of 61% under these conditions, 51 carcasses/ha still remained after clean-up, likely because of the high initial density of carcasses and the heavy vegetation in the area. Another thorough clean-up of the area found only 33% of the marked carcasses, with 48 carcasses/ha remaining. On both occasions, the clean-up crew thought they had thoroughly searched the area even though the second search took half the time. The fact that similar carcass densities remained after each search suggests that searcher fatigue may play a role in achieving better detection rates.

Scavenging appears to play an insignificant role in carcass removal during large botulism outbreaks that occur in heavily vegetated wetlands. In three of the non-clean-up wetlands, where radio-marked carcasses were placed, none were removed by scavengers and all developed maggots and decomposed (Table 3). In other lakes, where carcass density was lower as a result of clean-up efforts and low levels of botulism mortality, scavenging rates varied from 0-13%. Scavenging appeared to be promoted by low carcass densities and higher carcass visibility, which resulted from light vegetation and expanses of open or thinly vegetated shorelines. Coyotes, raptors, crows and other scavengers were frequently observed when shorelines were being searched for carcasses.

Rates of maggot development on radio-marked carcasses revealed that carcasses took an average of six days to develop to the stage of most intense fly larvae activity. Even a few carcasses occurring on a botulism-prone wetland have the potential to precipitate an outbreak, because sediment from wetlands with a history of avian botulism are likely to contain spores of type C *C. botulinum* (Wobeser et al. 1987) and 60-100% of carcasses on wetlands during our study produced botulism toxin (Part VI; T. Bollinger, unpublished data), which is similar to previously reported rates of toxin development (Haagsma et al. 1972; Duncan and Jensen 1976). To prevent outbreaks on these wetlands, regular searches would need to occur at least every four to six days to prevent carcasses from developing maggots. Waterbird mortality commonly occurs on wetlands due to such causes as inclement weather and hailstorms (Stout and Cornwell 1976), as well as disease and trauma affecting juveniles in nesting colonies (Part V; Soos and Wobeser 2006). Intensive searches and clean-up of entire wetlands would need to occur approximately 10 times over the course of the summer, to ensure that these periodic or low-level occurrence mortality events do not escalate into large botulism outbreaks.

Carcass clean-up on large, vegetated wetlands was largely unsuccessful. Only 7% of carcasses were removed from Whitewater Lake, an 8,200 ha wetland of which 4,500 ha are heavily vegetated. Effective clean-up was not possible, due to the large area of vegetated wetland to be searched on a regular basis and the high cost of doing so. Based on the results of our intensive clean-up study, approximately ½ hr/ha would be required to find 50-60% of the carcasses which,

if extrapolated to Whitewater Lake, corresponds to 2,250 hrs of airboat time every six days, or 47 airboats searching for 8 hrs/ day over the course of the summer.

In summary, carcass clean-up of botulism outbreaks is costly and time consuming. These efforts remove <50% of carcasses, at best, on small wetlands and <10% of carcasses on large, heavily vegetated wetlands. Given these rates, as well as the associated costs and the absence of a clear survival benefit (Evelsizer et al. 2010b), carcass clean-up should be discontinued in most situations, and other options for managing avian botulism outbreaks should be explored.

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Table 1. Characteristics of lakes used to evaluate effectiveness of botulism clean-up operations on eight lakes in Western Canada, 1998-2001. Locations and coordinates for lakes are provided in Figure 1 and Table 1 of the General Introduction.

Year	Lake Province[a]	Area (ha)	Carcass Removal Dates[b]	Boat Hrs[c] (Boat hrs/ha)	Carcasses Removed (Ducks)	Carcass Removal Cost (C$)[d]	Cost/ Carcass (/Duck) (C$)	Cost/ ha (C$)[e]
1998	Pakowki AB	4,815	14 May-14 Sept	NA	4,943 (4,443)	81,714.00	16.53 (18.40)	16.97
	Whitewater MB	8,203	8 June-9 Sept	690 (0.09)	19,106 (11,631)	89,793.00	4.70 (7.72)	10.95
1999	Kimiwan AB	3,986	28 April-27 Sept	NA	1,978 (1,630)	85,000.00	42.97 (52.15)	21.32
	Whitewater MB	8,203	12 May-16 Sept	1170 (0.15)	15,512 (9,950)	125,442.17	8.09 (12.61)	15.29
2000	Paysen SK	445	6 June-28 Aug	461 (1.04)	2,928 (1,428)	52,170.37	17.82 (36.53)	117.24
	Frank AB	420	31 July-25 Aug	228 (0.54)	2,351 (1,495)	60,000.00	25.52 (40.13)	142.86
2001	Kettlehut SK	253	8 June-23 Aug	192 (0.76)	87 (12)	36,356.46	417.89 (3,029.71)	143.70
	Chaplin SK	552	19 June-23 Aug	215 (0.38)	242 (38)	48,272.88	199.47 (1,270.34)	87.45

[a] AB = Alberta; MB = Manitoba; SK = Saskatchewan.
[b] Removal dates include the period from initial surveillance to the last day of the removal operation.
[c] Number of hrs removal crews (usually one driver and one "picker") spent on the water, searching for carcasses. This does not include numerous hrs spent off the water, with duties such as equipment maintenance and repairs.
[d] Excludes capital cost of equipment, but does include total costs associated with surveillance and removal operations that involved equipment maintenance, repairs, rental, staff wages, housing, staff expenses, and vehicle expenses. Dollar amounts are taken from removal coordinators' reports. The report for Kimiwan Lake was prepared by Susan Tiege, DUC and Margo Pybus, Alberta Fish and Wildlife. The 1998 Whitewater report was prepared by Murray Breemersch, while the 1999 Whitewater report was prepared by Darcy Pisiak, Manitoba Department of Natural Resources. Reports of removals in SK were prepared by Steve Stire, DUC and a report on Frank Lake came from Dave Kay, DUC.
[e] Cost of the removal operation per wetland ha.

Table 2. Total and vegetated areas of lakes used for assessments of carcass clean-up efficiencies. Also shown are numbers of marked carcasses deployed at each lake, along with the percentage of marked carcasses found with 95% confidence intervals (CI).

Year	Lake, Province[a]	Total area (ha)	Area of vegetation (ha)	Number of carcasses marked	Percent carcasses found (%)	95% CI (%)
1998	Pakowki, AB	4,815	318	131	28	20-35
	Whitewater, MB	8,203	4,429	440	7	4-10
1999	Kimiwan, AB	3,986	569	41	32[b]	18-46
	Whitewater, MB	8,203	4,429	360	7	4-9
2000	Frank, AB	420	240	242	29	23-35
	Paysen, SK	445	417	236	42 (45[b])	36-49
2001	Kettlehut, SK	253	253	7	0	Not applicable
	Chaplin, SK	552	198	16	25[c]	4-46

[a] AB = Alberta; MB = Manitoba; SK = Saskatchewan.
[b] Percent found, adjusting for a 6.7% scavenging rate based on Table 3.
[c] These estimates are likely low because scavengers removed a significant number of marked carcasses, making them unavailable for clean-up.

Table 3. Number of radio-marked carcasses that developed maggots on lakes with botulism outbreak clean-up (managed) operations and those without (control), in Alberta (AB) and Saskatchewan (SK), 2000-2001.

Year	Lake, Province [a]	Treatment	Number of marked carcasses	Number (%) developing maggots	Comments re: carcasses
2000	Crane, SK	Control	29	29 (100)	
	Kettlehut, SK	Control	36	36 (100)	
	Frank, AB	Managed	30	23 (77)	7 collected
	Paysen, SK	Managed	30	13 (43)	2 scavenged; 15 collected
2001	Chaplin, SK	Managed	2	1 (50)	1 scavenged
	Frank, AB	Control	11	11 (100)	
	Paysen, SK	Control	21	20 (95)	1 scavenged

Table 4. Number of days required for radio-marked carcasses to reach specific stages of decomposition (SOD) or to disappear (submerged). See Methods for categories of SOD.

	Days to reach		
	SOD 5	**SOD 6**	**Submerged**
Mean	5.8	9.9	11.9
Sample size	117	102	76
Standard deviation	2	1.9	4.1
Minimum	2	6	7
Maximum	13	17	28

Table 5. Estimates of birds killed by avian botulism and mean carcass densities (carcasses/ha) remaining during searches, based on carcass pickup rates and transect data.

Year	Lake Province[a]	Number carcasses collected	Percent carcasses found	Scavenging Rate[b]	Carcasses remaining on lake[c]*	Mean density* based on marked carcasses	Mean density* based on transects (SD)[d]
1998	Pakowki AB	4,943	28	Unknown, likely zero	12,710		2.31 (na)
	Whitewater MB	19,106	7	Unknown, likely 0%	253,840		2.31 (na)
1999	Whitewater MB	15,512	7	Unknown, likely 0%	206,088	3.34	2.15 (2.39)
	Kimiwan AB	1,978	32	Unknown, likely high	4,200		na
2000	Paysen SK	2,928	42	13% (2/15)	4,040	1.3	1.23 (1.12)
	Frank AB	2,351	29	0% (0/30)	5,756	6.22	4.03 (1.69)
2001	Kettlehut SK	87	0 scavenging	Unknown, likely high	~0		na
	Chaplin SK	242	25	50% (1/2)	726		na

* Over summer.

[a] AB = Alberta; MB = Manitoba; SK = Saskatchewan.
[b] The number of carcasses scavenged relative to number available is shown in parentheses.
[c] Based on percent found.
[d] na = information not available.

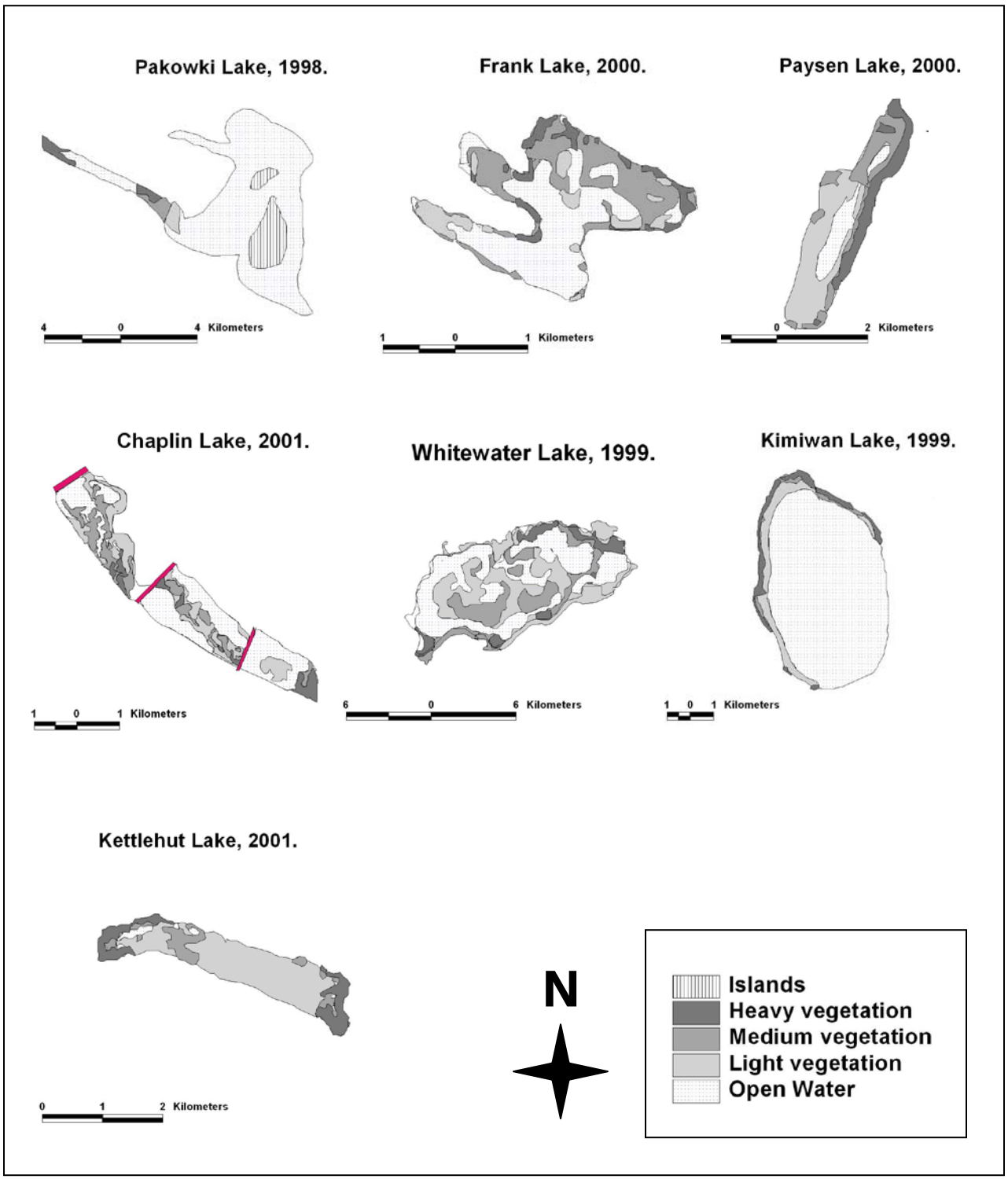

Figure 1. Maps of study lakes, indicating area covered by different densities of vegetation. See Table 1 for further details regarding lakes.

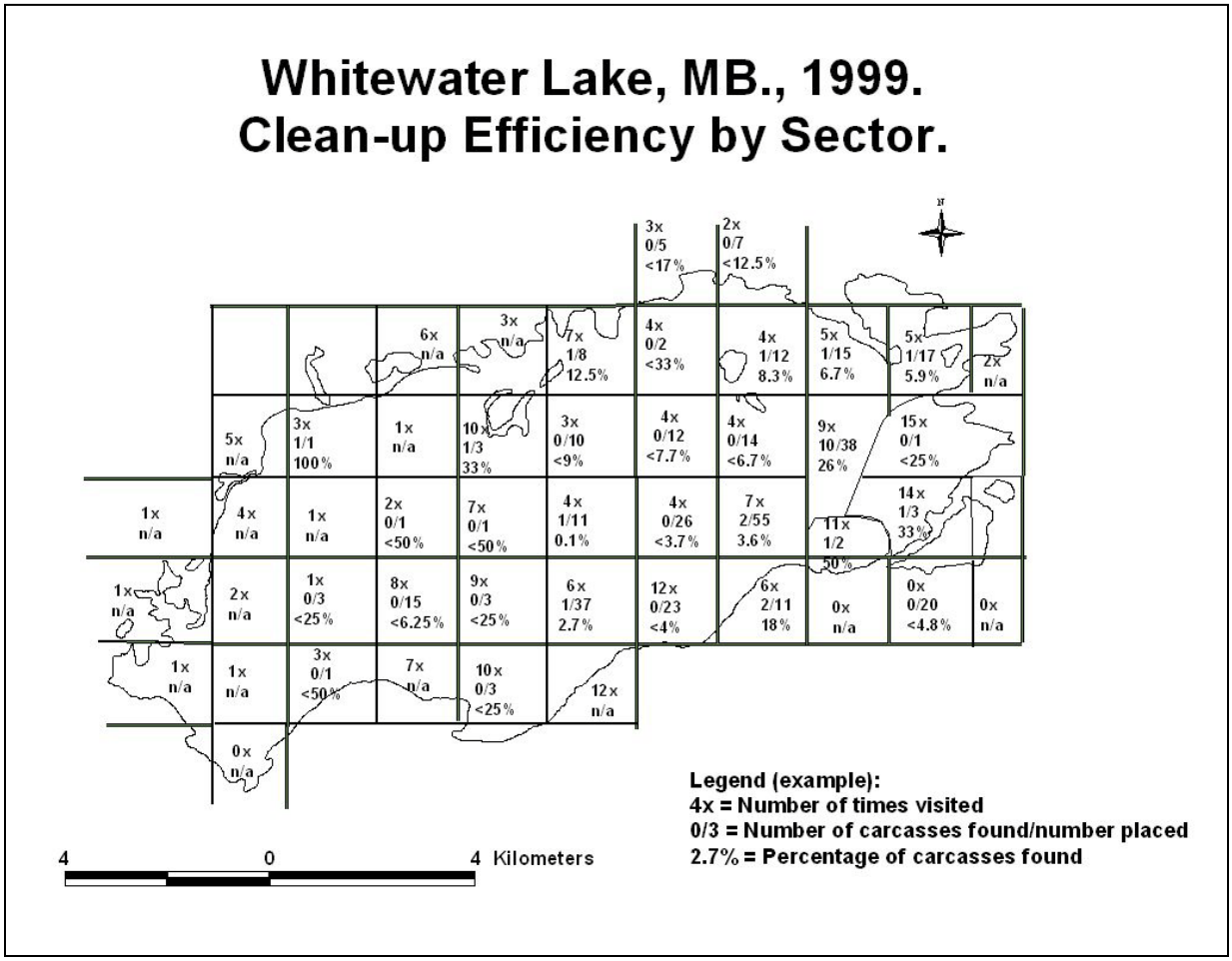

Figure 2. Map of Whitewater Lake, Manitoba showing its division into sectors to facilitate clean-up operations. The numbers within each sector, from top to bottom, indicate: the number of times the sector was visited by clean-up crews during the summer; the number of marked carcasses found/number placed; and the percentage of marked carcasses found.

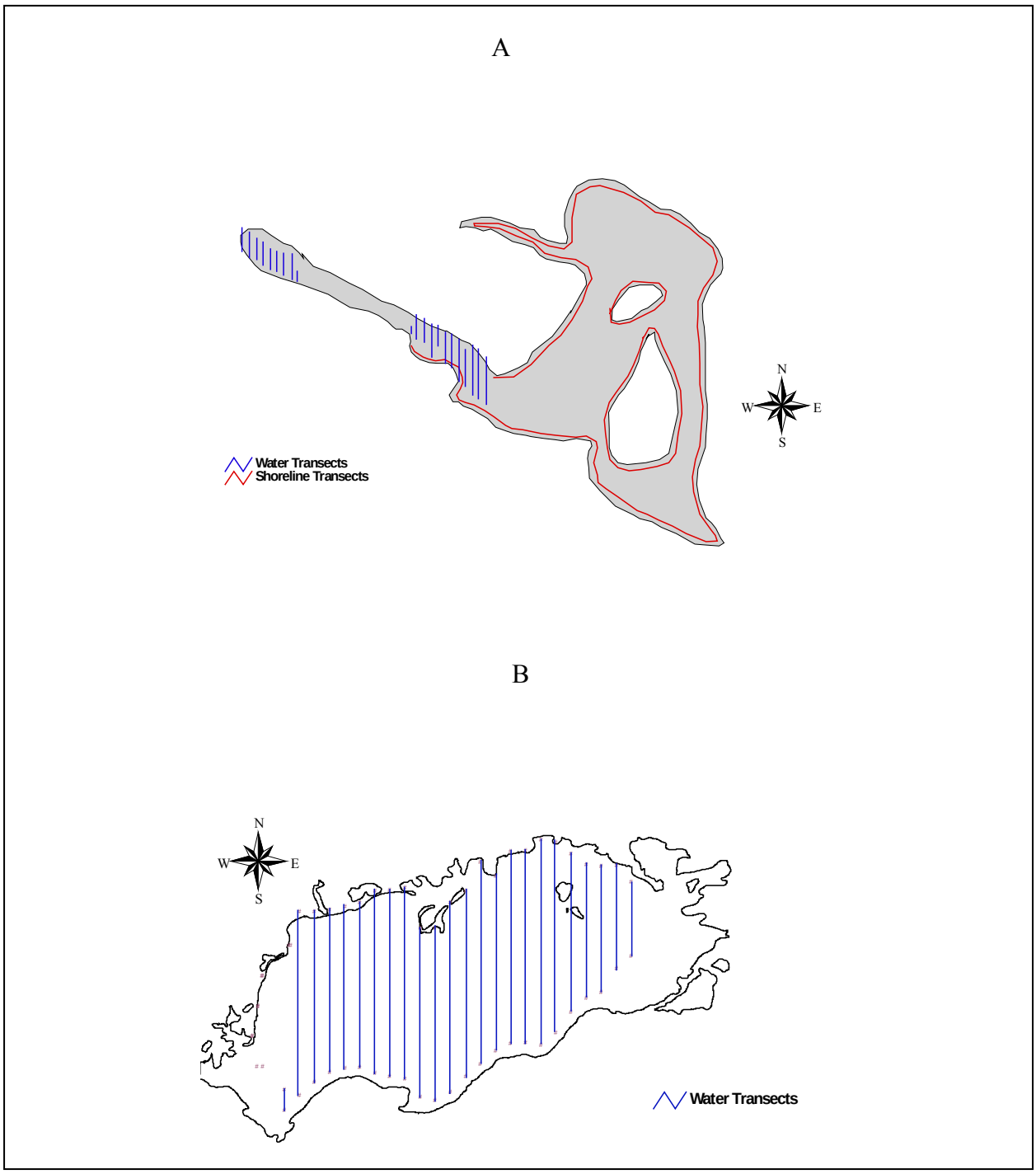

Figure 3. Map of Pakowki Lake, Alberta (A) and Whitewater Lake, Manitoba (B) showing typical distribution of water transects (blue lines) and land transects (red lines). The outline of Whitewater Lake was derived from a digitized topographic map. Although water levels were high, transects did not extend to shorelines depicted on the map. In Pakowki Lake, vegetation was restricted to the west arm (see A) and carcasses were extremely rare on open water, so transects were confined to the vegetated areas of the lake and shoreline.

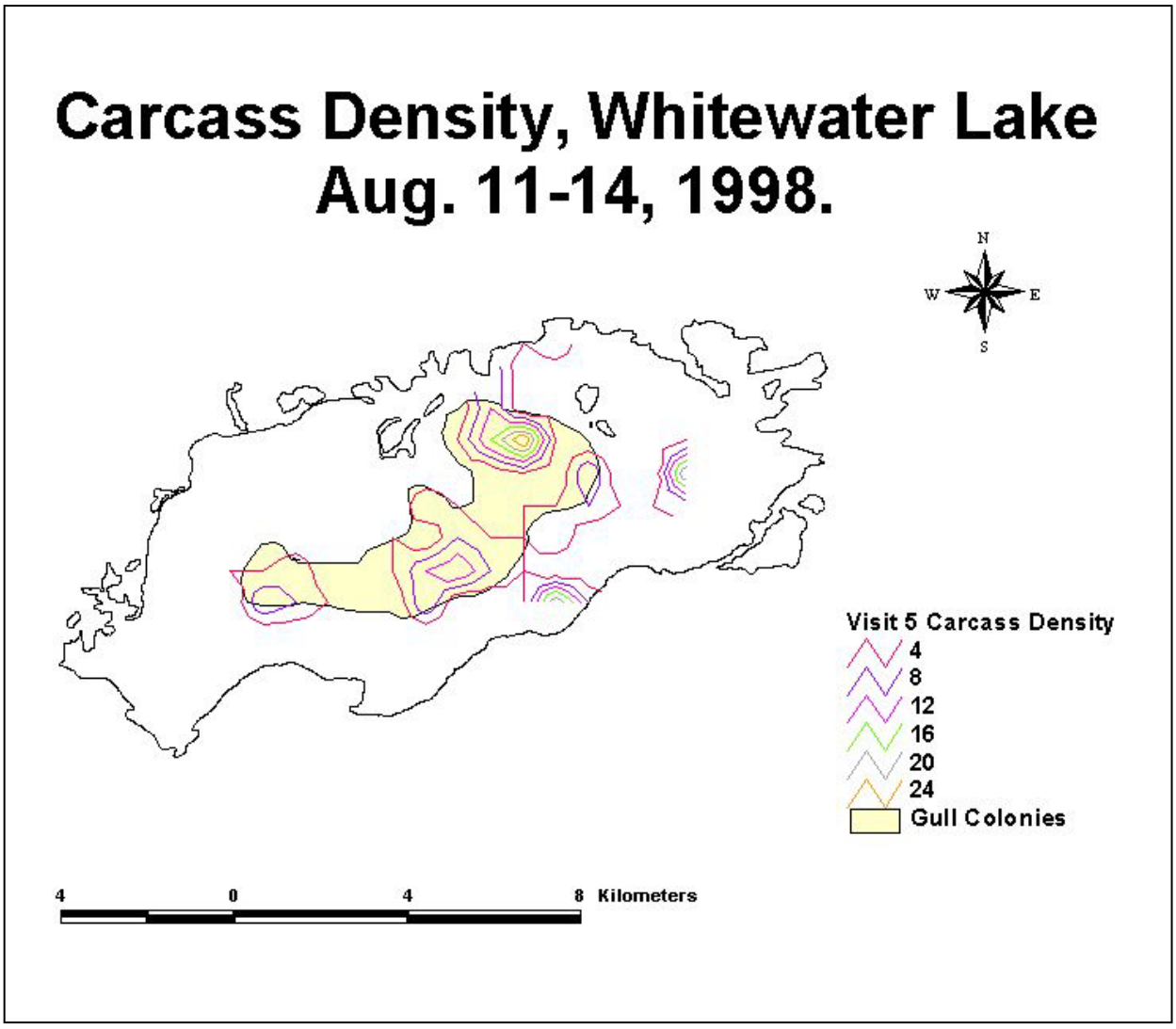

Figure 4. Variation of carcass density (carcasses/ha) within Whitewater Lake, Manitoba, as derived from line transects. Isoclines were derived using Spatial Analyst (ESRI Ltd)

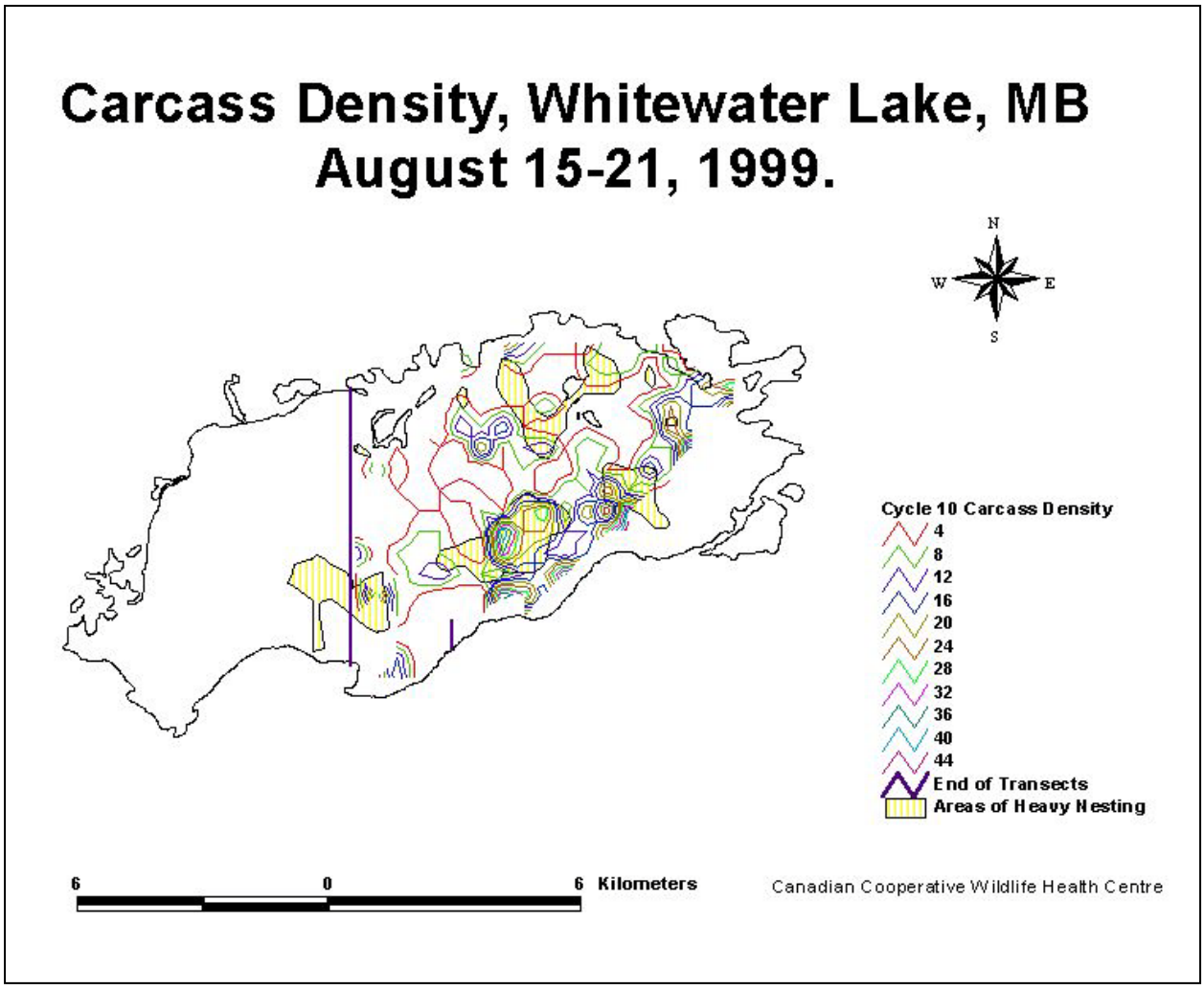

Figure 5. Variation in carcass density (carcasses/ha) across Whitewater Lake, Manitoba one week after estimates shown in Figure 4. Isoclines were derived using Spatial Analyst (ESRI Ltd). Note that point estimates exceed 30 carcasses/ha in several areas.

PART II

SURVIVAL OF RADIO-MARKED MALLARDS IN RELATION TO MANAGEMENT OF AVIAN BOTULISM

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An unabridged version of this paper appears in Journal of Wildlife Diseases 46, July 2010. Please contact Bob Clark (bob.clark@ec.gc.ca) for a copy of the full paper.

ABSTRACT

We radio-marked 419 moulting mallards (*Anas platyrhynchos*) on 11 botulism-prone lakes in Western Canada between 1999 and 2001 (July-August), and then monitored them for 30 days to test whether survival was higher on lakes with carcass removal. Botulism occurred on 10 lakes. On five lakes where carcasses were removed, greater-than-normal effort was made to conduct early, thorough surveillance and immediately remove carcasses; on six 'non-removal' lakes, no carcasses were removed. In 1999, estimated 30-day survival probabilities ranged from 0.15 (95% CI = 0.06-0.30) on one large lake with carcass removal to 0.47 (95% CI = 0.27-0.67) and 0.62 (95% CI = 0.44-0.77) on two non-removal lakes. As a result, we conducted work on smaller wetlands thereafter, reasoning that any management benefit would be easier to detect. In 2000, estimated 30-day survival probabilities were 0.31 (95% CI = 0.14-0.56) and 0.79 (95% CI = 0.61-0.90) on two carcass removal lakes versus 0.52 (95% CI = 0.36-0.68) and 0.74 (95% CI = 0.56-0.86) on two non-removal lakes. In 2001, botulism was detected on two non-removal lakes where survival probabilities were 0.84 (95% CI = 0.63-0.94) and 0.94 (95% CI = 0.78-0.98), as well as on one removal lake where survival probability was 1.0 (95% CI = 0.99-1.0), but was not detected on the other removal lake where no marked birds died from botulism (1.0, 95% CI = 0.99-1.0). Survival tended to be higher on lakes with lower carcass density.

INTRODUCTION

Where logistically feasible, botulism outbreaks have typically been "managed" by surveillance of wetlands during spring and summer, and by collecting and disposing of carcasses during botulism outbreaks (Locke and Friend 1987; Rocke and Samuel 1999). Removing carcasses before maggots develop may reduce bird losses by reducing the probability of uninfected birds ingesting toxic maggots, but this 'clean-up' practice can be expensive and time demanding (Part I; Wobeser 1987; Wobeser and Bollinger 2002).

Despite large investments in botulism control, results have been equivocal (Friend 1992). Reed and Rocke (1992) manipulated carcass density, and found that wing-clipped mallards (*Anas platyrhynchos*) held in enclosures with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in enclosures with no carcasses, indicating that absence of carcasses can improve survival. In a study of free-ranging mallards, birds exposed to >5 carcasses/ha during botulism outbreaks were >3.5 times more likely to die than were birds inhabiting carcass-free areas of wetlands (Part III; Evelsizer et al. 2010). However, effectiveness of removal operations is unknown under actual field conditions, where efficacy of removal operations could vary with the intensity of the operation, density and size of bird carcasses, and marsh vegetation. Cliplef and Wobeser (1993) evaluated effectiveness of carcass removal on a small Saskatchewan lake during a botulism outbreak and reported that 32.1% of tagged carcasses were recovered. Other researchers have reported rates of carcass detection as low as ~6% (Stutzenbaker et al. 1986). One carcass can produce thousands of maggots and, depending upon amount of toxin, as few as one to four maggots can kill a duck (Locke and Friend 1987; Hubalek and Halouzka 1991). Thus, a single carcass may produce enough toxin-laden maggots to kill hundreds of birds, which would then produce even more toxin-laden maggots (Wobeser 1997; Wobeser and Bollinger 2002).

Our main objective was to evaluate survival of wild moulting, radio-marked mallards during botulism outbreaks in Prairie Canada, to provide a known sample population of "ducks at risk". We determined probable cause(s) of mallard mortality, estimated survival rates for mallards on each wetland, compared survival among wetlands with surveillance and carcass removal (hereafter, removal lakes) to wetlands where carcasses were not collected (hereafter, non-removal lakes), and evaluated how survival was related to lake-wide estimates of carcass density.

METHODS

Study areas

Wetlands were chosen each year if they had recent recurrent botulism outbreaks, adequate water levels, and opportunity to maximize cooperation and assistance (airboats, and crews for surveillance and removal operations) from provincial agencies, the Canadian Wildlife Service, and Ducks Unlimited Canada. In 1999, work was conducted on two large (one with carcass removal) and one small lake with confirmed botulism outbreaks. On the basis of experience and preliminary results obtained from 1999, we focused work on smaller lakes in 2000 and 2001 to improve the likelihood of reducing carcasses to very low densities. In each of these two years,

two lakes were subjected to carcass removal while two served as non-removal (control) lakes. Three wetlands were revisited in 2000 and 2001, and treatments were crossed over.

All wetlands were shallow (maximum depth ≤ 2.5 m), but water levels were near basin capacity when work was conducted. Lakes had large areas of emergent vegetation utilized by moulting ducks; vegetation was composed of bulrush (*Scirpus spp.*), cattail (*Typha spp.*), and whitetop rivergrass (*Scholochloa festucacea*; specific to Whitewater Lake), which ranged from sparse to thick.

Carcass removal operations

On carcass removal wetlands, surveillance began as early as mid-May and, with the exception of Whitewater Lake in 1999 and Frank Lake in 2000, surveillance was usually initiated about two to four weeks earlier than normal (see Part I). Areas of open water and vegetation were searched using airboats, whereas shorelines were searched on foot or with all-terrain-vehicles (see below). To increase effectiveness of carcass removal, research crews informed clean-up personnel of places where mortality was occurring, so dead birds could be removed immediately. The removal operation at Whitewater Lake (1999) was delayed until early July but nonetheless had more manpower and airboats than had been employed previously. Wetlands were divided into 1 km^2 sectors and Global Positioning System (GPS) (Garmin Inc.™) units were used to help removal crews ensure complete wetland coverage. Although toxic maggots can develop on carcasses in as little as two to three days (Reed and Rocke 1992), removal crews were only able to revisit sectors once per week at Whitewater Lake due to its large size. For this reason, large wetlands such as Whitewater Lake were not used after 1999, and more thorough searches were accomplished twice per week on smaller wetlands.

Trapping and radio-marking mallards

Adult mallards in pre-moult and moult (emerging primaries <10 mm long) were captured and radio-marked prior to and during outbreaks on each wetland. We radio-marked mallards in pre-moult if outer primaries on one wing pulled out easily by thumb and index finger. This was done assuming that these birds were going to moult on the lake (and to ensure an adequate sample of radio-marked birds), which was a reasonable assumption because large numbers of moulting

mallards occurred on all study lakes. From 1 July to the second week of August, bait traps (see below) were placed simultaneously at several locations on each lake to capture moulting male and female mallards; a few were captured by drive-trapping. We attempted to balance the sex ratio of radio-marked birds at each lake, but this was not always feasible given timing of wing moult and sample size requirements (Table 1). Visibly healthy mallards were aged, sexed, weighed, and banded. They were also equipped with a back-mounted, prong and suture style radio transmitter (172-174 MHz), using standard techniques (Mauser and Jarvis 1991) under local anesthetic (1 ml Marcaine), and released within 30 min to reduce stress (Cox and Afton 1998). A small temperature-sensitive probe, encased in flexible plastic tubing, ran underneath the stainless steel anchor; when a drop (≥4 C) occurred in body temperature the transmitter pulse rate increased, indicating death. Transmitters were designed with a normal signal detection range of ~4 km (~2.5 km when submerged) and a life span of ~42 days, while retaining favourable features of small size and light weight (12 g). If a marked mallard died in July during banding, the transmitter was sterilized and re-used if sufficient life span (>30 days) remained. Methods were approved by the University of Saskatchewan's Committee on Animal Care (Protocol 19980040) on behalf of the Canadian Council of Animal Care.

Radio-tracking

Survival was determined by tracking mallards daily (04:00 - 09:00 CST), sometimes more frequently, for 30 days after release. After 30 days, surviving birds could not be tracked with certainty because most had completed moult and become mobile. Bird locations and status (dead or alive) were recorded each morning by tracking with a receiver linked to truck- or tower-mounted antennas. If the transmitter pulse rate increased, indicating death, the carcass was retrieved (usually within 2-6 hrs) with the aid of a hand-held tracking system from an air boat. The location of each dead bird was determined with a GPS unit and the carcass was frozen for necropsy. In some cases, birds were not found; these days were entered in the encounter history as being missing. Signals were occasionally lost permanently; possibly because these birds regained flight and left the wetland near the end of the tracking period, or the transmitter was shed or failed. In these cases, the encounter history was right censored (Williams et al. 2002).

Necropsies

Dead birds were included in survival analysis if they died from botulism. Botulism toxin causes no visible lesions; intoxicated birds often die from drowning, dehydration, or respiratory failure. Birds were diagnosed as having died of botulism if (1) botulism was diagnosed in other waterfowl on the lake using serum and mouse bioassay and (2) no other diseases or lesions were identified at necropsy. Botulism was the only substantial cause of mortality in ducks at all lakes. Birds demonstrating flaccid paralysis (see right) were consistently observed on all lakes. In 1999, nine of nine, six of six, and 13 of 14 serum samples tested positive for type C toxin at Whitewater, Old Wives and Eyebrow lakes, respectively. In 2000, five of five, six of seven, two of two, and four of four serum samples tested positive for type C toxin at Kettlehut, Paysen, Frank and Crane lakes, respectively.

Index of lake-wide carcass density from line transect data

Carcass densities on each of the lakes were estimated on a five to 14 day cycle, beginning no later than the first week of July and ending no earlier than 15 August, spanning the period over which radio-marked mallards were monitored. The exception was Frank Lake in 2000, where transect surveys did not commence until 23 July. Five to 12 estimates (cycles) of carcass density were obtained for each lake over the summer. Fixed line transects were established at 100-500 m spacing, depending on vegetation type and occurrence of carcasses, with the first transect randomly chosen at the beginning of each cycle. Using a GPS unit, an airboat was driven down the center of these transects while one or two observers stood at the front of the boat and counted all carcasses. For each carcass, the UTM coordinate of the boat was determined using the GPS, and perpendicular distance from the center of the boat (i.e., transect) to the carcass was estimated. We assumed that detection probability in front of the airboat was 1.0. Because submerged carcasses would not produce maggots, they were not considered. Density estimates were derived using the software DISTANCE (Thomas et al. 2006), as described in Part I. Best fit detection functions were derived for each cycle.

To obtain a lake-wide estimate of carcass density for each lake in each year, we computed weighted average density estimates (above) across all cycles in a given lake-year, where the weighting factor for each individual carcass density estimate (c_i) was the total number of radio-marked mallards known to be alive during that cycle. Number of birds alive in each cycle was expressed as a proportion (a_i) of the total number of birds known to be alive over all cycles.

Weighted carcass density for each lake was then calculated by summing the products of carcass density and proportion of radio-marked mallards alive in each cycle.

Survival analyses

Survival for 30 days post release was estimated using binomial modeling procedures for known fate data as implemented in Program MARK (White and Burnham 1999). Years were analyzed separately because of annual variation in the magnitude of botulism outbreak severity, annual variation in wetland conditions, and changes to our clean-up protocols after 1999. The same general *a priori* global starting model was fit to the data each year. Lakes were treated as separate input groups in program MARK; and capture and release date, sex, and body mass were included as individual covariates in the general starting model {S (lake+date+sex+mass)}. Survival was initially modeled in relation to "lake" to determine whether we could detect lake-specific differences in survival. Sex was used because we radio-marked male and female mallards (Table 1) and sex-specific diet preferences could potentially produce different survival rates (Rocke and Brand 1994). We also tested whether survival was related to body mass, independent of possible sex-related differences, because light-weight birds could be more susceptible to toxicosis. Capture date (days since 1 January) was included to determine whether survival varied through the radio-marking period. Models incorporating temporal variation in survival rates received no support, so we modeled survival rates as being constant over the 30-day encounter period.

We combined information obtained from all years for lake-wide estimates of weighted carcass densities, to test the prediction that survival would be negatively related to carcass density. This model set included effects of date, sex, body mass, lake-specific weighted carcass density, and additive combinations of these explanatory variables.

The model(s) with the lowest AIC_c (Akaike's Information Criterion with an adjustment for sample size) value was/were systematically selected from a set of candidate models after removing and re-entering individual covariates. Model weights (w_i), precision of parameter estimates for individual covariate(s), and differences in AIC_c values between models (ΔAICc) were considered. We report 30-day survival probabilities of flightless mallards exposed to botulism outbreaks, which were derived by model averaging to accommodate model selection uncertainty (Burnham and Anderson 2002). With this method, best-fit models with the highest model weight contribute most to average daily survival rate. Ninety-five percent confidence intervals (CI) were constructed based on the estimated unconditional variance and, as such, include a model selection uncertainty component (Burnham and Anderson 2002).

RESULTS

Radio transmitters were deployed on 419 mallards from 1999-2001, but for various reasons only 393 mallards were used to model survival variation; 101 transmitters (24%) failed or their signals were lost during the tracking period, so encounter histories for birds equipped with these radios

were right censored. One hundred twenty deaths (31% of the total sample of marked individuals) were attributed to botulism. The remaining 172 birds survived the 30-day tracking period.

Survival in 1999

A total of 107 birds was available for analyses. Lake differences in survival were common to eight best-approximating models, and these models collectively embraced 100% of the total support based on AIC_c weights (Table 2). The most parsimonious model included effects of lake and capture date, and was 3.6 times better-supported than the global model (Table 2). Based on this model, survival was negatively related to capture date (β = -9.08, 95% CI = -16.35 to -1.82).

Model-averaged estimates revealed wide variation in survival among the three lakes (Table 3). The lowest survival rate was recorded at Whitewater Lake, a carcass removal site, whereas survival was three to four times higher on the two non-removal wetlands, Eyebrow and Old Wives lakes (Table 3).

Survival in 2000

A total of 147 mallards was used in survival analyses. Seven top-ranked models included effects of lake, and four incorporated effects of body mass (cumulative weight for mass = 0.69; Table 2). Under the top model, heavier mallards had higher survival rates than did light-weight birds (β = 0.35, 95% CI = 0.03 to 0.68). Effects of date and mass were weak or absent. Survival probability was lowest at Frank Lake, a carcass removal site, followed by Crane Lake, a non-removal site. Survival was higher at Kettlehut Lake, a non-removal site, and higher still at Paysen Lake, a removal site, but 95% CIs overlapped for these two lakes (Table 3).

Survival in 2001

In total, 139 moulting mallards were used in survival analyses. Botulism was detected less often in 2001 and there was less variation in survival among lakes. Kettlehut, a removal lake, had no detectable botulism and Chaplin, the other removal lake, had low levels of botulism. Frank and Paysen lakes, which had no removal, also had low levels of detectable botulism. All best-approximating models included effects of lake (Table 2). Females survived better than did males (β = 0.89, 95% CI = 0.01 to 1.77), and heavier mallards also had higher survival rates (β = 1.59, 95% CI = 0.48 to 2.70). Survival rates approached 1.0 on Kettlehut and Paysen lakes, which were removal lakes with limited or no detectable botulism (Table 3).

Survival in relation to carcass density

We sought to determine whether survival was related to lake-wide estimates of carcass density, or other covariates, by combining all data in one modeling framework. Models with lake-year effects were well supported (Table 4). Based on the top-ranked model, heavier birds survived

longer than did light-weight mallards (β = 0.36, 95% CI = 0.13 to 0.59). Birds marked earlier in outbreak cycles tended to survive better as well (β = -0.20, 95% CI = -0.52 to 0.11), on the basis of the second-ranked model. The best-approximating model that incorporated effects of carcass density index had little support when compared with lake-year models (Table 4), but nonetheless survival rates did tend to decrease with increasing carcass density (β = -0.21, 95% CI = -0.35 to -0.08).

DISCUSSION

During botulism outbreaks, the number of dead birds collected can be counted, but mortality rate typically cannot be estimated reliably because the number of wild birds "at risk" is unknown. Our estimates indicate that the impact of botulism can be severe (Table 3). Furthermore, across lakes and years, there was a negative association between survival and carcass density, as reported recently by Evelsizer et al. (2010) and in Part III of this report, but carcass removal did not consistently enhance survival, even on smaller wetlands with intense surveillance. Lake-specific differences in prevalence and toxicity of botulism toxin or densities of susceptible birds possibly superseded the importance of carcass density effects in predicting survival (Table 4).

Based on results obtained in 1999, it was obvious that our ability to conduct an effective removal operation was futile on large wetlands with dense vegetation and other features like those encountered at Whitewater or Old Wives lakes. So, in 2000 and 2001, smaller lakes were used to improve the chances of reducing carcass density to very low levels needed to enhance survival and detect a positive effect of carcass removal operations. Effort on carcass removal lakes in 2000 and 2001 was considered very intense. Removal crews were trained, and equipment was prepared in early July, much earlier than in traditional responses to outbreaks. Our results imply that survival could not be increased by carcass removal and, therefore, the number of ducks potentially saved from botulism mortality was not related clearly to management effort.

Survival probability increased for heavier mallards at four lakes studied in 2000, with no sex-related differences, suggesting an interaction between body mass and impact of toxin. Furthermore, when all data were analyzed simultaneously (Table 4), light-weight birds marked later in the summer were more susceptible to botulism mortality. In general, survival was directly or indirectly (via sex effects) related to body mass, which is consistent with a toxin dose-host size effect. Thus, if our radio-marked samples were composed of mostly poor condition mallards because of bait trapping (Weatherhead and Ankney 1984), survival estimates may have been biased low. Unfortunately, we have no way of directly assessing this question.

We evaluated management effectiveness on wetlands where carcasses were removed and on those not subject to such management. Strict adherence to normal experimental protocols (*i.e.*, random allocation of treatment/control, replication, cross-over, data independence) was not possible, and no two wetlands or outbreaks were similar in all respects (Wobeser et al. 1987; Sandler et al. 1993). In 1999, we observed that radio-marked birds utilized large portions of wetlands, moved across lakes, and were not always confined to relatively small areas (T. Bollinger, unpublished data). In future studies, it may be possible to adopt a split-wetland

design, possibly with a treatment cross-over between years, and to use radio telemetry to monitor mortality risk in clean-up versus control locations within a lake. Finally, we did not explicitly include "clean-up treatment" effects in the modeling framework because of our inability to apply such a treatment in a consistent manner at all clean-up lakes. In short, the imposition of the treatment (e.g., management "dose") could not be done uniformly across clean-up lakes, and "treatment" changed between years.

Even though natural resource agencies spend considerable time and money during removal operations, other methods such as manipulating water levels, and/or vegetation or treating sick birds to recovery have been used (Hunter et al. 1970; Rosen 1971; Locke and Friend 1987; Sandler et al. 1993). However, these alternative methods may not be logistically feasible, especially on larger botulism-prone wetlands. Carcass removal operations have been the traditional and most advocated response to outbreaks of avian botulism in North America. This method attempts to break the carcass-maggot cycle via carcass removal and thereby attempts to reduce mortality of waterfowl. It also considers the view that a potentially beneficial action is better than doing nothing, and that visible action demonstrates good intentions (Peterson 1991). Our results are not consistent with the hypothesis that carcass removal reduces duck mortality in the varied conditions we encountered. Intensity of the outbreaks may be a function of spore density, bacteria, bacteriophage, substrates, transfer of toxin, bird usage or other factors working together in a given year and wetland (Rocke and Bollinger 2007).

We do not know whether duck mortality can be reduced on wetlands where carcasses are highly visible, or whether they can be detected and removed quickly (e.g., see Part I). Likewise, picking up and recovering sick birds may be effective in some cases but, to our knowledge, no one has critically evaluated this practice. Decisions about launching carcass removal operations will be based on socioeconomic and biological information. Our results strongly suggest that decisions to undertake clean-up operations on lakes that are similar to those we studied should not be based on the premise that survival of moulting ducks will always be improved.

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Table 1. Body mass (g) and date of radio-marking for male and female mallards on study lakes in Prairie Canada, 1999-2001. Locations and coordinates for lakes are provided in Figure 1 and Table 1 of the General Introduction. Shown are sample size (n), mean ($\bar{x}$) and standard deviation (SD).

Year **Lake, Province** [a]	**Males**					**Females**				
	Body mass			**Capture date**		**Body mass**			**Capture date**	
	n	$\bar{x}$	**SD**	$\bar{x}$	**SD (days)**	***n***	$\bar{x}$	**SD**	$\bar{x}$	**SD (days)**
1999										
Whitewater, MB	3	1275	109	1 Aug	3	38	1077	93	28 July	4
Old Wives, SK	24	1169	112	29 July	3	4	1006	123	31 July	6
Eyebrow, SK	16	1301	106	28 July	3	22	1102	118	30 July	4
2000										
Frank, AB	7	1186	106	27 July	2	21	1010	93	31 July	4
Kettlehut, SK	28	1286	120	19 July	6	9	1142	53	17 July	7
Paysen, SK	34	1242	102	17 July	5	3	1135	74	5 Aug	2
Crane, SK	27	1371	130	15 July	3	19	1092	93	23 July	7
2001										
Kettlehut, SK	20	1267	88	18 July	3	13	1057	100	19 July	4
Paysen, SK	28	1344	116	14 July	5	6	1161	75	20 July	9
Frank, AB	20	1230	140	13 July	9	18	1037	73	22 July	7
Chaplin, SK	19	1291	145	10 July	5	15	1087	127	18 July	8

[a] MB = Manitoba; SK = Saskatchewan; AB = Alberta.

Table 2. Models used to assess effects of lake, radio-marking date, sex, and body mass on 30-day survival of mallards during botulism outbreaks in Prairie Canada. The best fitting model has the lowest Akaike's Information Criterion (AIC_c) adjusted for sample size. Only models with model weight ≥0.05, and the global and null (intercept-only) models are shown for each year.

Year	Model	ΔAIC_c[a]	Weight[b]	K[c]
1999	Lake, date	0.00	0.354	4
	Lake, date, mass	0.60	0.262	5
	Lake, date, sex	1.53	0.165	5
	Lake, date, sex, mass	2.57	0.098	6
	Null	22.53	0.000	1
2000	Lake, mass	0.00	0.368	5
	Lake, date, mass	1.98	0.137	6
	Lake, sex, mass	2.01	0.135	6
	Lake, sex	2.43	0.110	5
	Lake	2.65	0.098	4
	Lake, date	3.62	0.060	5
	Lake, date, sex, mass	3.99	0.050	7
	Null	23.77	0.000	1
2001	Lake, sex, mass	0.00	0.499	6
	Lake, date, sex, mass	1.99	0.184	7
	Lake, mass	2.25	0.162	5
	Lake, date, mass	3.99	0.068	6
	Null	10.13	0.003	1

[a] The difference in value between AIC_c of the current model versus the best-fitting model (1999, {S(lake+date)}, AIC_c = 510.51; 2000, {S(lake+mass)}, AIC_c = 492.85; 2001, {S(lake+sex+mass)}, AIC_c = 105.64).
[b] Weight of evidence in favour of model, relative to those in candidate list; weights sum to 1.0.
[c] Number of estimable parameters in model. Note: analyses involved three lakes in 1999, four lakes in 2000, and four lakes in 2001. Date and mass were treated as continuous predictors.

Table 3. Estimated survival probability (to 30-days post-release) of moulting mallards radio-marked on 11 lakes on the Canadian Prairies, 1999-2001, in relation to carcass removal operations, and number of birds (n) included in survival analyses. Survival estimates are adjusted for covariate effects in top-ranked models (see Table 2).

Year	Lake, Province [a]	Treatment	*n*	Survival (95%CI)[b]
1999	Whitewater, MB	Removal	41	0.149 (0.065-0.304)
	Eyebrow, SK	Non-Removal	38	0.618 (0.443-0.767)
	Old Wives, SK	Non-Removal	28	0.466 (0.270-0.674)
2000	Frank, AB	Removal	28	0.313 (0.143-0.556)
	Paysen, SK	Removal	37	0.794 (0.609-0.905)
	Crane, SK	Non-Removal	45	0.525 (0.362-0.682)
	Kettlehut, SK	Non-Removal	37	0.743 (0.564-0.866)
2001	Chaplin, SK	Removal	34	1.00 (0.99-1.00)
	Kettlehut, SK[c]	Removal	33	1.00 (0.99-1.00)
	Frank, AB	Non-Removal	38	0.942 (0.778-0.987)
	Paysen, SK	Non-Removal	34	0.845 (0.630-0.946)

[a] MB = Manitoba, SK = Saskatchewan; AB = Alberta.
[b] Model-averaged estimates (unconditional 95% confidence intervals).
[c] Botulism was not detected at this site.

Table 4. Models used to evaluate effects of carcass density index (linear and quadratic terms), lake-year, radio-marking date, sex, and body mass on 30-day survival of molting mallards during botulism outbreaks, 1999-2001, in Prairie Canada. The best fitting model has the lowest Akaike's Information Criterion (AIC_c) adjusted for sample size. Only models with weight ≥0.05, the top-ranking model with carcass density effects and the null model are shown.

Year/Model	ΔAIC_c [a]	Weight [b]	K [c]
Lake-year, mass [d]	0	0.430	13
Lake-year, mass, date	0.44	0.346	14
Lake-year, mass, date, sex	1.95	0.162	15
Lake-year	2.41	0.117	12
Carcass density, carcass density², year, date, mass	14.29	0	7
Null	137.94	0	1

[a] The difference in value between AIC_c of the current model versus the best-fitting model.
[b] Weight of evidence in favor of model, relative to those in candidate list, weights sum to 1.0.
[c] Number of parameters (The model incorporating carcass density has 2 year estimates), including an intercept term.
[d] AIC_c is 1112.512.

PART III

RELATIONSHIPS BETWEEN LOCAL CARCASS DENSITY AND SURVIVAL OF MOULTING MALLARDS DURING OUTBREAKS OF AVIAN BOTULISM

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ABSTRACT

An inverse relationship between carcass density and survival has been reported in controlled studies of botulism (*Clostridium botulinum*, Type C) susceptibility in captive mallards (*Anas platyrhynchos*), but has not been verified in wild ducks during naturally-occurring botulism outbreaks. We radio-marked 204 moulting mallards on seven lakes in Western Canada during July and August 1999-2000, and monitored their survival daily for 30 days. Carcass searches were conducted simultaneously at 90 matched locations, for freshly dead and randomly selected, live radio-marked mallards. Carcass density (carcasses/ha) averaged about two times greater at dead than at live duck locations ($\bar{x}$ = 12.4, SE = 1.2 versus $\bar{x}$ = 5.0, SE = 0.7). Predicted risk of mortality increased rapidly with carcass density (case-control logistic regression: model-averaged $\beta_{\text{density}} = 0.167$, unconditional SE = 0.062). Mallards exposed to 5-11 and >11 carcasses/ha were 3.5 and 13 times more likely to die, respectively, than were mallards inhabiting carcass-free areas. Mortality risk was more closely related to density of maggot-laden carcasses than to maggot-free carcass densities. Our results were consistent with the assumption that reducing carcass density could enhance survival. However, we caution that survival rates may remain low on lakes in which areas with high carcass densities persist due to incomplete carcass removal.

INTRODUCTION

Maggot-laden carcasses are a known source of botulism (*Clostridium botulinum*, Type C) toxin for waterfowl (Duncan and Jensen 1976); and vertebrates that die from any cause in a wetland can initiate and perpetuate botulism through a carcass-maggot cycle (Wobeser 1997a; Rocke and Bollinger 2007). Thus, the principal goal of carcass removal (or 'clean-up') operations is to stop the carcass-maggot cycle and reduce mortality in healthy birds.

Reed and Rocke (1992) provided experimental confirmation that carcass removal could lower mortality, reporting that captive mallards (*Anas platyrhynchos*) held in 0.8 ha enclosures with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in pens with no carcasses. Here, we examined the relationships between carcass density and survival of free-ranging wild birds, by relating risk of mortality in wild radio-marked mallards, tracked during botulism outbreaks, to local variation in density and characteristics of carcasses on multiple lakes with and without carcass removal. Based on previous work (Reed and Rocke 1992), we predicted that mortality risk would be positively related to carcass density, and most strongly associated with density of maggot-laden carcasses.

METHODS

Mallards were captured, marked, and radio-tracked using standard methods. Briefly, adult mallards in pre-moult and wing feather moult were captured in bait traps and radio-marked (see below) from ~1 July to the second week of August, 1999 and 2000, at seven lakes on the Canadian Prairies (Table 1). Visibly healthy ducks were banded, equipped with back-mounted radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota, USA, Custom Model A4430; Mauser and Jarvis 1991) and released within 30 min. A thermister, connected to the transmitter anchor, was placed under the skin; and a ≥4 C drop in body temperature triggered an increase in transmitter pulse rate, indicating death. Marking methods were approved by the University of Saskatchewan Committee on Animal Care (Protocol 19980040).

Status (dead or alive) of each bird was determined daily using truck-mounted, fixed location, and hand-held telemetry. If a bird was dead, the carcass was retrieved as quickly as possible with the aid of a hand-held tracking system from an airboat. The location of each dead bird was recorded with a Global Positioning System (GPS) (Garmin Inc.™) and marked with a tall wooden stake,

and the carcass was frozen for necropsy. A search for other carcasses was then completed within a 50 m radius (0.785 ha plot) of the dead mallard's location as well as, within 24 hrs, at a randomly-selected, live radio-marked mallard's location. Live birds were drawn randomly (without replacement) each day a mallard died. We moved slowly to the live bird's morning position, using a hand-held tracking system from an airboat, and reduced our approach speed as signal strength increased and the bird began to move away from the boat.

At the start of each carcass search, coloured flagging tape was tied to vegetation, or a wooden stake was placed in four cardinal directions, 50 m from the central stake. A systematic search was conducted in water <1 m deep, by wading through the area with observers spaced 1 m apart in dense vegetation and ~3-5 m apart in sparse vegetation. Searches were conducted from an airboat in deeper water (see right). We recorded total number of carcasses found and, whenever possible, each carcass was assigned to a stage of decomposition (SOD), which was determined by comparing carcasses to color photographs and written descriptions (see Part I). SOD was not recorded at Old Wives Lake.

Gross necropsies were performed on radio-marked birds that died. Those that had lesions suggesting disease other than botulism were investigated using standard diagnostic techniques, including histology. Confirmation of botulism in waterfowl on individual wetlands was based on observations of birds with flaccid paralysis and testing of serum samples from sick birds for type C toxin using mouse bioassay.

Relationships between local carcass density and mortality risk were evaluated using case-control logistic regression analyses based on conditional maximum likelihood (PROC LOGISTIC, SAS Institute 1999), specifying bird status (i.e., dead versus alive) as a binary response variable and lake, carcass density, search date, and nested effects (e.g., density nested within lakes) as explanatory variables. Carcass density (carcasses/ha) was calculated as the number of carcasses found per area searched. Search date was expressed as number of days since 1 January.

Akaike's Information Criterion adjusted for sample size (AIC_c) was calculated using the estimated log-likelihood and number of parameters in the model (Burnham and Anderson 2002). Differences between AIC_c of the most plausible and other models (ΔAIC_c), and model Akaike weights (w_i), were used to compare models (Burnham and Anderson 2002). We also calculated model-averaged parameter estimates and unconditional standard errors (Burnham and Anderson 2002). Following Hosmer and Lemeshow (2000), we also assessed potential for nonlinear relations between carcass density and mortality risk using second and third order polynomials. We evaluated the influence of data distribution on estimates of mortality risk by grouping carcass density into four, five or six categories of equal sample size, or by balancing for sample size

among categories after creating a separate category for plots having no carcasses (~14% of plots). Risk of mortality in each category was assessed using odds ratios and 95% confidence intervals.

Finally, we conducted case-control logistic regression analyses that considered effects of carcasses with (SOD4-SOD6) and without (SOD1-SOD3) maggots. Because SOD was not recorded at Old Wives Lake, and Whitewater Lake had high overall mortality (Evelsizer 2002), we excluded these two lakes from this analysis. Models were constructed to estimate, separately and in combination, the effects of maggot-free and maggot-laden carcass densities on mortality risk. Model selection methods were as described above.

RESULTS

Botulism was the only substantial cause of mortality in ducks at all seven lakes. In 1999, nine of nine, six of six, and 13 of 14 serum samples tested positive for type C toxin at Whitewater, Old Wives and Eyebrow lakes, respectively. In 2000, five of five, six of seven, two of two, and four of four serum samples tested positive for type C botulism toxin at Kettlehut, Paysen, Frank and Crane lakes, respectively.

Searches were conducted at 90 matched dead and live bird locations from 26 July to 30 August in 1999, and from 15 July to 27 August in 2000 (Table 1). Overall, carcass density (carcasses/ha) was roughly two times higher at dead ($\bar{x}$ = 12.4, SE = 1.2, range 0-71) than at live ($\bar{x}$ = 5.0, SE = 0.7, range 0-42) bird locations.

Risk of mortality was best modeled by incorporating linear and nonlinear effects of carcass density (Table 2), such that mortality risk rose initially with carcass density and then levelled off ($\beta_{density}$ = 0.177, SE = 0.046; $\beta_{density}^2$ = -0.0021, SE = 0.0092). However, an equally plausible model indicated that mortality risk was positively ($\beta_{density}$ = 0.117, SE = 0.029) related to carcass density. These two models accounted for ~60% of summed *w*. Other plausible models, which incorporated more complex nonlinear density effects and date, had less support and parameter estimates were imprecise ($\beta_{density}^3$ = 0.00005, SE = 0.00007; β_{date} = 0.328, SE = 0.568). The model-averaged estimates of the relationship between carcass density and mortality risk were $\beta_{density}$ = 0.167 (unconditional SE = 0.062) and $\beta_{density}^2$ = -0.0031 (unconditional SE = 0.0028). Models that included effects of density nested within lakes had no support (i.e., $\Delta AIC_c \geq 20.000$ and $w \leq 0.001$ for the best of these models) and thus are not considered further.

Carcass density was grouped into four categories, one consisting of plots with no carcasses and three others of nearly equal sample size. Mallards exposed to 5-11 carcasses/ha and >11 carcasses/ha had roughly 3.5 (95% CI = 0.9 –13) times and 13 (95% CI = 3.5 – 48) times greater odds of dying, respectively, relative to birds inhabiting areas with no carcasses.

A case-control logistic regression model that incorporated effects of maggot-laden carcass density (w = 0.504; AIC_c = 118.497, K = 2, n = 60 pairs) was three times more plausible based on *w* than a model with both maggot-laden and maggot-free carcasses (i.e., total carcass density; w = 0.167, ΔAIC_c = 2.214, K = 3). A model with effects of maggot-free carcass density alone was

not well supported ($\Delta AIC_c = 4.513$, K = 2, $w = 0.053$). Thus, mortality risk was more closely associated with maggot-laden than maggot-free carcass density.

DISCUSSION

Our results were consistent with the assumption that survival of free-ranging ducks can be improved by reducing density of carcasses during outbreaks of avian botulism. Reed and Rocke (1992) reported that captive mallards placed in large enclosures with 12 carcasses/ha were 4.5 times more likely to die of botulism than were birds in pens with no carcasses. Relative to birds monitored in carcass-free areas, we found that wild mallards exposed to >11 carcasses/ha were ~13 times more likely to die. The nonlinear relationship that we observed between mortality risk and carcass density implies that carcass removal may be most effective at lower to intermediate carcass densities. When carcass densities are very high, reducing carcass density may have a limited impact on mortality risk. Thus, when feasible, early surveillance and carcass removal may be a more effective strategy for botulism management than attempting to reduce high carcass densities after an outbreak has initiated. Differences in toxicity, amount of available toxin, environmental characteristics, live bird density, or other as yet unidentified factors likely contributed to variation in risk of mortality (Wobeser 1997b; Rocke and Brand 1994; Rocke et al. 1999).

Because our focus was on flightless moulting birds, we assumed that carcass density estimates represented conditions experienced by radio-marked mallards during the timeframe over which botulism intoxication could occur. However, because birds can live for hours to days after ingesting type C toxin, depending on the dose consumed (Duncan and Jensen 1976), we could not determine precisely where and when ducks ingested toxin. Likewise, we assumed that factors influencing carcass detection probability were unbiased with respect to a bird's status on each lake.

In a related study, survival of radio-marked mallards was not enhanced by carcass removal operations (Part II; Evelsizer 2002), possibly because maggot-laden carcasses remained in some "hot spot" areas of lakes during removal operations. Consistent with this last suggestion, maggot-laden carcasses were abundant in some localized areas of all clean-up lakes (Table 1; Figure 1). It seems likely that removal operations would not substantially reduce mortality unless extremely low carcass densities can be maintained on most areas of lakes during an outbreak. Our results clearly demonstrate that mortality risk could potentially be reduced with carcass removal.

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Table 1. Estimates of carcass density (carcasses/ha) near the locations of dead and live radio-marked mallards on seven lakes, 1999-2000. Shown are number of searches (N), and mean ($\bar{x}$) and range of carcass density. Maggot-laden carcasses were not recorded at Old Wives Lake (NA). Carcasses were actively removed from Whitewater, Frank and Paysen lakes as part of clean-up operations. Lake locations and coordinates are provided in Figure 1 and Table 1 of the General Introduction.

Lake Province[a]	Dead birds						Live birds					
	All carcasses[b]			Maggot-laden carcasses[c]			All carcasses[b]			Maggot-laden carcasses[c]		
	N	$\bar{x}$	Range	N	$\bar{x}$	Range	N	$\bar{x}$	Range	N	$\bar{x}$	Range
Whitewater MB	12	16	3-37	8	4	0-13	12	2	0-10	8	0	-
Old Wives SK	13	15	1-51	NA	-	-	13	2	0-9	NA	-	-
Eyebrow SK	12	15	5-29	7	13	3-28	12	6	0-17	7	4	0-9
Crane SK	16	11	0-71	16	8	0-64	16	6	0-19	16	4	0-9
Frank AB	21	10	1-21	21	4	0-9	21	5	0-13	21	2	0-7
Paysen SK	8	8	0-38	8	4	0-20	8	6	0-24	8	3	0-11
Kettlehut SK	8	14	0-33	8	8	0-18	8	9	0-42	8	7	0-35

[a] MB = Manitoba, SK = Saskatchewan; AB = Alberta.
[b] All carcasses (SOD 1-6; see definitions in Methods) found in search plots.
[c] Carcasses with visible maggots (SOD 4-6; see definitions in Methods).

Table 2. Candidate set of case-control logistic regression models used to evaluate risk of mortality due to avian botulism for radio-marked mallards from seven lakes in Prairie Canada during July and August, 1999-2000. Models considered effects of carcass density (linear, squared and cubic terms), marking and release date (date), lake and density nested within lakes (see Methods) on mortality risk; only the five top-ranked models are shown.

Model	AIC_c [a]	ΔAIC_c [b]	w [c]	K [d]
Density, density^2	99.786	0	0.349	3
Density	100.505	0.719	0.244	2
Density, density^2, density^3	101.194	1.408	0.172	4
Density, density^2, date	101.686	1.900	0.135	4
Density, date	102.284	2.500	0.100	3

[a] Akaike's Information Criterion, adjusted for sample size.
[b] Difference in AIC_c between current model and best approximating model (AIC_c = 99.786).
[c] Weight of evidence in favour of model relative to others in the candidate list; model weights sum to 1.0.
[d] Number of parameters.

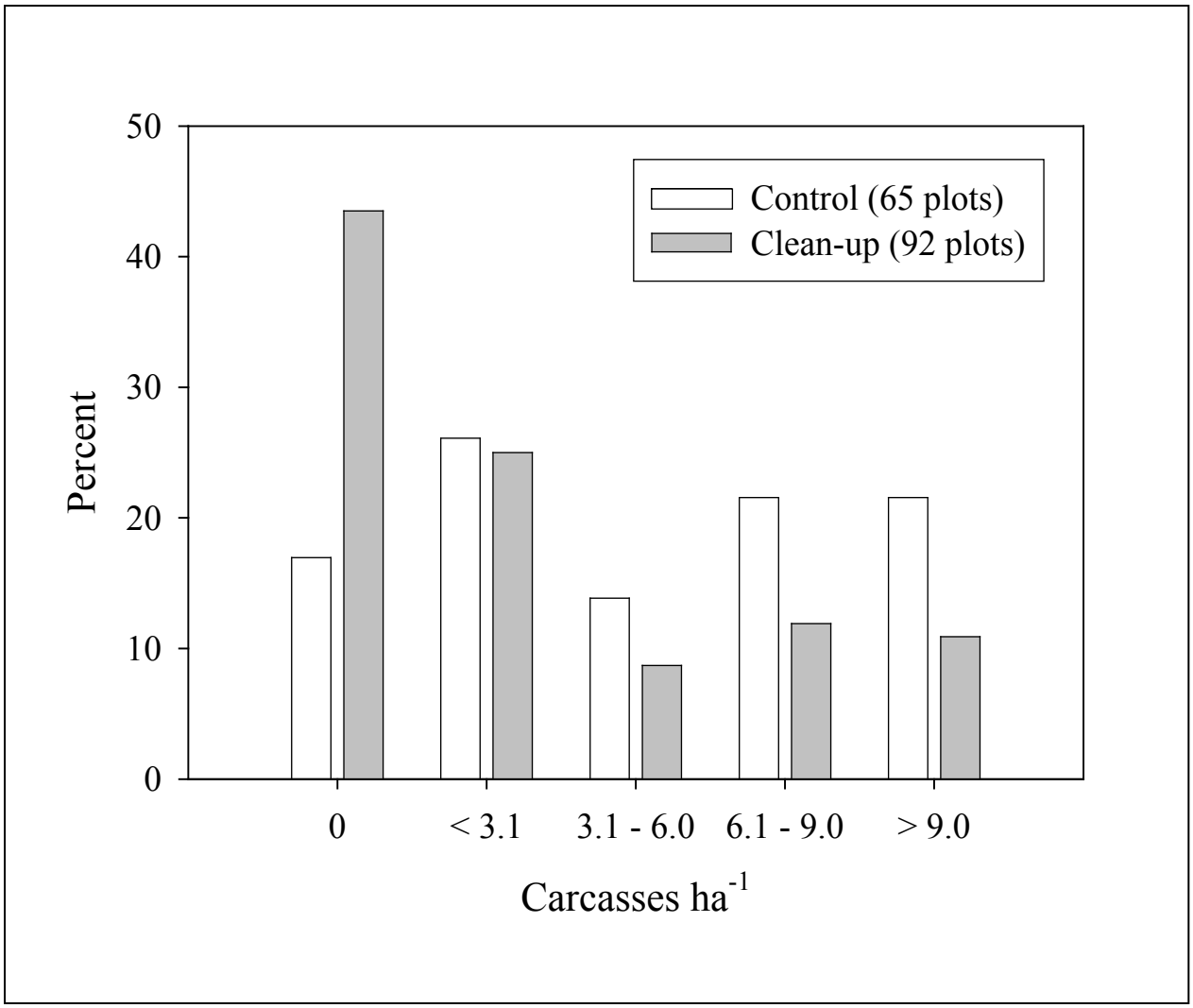

Figure 1. Density of maggot-laden carcasses in relation to carcass clean-up on Saskatchewan lakes, 1999-2000. Shown is percentage of plots with different carcass density levels for lakes with carcass removal (clean-up) and for control lakes.

PART IV

LATE-SUMMER SURVIVAL OF MALLARDS EXPOSED TO AVIAN BOTULISM: AN INVESTIGATION USING DIRECT BAND RECOVERY ANALYSES

KEVIN W. DUFOUR AND TRENT K. BOLLINGER

ABSTRACT

Catastrophic losses of waterfowl to avian botulism (*Clostridium botulinum*, Type C) are well documented, but few studies have attempted to quantify impacts at the population level. We used band-recovery data from adult mallards (*Anas platyrhynchos*), trapped at nine botulism outbreak sites in Prairie Canada (late June to early August, 1998-2000), to determine the extent to which exposure to botulism during the post-breeding season resulted in reduced late-summer survival. Specifically, we tested the prediction that direct recovery rates (indicative of survival during the period between banding and harvest) would be lower among birds banded at outbreak sites (n = 6,594) than among conspecifics banded at non-outbreak control sites (n = 15,241). As a secondary objective, we tested the prediction that direct recovery rates should be higher among individuals banded at outbreak sites with intensive carcass clean-up operations than among those banded at outbreak sites with no such clean-up. Banding data from two of the three Prairie provinces (Saskatchewan and Manitoba) were uniformly consistent in supporting the predicted association between exposure to botulism and direct recovery probability: recovery rates of mallards banded at outbreak sites in these areas were 14-44% lower than expected based on comparisons with control data. This pattern was evident among both males and females, and occurred in all three years of study. In contrast, results from a third province (Alberta) were mixed and generally did not support the predicted association. Overall, our results indicate that exposure to botulism during the post-breeding season can have a measurable impact on survival at the population level. However, our findings from Alberta suggest a need for caution when generalizing from these results. Finally, our comparison of recovery rates of mallards banded at outbreak sites with and without intensive carcass removal provided little evidence to suggest that carcass removal was effective in reducing botulism-related mortality. Future investigations should seek to: (1) refine our ability to predict when and where botulism-related mortality is likely to be most severe, and (2) identify and evaluate alternative management strategies.

INTRODUCTION

Avian botulism (*Clostridium botulinum*, Type C) is an issue of major concern to waterfowl managers in North America, in part because the frequency and magnitude of botulism outbreaks has increased in recent years. Although catastrophic losses of waterfowl to botulism are well documented, little is known about the impact of the disease at the population level. In particular, estimates of botulism-related mortality rates are lacking, as the number of individuals present at outbreak sites (i.e., number at risk) is rarely quantified.

Our first aim was to use band-recovery data from mallards (*Anas platyrhynchos*) trapped at nine major outbreak sites in Prairie Canada, to test the hypothesis that individuals exposed to outbreak levels of botulism during the post-breeding season suffer reduced late-summer survival as a consequence. Specifically, we tested the prediction that direct recovery rates (indicative of survival between banding and the hunting season) would be lower among individuals banded at outbreak sites than among conspecific individuals banded at non-outbreak control sites.

We also summarize results of an analysis aimed at assessing the extent to which carcass removal is effective in reducing mortality among post-breeding mallards. Specifically, using banding data assembled from a three-year field investigation, we tested the prediction that the rate of direct recovery by hunters (indicative of survival during the period between banding and harvest) should be higher among birds banded at outbreak sites with intensive clean-up operations than among birds banded at outbreak sites with no such clean-up. This second direct recovery analysis complements that shown in Part II of this report.

METHODS

Data collection

This study was conducted as part of a broader investigation, initiated in 1998 by the Prairie Habitat Joint Venture (PHJV) Avian Botulism Working Group. Field protocols for that broader investigation included the capture and banding of mallards and other dabbling ducks at selected botulism outbreak sites in each of the three Canadian Prairie provinces. Banding data used in the analyses presented here were derived from fieldwork conducted at the following botulism outbreak sites: Pakowki Lake, Alberta (AB) and Old Wives Lake, Saskatchewan (SK) in 1998; Kimiwan Lake, AB, Eyebrow Lake, SK, Old Wives Lake, SK, and Whitewater Lake, AB in 1999; and Frank Lake, AB, Crane Lake, SK, Paysen Lake, SK, Kettlehut Lake, SK, and Whitewater Lake, Manitoba (MB) in 2000 (locations and coordinates of lakes are shown in Figure 1 and Table 1 of the General Introduction). In each of the three years, a subset of outbreak sites was subjected to surveillance and carcass collection as part of an experimental protocol aimed at assessing the effectiveness of traditional management practices. Additional banding information was obtained from "control" wetlands that lacked botulism outbreaks (details below).

In each year of study, post-breading mallards were: captured using wire bait traps; classified according to sex and age (hatch-year [HY] vs. after-hatch-year [AHY]); fitted with a standard U.S. Fish and Wildlife Service aluminum leg band (see right); and then released. Capture dates spanned 22 June - 1 August in 1998, 8 July - 9 August in 1999, and 5 July - 24 August in 2000, with most birds being captured in July or early August. Birds were classified as recovered if they were shot and retrieved during the hunting season immediately following banding and the band was reported to the U.S. Bird Banding Laboratory or the Canadian Bird Banding Office. Because few HY birds were banded in any given year, analyses were restricted to AHY individuals (hereafter "adults").

Assessing the impact of exposure to botulism on late-summer survival required band recovery data from non-outbreak control sites for comparison. For the control sample, banding data were collected as part of the Western Canada Cooperative Waterfowl Banding Program, administered jointly by the Canadian Wildlife Service (CWS) and the U.S. Fish and Wildlife Service (USFWS). Because focal botulism outbreak sites were distributed among three broad geographic regions (AB, SK, and MB), three traditional cooperative banding sites were selected for use as controls: Brooks, AB; Last Mountain Lake, SK; and Big Grass Marsh, MB. These sites were chosen on the basis of their proximity to focal botulism outbreak sites (generally <200 km from all botulism study sites in the same region) and because they consistently produced large samples of banded mallards on an annual basis. Field crews associated with the cooperative banding program actively avoided banding at sites where avian botulism was evident. Thus, banding data obtained under the cooperative program were suitable for our purposes.

Direct recoveries of mallards banded on botulism wetlands with and without clean-up

Banding data used in this study were derived from fieldwork conducted during 1998-2000, at nine major botulism-prone wetlands in Prairie Canada (two in 1998, four in 1999, and five in 2000; Table 1). An explicit objective of the PHJV Avian Botulism Working Group study was to evaluate the effectiveness of traditional management practices at reducing losses to avian botulism. Thus, in each year of study, carcass removal (or 'clean-up') was employed on roughly half of the focal wetlands (one in 1998, two in 1999, and two in 2000; Table 1), whereas no removal was attempted on the remaining wetlands. All focal wetlands were characterized by a history of avian botulism, and botulism outbreaks were confirmed in the year of study in all cases (methods described in Part I).

We used the rate of direct recovery as a relative index of survival during the period between banding and the onset of the hunting season (Samuel et al. 1992). To statistically evaluate among-site variation in recovery rates, we developed binary logistic regression models, specifying: recovery status (recovered vs. not recovered) as the binary response variable; date of banding as a covariate; and sex and location as categorical predictors. Initial models also included a sex-by-location interaction term, to allow for the possibility that the pattern of among-site variation in recovery probability differed between the sexes. In all analyses, when an interaction term was not significant ($P > 0.10$), it was removed and a reduced model was developed. All analyses were conducted separately by year.

It is worth noting that the analytical design described above effectively pairs botulism outbreak and non-outbreak control sites occurring within the same geographic region (i.e., province). For most year-region combinations (six of eight), each treatment group was represented by a single site. However, SK was represented by two botulism study sites in 1999 and three botulism sites in 2000. For purposes of analysis, we combined data from SK sites in each of these two years, based on the assumption that recovery rates of birds banded at these sites were similar. A preliminary analysis confirmed that recovery rate differences among outbreak sites in SK did not differ for both 1999 and 2000 (K. Dufour, unpublished data).

RESULTS

Over three years of study, trapping at botulism outbreak sites yielded a total banded sample of 6,594 adult mallards (annual totals for 1998, 1999, and 2000 were 1,057, 1,679, and 3,858, respectively; Table 1). Over the same period, 15,241 adult mallards were trapped and released at CWS/USFWS banding stations selected for use as controls (Table 1). Both samples were heavily skewed toward males, with males comprising 84 and 79% of the total banded sample for botulism and control sites, respectively (Table 1).

Recovery rate comparisons - 1998

We predicted that recovery rates of mallards banded at botulism outbreak sites would be lower than those of mallards banded in the same year at non-outbreak control sites. Consistent with this prediction, recovery rate estimates for control sites in 1998 exceeded point estimates for outbreak sites in three of four separate comparisons (by sex and region; Figure 1). However, the initial logistic regression model revealed a marginally significant treatment-by-region interaction ($\chi^2 = 2.79$, df = 1, $P = 0.095$), suggesting regional variation in recovery rate differences between outbreak and control sites. Analyses conducted separately by region indicated a significant treatment effect in SK but no apparent treatment effect in AB (Table 2). Where a treatment effect was evident (i.e., in SK), recovery rates of birds banded at botulism outbreak sites were 44% lower (sexes pooled) than expected based on comparison with control data (Figure 1). In both SK and AB, recovery rates of males exceeded those of females (Figure 1; Table 2); a result that likely reflects sex differences in vulnerability to hunting (Anderson 1975, Nichols et al. 1990).

Recovery rate comparisons - 1999

Data from 1999 were consistent with our initial prediction, in that recovery rate estimates for control sites exceeded point estimates for outbreak sites in five of six separate comparisons (Figure 2). However, similar to 1998, the initial logistic regression model suggested an interaction effect between treatment and region ($\chi^2 = 4.57$, df = 2, P = 0.102). Inspection of the actual recovery rates indicated that the interaction was largely attributable to an anomalous pattern in the data from AB. Specifically, whereas recovery rate estimates for outbreak sites in SK and MB were consistently lower than corresponding estimates obtained from control sites, results from AB were mixed (Figure 2). Accordingly, when recovery data from AB were analyzed separately from data obtained from the other two provinces, a significant effect of botulism was found for SK and MB, but not for AB (Table 3, Figure 2). Recovery rates of birds banded at outbreak sites in SK and MB were, respectively, 44 and 18% lower (sexes pooled) than expected based on comparisons with control data (Figure 2).

Recovery rate comparisons - 2000

Results from the 2000 field season were similar to those obtained in 1999: the initial logistic regression model revealed a significant treatment-by-region interaction ($\chi^2 = 7.48$, df = 2, P = 0.024) and inspection of recovery rate estimates suggested that the interactive effect was largely due to the pattern in AB differing from that observed in the other two provinces (Figure 3). Thus, we again treated data from AB separately in the final analysis. Results indicated a highly significant treatment effect in SK and MB (Table 4), with recovery rates of birds banded at outbreak sites being 14-35% lower (sexes pooled) than expected based on comparisons with control data (Figure 3). Interestingly, the opposite pattern was evident in AB (Figure 3), although recovery rate differences between outbreak and control sites in that province were only marginally significant (Table 4).

Clean-up versus no clean-up on botulism-prone wetlands

Over all years, field crews working at botulism outbreaks sites trapped and released 6,594 adults mallards (annual totals for 1998, 1999, and 2000 were 1,057, 1,679, and 3,858, respectively; Table 5). Partitioning data by sex and site, direct recovery rates ranged from 0-5.7% in 1998, 0-8.0% in 1999, and 0-16.7% in 2000 (Table 5). For reasons explained above, recovery rates of males almost invariably exceeded those of females (Table 5).

If carcass removal is effective at reducing botulism-related mortality, one would expect recovery rates of birds banded at managed sites to be greater than those of birds banded at unmanaged sites. Results from 1998 were consistent with this expectation, in that recovery rates of birds banded at Pakowki Lake, AB (a removal site) exceeded those of birds banded at Old Wives Lake, SK (a non-removal site; Table 5). However, formal analysis indicated that, after controlling effects of sex and date of banding, differences between the two sites were no greater than expected based on sampling error (P = 0.651; Table 6). Similar results were obtained for 1999 (Tables 5 and 6).

Results from the 2000 field season were slightly more complex, in that the initial logistic analysis revealed a significant sex-by-site interaction (P = 0.008; Table 6). Accordingly, for that year, we assessed among-site variation in recovery probability separately by sex. With respect to our central objective, results were mixed and generally did not support the predicted association between carcass removal and direct recovery rate. Among males, the recovery rate of birds banded at one removal site (Frank Lake, AB) was the highest observed in that year (Table 5), but the opposite was true of a second removal site (Paysen Lake, SK; Table 5). Further, logistic analysis indicated that, overall, variation among sites was no greater than expected (P = 0.23; Table 6), despite reasonably large samples of banded individuals. Among females, the pattern of recovery rate variation was consistent with our original prediction (Table 5), but sample sizes in this instance were extremely limited (e.g., only five recoveries from removal sites). Perhaps not surprisingly, formal analysis again failed to distinguish among-site variation from random sampling error (P = 0.20; Table 6).

DISCUSSION

Our results lend considerable support to the suggestion that, for mallards, exposure to botulism during the post-breeding season can have a measurable impact on survival at the population level. In particular, analyses of banding data from SK and MB were uniformly consistent in supporting the predicted association between exposure to botulism and direct recovery probability. To the extent that recovery probability provides a relative index of late-summer survival, data from SK and MB suggested a 14-44% reduction in survival among mallards using botulism outbreak sites. This finding seems especially significant given that, in the absence of disease, late-summer survival rates of post-breeding mallards are thought to approach unity.

In contrast to the pattern observed in SK and MB, results from AB were mixed and generally did not support the prediction that mallards banded at outbreak sites would show reduced rates of direct recovery. Reasons for this discrepancy are unclear. Conceivably, differences in results obtained from the two areas might be attributable to regional variation in factors such as timing of post-breeding activities and/or timing and extent of post-breeding movements. For instance, if mallards in AB had, on average, completed post-breeding activities earlier in the season (resulting in a disproportionately high number of post wing-moult individuals in the banded sample), many banded individuals might have emigrated from botulism study areas before the onset of the propagation phase of the disease, thus reducing their overall level of exposure. Alternatively, botulism outbreaks at the AB study sites may have been less severe than those occurring at study sites in the other two provinces. It is possible that outbreaks at Kimiwan Lake and Frank Lake (both in AB) might have been less severe than initially assumed.

To our knowledge, only one other study has attempted to quantify effects of exposure to botulism on rates of late-summer survival in waterfowl. Evelsizer et al. (Part II) deployed radio-marked mallards on the same botulism outbreak sites as those used in the present study, and monitored individual survival for a period of 30 days. They found that 30-day survival rates ranged from ~0.15 to >0.70, depending on the wetland examined. An important distinction

between the present study and the radio-telemetry investigation is that the latter focused exclusively on moulting (i.e., flightless) individuals, so radio-marked birds were essentially "grounded" and therefore present at botulism outbreak sites throughout the entire 30-day monitoring period. In contrast, our banded samples included birds in all three major stages of wing moult (i.e., pre-moult, moulting, and post-moult), and it is likely that many birds used in our analyses were present on the study area (and therefore exposed to the outbreak) for a much shorter period of time. That we were able to detect recovery rate differences between outbreak and non-outbreak control sites despite these considerations suggests a strong influence of botulism on survival probability.

Although it seems clear that exposure to botulism can have a measurable impact on survival at the population level, several research needs are evident. First, studies are needed to determine the extent to which existing results for mallards can be generalized to other avian species, particularly those of potential management concern (e.g., northern pintails [*Anas acuta*]). Second, as indicated earlier, it is unclear why patterns of band recoveries from outbreak and non-outbreak wetlands might vary on a regional basis. In the context of the present study, it would be instructive to know why results obtained from AB were so at odds with results obtained from the other two Prairie provinces. Finally, it is important to recognize that effects of botulism on continental populations will depend not only on the mortality rates of exposed individuals, but also on the proportion of individuals in the population that are routinely exposed. While some information on mortality rates is now available (e.g., Evelsizer et al. 2010), virtually nothing is known about the size of the population at risk. Unfortunately, the secretive nature of moulting and staging waterfowl, coupled with challenges associated with predicting botulism outbreaks, will make this information very difficult to obtain. Nevertheless, such information is necessary if we are to fully understand the impact of botulism at the population level.

Finally, our results provide little compelling evidence to suggest that carcass removal is effective at reducing waterfowl mortality due to avian botulism. After controlling effects of potentially confounding variables, we were unable to detect measurable differences in direct recovery rates among mallards banded at outbreak sites with and without intensive carcass clean-up. Similar to results of the present study, findings in Part II indicate no consistent difference in survival rates of birds moulting on wetlands with and without intensive carcass removal. Bollinger et al. (Part I) further demonstrated that the efficiency of carcass removal operations on Prairie wetlands is typically far from absolute. Using samples of marked carcasses, they estimated that only 7-51% of carcasses are actually retrieved, depending of the size of the wetland and the extent of vegetation. Collectively, these recent investigations call into question the viability of carcass removal as a management strategy, at least for large, heavily vegetated wetlands in Prairie Canada. Additional research is needed to identify alternative management strategies and to evaluate their efficacy.

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Table 1. Number of adult (after-hatch-year) mallards trapped and released during July-August at selected botulism outbreak sites in Prairie Canada, 1998-2000. Also tabulated are corresponding totals for banding stations selected for use as non-outbreak control sites. Locations and coordinates for most* lakes are provided in Figure 1 and Table 1 of the General Introduction.

			Numbers Banded		
Year	**Lake, Province**	**Type**	**Males**	**Females**	**Total**
1998	Pakowki, AB	Botulism	490	290	780
	Brooks, AB	Control	1,725	687	2,412
	Old Wives, SK	Botulism	241	36	277
	Last Mountain, SK	Control	1,823	625	2,448
1999	Kimiwan, AB	Botulism	323	153	476
	Brooks, AB	Control	1,380	333	1,713
	Eyebrow, SK	Botulism	145	65	210
	Old Wives, SK	Botulism	475	19	494
	Last Mountain, SK	Control	449	104	553
	Whitewater, MB	Botulism	470	29	499
	Big Grass Marsh, MB	Control	1,596	293	1,889
2000	Frank, AB	Botulism	155	17	172
	Brooks, AB	Control	2,413	433	2,846
	Crane, SK	Botulism	466	32	498
	Paysen, SK	Botulism	764	24	788
	Kettlehut, SK	Botulism	1,046	28	1,074
	Last Mountain, SK	Control	1,074	273	1,347
	Whitewater, MB	Botulism	945	381	1,326
	Big Grass Marsh, MB	Control	1,565	468	2,033
		Total	17,545	4,290	21,835

* Except for Brooks, the coordinates for which are 50.35N - 111.53W.

[a] AB = Alberta; SK = Saskatchewan; MB = Manitoba.

Table 2. Logistic analyses evaluating effects of date of banding, sex, and treatment (botulism vs. control) on the probability of direct recovery for mallards banded at botulism outbreak and non-outbreak control sites in 1998. Outbreak sites include Pakowki Lake, Alberta (AB) and Old Wives Lake, Saskatchewan (SK). Control sites for AB and SK were Brooks and Last Mountain Lake, respectively. Second-order interactions were not significant (P > 0.10) and were therefore removed from each model.

Province	n	Source	Coefficient	χ^2	df	P
AB	3,192	Date	0.00	0.00	1	0.945
		Sex	0.78	14.44	1	<0.001
		Treatment	-0.12	0.11	1	0.745
SK	2,725	Date	-0.01	0.79	1	0.373
		Sex	0.89	16.66	1	<0.001
		Treatment	-1.04	4.58	1	0.032

Table 3. Logistic analyses evaluating effects of date of banding, sex, and treatment (botulism vs. control) on the probability of direct recovery for mallards banded at botulism outbreak and non-outbreak control sites in 1999. Outbreak sites included: Kimiwan Lake, Alberta (AB); Eyebrow Lake, Saskatchewan (SK); Old Wives Lake, SK; and Whitewater Lake, Manitoba (MB) (data from the two SK sites were combined for analysis). Control sites for AB, SK, and MB were Brooks, Last Mountain Lake, and Big Grass Marsh, respectively. Second-order interactions were not significant (P > 0.10) and were therefore removed from each model.

Province	n	Source	Coefficient	χ^2	df	P
AB	2,189	Date	0.01	0.47	1	0.495
		Sex	0.49	4.10	1	0.043
		Treatment	0.37	0.57	1	0.451
SK / MB	3,645	Date	-0.01	1.17	1	0.279
		Sex	0.36	2.68	1	0.102
		Province	0.06	0.13	1	0.722
		Treatment	-0.67	5.44	1	0.020

Table 4. Logistic analyses evaluating effects of date of banding, sex, and treatment (botulism vs. control) on the probability of direct recovery for mallards banded at botulism outbreak and non-outbreak control sites in 2000. Outbreak sites included: Frank Lake, Alberta (AB); Crane Lake Saskatchewan (SK); Paysen Lake, SK; Kettlehut Lake, SK; and Whitewater Lake, Manitoba (MB) (data from the three SK sites were combined for analysis). Control sites for AB, SK, and MB were Brooks, Last Mountain Lake, and Big Grass Marsh, respectively. Second-order interactions were not significant ($P > 0.10$) and were therefore removed from each model.

Province	n	Source	Coefficient	χ^2	df	P
AB	3018	Date	0.01	1.28	1	0.258
		Sex	0.41	3.72	1	0.054
		Treatment	0.58	3.22	1	0.073
SK / MB	7066	Date	0.00	0.15	1	0.695
		Sex	0.63	21.39	1	<0.001
		Province	0.09	0.90	1	0.342
		Treatment	-0.37	9.14	1	0.003

Table 5. Direct recovery rates of mallards trapped and released during late summer (July - early August) at selected botulism outbreak sites in Prairie Canada, 1998-2000. Managed sites (those subject to surveillance and carcass removal) are indicated with *.

Year	Sex	Lake, Province[a]	Number Banded	Number Recovered	Percent Recovered
1998	Male	Pakowki Lake, AB*	490	28	5.7
		Old Wives Lake, SK	241	12	5.0
	Female	Pakowki Lake, AB*	290	10	3.4
		Old Wives Lake, SK	36	0	0.0
1999	Male	Kimiwan Lake, AB*	323	26	8.0
		Eyebrow Lake, SK	145	8	5.5
		Old Wives Lake, SK	475	21	4.4
		Whitewater Lake, MB*	470	31	6.6
	Female	Kimiwan Lake, AB*	153	5	3.3
		Eyebrow Lake, SK	65	2	3.1
		Old Wives Lake, SK	19	0	0.0
		Whitewater Lake, MB*	29	1	3.4
2000	Male	Frank Lake, AB*	155	18	11.6
		Crane Lake, SK	466	37	7.9
		Paysen Lake, SK*	764	53	6.9
		Kettlehut Lake, SK	1046	89	8.5
		Whitewater Lake, MB	945	77	8.1
	Female	Frank Lake, AB*	17	1	5.9
		Crane Lake, SK	32	1	3.1
		Paysen Lake, SK*	24	4	16.7
		Kettlehut Lake, SK	28	0	0.0
		Whitewater Lake, MB	381	13	3.4

[a] AB = Alberta; SK = Saskatchewan; MB = Manitoba.

Table 6. Results of logistic regression models evaluating among-site variation in direct recovery probability of mallards, after controlling effects of sex (1998 and 1999 only) and date of banding. For 2000, models were developed separately by sex because preliminary analyses revealed a significant sex-by-site interaction ($\chi^2 = 13.82$, df = 4, P = 0.008). No such interaction was evident in either 1998 ($\chi^2 = 0.22$, df = 1, P = 0.64) or 1999 ($\chi^2 = 0.61$, df = 3, P = 0.89).

Model	n	Source	χ^2	df	P
1998 - sexes combined	1,057	Date	0.06	1	0.807
		Sex	3.14	1	0.076
		Site	0.20	1	0.651
1999 - sexes combined	1,679	Date	3.66	1	0.056
		Sex	4.47	1	0.035
		Site	1.84	3	0.606
2000 - males	3,376	Date	1.61	1	0.205
		Site	5.64	4	0.227
2000 - females	482	Date	0.60	1	0.440
		Site	5.96	4	0.202

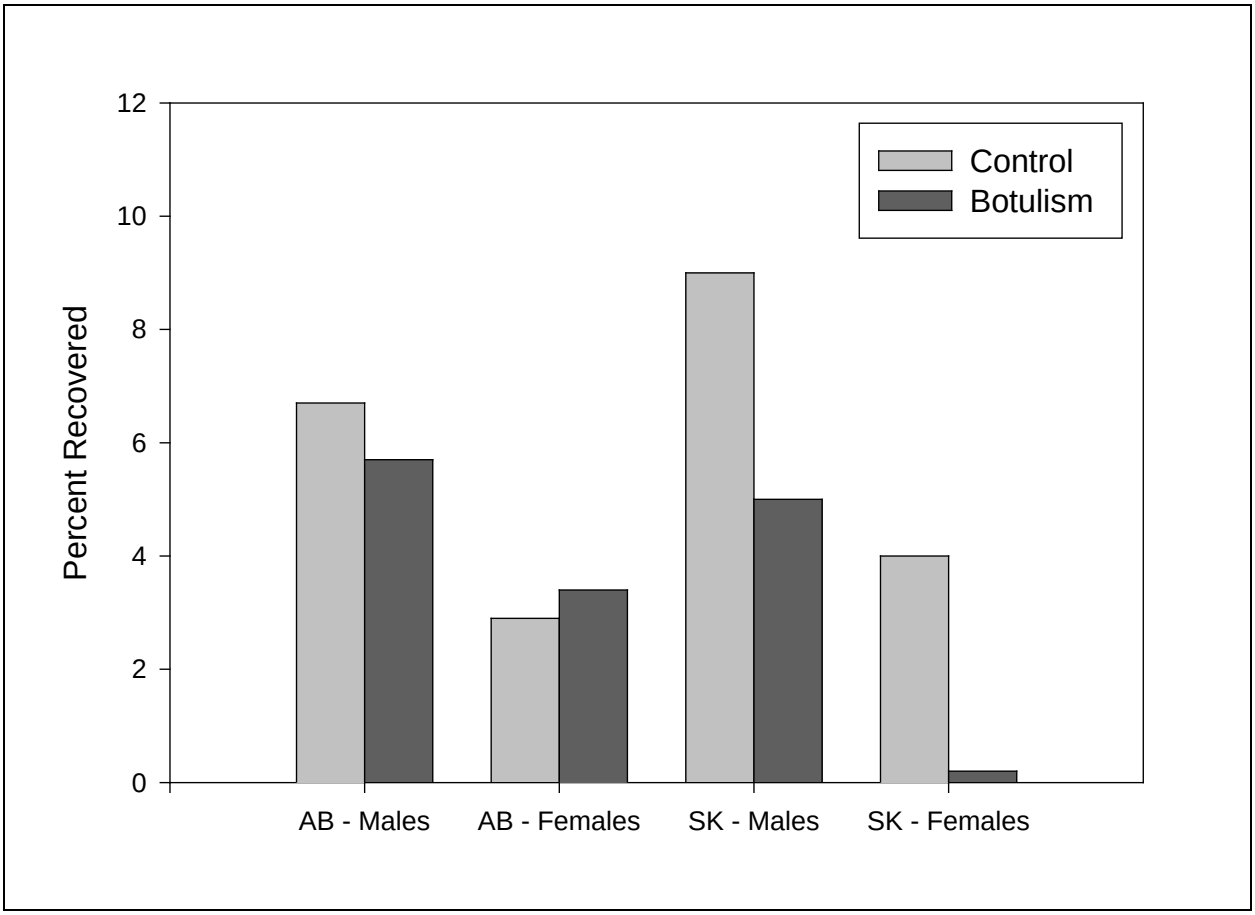

Figure 1. Direct recovery of mallards banded at botulism outbreak and non-outbreak control sites Alberta (AB) and Saskatchewan (SK), 1998. Outbreak and control sites for AB are Pakowski Lake and Brooks, respectively. Outbreak and control sites for SK are Old Wives Lake and Last Mountain Lake, respectively. The number of banded individuals for each site is given in Table 1.

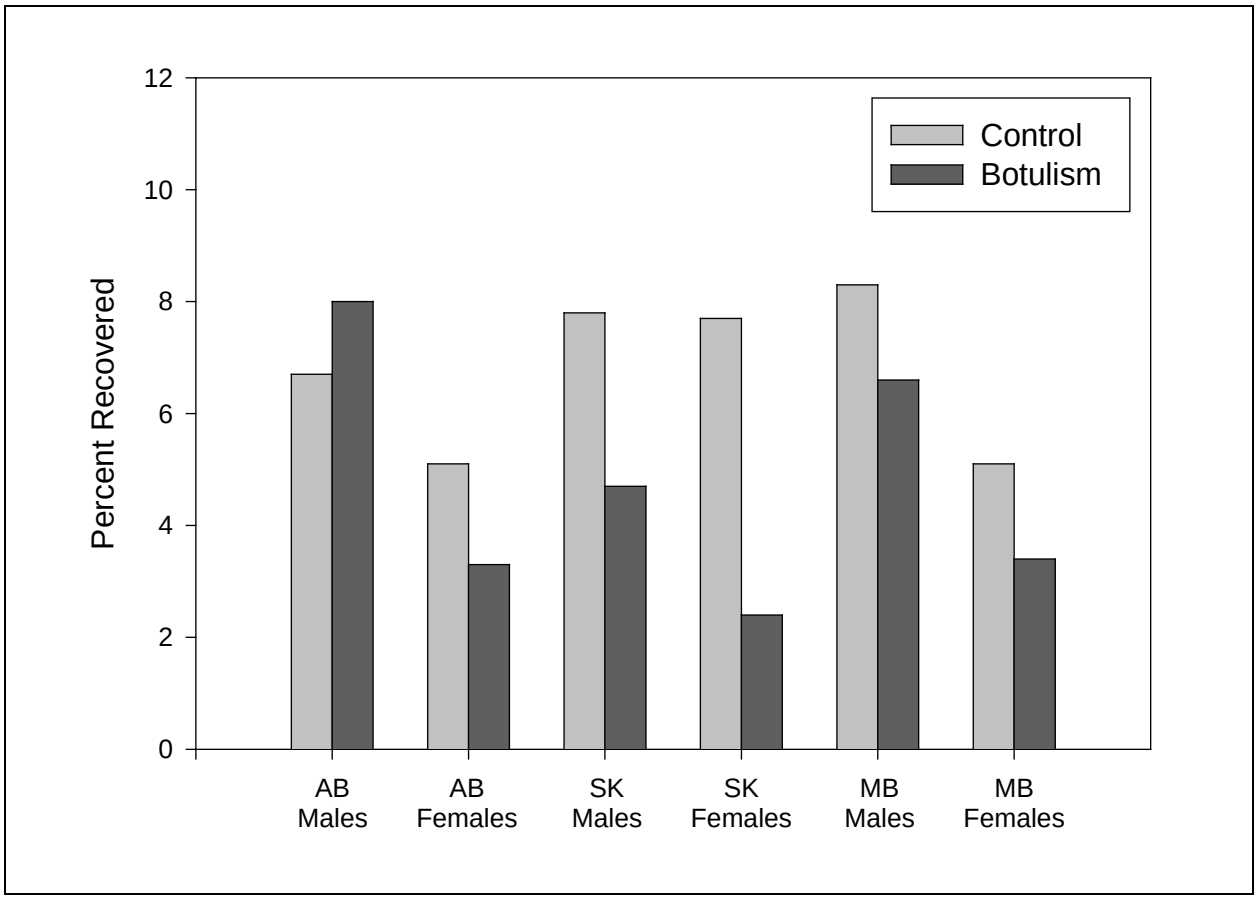

Figure 2. Direct recovery rates of mallards banded at botulism outbreak sites and non-outbreak control sites in each of the three Prairie provinces, 1999. Outbreak sites include: Kimiwan Lake, Alberta (AB); Eyebrow Lake, Saskatchewan (SK); Old Wives Lake, SK; and Whitewater Lake, Manitoba (MB) (data from the two SK sites were combined for analysis). Control sites for AB, SK, and MB were Brooks, Last Mountain Lake, and Big Grass Marsh, respectively. The number of banded individuals for each site is given in Table 1.

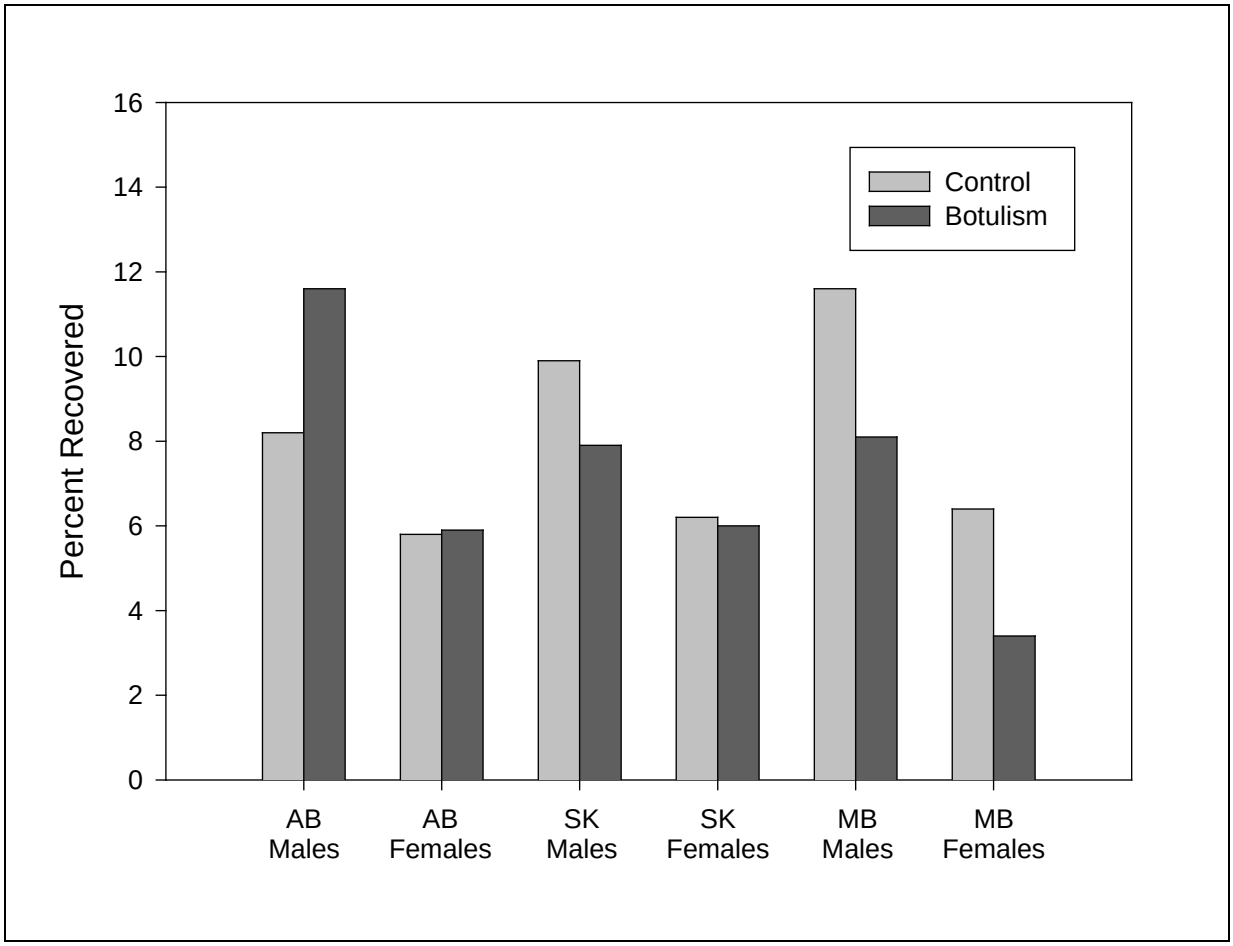

Figure 3. Direct recovery rates of mallards banded at botulism outbreak sites and non-outbreak control sites in each of the three Prairie provinces, 2000. Outbreak sites include: Frank Lake, Alberta (AB); Crane Lake, Saskatchewan (SK); Paysen Lake, SK; Kettlehut Lake, SK; and Whitewater Lake, Manitoba (MB) (date from the three SK sites were combined for analysis). Control sites for AB, SK, and MB were Brooks, Last Mountain Lake, and Big Grass Marsh, respectively. The number of banded individuals for each site is given in Table 1.

PART V

IDENTIFICATION OF PRIMARY SUBSTRATE IN THE INITIATION OF AVIAN BOTULISM OUTBREAKS

CATHERINE SOOS and GARY WOBESER

This section appears, unabridged, in Journal of Wildlife Management 70:43-53, 2006. Please contact Catherine Soos (catherine.soos@ec.gc.ca) for a copy of this paper.

ABSTRACT

The source of substrate for initial proliferation and toxigenesis of *Clostridium botulinum*, type C prior to outbreaks of avian botulism is typically unknown. We investigated factors involved in the initiation of avian botulism outbreaks at Eyebrow Lake, Saskatchewan, focussing on the role of Franklin's gull (*Larus pipixcan*) mortality as a source of initial substrate for *C. botulinum*. From 1999 to 2001, hatch-year gull carcasses were the predominant source of toxin-laden maggots found prior to outbreaks of avian botulism in waterfowl. Peak carcass density of gulls occurred one to two weeks prior to the onset of botulism outbreaks in waterfowl. Nest density at the beginning of the breeding season was a significant predictor of juvenile gull carcass density. Both the proportion of gull carcasses developing maggots and the proportion of maggot samples containing toxin increased as the season progressed, and carcasses were 22.7 times more likely to develop toxin-laden maggots at mean daily water temperatures ≥20 C than at <20 C. This was primarily a result of carcasses being 22.2 times more likely to become maggot-laden at ≥20 C, whereas there was no difference in the occurrence of toxin within maggots developing below or above 20 C. With increasing water temperature, carcasses developed maggots significantly more rapidly, and were available for a shorter period of time. High densities of toxic material from hatch-year gull carcasses prior to the onset of botulism coincided with high densities of susceptible birds, hence gull mortality had the potential to be a major initiating factor for botulism outbreaks at Eyebrow Lake. If management is deemed necessary on lakes enzootic for botulism, intensive surveillance should begin well before the anticipated onset of outbreaks, to identify and remove sources of primary substrate. Managers might also consider developing and evaluating strategies to prevent the occurrence of high carcass density, particularly in areas of high nest density of species that may generate high juvenile carcass densities.

INTRODUCTION

Avian botulism (*Clostridium botulinum*, type C) is an important disease of wild waterfowl throughout the world, particularly in North America. The disease is caused by ingestion of neuroparalytic toxin produced by type C *C. botulinum*, an anaerobic bacterium that requires protein-rich substrate for growth. *C. botulinum* produces toxin only when infected with a bacteriophage containing the genetic code for type C_1 toxin (Eklund et al. 1971). High temperatures and shallow, stagnant, saline water with low dissolved oxygen were believed to be favourable for outbreaks (Bell et al. 1955). However, many wetlands with these characteristics have no history of botulism, and outbreaks often occur on deep, well-oxygenated lakes, or in late winter or early spring (Rocke et al. 1999). Water temperature, pH, salinity, redox potential, and invertebrate biomass influence the occurrence of outbreaks (Rocke et al. 1999, Rocke and Samuel 1999), but some factors have not been consistently different between lakes with and without outbreaks (Rocke et al. 1999).

Botulism outbreaks have been described as having an 'initiation phase', during which toxin produced within unknown substrate becomes available to birds, and a 'propagation phase', during which carcasses of birds killed by botulism (see below) become substrate for production of further toxin that reaches healthy birds via the carcass-maggot cycle (Ball et al. 1998). The occurrence of botulism is likely not limited by the availability of spores or susceptible birds, both of which are abundant on wetlands (Smith and Young 1980; Smith 1982; Williamson et al. 1999), but by the availability of suitable protein-rich substrate for bacterial growth and toxin production (Ball et al. 1998). Vertebrate carcasses are optimal substrate for *C. botulinum* toxigenesis, and for the development of blowfly larvae that provide a vehicle for the transfer of botulinum toxin when ingested by susceptible birds (Bell et al. 1955).

The primary sources of substrate that initiate outbreaks remain unknown, although hundreds of botulism outbreaks have been documented in North America since the early 1900s. Sudden availability of carcasses as a result of hailstorms (Ball et al. 1998), blue-green algal (*Cyanobacteri*a *spp.*) blooms (Murphy et al. 2000), or fishkills (Ball et al. 1998) might provide substrate to initiate some botulism outbreaks. However, many wetlands experience botulism annually, suggesting that a predictable source of initial substrate exists on these wetlands. Identification of an association between substrate availability (or abundance) and the onset of botulism would allow prediction of outbreaks on such wetlands, and development of methods for surveillance and prevention.

During the mid-1990s, extensive mortality of Franklin's gulls (*Larus pipixcan*) (FGs) was observed on several Canadian marshes, preceding or coincident with botulism outbreaks in waterfowl (T. Bollinger, Canadian Cooperative Wildlife Health Centre [CCWHC], personal communication). The dead FGs were predominantly hatch-year (HY) birds that had died of starvation, bacterial or parasitic infections, or predation (CCWHC, unpublished data). It was hypothesized that FG carcasses might provide substrate to initiate outbreaks of botulism on these wetlands.

The focus of this study was to determine the role of FG mortality in the initiation of avian botulism at Eyebrow Lake, Saskatchewan, a wetland in which botulism is considered enzootic (Wobeser et al. 1983, Wobeser 1997a). Our objectives were to determine: (1) the density of nests within the FG colony; (2) the extent and pattern of mortality of FGs and other species prior to botulism outbreaks; (3) the suitability of HY FG carcasses for production of *C. botulinum* toxin; and (4) the possible role of temperature in the development of maggots and toxin-laden maggots within HY FG carcasses.

STUDY AREA

Eyebrow Lake is a 900 ha wetland, managed by Ducks Unlimited Canada, in the mid-grass Prairie region of south-central Saskatchewan. Created in the early 1970s by construction of impoundments and water control structures (Saigeon 1995), the lake has three basins (A, B, C in box a of Figure 1) in which water levels can be controlled. The study took place in basin C, which was maintained at full supply level (FSL) each year of this study. Maximum water depth of basin C at FSL is approximately 1 m in late spring or early summer. Predominant emergent plants are bulrush (*Scirpus spp*.) and cattail (*Typha spp.*). The lake is utilized by thousands of FGs, waterfowl, shorebirds, and other species for breeding, moulting, or staging, and is a nationally significant site for congregatory species according to the Important Bird Areas Criteria (Important Bird Areas of Canada, http://www.ibacanada.com). Botulism has occurred annually at Eyebrow Lake for at least the last two decades, with intermittent large epizootics (Wobeser 1997a).

METHODS

Transects

Line transect methods (Gates 1979; Burnham et al. 1980) were used to estimate density of FG nests and density of vertebrate carcasses. From 1999 to 2001, parallel transects were placed 100 m apart using colour-coded wooden stakes to demarcate each line (Figure 1). Transects were established before FGs chose nest sites each year, but were placed in areas where gulls had nested in the past. Total length of transects (L) for 1999, 2000, and 2001 was 18.7, 6.9, and 3.8 km, respectively, as measured with Arcview 3.1 (Environmental Systems Research Institute, New York) from coordinates obtained along each transect, using Global Positioning System

(GPS) receivers (eTrex Venture, eTrex, or Garmin 38, Opathe, Kansas). A canoe or kayak was used to search transects during annual nest surveys and weekly mortality surveys.

Estimation of density and abundance of FG nests

Surveys to count FG nests were performed after nest-building was complete each year, on 19-27 May in 1999, 23-30 May in 2000, and 31 May in 2001. Nests within 5 m on both sides of each transect line were counted, and were excluded if more than half of their diameter extended beyond 5 m. Hence, the strip half-width, denoted as *w*, was 5 m for nest density estimation, giving rise to a total strip width (2*w*) of 10 m. Perpendicular distance to each nest from the centre of the transect (or 'centreline') was recorded in 2001. In each year, area of the FG colony was estimated in Arcview 3.1 using GPS coordinates of the colony perimeter. Nest density was estimated using the strip transect method (Burnham and Anderson 1984), a modification of the line transect method, which assumes that all objects are detected within the strip width. This assumption was initially believed to be appropriate because of the narrow strip half-width of 5 m and the large size of FG nests (1-1.5 m in diameter above water surface). To test this assumption, the line transect method, which accounts for reduced detectability with increasing distances from the centerline (Burnham et al. 1980), was employed in 2001 to estimate the probability of nest detection ($\hat{P}_{a_{nest}}$) and nest density (see below). $\hat{P}_{a_{nest}}$ obtained for 2001 was subsequently used to adjust estimates of nest density and abundance for 1999 and 2000, using methods described by Buckland et al. (2001). It was assumed that there was little variation in $\hat{P}_{a_{nest}}$ among years because nests were counted by the same primary observers during the same time of year, and under similar weather conditions and vegetation density. Variance was estimated using the Delta method, standard error was obtained with the square root of variance, and 95% confidence intervals were calculated using $z_{\alpha} = 1.96$ (see Buckland et al. 2001).

During the nest survey in 1999, GPS coordinates of nests along transects were recorded. Arcview maps of these nest coordinates were used to divide the study area into discrete sections, referred to as 'sub-areas', based on a qualitative assessment of nest density (i.e., zero, light, medium, or high nest density) (box b in Figure 1). Each transect line was divided into discrete segments based on whether segments were within or outside (i.e., zero nest density) the gull colony. To quantitatively assess this subjective method of sub-area classification, nest density (with measures of precision) for each sub-area was estimated using the strip transect method, and adjusted with $\hat{P}_{a_{nest}}$ for 2001 as described above. Area and *L* (the sum of all transect segment lengths) for each sub-area were employed in these estimations. These sub-areas were subsequently employed to examine the relationship between nest density and carcass density (see below).

Estimation of carcass density and abundance

Transects were searched for carcasses weekly from mid-May to late July each year (i.e., weeks of 23 May – 25 July in 1999, weeks of 21 May – 23 July in 2000, and weeks of 20 May – 22 July in 2001). Searches were concentrated within, but not restricted to, 5 m of the centerline. For

each dead or sick animal found, species, date, GPS coordinates, stage of carcass decomposition, and perpendicular distance from centerline (rounded to the nearest m) were recorded. Stage of carcass decomposition was described as: fresh (i.e., recently dead); sodden (i.e., intact but rotten carcass, usually wet); early maggot development (i.e., early larval fly stages that have not penetrated the carcass); or profuse maggot development (i.e., large numbers of plump larval stages that have penetrated the carcass abdomen, occupying >50% of the abdominal cavity). For species with a sufficiently large sample size (n > 60), probability of carcass detection ($\hat{P}_{a_{carc}}$) and carcass density were estimated using line transect methods as described below. Although providing conservative estimates, the strip transect method (which assumes that $\hat{P}_{a_{carc}} = 1$) was used to compare carcass density estimates among all species (including those with smaller sample sizes) and to examine weekly trends in mortality. All estimates were calculated using program Distance 4.0, release 2 (Thomas et al. 2003), as described below.

Line transect methods using Distance 4.0

To estimate FG nest density in 2001 and carcass density in 1999-2001, Distance 4.0 was used to model the detection function (Burnham et al. 1980). Data sets for nest density estimation in 2001 and carcass density estimation for 1999-2001 were treated similarly. Nest density was estimated using data obtained from a single visit to transects in 2001, whereas data from weekly visits to transects were pooled to estimate overall carcass density each year. Distance data were truncated to remove outliers where relevant (carcass data only), and were grouped to reduce effects of heaping (rounding errors) or violations of the assumption of complete censusing of the centerline as recommended by Buckland et al. (2001). The shape of the detection curve was estimated by fitting grouped distance data to models (e.g., uniform + cosine, uniform + simple polynomial, half-normal + cosine, half-normal + hermite polynomial, hazard rate + cosine, and hazard rate + simple polynomial) recommended by Buckland et al. (2001). The best-fitting model was chosen to estimate nest or carcass density and abundance, primarily using Akaike's Information Criterion (AIC). χ^2 goodness of fit of the model was employed to assess the adequacy of the fitted model. To improve measures of precision, and estimate weekly carcass density, models for each year were modified to include post-stratification analysis by week. Density estimates for each stratum (week) were obtained using the global detection function and $\hat{P}_{a_{carc}}$ of pooled strata, while weekly confidence intervals were a function of the variation of carcass density among transects within each stratum. To calculate total carcass density up to the first detected cases of botulism in waterfowl, $\hat{P}_{a_{carc}}$ and standard error of $\hat{P}_{a_{carc}}$ obtained for the entire season of each year were employed as multipliers in a model using a uniform key function with zero adjustment terms (Buckland et al. 2001; Thomas et al. 2003). Methods to calculate total and weekly carcass density for each species using the strip transect method in Distance 4.0 were similar to those described above, except that a uniform key function, with zero adjustment terms and no multipliers (thereby setting $\hat{P}_{a_{carc}}$ to 1), was employed.

For 1999 data only, FG carcass density was estimated for each sub-area categorized by nest density. Procedures were similar to those described for nest density, except values were adjusted using $\hat{P}_{a_{carc}}$ obtained for 1999. For each transect segment within the colony, an index of FG

mortality rate was calculated by dividing the detected number of sick and dead HY FGs by the product of hatch rate and number of nests counted (i.e., estimated number of hatched chicks). A hatch rate of 2.48 chicks per nest (C. Soos, unpublished data) was used in the calculations.

Sampling from carcasses and sick birds on transects

Samples of maggots were collected from all profusely maggot-laden carcasses found on transects, and were frozen at -20 C until processed for detection of *C. botulinum* toxin. Carcasses with early maggot development were not tested because toxin is unlikely to be found in earlier larval stages that have not penetrated the carcass abdomen. To promptly detect the onset of botulism on Eyebrow Lake, all sick waterfowl or other birds detected anywhere within basin C were tested for botulism. Prior to euthanasia by cervical dislocation, blood samples were collected by jugular venipuncture, and placed into CaEDTA or lithium heparin tubes. Tubes were centrifuged, and serum or plasma samples were harvested and frozen at -20 C or -75 C either immediately or following temporary storage in liquid nitrogen (-196 C), until tested for *C. botulinum* toxin. With the exception of carcasses referred to in the following section, all euthanized birds and fresh carcasses found on transects were collected, weighed and processed for necropsy and histopathology as part of a related study (Soos 2004). Carcasses that were not fresh were not collected for necropsy, but, if still relatively buoyant, were marked with bright paint or removed to avoid recounting them the following week.

Fate of FG carcasses

From late May to late July each year, recently dead HY FG carcasses collected from transect surveys (1999 and 2000), and apparently healthy HY FGs captured and euthanized by cervical dislocation (2001) were placed within the marsh, in an area adjacent to the nesting colony outside the transect area. Each carcass was tethered to a flagged bamboo pole with a 1-1.5 m length of cotton thread (breaking strength <1 kg). In 2001, carcasses were weighed prior to being placed in the marsh. Disappearance of carcass, state of carcass decomposition, time to profuse maggot development (i.e., the number of days for approximately >50% of the carcass abdomen to become laden with maggots), and time to sinking were recorded every one to four days in 1999 and 2000, and daily in 2001. Maggot samples were collected and frozen at -20 C until tested for the presence of *C. botulinum* toxin.

Identification of *C. botulinum* toxin

For each maggot sample, 1 g was ground with 9 ml of a penicillin-streptomycin solution (10,000 IU/ml penicillin, 10,000 µl/ml streptomycin). Following centrifugation at 4,000-5,000 rpm for 10 min, the supernatant was frozen until tested for toxin. Adult male CD1 mice (20-30 g), housed individually or in pairs in 15 x 25 cm cages containing corn cob litter (Bed-O'Cobs, The Andersons, Maumee, Ohio, USA), were provided with food (Purina Rodent Chow 3000, Ralston Purina, St. Louis, Missouri, USA) and water *ad libitum*. In 1999, two mice (test and control) were employed for each maggot or serum/plasma sample tested as described by Quortrup and

Sudheimer (1943). The control mouse was injected intraperitoneally with 0.1 ml of type C *C. botulinum* antitoxin (National Wildlife Health Center, Madison, Wisconsin, USA) 30-60 min prior to injecting both mice with either 0.1 ml of maggot solution or 0.5-1 ml of serum or plasma intraperitoneal injection. Following injection, mice were monitored for four to five days. A sample was considered to be positive if the test mouse died or had signs of paresis or respiratory distress while the corresponding control did not (Quortrup and Sudheimer 1943). For samples obtained in 2000 and 2001, a single mouse was used initially for each sample; control mice were employed only for those samples in which the first mouse tested positive. Mice with respiratory distress were euthanized immediately with halothane in an enclosed chamber. All mice were euthanized at the end of the trial.

Water temperature

Water temperature in basin C was recorded hourly with thermistors (Optic StowAway Temp loggers, Onset, Bourne, Massachusetts, USA) submerged within 10-20 cm of the water surface, in densely vegetated areas within the marsh.

Statistical Analyses

The *z*-test was employed for comparisons between two density estimates (e.g., comparing estimates of carcass density within and outside the FG colony). The generalized χ^2 statistic (Sauer and Williams 1989) was employed using program CONTRAST (Sauer and Hines 1989) to compare estimates of nest density or carcass density among nest density categories assigned in 1999. For 1999 only, a multilevel model was used to evaluate the relationship between nest density and HY FG carcass density using MLwiN version 1.1 (Rasbash et al. 2002). For this analysis, uncorrected nest and carcass density estimates for each transect segment were employed, and segments with zero nesting on the same transect line were combined. Transect was employed as a random effect in the model, to account for the possibility that segments from the same transect were not independent of each other. The value for R^2 was calculated as described by Snijders and Bosker (1999) for multilevel models. A Kruskall-Wallis test was performed to compare mortality rate indices of transect segments among low, medium, and high nest density categories (Norušis 2002).

Logistic regression was employed with a random intercept model, using trial and year as random effects to determine the effect of temperature on the likelihood of FG carcasses developing maggots containing *C. botulinum* toxin (Rasbash et al. 2002). Analyses were repeated to examine the effects of water temperature on probability of maggot development alone, and on probability of toxin concentration within maggot samples. For these analyses, the mean of daily mean temperatures of the first seven days of each trial was employed as a dichotomized categorical variable using 20 C as a cut-off value (≥20 C = '1' and <20 C = '0'). The effects of water temperature (mean of daily mean temperatures of first seven days of trials) on number of days to maggot development and number of days to sinking were analyzed using a mixed general linear model (PROC MIXED; SAS Institute 2001). Model specifications included a random effect for year and trial nested within year. To compare the occurrence of toxin within maggot

samples from FG carcasses collected on transects prior to and following the onset of botulism, logistic regression was employed with a random intercept model using year as a random effect (Rasbah et al. 2002). Results were considered statistically significant at $P \leq 0.05$.

RESULTS

Density and abundance of FG nests

The area of the FG colony was 172, 141, and 156 ha in 1999, 2000, and 2001, respectively (Figure 1). In 2000 and 2001, transects were located exclusively within the colony, whereas in 1999, 9.3 km of the 12 transects (and 8.7 km of the 11 transects surveyed for carcasses) overlapped the colony (Figure 1). The number of nests counted on transects was 1,091, 1,508, and 637, and the estimated nest density on the colony (using the strip transect method with $w = 5$ m) was 117,243, and 170 nests/ha in the three years, respectively. For 2001, the detection function estimated with a uniform + cosine model provided the best fit for grouped distance data, based on the lowest AIC and highest goodness of fit P ($\chi^2 = 0.28$, $P = 0.6$). According to this model, $\hat{P}_{a_{nest}}$ in 2001 was 0.88 (95% CI = 0.80-0.98); hence, on average, 12% of nests were not detected when counting nests within the 10 m strip width. Although adjusted nest density and abundance appeared higher in 2000 and lower in 1999, confidence intervals overlapped (Table 1).

There was a significant difference in nest density among sub-areas subjectively classified as zero (0 nests/ha, SE = 0), low (18 nests/ha, SE = 10), medium (68 nests/ha, SE = 25), and high nest density (238 nests/ha, SE = 107; $\chi^2 = 15.6$, df = 3, $P = 0.0014$; Figure1), supporting the use of these nest density categories in further analyses.

Species composition of carcasses on transects

A botulism outbreak occurred in waterfowl beginning within the first week of July in each year of the study. FGs comprised 85-93% of the carcasses observed on the gull colony prior to the recognition of botulism outbreaks (Table 2). Most of the FG carcasses were HY birds (97.3%, 93.3%, and 80% in 1999, 2000, and 2001, respectively). Few American coots (*Fulica americana*) and eared grebes (*Podiceps nigricollis*) were found dead prior to botulism outbreaks (Table 2). Within the nesting colony, carcasses containing maggots were predominantly FGs (61/69 or 88%; Table 2), 93% (57/61) of which were HY. Carcasses found outside the colony in 1999 were predominantly eared grebes (Table 2), almost all of which were HY (11/12); no maggot-laden HY grebes were detected. Only five maggot-laden carcasses were found outside the colony prior to the botulism outbreak in 1999 (Table 2).

Carcass density

Modelling of the distance data for species other than FGs was not performed because of the small number of carcasses found (Table 2). To compare total and weekly carcass densities among species, unadjusted estimates of density (using the strip transect method with w = 10 m) were employed. The total number of carcasses found per ha (including all species) prior to the botulism outbreak in 1999 was markedly higher within (166 carcasses on 8.7 km = 9.5/ha) than outside (19 carcasses on 8.1 km = 1.2/ha) the FG colony, primarily a result of higher carcass density of HY FGs within compared to outside the colony (Table 3, $z = 5.45$, $P < 0.001$). The density of American coot and eared grebe carcasses did not differ within versus outside the colony (Table 3; $z_{amco} = -0.25$, $P = 0.80$; $z_{eagr} = -0.17$; $P = 0.87$). Each year, carcass density of HY FGs within the colony was significantly higher than carcass density of American coots (1999: $z = 5.29$, $P < 0.001$; 2000: $z = 7.06$, $P < 0.001$; 2001: $z = 3.56$, $P = 0.0004$) and eared grebes (1999: $z = 5.01$, $P < 0.001$; 2000: $z = 7.22$, $P < 0.001$; 2001: $z = 3.66$, $P < 0.001$). We assumed that probability of carcass detection within the 10 m strip half-width was identical among species. The observed magnitude of difference between the density of FG carcasses and that of other species likely would be affected minimally by incorporating $\hat{P}_{a_{carc}}$ for each species in these calculations.

Temporal pattern of HY FG mortality

The pattern of HY FG mortality on the colony was similar in each year (Figure 2). Mortality of HY birds began with onset of hatching, and peaked in mid-June. In 1999, there appeared to be a second peak in mortality in late June (box a in Figure 2), which occurred primarily in recently fledged FGs (Soos 2004). Carcass density during peak mortality appeared higher in 2000 than in 1999 and 2001 (Figure 2, and see below). Each year, the apparent decline in mortality of HY FGs after late June coincided with fledging, and with a rapid decline in the number of FGs present as they began southward migration. Botulism among waterfowl was first detected within one to two weeks after peak mortality of HY FGs each year (7, 4, and 3 July in 1999, 2000, and 2001, respectively; Figure 2).

Estimation of HY FG carcass density using Distance 4.0

Sample sizes of sick and dead HY FGs were sufficient to model the detection function and estimate $\hat{P}_{a_{carc}}$ and carcass density for each year (n = 210, 222, and 69 within a 20 m strip half-width in 1999, 2000, and 2001, respectively). For each year, the best model to estimate the detection function for grouped distance data, truncated to 10 m from the centerline, was the hazard rate key function + cosine adjustment term (1999: $\chi^2 = 0.0013$, $P = 0.97$; 2000: $\chi^2 = 0.0008$, $P = 0.98$; and 2001: $\chi^2 = 0.0053$, $P = 0.94$). Based on these models, on average 54-66% of HY FG carcasses were undetected within the 10 m strip half-width on transects each year (Table 4). Estimated overall carcass density of HY FGs on the colony for the entire pre-fledgling period (early June to late July) was similar in each year (Table 4; $\chi^2 = 1.231$, $P = 0.54$). There also was no difference in total carcass density of HY FGs up to the onset of botulism

outbreaks in waterfowl (Table 4; $\chi^2 = 4.29$, $P = 0.12$). Estimated peak density of FG carcasses one to two weeks prior to the recognition of botulism (Figure 2) was nearly significantly different among the three years (Table 4; $\chi^2 = 5.84$, $P = 0.054$), primarily because density in 2000 was higher than that observed in either 1999 ($\chi^2 = 5.03$, $P = 0.025$) or 2001 ($\chi^2 = 4.29$, $P = 0.038$).

In 1999, estimates of the total density of HY FG carcasses prior to botulism outbreaks were significantly different among sub-areas classified as zero (0.18 carcasses/ha, SE = 13.8), low (3.5 carcasses/ha, SE = 2.9), medium (9.7 carcasses/ha, SE = 5.4), and high (43.9 carcasses/ha, SE = 9.0; $\chi^2 = 18.52$, df = 3, $P = 0.003$) nest density. Nest density was a significant predictor of HY FG carcass density ($\chi^2 = 43.69$, df = 1, $P < 0.001$, $R^2 = 0.61$), and transect line had no significant clustering effect on transect segments ($\chi^2 = 0.014$, df = 1, $P = 0.906$). Mortality rate index was not significantly different among nest density categories (Kruskall-Wallis $\chi^2 = 5.15$, df = 2, $P = 0.076$).

Maggot samples from transects

Of the maggot samples collected from HY FG carcasses prior to botulism outbreaks in waterfowl, 0/12, 2/6, and 2/8 were positive for *C. botulinum* toxin in 1999, 2000, 2001, respectively, whereas 3/4, 5/6, and 13/17 of samples contained toxin after the onset of botulism in each year, respectively. Analysis of pooled data employing year as a random effect revealed that maggots collected after the final week of June were 19.3 times more likely to contain toxin than maggots collected prior to that time period (95% CL = 4.8-78.1, $\chi^2 = 17.17$, df = 1, $P < 0.001$). In 2000 and 2001, toxin was present in FG carcasses at least 10-11 days prior to the first detected cases of botulism in waterfowl. None of the maggot samples collected from other species prior to botulism outbreaks tested negative for toxin (Table 2 lists total numbers of carcasses with early or profuse maggot development for each species; however, only carcasses with profuse maggot development were tested for toxin, i.e. four American coots (one within and three outside colony) and one eared grebe (outside colony) in 1999, one black-crowned night heron in 2000, and one muskrat in 2001 were tested).

Fate of FG carcasses

Only two of 120 HY FG carcasses placed in the marsh were removed by a scavenger (northern harrier [*Circus cyaneus*]), and one carcass disappeared following a windy day (Table 5). All other carcasses remained in position and sank, on average, 9.1 days after placement (SD = 3.4, range = 2-20 days). Mean time required for profuse maggot development was 4.6 days (SD = 1.7, range = 1-8 days). Time to maggot development and to carcass sinking both decreased with water temperature ($\beta = -0.60$ day/ C, 95% CI = -0.83 to -0.37, df = 54, $P < 0.001$; and $\beta = -0.95$ day/ C, 95% CI = -1.35 to -0.55, df = 103, $P < 0.001$, respectively).

Overall, 56.0%, 23.3%, and 67.5% of carcasses became maggot-laden in 1999, 2000, and 2001, respectively, and 12.5%, 60%, and 74.1% of maggot samples tested were positive for *C. botulinum* toxin each year (Table 5). Carcasses were 22.7 times more likely to develop toxin-

laden maggots when mean daily water temperatures were ≥20 C than when temperatures were <20 C (95% CI = 3.0-170.4; $\chi^2 = 9.24$; $P = 0.002$). This was primarily because carcasses were 22.2 times more likely to become maggot-laden when water temperatures were ≥20 C (95% CI = 2.4-209.2; $\chi^2 = 8.24$; $P = 0.007$). Of the maggot-laden carcasses, there was no significant difference in the occurrence of toxin within maggots developing below or above 20 C ($\chi^2 = 0.081$; $P = 0.78$). In each year, the proportion of carcasses developing maggots or toxic maggots appeared to increase with trial, likely because of increasing temperature and increasing mass of HY FGs as the season progressed. The HY FGs used in trials in 2001 were representative of HY FGs found on transects, as they exhibited similar trends in mean mass over time (data not shown; Soos 2004). The timing of toxin availability in carcasses was also similar to that observed on transects. In 2000 and 2001, toxin was first detected in HY FG carcasses from trials initiated 11-15 days prior to the onset of botulism in waterfowl (Table 5). No maggot samples prior to botulism were available for testing from trials performed in 1999 (Table 5).

DISCUSSION

The initial source of substrate for proliferation and toxigenesis of *C. botulinum* in the initiation phase of avian botulism outbreaks is typically unknown. A botulism outbreak occurred among waterfowl at Eyebrow Lake, Saskatchewan, beginning in the first week of July each year of the study. Dead HY FGs were, by far, the most abundant vertebrate carcasses found prior to each outbreak. The pattern of HY FG mortality was similar each year, with mortality peaking one to two weeks prior to the first detected case of botulism in waterfowl. HY FG carcasses were suitable substrate for production of toxin by *C. botulinum* type C, and were the predominant source of substrate for both toxin production and maggot development on transects, beginning at least 10-11 days prior to the first detected cases of botulism in waterfowl.

The density or abundance of carcass substrate during the initiation phase of avian botulism outbreaks is likely an important factor determining whether an outbreak will progress to the propagation phase. High carcass density may perpetuate the carcass-maggot cycle by overwhelming the scavenging system, and increasing the contact rate between toxin-laden maggots and susceptible birds (Wobeser 1997b). The estimated density of HY FG carcasses during the peak of mortality ranged from 8.4-18.7 carcasses/ha (Table 4), and scavenging did not play an important role in reducing carcass density in the marsh (Table 5). Enclosures with 12.5 duck carcasses/ha were, on average, 4.5 times more likely to develop botulism outbreaks than enclosures with no carcasses (Reed and Rocke 1992), and radio-tracked mallards were 12.5 times more likely to die within areas containing 12 carcasses/ha than within areas with zero carcasses/ha on lakes high in risk for botulism outbreaks (Evelsizer 2002).

The probability of maggot development within carcasses (P_m) and the probability of toxin concentration within maggots (P_{tox}) may also be important factors affecting the occurrence of botulism outbreaks (Wobeser 1997a). The reproductive rate for avian botulism (R_o) is the average number of birds dying of secondary poisoning (M_2) divided by the number of animals dying for any reason (M_1), and $M_2 = M_1(P_m)(P_{tox})(\beta)$ (modified from Wobeser 1997a), hence $R_o = \beta(P_m)(P_{tox})$. The intoxication coefficient, β, is the product of contact rate between susceptible

birds and toxic material, C, and proportion of contacts resulting in intoxication and death, P_i (Wobeser 1997a). If R_o >1, then a botulism outbreak may occur (Wobeser 1997a). For example, during the week prior to the first detected cases of botulism in 2001, HY FG carcasses had a 0.9 (9/10) probability (P_m) of developing maggots, which had a 0.67 (6/9) probability (P_{tox}) of containing toxin (see Table 5, trial initiated 30 June 2001). R_o for HY FG carcasses was therefore (0.9)(0.67)(β) or 0.6β, hence for R_o to be >1, β was >1.7. If each contact resulted in death, >1.7 contacts per HY FG carcass (on average) would have been required to initiate a botulism outbreak in 2001. Contact rate was likely high during the week prior to botulism in 2001 because there was approximately 93 kg of toxic maggot-laden carcass material from HY FGs within the colony (estimated using the product of adjusted carcass density [4.6 carcasses/ha], mean carcass mass [214 g/carcass], P_m [0.9], P_{tox} [0.67], and colony area [156 ha]), concurrent with a high density of susceptible birds.

For a given lake and population density of susceptible birds, there may be a 'threshold' carcass density, below which botulism outbreaks are unlikely to occur. We could not assess this because a botulism outbreak (preceded by high FG carcass density) occurred every year of this study. If carcass density is a key factor initiating botulism outbreaks, attempts should be made to investigate the relationship between population density and the density of toxic material (hence taking into account P_m, P_{tox}, and carcass mass). Other factors such as aquatic invertebrate abundance, temperature, redox potential, pH, and salinity should be considered; however, further research is required to evaluate their relationship with density of toxic material, or the effects of abiotic factors on P_m and P_{tox}.

Temperature is important in the ecology of botulism outbreaks because it influences *C. botulinum* type C proliferation and toxigenesis, as well as blowfly (*Calliphora spp.*) activity. Optimal temperatures for proliferation and toxigenesis are >30 C (Cato et al. 1986; Smith and Turner 1987); however, low levels of toxin production may occur at 12.5 C (Haagsma 1973). In the current study, gull carcasses were 22.2 times more likely to develop maggots when average water temperature was ≥20 C than when it was <20 C, whereas the occurrence of toxin within maggot samples did not differ above and below 20 C. The presence of toxin was only measured in maggots and not in carcasses, so that the effect of temperature on toxin production within carcasses that did not develop maggots was unknown. It was not known whether carcass temperature was independent of ambient temperature during periods of high maggot activity, as occurs in duck carcasses (Wobeser and Galmut 1984). HY FG carcasses, being much smaller than duck carcasses, have a larger surface area to volume ratio, and are likely more affected by environmental temperatures. With increasing water temperature, gull carcasses became infested with maggots more rapidly (0.60 day earlier for each rise in C), but were available for a shorter period of time before sinking (0.95 day less per increase in C). These effects were probably due to increased blowfly activity and more rapid larval development, with more rapid consumption of carcasses at higher temperatures.

It is also possible that the presence of HY FG carcasses increased spore density in the environment and in tissues of animals living in Eyebrow Lake (particularly in areas of high carcass density). This was evidenced by the increase in P_{tox} over time observed in trials (Table 5) and on transects (e.g., maggots from FG carcasses were 19.3 times more likely to contain toxin when collected after the final week of June compared to samples collected earlier).

While we did not assess all possible sources of initial substrate (e.g., invertebrate carcasses or proteinaceous material), we believe that HY FG carcasses increased the risk of botulism outbreaks at Eyebrow Lake in 1999-2001, given; their extensive mortality; their suitability as substrate for *C. botulinum* proliferation and toxigenesis; the substantial biomass of toxic material provided by their carcasses; and the consistent timing of mortality and availability of toxin-laden maggots prior to botulism outbreaks.

Management Implications

The most widely used technique to manage avian botulism has been collection and disposal of carcasses during the propagation phase of outbreaks. Carcass removal is labour-intensive, costly (Ball et al. 1998), inefficient at reducing carcass densities (Cliplef and Wobeser 1993), and ineffective at reducing duck mortality rates due to botulism (Evelsizer 2002). Once botulism outbreaks have progressed to the propagation phase, mortality is likely to exceed the rate at which carcasses can be collected. Carcass removal might be more successful if performed during the initiation phase of outbreaks, when carcass density and mortality are lower. This requires early surveillance. Outbreaks of botulism on Eyebrow Lake from 1988-1992 were first detected between 14-28 July, with a median date of 21 July (Saigeon 1995). However, during each year of this study, the first cases of botulism were detected within the first week of July, and mortality had expanded into a large outbreak by mid to late July. If management is deemed necessary on lakes where botulism occurs frequently, intensive surveillance should begin prior to the anticipated onset of botulism, and should concentrate on identifying sources of substrate and locating areas with high carcass densities, such as nesting areas of colonial species. Such areas could be targeted for carcass removal long before the expected onset of botulism outbreaks. Management strategies might also focus on preventing birds from nesting in high densities, or on reducing hatch rate in abundant species that generate high juvenile carcass densities. Such strategies should be evaluated for their effectiveness in reducing mortality caused by botulism, prior to being incorporated into management plans.

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Table 1. Adjusted estimates of Franklin's gull nest density (nests/ha) and abundance in late May of 1999, 2000, and 2001. Estimates for 2001 were obtained from the model created in Distance 4.0. The probability of nest detection ($\hat{P}_{a_{nest}}$) for 2001 (0.88) was employed to adjust nest density estimates for 1999 and 2000. Surveys were performed on 19-27 May in 1999, 23-30 May in 2000, and 31 May in 2001.

Year	Adjusted nest density			Adjusted no. of nests			CV
	$\hat{D}$	SE	95% CL	$\hat{N}$	SE	95% CL	
1999	133	48	61 - 290	22,789	8,204	10,433 - 49,777	0.36
2000	276	41	207 - 367	38,949	5,726	29,249 - 51,864	0.15
2001	192	22	147 - 252	30,056	3,499	22,984 - 39,302	0.12

Table 2. Species composition of carcasses found within a 10 m strip half-width on transects prior to botulism outbreaks each year, from surveys conducted during the weeks of 23 May – 27 Jun in 1999, 21 May – 25 Jun in 2000, and 20 May – 24 Jun in 2001. Number of carcasses with early or profuse maggot development are indicated in parentheses for each species.

Species	Off colony	Within colony		
	1999	1999	2000	2001
Franklin's gull	1 (1)	149 (26)	223 (26)	60 (9)
American coot	6 (3)	5 (4)	10 (1)	3 (0)
Eared grebe	12 (1)	11 (1)	6 (0)	2 (0)
Other[a]	0 (0)	1(0)	1 (1)	6 (1)
Total	19 (5)	166 (31)	240 (28)	71 (10)

[a] Other species consisted of a redhead (*Aythya americana*) in 1999, a black-crowned night heron (*Nycticorax nycticorax*) in 2000, and a common tern (*Sterna hirundo*), a yellow-headed blackbird (*Xanthocephalus xanthocephalus*), and four muskrats (*Ondatra zibethicus*) (of which one was maggot-laden) in 2001.

Table 3. Unadjusted estimates of total carcass density (carcasses/ha) within Franklin's gull (FG) colony on Eyebrow Lake, Saskatchewan, prior to the discovery of botulism in waterfowl, from surveys conducted during the weeks of 23 May – 27 Jun in 1999, 21 May – 25 Jun in 2000, and 20 May – 24 Jun in 2001. Estimates were calculated with the strip transect method, using a 10 m half-strip width. The 95% confidence limits are shown in parentheses.

Species	Off colony	Within colony		
	1999	1999	2000	2001
Hatch-year FG	0.058 (0.005-0.7)	8.4 (5.8-12.1)	15.1 (11.5-19.9)	6.4 (3.7-11.1)
American coot	0.35 (0.1-1.0)	0.29 (0.1-0.7)	0.73 (0.4-1.4)	0.40 (0.08-2.0)
Eared grebe	0.69 (0.3-1.4)	0.63 (0.3-1.4)	0.44 (0.2-0.9)	0.27 (0.06-1.1)

Table 4. Estimates of carcass detection probability ($\hat{P}_{a_{carc}}$) and carcass density (carcasses/ha) of hatch-year Franklin's gulls (HY FG) within a colony on Eyebrow Lake in 1999, 2000, and 2001 using Distance 4.0. The 95% confidence limits are shown in parentheses.

Year	$\hat{P}_{a_{carc}}$	Overall carcass density[1]	Total carcass density to botulism outbreak[b]	Peak carcass density[c]
1999	0.34 (0.29-0.41)	35.1 (25.2-48.9)	24.3 (16.2-36.3)	9.0 (5.1-15.9)
2000	0.46 (0.41-0.51)	35.1 (26.5-46.6)	32.9 (24.4-44.5)	18.7 (12.4-28.2)
2001	0.35 (0.27-0.45)	26.4 (15.8-44.0)	18.3 (10.0-33.8)	8.4 (3.2-21.9)

[a] This is an estimate of the 'cumulative' density of HY FG carcasses detected throughout the entire fledgling period from early June to late July (i.e., during the weeks of 6 Jun – 15 Jul in 1999, 4 Jun – 23 Jul in 2000, and 3 Jun – 22 Jul in 2001). The word cumulative is in quotations to emphasize that carcasses do not persist throughout the entire period, but sink on average 9.1 days (SD = 3.4) after death.
[b] Includes all HY FGs detected from early June to the first detected case of botulism each year, (i.e., 23 May – 27 Jun in 1999, 21 May – 25 Jun in 2000, and 20 May – 24 Jun in 2001).
[c] Estimates of peak carcass density correspond to peaks in Figure 2 (i.e., weeks of 27 Jun in 1999, 18 Jun in 2000, and 17 Jun in 2001). These estimates may be conservative because they represent newly detected carcasses in a single week, and do not include carcasses from the have persisted had they not been collected.

Table 5. Fate of recently dead hatch-year Franklin's gull carcasses (HY FG) placed in Eyebrow Lake in 1999, 2000, and 2001. Ten carcasses were employed for each trial unless otherwise stated.

Year	Start date	Early maggot development only	Maggot infested	No. positive/ no. tested for toxin	No. days to maggot infestation $\bar{x}$ (range)	No. days to sinking $\bar{x}$ (range)	Temperature (C) Average Daily[a] $\bar{x}$	Max
1999	15 June[b]	5	0	0/0	NA[c]	8.7 (6-10)	22.3	26.0
	29 June[d]	1	0	0/0	NA	9.8 (7-13)	19.5	22.5
	6 July	2	8	0/6	5.3 (3-6)	7.6 (6-8)	22.6	27.9
	15 July	0	10	0/5	4.8 (4-5)	7.0 (7)	21.8	25.9
	19 July	0	10	2/5	2.2 (1-4)	5.0 (4-6)	24.3	29.3
2000	14 June	0	0	0/0	NA	13.9 (8-20)	16.5	18.4
	19 June	1	3	1/1	8 (8)	14.5 (10-15)	17.2	19.1
	21 June	3	4	2/4	6.5 (3-8)	11.3 (7-13)	17.2	19.3
2001	15 June	0	0	0/0	NA	6.1 (2-7)	19.5	22.8
	22 June	1	9	6/9	4.7 (4-7)	7.9 (7-8)	21.3	25.8
	30 June[e]	0	9	6/9	4.7 (3-6)	8.7 (6-9)	20.8	28.0
	6 July[e]	0	9	8/9	4.0 (3-7)	8.7 (7-10)	22.8	26.7

[a] Temperatures represent average daily mean or maximum temperatures of first seven days of each trial.
[b] Eleven carcasses were employed in this trial; four were put out on 17 June. One carcass disappeared following windy day.
[c] Not applicable (i.e., carcasses did not become maggot-infested).
[d] Nine carcasses were employed in this trial.
[e] One carcass from each of the latter two trials in 2001 was removed by a northern harrier (*Circus cyaneus*) within one day of placement in the marsh.

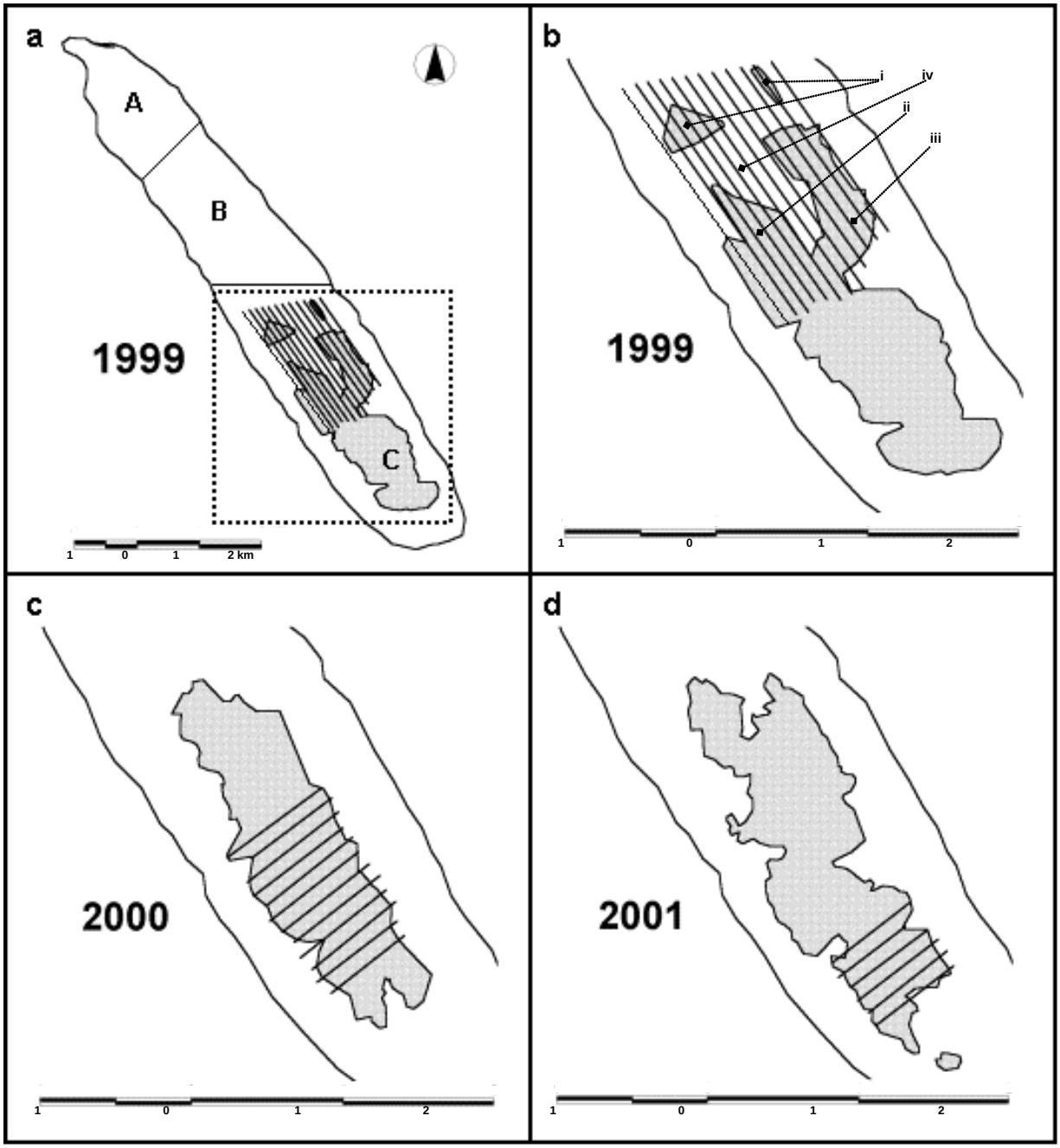

Figure 1. Map of Eyebrow Lake, Saskatchewan, showing location of basins (A, B, C divided by dykes) and Franklin's gull colony in 1999, 2000, and 2001. Solid outermost outline represents the shoreline of Eyebrow Lake, and shaded area on lake represents the area of the gull colony. Parallel lines indicate location of transects surveyed each year. Box with dashed outline in (a) represents the area on the lake shown in (b) through (d). In (b), numerals i, ii, iii, and iv represent sub-areas within the study area categorized as light, medium, high, and zero nest density, respectively. The border between sub-areas ii and iii was located between the 6^{th} and 7^{th} transects (from east to west). Western-most transect (1.9 km) in 1999 was not included in surveys for mortality. Northern-most transect (0.7 km) in 2000 was not included in nest survey.

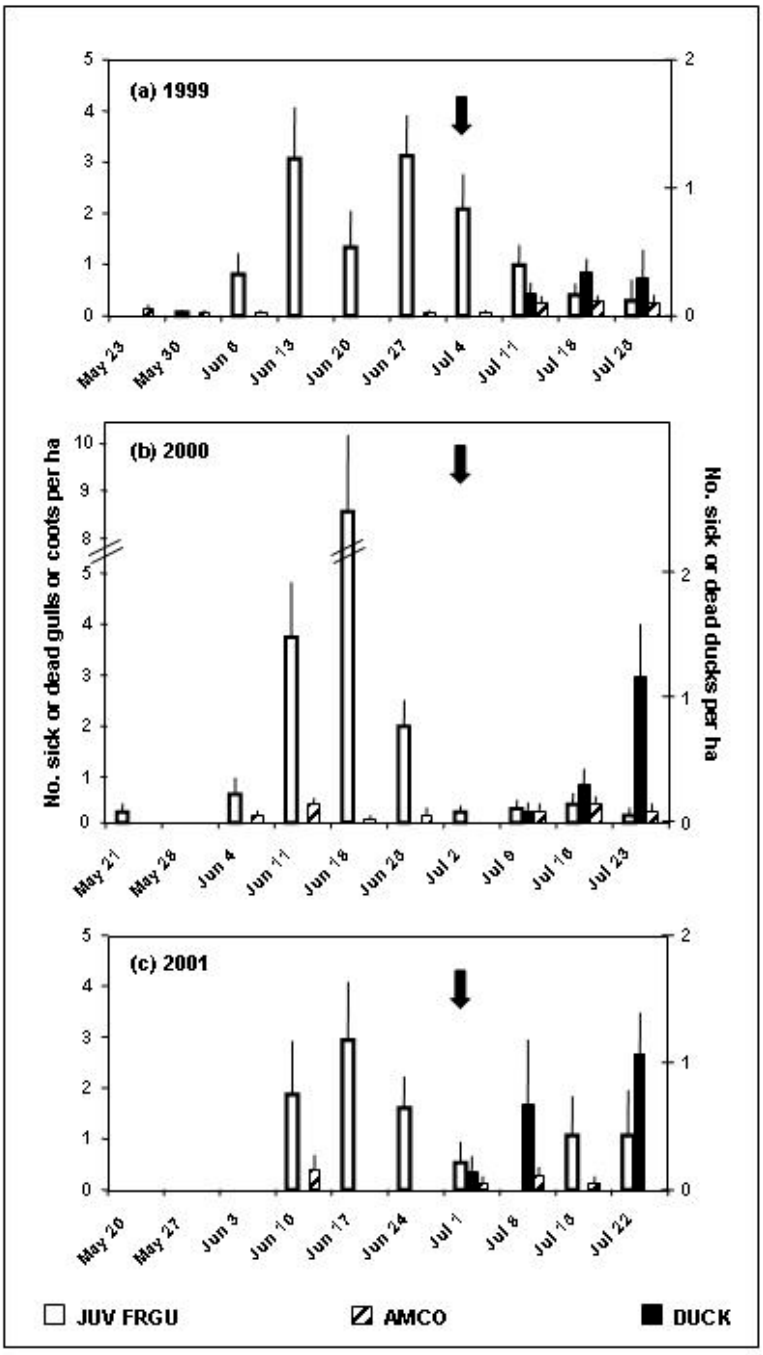

Figure 2. Weekly pattern of mortality of hatch-year Franklin's gulls, American coots, and dabbling ducks on the gull colony in (a) 1999, (b) 2000, and (c) 2001. Number of sick or dead birds per ha (+ 1 SE) was estimated with strip transect methods, using a 10 m half-strip width. Black arrows indicate weeks during which the first botulism cases in waterfowl were detected within the study area. The first case of botulism was detected along a transect line approximately 100 m outside the gull colony in 1999, within the colony but between transects in 2000, and within the colony along a transect line in 2001.

PART VI

VARIABILITY OF TYPE C *CLOSTRIDIUN BOTULINUM* TOXIN PRODUCTION ON CANADIAN PRAIRIE LAKES: A PREDICTOR OF MALLARD SURVIVAL

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ABSTRACT

Maggots containing *Clostridium botulinum,* type C toxin play an important role in the propagation of botulism outbreaks in wetlands. To evaluate factors contributing to the development of toxin in bird carcasses, we collected samples of maggots from lakes with and without botulism outbreaks, and tested for presence and concentration of *C. botulinum* toxin. Carcasses from lakes with a history of previous botulism outbreaks were ~15 times more likely to produce type C toxin than were carcasses on lakes without previous botulism outbreaks. Carcasses of birds that died of botulism were 4.7 times more likely to produce toxin than were birds, from the same lake, that died of gunshot. In addition, we used logistic regression to model the relationship between moulting mallard survival rate and carcass density, probability carcasses contain spores (produce toxin, P_S), probability carcasses produce high toxin concentrations (P_T) and the probability carcasses persist and produce maggots (P_M). Carcass density, P_S, P_T and the interaction term P_S*P_T were significant predictors of survival. The odds ratio for the risk factor density was 1.77 (95% CI = 1.15-2.73), indicating that the addition of one carcass/ha increased the odds of botulism mortality by ~1.8 times. Based on comparisons of pseudo-R^2 values, there was a 23% relative improvement in model fit by adding the P_S and P_T terms, including their interaction term, compared to a model containing density alone. Understanding the ecological factors that affect spore density, distribution and persistence on wetlands may lead to more cost effective measures for managing avian botulism.

INTRODUCTION

Avian botulism is an important disease of waterfowl worldwide. In North America, die-offs typically occur during summer and early fall, under hot conditions, and they often coincide with large aggregations of birds during moult and pre-migration staging. Botulism outbreaks on such lakes can result in losses of thousands to hundreds of thousands of birds during a single season (Canadian Cooperative Wildlife Health Centre database), although smaller outbreaks also frequently occur.

Avian botulism is caused by ingestion of a paralytic neurotoxin produced by type C *Clostridium botulinum*, which exists in two forms. The spore or resting form is found in soils associated with wetland environments, where it resists environmental degradation and can persist for decades. The vegetative form occurs under anaerobic conditions, with a nutrient rich substrate and elevated temperatures. Type C toxin is produced by rapidly dividing Clostridial organisms that contain a plasmid with the gene for the toxin. Decaying vertebrate carcasses provide a highly suitable substrate for vegetative growth and toxin production. Decomposing carcasses also provide a substrate for growth and development of sarcophageous fly larvae, which accumulate high concentrations of type C toxin if present and can transfer the toxin to other vertebrates that feed on these maggots. This is frequently referred to as the carcass-maggot cycle and is a widely accepted mechanism of botulism occurrence (Rocke and Bollinger 2007).

Causes of waterbird mortality that may precipitate a botulism outbreak are varied, and include disease and weather-related mortality, particularly due to hail storms during the summer months (Stout and Cornwell 1976). Powerline collisions over wetlands (Malcolm 1982) and mortality of juvenile Franklin's gulls (*Larus pipixcan*) on nesting colonies (Soos and Wobeser 2006) are two examples where direct links to subsequent botulism outbreaks have been demonstrated. It is frequently stated that *C. botulinum* type C spores are abundant on wetlands and are unlikely to be limiting factors in botulism outbreaks (Soos and Wobeser 2006).

A review of botulism stated that: "Spores of type C botulism strains are widely distributed in wetland sediments; they can be found in the tissues of most wetland inhabitants, including aquatic insects, mollusks, and crustacea and many vertebrates, including healthy birds" and that because "botulinum spores and the phages that carry the toxin gene are so prevalent in wetlands, they are not considered to be a limiting factor in the occurrence of outbreaks in waterbirds" (Rocke and Friend 1999). However, there are few data to support these statements and some reports suggest that the occurrence and prevalence of type C spores in wetland sediments is highly variable. For instance, Wobeser et al. (1987) summarized the literature on the occurrence of *C. botulinum* type C in wetland soils, noting that its prevalence ranged from zero to as high as 78% across studies. Although the factors responsible for this high variability are unknown, they reported a strong association between the occurrence or prior history of avian botulism and the detection of *C. botulinum* type C. They also detected spores in wetland basins that had been dry for several years, demonstrating the persistence of these bacteria. Sandler et al (1993) reported 52% prevalence of *C. botulinum* type C in sediment samples from botulism-prone wetlands within one Wildlife Refuge in California. Although they found no difference in prevalence between wetlands with or without botulism mortality, or between wetlands with a history of high or low levels of botulism occurrence, they did detect higher prevalence in permanent versus

seasonal wetlands. Williamson et al. (1999) found the toxin gene in 16 of 18 wetland sediments and confirmed its presence in both outbreak and non-outbreak wetlands. However, the botulism history for non-outbreak marshes was not given, and too few samples were analyzed from individual wetlands to make comparisons among them.

Wobeser (1997) proposed a simple model to account for transfer of toxin to susceptible hosts. Making an analogy to infectious diseases, he suggested that the basic reproductive rate (R; the average number of secondary infections arising from a single infection in a naïve population) could be used to describe the number of secondary intoxications attributed to a single carcass as:

$$R = M_2/M_1,$$

where M_2 is the number of individuals dying of secondary intoxications arising from M_1, and M_1 is the number of carcasses on a wetland. Disease incidence increases if $R > 1$, whereas it declines if $R < 1$. Furthermore, M_2 was defined as:

$$M_2 = M_1(P_s)(P_m)(\beta),$$

where P_s is the proportion of carcasses that contain spores of toxigenic *C. botulinum*, and P_m is the proportion of carcasses that become infested with maggots and persist until toxin-laden maggots emerge. The β term is an intoxication coefficient that consists of two components: 1) the frequency of contacts between live birds and toxic material (C) and 2) the proportion of such contacts that result in intoxication (P_i). In this model, the proportion of carcasses that contain spores is a key determinant of botulism outbreaks.

Our objectives were to determine: 1) whether the prevalence of toxin-laden carcasses and levels of toxin varied among wetlands, during botulism outbreaks and between years, and 2) if, during an outbreak, birds that died of botulism had a higher probability of producing type C than birds that died for other reasons (e.g., gunshot). This would suggest that the bacterium is "transmitted" along with the toxin, leading to further objectives to determine: 3) whether ducks from wetlands with a previous history of botulism were more likely to produce type C toxin than ducks from wetlands with no previous history of botulism (i.e., whether the bacterium is acquired locally) and 4) the role that factors such as P_S and P_M play in determining the occurrence of botulism outbreaks and survival of waterfowl.

METHODS

Work was conducted from 1999 to 2003 at wetlands or lakes on the Canadian Prairies. All wetlands provided habitat for dabbling ducks during the summer months and were used to varying degrees for brood rearing, moulting and migration. They were chosen because they had sufficient numbers of ducks for our sampling efforts and because they were subjected to ongoing monitoring, which enabled us to determine if they had previously experienced botulism outbreaks. From 1999 to 2001 inclusive, this research was part of a larger study evaluating aspects of botulism management and impacts, including clean-up efficiency, estimating

mortality, effects on populations, and survival rates. In 2003, samples were collected specifically for this research.

Sample collection during botulism outbreaks

While undertaking line transect surveys using an airboat (see left), maggot samples were collected from decomposing carcasses throughout July and August from 1999 to 2003. These birds were assumed to have died of botulism, because botulism was confirmed to be present on the wetland and was the leading cause of mortality (also see Part II). Maggot samples were collected from carcasses on wetlands at the maximum stage of maggot infestation, when maggots were large and found throughout the carcass. A minimum of 3 g of maggots was collected into small bags, then placed in a cooler with ice-packs and frozen at 10 to 20 C within 12 hours of collection.

Sampling healthy mallards for presence of spores

Moulting mallards were collected from lakes with various histories of botulism, on the same day in early August, by trapping and shooting. These mallards were then placed on the shore of a wetland, where they were individually placed in shallow plastic-lined cardboard boxes and allowed to decompose. An electric fence enclosed these carcasses to deter scavengers; avian scavengers were rare and did not disturb the carcasses. Carcasses were visited daily, at which time cloacal temperatures were taken and maggot samples were collected as described above.

Testing for toxin

Maggot samples were thawed and 1 g was placed in a stomacher bag (Seward Model 80 bag) along with 10 ml of Penicillin and Streptomycin solution (0.5 g penicillin G potassium 10 MU plus 5 g Streptomycin B sulfate in 500 ml 0.85% saline). Maggots and solution were macerated for 1-2 min using a Stomacher (Seward Model 80). The resulting slurry was placed, by pipette, into 15 ml centrifuge tubes and centrifuged for 10 min @ 4343 G. The supernatant, considered a 1/10 dilution, was collected and frozen at 20 C or tested immediately for type C botulism toxin using a mouse bioassay (Quortrup and Sudheimer 1943). Each initial 1/10 dilution sample was tested on two adult CD1 mice of similar size. One mouse was given 0.1 ml of type C antitoxin by intraperitoneal injection. After 30 min, both mice were injected intraperitoneally with 0.1 ml of the maggot antibiotic supernatant solution. Mice were monitored regularly for 4 days to detect

clinical signs of botulism (i.e., use of abdominal muscles for respiration). Mice that were recumbent and showing advanced signs of botulism were euthanized with an overdose of halothane. Although these mice were euthanized, we assumed they would have died of botulism.

A subset of samples containing toxin was titrated with sterile normal saline and tested on pairs of CD1 mice as follows (Duncan and Jensen 1976). If both mice died at 1/10,000 dilution, the minimum lethal dose (MLD) was considered to be $\geq$ 10,000 MLD/g of larvae. This was classified as a high concentration in results. If both mice died at 1/1000, but not 1/10,000 dilution, the concentration was considered to be between 1,000 and 10,000 MLD/g of larvae and was classified as a medium concentration. Finally, if both mice did not die at the 1/1000 dilution, the concentration was considered to be <1,000 MLD/g of larvae and a low concentration. Based on values tabulated by Wobeser (1997), indicating the lethal dose of type C toxin for a mallard was ~ 5 x 10^4 mouse intraperitoneal LD_{50} units, a concentration of type C toxin >10,000 MLD_{100}/g of larvae was considered to be high in our analysis, given the small amount of ingested larvae that would cause death.

Proportion of carcasses that develop fly larvae

To determine the proportions of carcasses removed by scavengers and clean-up operations, and to monitor rates of decomposition, radio-transmitters (both Mauser and intra-abdominal types) were firmly attached to freshly dead carcasses and left on wetlands during clean-up operations as well as on four wetlands without clean-up. Radio-marked carcasses were monitored daily, at which time their rate of decomposition and fate were recorded. Clean-up crews collected -radio-marked carcasses if found.

Estimates of carcass densities

Carcass densities on each lake were estimated on a 5-14 day cycle beginning no later than the first week of July and ending no earlier than 15 August, which spanned the period over which radio-marked mallards were monitored. The exception was Frank Lake in 2000, where surveys did not commence until 23 July. Five to 12 estimates (cycles) of carcass density were obtained for each lake over the summer. Fixed line transects were established at 100-500 m spacing depending on vegetation type and occurrence of carcasses, with the first transect randomly chosen at the beginning of each cycle. Using a Global Positioning System (GPS) unit (Garmin Inc.™), an airboat was driven down the center of these transects while one or two observers stood at the front of the boat, counting all carcasses. For each carcass, the UTM coordinate of the boat was determined using the GPS, and perpendicular distance between the center of the boat (*i.e.*, the transect) and the carcass was estimated.

Density estimates were derived using the software DISTANCE (Thomas et al. 2006). Perpendicular distance data were grouped into various intervals based on frequency histograms, and right truncated if outliers were present. Depending on lake and cycle, the following models were used to derive detection functions and estimate densities: half normal adjusted with cosine

series or simple polynomial series; hazard rate adjusted with cosine series; and uniform adjusted with cosine series. Models were selected based on likelihood ratio tests (Buckland et al. 1993).

Estimates of 30-day survival rate

Results of this work and detailed methodology are published elsewhere (Part II; Evelsizer et al. 2010). Briefly, adult moulting mallards were captured using bait traps and drive-trapping, from 1 July to the second week of August. Visibly healthy mallards were equipped with a back-mounted, prong and suture style radio-transmitter (172-174 MHz) using standard techniques (Mauser and Jarvis 1991), under local anesthetic (1 ml Marcaine), and then released within 30 min to reduce stress (Cox and Afton 1998). A small temperature-sensitive probe encased in flexible plastic tubing ran underneath the stainless steel anchor; when a drop (≥4 C) occurred in body temperature the transmitter pulse rate increased, indicating death.

Survival was determined by tracking mallards daily (04:00 - 09:00 CST), sometimes more frequently, for 30 days after release. After 30 days, surviving birds could not be tracked with certainty because most had completed moult and became mobile. Bird locations and status (dead or alive) were recorded each morning by tracking with a receiver linked to truck or tower-mounted antennas. If the transmitter pulse rate had increased, indicating death, the carcass was retrieved usually within 2-6 hours with the aid of a hand-held tracking system from an airboat. The location of each dead bird was determined with a GPS unit and the carcass was frozen for necropsy. When signals were lost, this could have occurred because birds regained flight and left the wetland near the end of the tracking period, or because the transmitter was shed or failed.

Statistical Analysis

Logistic regression was used to evaluate the association between carcasses developing toxin on a particular lake and that lake's history of prior botulism occurrence. Similarly, logistic regression was used to determine the association between carcasses developing toxin and whether the carcasses were a result of death due to botulism or some other cause of death. Logistic regression analysis was used to evaluate the association between botulism mortality and the following potential risk factors: carcass density; probability of a carcass on a wetland containing toxin (P_S); probability of a carcass on a wetland having high levels of toxin (P_T); and the probability of a carcass on a wetland persisting to the stage of maggot development (P_M). Only lakes with measured carcass densities and survival rates were used in this analysis. Backwards elimination techniques were used and the variable P_M was rejected due to a lack of variability in the parameter. With the remaining variables, a biologically relevant two-way interaction term was added to the model. To compare models, a pseudo R^2 term was derived, comparing the fit of selected models to that of the intercept-only model (Dohoo et al. 2003). All analyses were performed using SPSS versions 16.0 (SPSS Inc., Chicago). Methods were approved by the University of Saskatchewan's Committee on Animal Care (Protocol 19980040) on behalf of the Canadian Council of Animal Care.

RESULTS

The proportion of carcasses producing type C toxin varied from 0 to 100% prevalence across lakes (Table 1). Although the prevalence of toxin production on a particular wetland was generally similar among years, there was one notable exception. In 2000, Kettlehut Lake had a prevalence of toxin production of 93% for botulism carcasses and 87% for carcasses of euthanized mallards that were allowed to decompose. This is in sharp contrast to 2001, when there was no botulism outbreak detected on Kettlehut Lake and the prevalence of toxin production from euthanized mallards was only 15%. Water levels were low in Kettlehut Lake in 2001 compared to 2000, reducing the surface area covered by water from an average of 304.8 ha in 2000 to 252.5 ha in 2001. This would have altered the areas of the lake used by waterfowl and perhaps reduced their exposure to *C. botulinum* spores in wetland sediments in 2001.

There was a significant difference, in the proportion of euthanized bird carcasses that produced toxin, between lakes with a history of botulism outbreaks and those with no previous history of botulism occurrence (Wald's chi-square 30.68; $P< 0.0001$). The prevalence of toxin production on non-botulism lakes ranged from 0 to 38% (median = 17%) and toxin concentrations were often low (Table 1); however, there was no difference in the proportions of carcasses that produced high levels of toxin between botulism and non-botulism lakes (Pearson's chi-square = 2.45, $P = 0.12$). The prevalence of toxin production on lakes with a history of botulism ranged from 15 to 100% (median = 71%). Waterfowl dying on a lake with a recent history of botulism were ~15 times more likely (odds ratio = 15.3, 95% CI = 5.8 to 40.0) to produce type C *botulinal* toxin than were birds dying on a lake without a previous history of botulism.

There was also a significant difference, in the probability of developing toxin, between carcasses of birds that died of botulism and those that were euthanized on the same botulism-prone lakes (Wald's chi-square = 7.05; $P<0.008$). Prevalence of toxin production ranged from 40 to 100% (median = 72%) for euthanized mallards, whereas prevalence of toxin production in birds that died of botulism ranged from 60 to 100% (median = 92%). The odds ratio of toxin production for birds dying of botulism, compared to those dying of other causes on lakes with recent or ongoing botulism occurrence, was 4.7 (95% CI = 2.3 to 10.0).

The proportion of carcasses that were not scavenged or removed during clean-up operations, and therefore persisted to the stage of decomposition where maggots developed on the carcass, also varied among lakes (Table 2). Virtually all carcasses on wetlands without carcass clean-up developed maggots, whereas 43 - 77% of carcasses developed maggots on clean-up lakes. Most carcasses were removed by clean-up crews, but others were removed by scavengers.

Risk of outbreak occurrence was assessed by combining: probabilities of spore presence; occurrence of high toxin potency; and maggot development in carcasses (Table 3). Carcass densities and 30-day survival probabilities for Boulder, Lucky, Indi and Brightwater lakes (identified with asterisks in Table 3) were based, respectively, on failure to observe carcasses during the collection period and typically high survival probability during moulting periods.

Carcass density, P_S, P_T and the interaction term P_S*P_T were found to be significant in the logistic regression model (Table 4). The odds ratio for the risk factor density was 1.77 (95% CI = 1.15-

2.73), indicating that the addition of one carcass/ha increased the odds of botulism mortality by ~1.8 times. Based on comparisons of pseudo-R^2 values, there was a 23% relative improvement in model fit by adding the P_S and P_T terms, including their interaction term, compared to a model containing density alone (Table 4).

DISCUSSION

An understanding of factors that precipitate and perpetuate botulism outbreaks is required to prevent and manage this disease. Previous research has shown that the carcass-maggot cycle is often the most significant factor in the spread of botulism on a wetland. Our research demonstrated that carcass density is a significant predictor of moulting mallard survival on wetlands, with each additional mean carcass per ha increasing the odds of botulism mortality by 1.8 times. The addition of P_S, P_T and the interaction term improved the model by 23%. Logistics and costs prevent carcass clean-up from being an effective management response to botulism outbreaks on most wetlands with significant botulism die-offs (Parts I and II) despite of the survival benefits of lower carcass densities (see Part III).

Given the important role of fly larvae in the spread of botulism, researchers have investigated the prevalence and levels of toxin in maggots collected from carcasses during outbreaks. Duncan and Jensen (1976) found 120/142 or 84.5% of fly larvae samples from carcasses contained type C toxin and, from 15 July to 2 October, 92% contained toxin with the concentration of toxin increasing dramatically after 15 July. Twenty-one (15%, n = 142) of these samples had a minimum lethal dose of $\geq$ 100,000 MLD_{50}/g of larvae. Haagsma et al. (1972) reported that ~96% of fly larvae samples contained toxin during a type C botulism outbreak in the Netherlands. Reed and Rocke (1992) placed previously frozen carcasses on a wetland and monitored fly larvae and toxin development, and found on average 41% (SE = 9%) of maggots developed toxin and on average 16% (SE = 6%) of maggots from euthanized birds and 10% of maggots from botulism-intoxicated birds had levels of toxin $\geq$ 100 MLD/g of maggots.

For botulism to spread, ingested maggots must also contain sufficient concentrations of toxin to kill a bird. For botulism to spread beyond this newly intoxicated bird, the bacterium with the type C gene must either be transmitted with the maggots or the bird must already contain previously ingested spores of *C. botulinum*. Our research shows that between zero and 38% of carcasses that decompose on lakes without a history of botulism produce toxin; and because many of these carcasses produce low levels of toxin (as determined by the MLD from maggots), the probability of a decomposing carcass producing significant amounts of toxin drops to between 0 and 7% (Table 3). Of the six lakes that fit this category in our study, three would have had a probability of 0%, one with 5% and two with 7% probability. This is in sharp contrast to the proportion of carcasses that contain toxin at high levels on wetlands experiencing botulism outbreaks. When comparing lakes with differing histories of botulism, the odds ratio indicates that carcasses of waterfowl dying on lakes with a recent history of botulism are 15 times more likely to produce type C toxin than they would if birds had died on a lake without a history of botulism. These findings indicate that most mallards, and likely other waterfowl, are ingesting spores locally during the course of feeding activities. The high level of spore contamination of

sediments in basins of these botulism lakes has been demonstrated by Wobeser et al. (1987). In the wetlands that they studied, 59% of soil samples collected from marshes with a known history of botulism produced toxin, whereas only 6% of soil samples from marshes with no history of botulism produced toxin.

Our studies also suggest that exposure to spores in the environment can change significantly from one year to the next. During a botulism die-off on Kettlehut Lake in 2000, 87% of carcasses from euthanized birds produced toxin compared to only 15% of carcasses from euthanized birds in 2001. In 2001, carcasses were infrequently observed on Kettlehut Lake despite intensive surveillance efforts similar to those in 2000. Sick birds were not observed in 2001 and botulism was never confirmed. Water levels were low on Kettlehut in 2001 compared to 2000, changing the total surface area and likely the feeding areas used by waterfowl. Thus, distribution of spores of *C. botulinum* type C within a wetland may vary spatially and over time. Factors affecting distribution of spores in wetlands warrants further investigation.

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Table 1. Prevalence and concentration of type C botulism toxin in maggots collected from waterfowl carcasses dying from botulism versus collection via shooting at selected wetlands. Also shown are percentages of birds by categories (Low, Medium, High) of toxin concentration determined by minimum lethal dose to mice (see Methods). Locations and coordinates of lakes with botulism are shown in Figure 1 and Table 1 of the General Introduction, while the coordinates for those with no previous history of botulism (*) are listed below the table.

Year	Lake, Province[a]	Cause of Death	Proportion with toxin (%)	Toxin concentration (%)		
				Low	Medium	High
1998	Old Wives, SK	botulism	21/25 (84)	12/20 (60)	4/20 (20)	4/20 (20)
	Whitewater, MB	botulism	52/55 (95)	7/30 (23)	6/30 (20)	17/30 (57)
	Pakowki, AB	botulism	11/11 (100)	2/10 (20)	1/10 (10)	7/10 (70)
1999	Old Wives, SK	botulism	19/21 (90)	11/19 (58)	7/19 (37)	1/19 (5)
		euthanized	11/25 (44)	8/11 (73)	2/11 (18)	1/11 (9)
	Whitewater, MB	botulism	21/21	4/20 (20)	3/20 (15)	13/20 (65)
	Eyebrow, SK	botulism	22/22	10/19 (53)	6/19 (31)	3/19 (16)
		euthanized	18/25 (72)	9/18 (50)	4/18 (22)	5/18 (28)
	Lucky*, SK	euthanized	4/15 (26)	3/4	1/4	
2000	Kettlehut, SK	botulism	28/30 (93)	11/15 (73)	1/15 (7)	3/15 (20)
		euthanized	26/30 (87)	9/20 (45)	8/20 (40)	3/20 (15)
	Paysen, SK	botulism	15/18 (83)	7/15 (47)	5/15 (33)	3/15 (20)
		euthanized	23/26 (88)	12/20 (60)	4/20 (20)	4/20 (20)
	Crane, SK	botulism	12/13 (92)	6/12 (50)	4/12 (33)	2/12 (17)
		euthanized	30/30	11/20 (55)	9/20 (45)	
	Frank, AB	botulism	15/20 (75)	5/15	5/15	5/15
	Whitewater, MB	botulism	10/10	3/10	5/10	2/10
	Boulder*, SK	euthanized	6/30 (20)	4/6 (67)		2/6 (33)
	Barber*, SK	euthanized	1/9 (11)	1/1		
	Eyebrow, SK	euthanized	2/3 (67)	2/2		
2001	Kettlehut[b], SK	euthanized	3/20 (15)	3/3		
	Paysen, SK	botulism	20/20	2/20 (10)	8/20 (40)	10/20 (50)
		euthanized	14/20 (70)	11/14 (79)	3/14 (21)	0/14

	Chaplin, SK	botulism	3/5 (60)	1/3	0/3	2/3
		euthanized	8/20 (40)	7/8 (88)	1/8 (12)	0/8
	Frank, AB	botulism	16/20 (80)	9/16 (56)	3/16 (19)	4/16 (25)
	Whitewater, MB	botulism	19/20 (95)	12/19 (63)	6/19 (32)	1/19 (5)
	Barber*, SK	euthanized	0/5			
	Indi*, SK	euthanized	0/20			
2003	Eyebrow, SK	euthanized	28/28	17/20 (85)	3/20 (15)	
	Brightwater*, MB	euthanized	6/16 (38)	6/6		
	Indi*, SK	euthanized	4/24 (17)	4/4		
	Whitewater, MB	euthanized	44/47 (94)	19/22 (86)	3/22 (14)	
	Pakowki, AB	euthanized	25/29 (86)	13/20 (65)	7/20 (35)	

* Lucky Lake = 51.06880N -107.08844W; Boulder Lake = 51.59974N -105.23776W; Barber Lake = 51.36800N -107.65903; Indi Marsh = 51.69440N -106.51620W; Brightwater Reservoir = 51.59660N -106.53740W.

[a] SK = Saskatchewan; MB = Manitoba; AB = Alberta.
[b] Botulism-prone lake but no botulism in current year.

Table 2. Numbers of carcasses developing maggots at selected Canadian prairie wetlands in 2000-2001. The "control" treatment indicates no carcass cleanup was undertaken whereas "managed" treatment indicates intensive surveillance and carcass cleanup occurred. Fresh duck carcasses were marked with radio transmitters, placed where they were initially found and monitored daily until they sank, disappeared or were recovered on managed lakes only by cleanup crews.

Year	Lake, Province [a]	Treatment	Number of marked carcasses	Number (%) developing maggots	Comments re: carcasses
2000	Crane, SK	Control	29	29 (100)	
	Kettlehut, SK	Control	36	36 (100)	
	Frank, AB	Managed	30	23 (77)	7 collected
	Paysen, SK	Managed	30	13 (43)	2 scavenged; 15 collected
2001	Chaplin, SK	Managed	2	1 (50)	1 scavenged
	Frank, AB	Control	11	11 (100)	
	Paysen, SK	Control	21	20 (95)	1 scavenged

[a] SK = Saskatchewan; MB = Manitoba; AB = Alberta.

Table 3. Annual and lake-specific probability estimates for presence of spores (PS), production of botulinum toxin (PT [a]) and maggots (PM), and the product of these three probabilities (PS*PT*PM). Also shown are density of toxic carcasses on a wetland (corrected carcass density) and survival probability for radio-marked moulting mallards.

Lake, Province	Year	PS	PT	PM	PS*PT*PM	Corrected carcass density	Survival probability
Whitewater, MB	1999	1.0	0.80	1.0	0.80	3.94	0.046
	2000	1.0	0.70	1.0	0.70	Not known	Not known
	2001	0.95	0.37	1.0	0.35	Not known	Not known
Kettlehut, SK	2000	0.93	0.27	1.0	0.25	3.23	0.597
	2001	0.15	0.33	1.0	0.05	0	1.0
Paysen, SK	2000	0.83	0.53	0.43	0.19	1.23	0.576
	2001	0.70	0.21	0.95	0.14	0.77	0.886
Frank, AB	2000	0.75	0.67	0.77	0.39	4.56	0.369
	2001	0.80	0.44	1.0	0.35	2.14	0.860
Chaplin, SK	2001	0.40	0.12	0.5	0.02	0	0.962
Boulder*, SK	2000	0.20	0.33	1.0	0.07	0	~1.0
Lucky*, SK	1999	0.26	0.25	1.0	0.07	0	~1.0
Indi*, SK	2001	0	0	1.0	0.00	0	~1.0
	2003	0.17	0	1.0	0.00	0	~1.0
Brightwater*, SK	2003	0.38	0	1.0	0.00	0	~1.0
Old Wives, SK	1999	0.90	0.41	1.0	0.37	6.57	0.043
Eyebrow, SK	1999	1.0	0.47	1.0	0.47	2.97	0.386
	2003	1.0	0.15	1.0	0.15	Not known	Not known
Crane, SK	2000	0.92	0.50	1.0	0.46	3.41	0.486

[a] Proportion of toxic carcasses that contain maggots with concentrations $\geq$ 1000 minimum lethal dose.
[b] MB = Manitoba; SK = Saskatchewan; AB = Alberta.

* Lakes with no previous history of botulism.

Table 4. Lake-adjusted associations between risk factors and the 30-day probability of dying of botulism.

	Variable	Regression coefficient (β)	95% Confidence Interval for β		P-value	Quasi Likelihood under Independence Model Criterion (QIC value)
			Lower	Upper		
Unconditional models:						
	PS	4.87	1.80	7.95	0.002	
	PT	3.20	1.07	8.71	0.003	
	Carcass Density	0.50	0.15	0.84	0.005	
Main effects and interaction (#1:)						514
	PS	-0.93	-5.02	3.16	0.660	
	PT	-8.28	-18.34	1.79	0.110	
	Carcass Density	0.57	0.14	1.00	0.010	
	PS*PT	13.22	3.05	23.39	0.011	
Main effect (#2):						1,535
	Density	0.90	0.47	1.33	0.000	
	Intercept only					4,912

Pseudo R^2 for model #1: (4,912-514)/4,912 = 0.90

Pseudo R^2 for model #2: (4,912-1,535)/4,912 = 0.69

Comparison of Pseudo R^2: (0.90-0.69)/0.90 = 0.23

MAIN CONCLUSIONS AND RECOMMENDATIONS

A. Evaluation of effectiveness of carcass clean-up

The ability of search crews to discover dead ducks was severely hampered by dense vegetation, as indicated by low recovery rates of marked carcasses (Part 1). Primarily as a result of this limitation, the number of carcasses remained high in many areas of wetlands after crews had finished thoroughly searching targeted areas. Therefore, despite intensive effort, it was not possible to enhance survival of radio-marked mallards (*Anas platyrhynchos*) on moderate to large vegetated wetlands (Part II). Furthermore, results of direct recovery analyses of banded mallards generally confirmed the findings obtained from radio-marked birds; recovery rates were similar for birds banded on botulism wetlands with and without clean-up operations (Part IV). Finally, we discovered that the assumption underlying the rationale for carcass clean-up was valid; risk of mortality was higher for mallards exposed to higher carcass densities (Part III). But, even with intensive carcass removal operations, it was not possible to reduce carcass densities to low levels needed to improve survival rates in a cost-effective manner. However, this result does imply that carcass removal could potentially be more cost-effective in situations where carcasses are easily found and can be removed quickly on a frequent basis.

Recommendation 1:
It was recommended that the Prairie Habitat Joint Venture (PHJV) stop routine clean-up operations, and this was adopted in 2001.

Recommendation 2:
If botulism becomes a significant threat to shorebirds (especially species at risk), a botulism working group (with a renewed mandate; see below) should consider and evaluate shoreline clean-ups to reduce carcass densities in these areas (see Part III).

B. Botulism Impacts on Populations

Results of this study lend considerable support to the suggestion that, for mallards, exposure to botulism during the post-breeding season can have a measurable impact on survival at the population level (Part IV). However, these results also provided little evidence that carcass removal resulted in any measurable impact on direct recovery rates of mallards from outbreak sites with and without clean-up (i.e., contrary to expectation, there was no increase in late-summer survival). It remains unclear how differences in toxin toxicity, amount of available toxin, environmental characteristics, live bird density, or other as yet unidentified factors likely contributed to differences in risk. Any additional work arising from the recommendations below could be pursued by individual agencies or universities based on their respective mandates.

Recommendation 3:
Studies are needed to determine the extent to which existing results for mallards can be generalized to other avian species, particularly those of potential management concern (e.g., northern pintails [*Anas acuta*]).

Recommendation 4:
Population modeling work that assesses the impact of reduced survival due to disease (such as botulism) should be undertaken and/or continued, particularly on species of management concern (e.g., northern pintails).

Recommendation 5:
It is important to recognize that effects of botulism on continental populations will depend not only on the mortality rates of exposed individuals, but also on the proportion of individuals in the population that are routinely exposed. Acquiring information on the size of the population at risk is essential.

C. Ecology of botulism

The most probable sources of *C. botulinum* toxin prior to and during botulism outbreaks were maggots produced in bird carcasses (e.g., gull [*Larus*] and grebe [*Podiceps*] chicks; ducks). Consistent with earlier published reports, likelihood of maggot development was positively related to air and water temperatures (Part V). Furthermore, botulism production risk was also related to a wetland's history of botulism and the potency of botulism toxin (Part VI). Any additional work arising from the recommendations below could be pursued by individual agencies or universities based on their respective mandates.

Recommendation 6:
Further work is needed to determine how spore and toxin production and potency interact to affect bird survival rates, and to ascertain whether these factors could be managed to reduce occurrence or severity of botulism outbreaks.

Recommendation 7:
Quantitative evaluations should be undertaken of the relative importance of different risk factors that could contribute to the initiation and perpetuation of botulism outbreaks.

D. Communications and next steps

Over the course of this study, considerable effort was put into communicating why work was being undertaken and regularly reporting study results. As findings became available, immediate decisions were made and actions were taken to affect botulism management decisions. Therefore, some of the recommendations listed above were rapidly implemented. Since the decision was made to cease carcass clean-up operations, to our knowledge, there has been no adverse reaction (in Canada or in the U.S.) to the lack of clean-up. In view of all study findings, in future, a thorough review of new knowledge would help to guide alternative approaches to botulism management.

Recommendation 8:
The model of botulism ecology, as formulated for the 1998 botulism report, should be reviewed and used as a basis for guiding future management and research. New models should also be tested and updated periodically as new information becomes available. In future, botulism research should evaluate alternative management strategies for reducing risks of avian botulism (e.g., manipulating wetland water levels), based on relative costs and benefits of each approach.

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APPENDIX 1

AVIAN BOTULISM IN ALBERTA: A HISTORY

PYBUS, M. J.

Avian botulism in Alberta: a history

by
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Alberta
SUSTAINABLE RESOURCE DEVELOPMENT

AVIAN BOTULISM IN ALBERTA: A HISTORY

compiled by M.J. Pybus

from records provided by Fish and Wildlife Division,
Canadian Wildlife Service and Ducks Unlimited Canada

***Summary:** Mortality of wild waterfowl has been reported on at least 33 different lakes in Alberta since 1924. Some of these lakes no longer exist or currently do not support conditions suitable for initiation of dieoffs. Between 1980 and 1993, losses occurred repeatedly on 5 lakes (Beaverhill, Grantham, San Francisco, Utikuma, and Whitford); however, reported botulism problems occurred on at least one lake in all but 5 years (1982, 1983, 1985, 1987, 1993). From 1994 to 2002, the pattern changed markedly with significant numbers of dead waterfowl found only on Pakowki Lake from 1994 to 1997, followed by widespread losses on various lakes (but not Pakowki) from 1998 to 2000 and only one lake in 2001. The mid 1980s were relatively dry years in the prairie regions and losses were relatively minor. In the early 1990s, the prairies entered a wet phase and record high numbers of May ponds were observed. In particular, Pakowki Lake filled with spring runoff and was used by migrant and resident waterfowl. In the late 1990s, the prairies again were relatively dry. Botulism losses decreased in the prairies and increased in the parkland and boreal areas. An apparent increase in disease losses in northern areas in recent years may reflect increased surveillance and control effort.*

Estimates of the actual losses per lake are extremely difficult to assess and are thought to be completely unreliable. As such, this report contains only the raw numbers of dead birds collected or observed at each lake. Generally, observed losses range from 700 to 2500 birds per lake, primarily dabbling ducks. There are a few notable exceptions where losses greatly exceeded this range. Pakowki, Hay Zama, and Utikuma are large staging lakes for a variety of waterfowl and they tend to have large problems whenever mortality occurs. Kimiwan is an anomaly in that it had a large mortality in 1998, but little or no mortality in other years. Losses generally begin in mid to late July and continue until early or mid- September, particularly in periods of prolonged hot dry weather. Blue-green algae toxins in the south and Newcastle Disease Virus in the north are confounding factors in some dieoffs and likely cause losses additive to those caused by botulism poisoning.

In 1992, Alberta began implementing a provincial Waterfowl Disease Contingency Plan (Anonymous 1992) that provided an overall approach to disease cleanup. It identified a communications network and general responsibilities of the three primary agencies concerned with waterfowl management/ conservation in Alberta (Environment Canada, Alberta Fish and Wildlife, and Ducks Unlimited Canada). In 1999, the Alberta Conservation Association joined as a full partner to the provincial contingency plan. In the late 1990s, regional surveillance plans and specific contingency plans for problem lakes were developed cooperatively among the partners. A plan for dealing with disease on Pakowki Lake (Pybus et al. 1995) was used as a model for other lakes.

Background

Munro (1927) provides the earliest record of what appears to be botulism poisoning in waterfowl and shorebirds in Alberta. Large numbers of dead birds were seen at Lake Newell in the summer months of 1924 and 1925. The clinical signs, distribution of carcasses, and species composition were consistent with botulism intoxication. Blue-green algae also were present in large amounts. Unfortunately it seems we did not progress much in the intervening 70 years, for a dieoff at Pakowki Lake in 1994 (Pybus 1994) appeared to be almost identical to the one at Lake Newell. However, since 1994, there has been considerable effort expended in trying to understand the underlying causes of avian botulism, to assess the potential management responses, and to estimate the impacts of botulism on local and flyway populations of waterfowl. In 1995, the partners to the provincial contingency plan in Alberta established the Pakowki Lake Working Group. Representatives from various disciplines associated with wetland management and ecology reviewed current information and management options for potentially limiting avian losses at Pakowki Lake (Pybus 1997). It became apparent that lack of information was itself a limiting factor in assessing various options. In 1997, the Prairie Habitat Joint Venture of the North American Waterfowl Management Plan established an Avian Botulism Working Group with interprovincial and international representation. At the time of writing (July 2002) this group has provided an interrim report and recommendations (PHJV 1998) and final recommendations relating to the lack of evidence that carcass cleanup made any difference in the survival of radio-marked moulting mallards (Bollinger et al. In prep)

The current report is provided as background to these initiatives. It contains a summary of available information provided by Ducks Unlimited Canada (DUC), Environment Canada (CWS), and Alberta Fish and Wildlife (F&W) up to July 2002. The document updates a previous review (Pybus 1995) with a few additional early records plus a summary of documented losses through to 2001.

Early records referring to 'waterfowl sickness' may include disease agents other than botulism. Starting in 1980, only those problems known or considered likely to be avian botulism intoxication are included.

Alberta Records of Waterfowl Sickness 1924-1979.

from materials provided by Wm. Wishart, Wildlife Biologist Emeritus, Alberta Fish and Wildlife.

LOCATION	DATE	SOURCE
L. Newell ("considerable losses")	1924, 1925 August botulism + bl-gr algae	Munro, J.A. 1927. Can Field Nat. 41:77-84.
Many Island L., Pakowki L.	1928	R.C. Duthie, Vet. Surgeon, general rpt., Oct. 6, 1934
Namaka L.("1000s of birds"), Stobart L.	1933, July, Aug botulism confirmed	Duthie, R.C., Oct 18, 1933: general rpt. Also: Shaw, R.M. & G.S. Simpson. 1936. J. Bact. 32:79-88.
Stobart L. Kings L. Welstead L.(WBNP)	1934, July, Sept	Sciple, G.W.. Avian Botulism. Sp. Sci. Rpt (Wildl.) #23, USFWS, Aug 1953.
Stirling L.	1935	Lethbridge Herald, Sept 15, 1958.
Namaka L., L. Newell Stobart L.	1938, July, Aug	DUC Rpt, 1939. Duck Disease Outbreaks in Western Canada.
Fincastle Lakes	1938, Aug	McLeod and Bondar. 1952. Can. J. Publ. Hlth 43: 347-350.
Big Hay Lake (1000s of Bonaparte's gulls)	Aug 1938 & 39	Soper, J.D. 1939. Report on Big Hay Lake Public Shooting Ground, Alberta. National Parks Bureau, Ottawa
Gambling L., Stobart L.	1939, Aug	DUC Rpt, 1939. Duck Disease Outbreaks in Western Canada.
San Francisco L	1948 ?	Turner, Sharp, Jones
Milo L.	1950, June (algae?)	Sharp, Freeman, Gollop
Stobart L.	1951, Aug 1952, Aug	Geo. Freeman, DUC
Frank L.	1954, Aug	Geo. Freeman, DUC
Many Island L.	1955, Aug	Geo. Mitchell F&W
Kings L.	1958, July, Aug	C. Sawyer, F&W
Lac la Biche Hay L. Murray Lakes	1958, Sept bl/gr algae at LLB?	Sharp, DUC Webb, F&W Bertelsen, F&W
Stobart L.	1959, Aug 1960, July, Aug	Geo. Freeman, DUC
Beaverhill L. ,Big Hay L. Cygnet L., Smoky L. Watt L., Whitford/Rush	1961, Aug	Wishart, F&W Webb & Vet Lab
Jessie L. Woods L.	1962, Aug	Bradshaw, F&W Adams, F&W
Smoky L. (14,723) Big Hay L.	1963, Aug	Schmitke, F&W
Smoky L. (≈4000)	1965 botulism	Presidente, F&W
12 Mile Cr (Leth)	1966 botulism	Armstrong, F&W
Beaverhill L. Smoky L. (2488)	1967 botulism	Novak, F&W
Pakowki	1975, Aug 16	Windberg memo, F&W 132 carcasses/100 yd along Rge Rd 885; 15/100 yd on north shore
Beaverhill	1975, Aug 25	Hanson, AB Agriculture lab. Rpt
Beaverhill Whitford	1976, Aug botulism confirmed	Cole memo, F&W A few hundred ducks & shorebirds in NE Beaverhill. A few birds on Whitford.
Buffalo	1979	AB Agric. lab rpt. Hundreds of dead birds of different species. 256 carcasses on island.

SUMMARY OF ALBERTA BOTULISM OUTBREAKS 1980-2001

YEAR	# LAKES	LOCATION	# collected/seen
1980	5	Beaverhill	3003
		Buffalo	1990
		Rush	<100
		Pakowki	8300
		Whitford	129
1981	6	Beaverhill	610
		Buffalo	821
		Hay	1430
		King's	1550
		Smoky	1010
		Whitford	1550
1982	0		
1983	0		
1984	4	Beaverhill	11
		Bittern	250
		Buffalo	40
		Whitford	2071
1985	0		
1986	1	Miquelon	unknown
1987	0		
1988	2	San Francisco	1783
		Whitford	1300
1989	3	Hay Zama	6022
		San Francisco	350
		Whitford	1500
1990	4	Grantham	837
		Hay Zama	3159
		San Francisco	592
		Utikuma	≈ 754*
1991	4	Beaverhill	3400
		Big	<100
		Grantham	<100
		Utikuma	4000
1992	5	Beaverhill	220
		Big Hay	30
		Grantham	237
		Magloire	100
		Utikuma	7242*
1993	0		
1994	1	Pakowki	31,517
1995	1	Pakowki	100.825
1996	1	Pakowki	12.342
1997	1	Pakowki	45,048
1998	13	Beaverhill	15.372
		Frank	460
		Hay Zama	≈4000
		Lac Cardinale	≈200
		Joseph	≈100
		Kimiwan	44.510
		Lac Ste Anne	≈140
		Namaka ?	≈50
		Pakowki	4943
		Stobart Lake	10.939
		Utikuma	80.000
		Whitford	790
		Winagami	760
1999	6	Beaverhill	235
		Frank	441
		Kimiwan	1980
		Utikuma	15.128
		Whitford	400
		Winagami	354
2000	7	Beaverhill	≈800
		Buffalo	≈2000
		Frank	2351
		HayZama	≈60,000
		Utikuma	7291
		Whitford	<100
		Winagami	551
2001	1	Buffalo	≈600

* Newcastle Disease Virus also confirmed on Utikuma

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Appendix 1: detailed information from unpublished reports.

(Additional information is available in the identified reports)

1) 1980 (Calverley 1980)

Lake	**Detection Date**	**# observed/ collected**	**assessment methods**	**primary species (%)**	**#man-days**
Beaverhill	Aug. 28	3003	cleanup	widgeon, gadwall, mallard, shoveler	20
Buffalo	Aug 5	1990	cleanup	widgeon, mallard, shoveler, bl-wing teal	22
Pakowki	Aug18	8300	cleanup	gr-wing teal, shoveler, pintail, malard	44.5
Rush	Aug 5	26	aerial		
Whitford	Aug 5	129	aerial		
1980 **TOTAL**		13,293			86.5

2) 1981 (Calverley 1981)

Lake	**Detection Date**	**# observed/ collected**	**assessment methods**	**primary species (%)**	**#man-days**
Beaverhill	Aug 18	610	aerial & cleanup	gr-win teal (48) pintail (15) shoveler (11)	
Buffalo	Aug 19	821	cleanup		18
Hay Lks	Sept 2	1430	cleanup		8
King's	Sept 2	1550	cleanup	pintail (40) gr-wing teal (30) mallard (20)	5
Smoky Lk	Sept 2	1010	aerial & cleanup	gadwall(23) bl-wing teal (20) shoveler(19)	10
Whitford	Aug 27	1550	aerial & cleanup	pintail(27) mallard(19) gr-wing teal(17)	9
1981**TOTAL**		6971			69.5

3) 1982, 1983 DUC systematic monitoring not implemented due to low potential for botulism (high water levels). No problems were identified through other channels. However, there is a report of possible botulism on Lost Lake (Hoffman memo, F&W: 300+ dead birds in August).

4) 1984 (Calverley 1985)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
Beaverhill	Aug 29	11	aerial		<0.5
Bittern	Aug 29	250	cleanup		
Buffalo	Aug 29	40	cleanup		1
Whitford	Aug 29	2071	aerial & cleanup		10
1984 **TOTAL**		2372			---

5) 1985 no reported losses

6) 1986 F&W memo indicating botulism in juvenile California Gulls at Miquelon Lake, late July and early August. Birds submitted to laboratory for evaluation. No indication of estimated losses.

7) 1987 no reported losses

8) 1988 (F&W files)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
San Francisco	Aug 10	1783	cleanup	gr-wing teal, mallard, pintail, coot	29*
Whitford	Aug 29	1300	cleanup	gr-wing teal (80) mallard (15)	
1988 **TOTAL**		3083			---

* DUC only, additional time from CWS and F&W

9) 1989 (F&W files)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
Hay Zama	Aug 31	6022	cleanup	gr-wing teal (30) mallard (30) coot (30)	
San Francisco	Aug 20	350	cleanup		
Whitford	Aug 10	1500	cleanup	gr-wing teal, mallard shorebirds	
1989 **TOTAL**		7872			

10) 1990 (F&W files)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
Grantham	July 30	837	cleanup	gr-wing teal (30) pintail (25) coot (14)	35
Hay Zama	Aug 14	3159	cleanup	gr-wing teal (34) mallard(30) pintail(20)	31
San Francisco	July 31	592	cleanup		10
Utikuma	Aug 7	754*	cleanup		19
1990 **TOTAL**		5342			

* low search efficiency. Botulism and NDV.

NOTE: Pakowki allegedly affected but not substantiated (Dube 1990)

11) 1991 (F&W files)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
Beaverhill	Aug 9	3400	cleanup	pintail, gr-wing teal	
Big Lake	Aug 5	50-60	cleanup		
Grantham		few	visual		
Utikuma	Aug 16	4000	cleanup		40
1991 **TOTAL**		7455			

12) 1992 (F&W files)

Lake	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days
Beaverhill	≈Aug 25	220	cleanup		
Big Hay	≈Aug 25	30	cleanup		
Grantham	Aug 10	237	cleanup	mallard (30) gr-wing teal (19) pintail (15)	14
Magloire	Aug 11	100	cleanup		2
Utikuma	July 9	7242*	cleanup	mallard, widgeon, lesser scaup, gadwall	58
1992 **TOTAL**		7829			

* Newcastle Disease Virus also confirmed on Utikuma.

13) 1993 no reported botulism

14) 1994 to 1997 **Pakowki Lake** (the only lake with identified mortality)

YEAR	Detection Date	# observed/ collected	assessment methods	primary species (%)	#man-days	reference
1994	Aug 19	31,517	cleanup	mallard (22) gadwall (21) shoveler (19)	195	Pybus 1994
1995	July 10	100,825	cleanup	gr-wing teal (52) pintail (20) shoveler (10)	691	Pybus and Eslinger 1996
1996	July 19	12,342	cleanup	shoveler (20) gr-wing teal (19) pintail (18)	239	Pybus and Anderson 1997
1997	July 16	45,048	cleanup	gr-wing teal (35) pintail (20) shoveler (12)	339	Peers et al. 1998

15) **1998**

Lake	Location	Detection Date	# Collected	*Primary Species*	Diagnosis	Reference
Beaverhill	53°27'N 112°32'W	Aug. 12	15,372	dabbling ducks	avian botulism	Barr and Pybus 1999
Cardinal	56°18'N 117°54'W	Aug. 23	≈200	dabbling ducks	probable botulism[1]	Moyles 1998
Frank	50°14'N 113°W	Aug. 17	460	dabbling ducks	avian botulism	Sadler 1998a
Hay Zama	58°40'N 119°W	Aug. 10	≈4,000	dabbling ducks	avian botulism	Pybus and Morton 1998
Joseph	53°00'N 113°.3'W	Aug. 26	≈100	ring-billed gulls	probable botulism[1]	Pybus and Girvan 1998
Kimiwan	55°45'N 116°55'W	Aug. 28	44,510	dabbling ducks	probable botulism[1]	Arbuckle et al. 1999
Lac Ste Anne	53°42'N 114°25'W	Aug. 23	≈140	various gulls	undetermined[2]	Pybus and Wollis 1999
Namaka	50°50'N 113°11'N	Sept 23	≈50	dabbling ducks	blue-green algae[3]	Sadler pers. comm.
Pakowki	49°20'N 111°W	July 22	4,943	dabbling ducks	avian botulism	Peers 1999
Stobart	50°50'N 113°10'W	Aug. 18	10,939	dabbling ducks	avian botulism	Sadler 1998b
Utikuma	55°55'N 115°25'W	Aug. 10	80,000	diving ducks	avian botulism	Heckbert and Pybus 1999
Whitford	53°53'N 112°15'W	Aug. 12	790	dabbling ducks	avian botulism	Moore and Pybus 1999
Winagami	55°38'N 116°45'W	Aug. 30?	760	dabbling ducks	probable botulism[1]	Arbuckle et al. 1999
cumulative	***total***		162,264			

[1] based on field evidence and clinical signs/recovery of sick birds; [2] probably NOT avian botulism;

[3] probable blue-green algal poisoning based on field evidence

16) **1999**

Lake	Location	Detection Date	# Collected	Primary Species	Diagnosis	Reference
Beaverhill	53°27'N 112°32'W	Sept. 8	235	dabbling ducks	avian botulism	Barr 2000
Frank	50°14'N 113°W	July 30	441	dabbling ducks	avian botulism	Peers 2000
Kimiwan	55°45'N 116°55'W	April 28	113	dabbling ducks	avian botulism	Tiege and Pybus 1999
		June 29	1867	dabbling ducks	avian botulism	
Utikuma	55°55'N 115°25'W	July 20	15,128	diving ducks	avian botulism	Pugh & Heckbert 2001
Whitford	53°53'N 112°15'W	July 15	400	dabbling ducks	avian botulism	Moore 2000
Winagami	55°38'N 116°45'W	Aug. 31	354	dabbling ducks	avian botulism	Tiege and Pybus 1999
cumulative	***total***		18,538			

17) **2000**

Lake	Location	Detection Date	# Collected	Primary Species	Diagnosis	Reference
Beaverhill	53°27'N 112°32'W	July 26	≈800	dabbling ducks	avian botulism	
Buffalo	52°30'N 112°45'W	Aug 14	≈2000	dabbling ducks	avian botulism	
Frank	50°14'N 113°W	July 18	2351	dabbling ducks	avian botulism	
Hay Zama	58°40'N 119°W	Aug 3/4	≈60,000	dabbling ducks	avian botulism	
Utikuma	55°55'N 115°25'W	July 18	7291	dabbling ducks	avian botulism	
Whitford	53°53'N 112°15'W	July 25	<100	dabbling ducks	probable botulism	
Winagami	55°38'N 116°45'W	Aug 14	551	dabbling ducks	suspect botulism	
cumulative	***total***					

18) **2001**

Lake	Location	Detection Date	# Seen	Primary Species	Diagnosis	Reference
Buffalo	52°30'N 112°45'W	July 26	≈600	dabbling ducks	avian botulism	Bjorge 2001

APPENDIX 2

EXPOSURE OF MALLARD DUCKLINGS TO MICROCYSTIN-LR

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INTRODUCTION

Although blue-green algae (*cyanobacteria spp.*) are very common in wetlands, and mortality of waterfowl has been attributed to toxicity from algae, there is very little information on the toxicity of specific toxins for birds, including waterfowl. Algal toxins are of two general types: neurotoxins and hepatotoxins. In general, hepatotoxic blooms occur more commonly than do neurotoxic blooms (Carmichael et al. 1985). Neurotoxins are associated with *Anabaena* and *Aphanizomenon spp*. cyanobacteria. One neurotoxin (anatoxin-a) is known to be toxic for mallards (*Anas platyrhynchos*) (Carmichael and Biggs 1978) and produces clinical signs similar to those produced by cholinesterase-inhibiting insecticides. However, this toxin is not reported commonly in Prairie lakes (e.g., Kotak et al. (1993) did not find it in any of 39 bloom samples collected from Alberta lakes and dug-outs). Hepatotoxins are produced by a number of different algae. The most common of these are a group of toxins called microcystins that are produced by *Microcystis spp*., as well as by some other cyanobacteria. Microcystin-LR is likely the most common form of microcystin. Kotak et al. (1993) detected microcystin-LR in 37 of 39 algal bloom samples collected in Alberta. Microcystin-LR is highly toxic for mammals, causing very rapid liver damage and death within a few hours. The toxic dose for mice by intraperitoneal injection is approximately 50-100 μg/kg. There is very little information on toxicity of microcystins for birds, and liver damage of the type that occurs in mammals has not been described in this group. Gorham (1960) and Konst et al. (1965) reported that domestic ducks were very resistant to toxins produced by *M. aeruginosa*. Takahashi and Kaya (1993) reported that another microcystin (microcystin-RR) was toxic for quail when injected intraperitoneally, but it caused enlargement of the spleen rather than causing the liver damage that occurs in mammals.

The association between these toxins and poisoning of birds is confusing, because commonly occurring hepatotoxins have not been clearly linked with toxicity in birds, but at least one of the less commonly occurring neurotoxins is known to be toxic for ducks. There are about 20 reports in the literature in which death of waterfowl has been associated with a blue-green algal bloom (Wobeser 1997). The *cyanobacteria* present were characterized poorly in many of these occurrences, but in 12 of 20 occurrences either *Anabaena* or *Aphanizomenon spp.* were described, so that neurotoxins may have been present. *Microcystis spp*. were described in seven instances, but in three of these cases, *Anabaena* or *Aphanizomenon spp.* were also present. The objective of this pilot study was to determine the toxicity of purified microcystin-LR (Calbiochem catalogue # 475815) for mallard (*Anas platyrhynchos*) ducklings.

EXPERIMENT 1

Methods

Day-old ducklings were obtained from a commercial supplier (Whistling Wings, Hanover, Illinois), held in the laboratory for one week for acclimation, and then divided into groups of five birds for exposure to toxin. Although Microcystin-LR had been successfully isolated from culture, the yield was less than expected so commercial supplies were used to expedite the study.

Toxin was administered orally using a stomach tube. The dosages tested were 0 (controls, which received water), 62.5, 125, 250, 500 and 1000 μg/kg body weight. Birds were: tagged; weighed before receiving toxin; monitored for four days after receiving toxin; euthanized; and then necropsied. Weight gain over the four-day period, as well as liver weight and spleen weight as a proportion of body weight, were calculated. Tissues (liver, spleen, lung, kidney, bursa of Fabricius) were fixed, processed and examined histologically.

Results

There was no evidence of any clinical disease in ducklings that received toxin. Their rate of growth, liver mass, and spleen mass were not different from control ducklings, and no microscopic lesions were detected in their tissues.

To ensure that the toxin administered to the ducklings was actually toxic, mice were exposed to 100 μg/kg by intraperitoneal injection. The mice became sick within 20 min and died in less than two hours. They had significantly increased liver mass and severe acute liver necrosis, typical of the lesions described in mammals exposed to this toxin.

EXPERIMENT 2

Methods

To test the toxicity of microcystin-LR by intraperitoneal injection for ducklings, additional toxin was ordered from the supplier. It was administered to groups of five 21-day-old ducklings. Because of the size of the ducklings (~500g) and the cost of the toxin (C$300.90 for 500μg), only two levels of toxin (50 and 100 μg/kg) were administered, and control birds were injected with sterile saline. The birds were followed for four days and handled as described above.

Results

There was no evidence of any effect on growth of the ducklings, or on any organ, compared with controls.

DISCUSSION

All that can be concluded from these trials is that ducks are less sensitive than mice to microcystin-LR. It is likely that the material is toxic for ducks at some level, and further trials using larger doses of toxin could be performed to test this. However, it is unrealistic to do this using commercially available toxin, given its cost, particularly if adult-sized birds were to be

used, as would be more appropriate for assessing field poisoning. Konst et al. (1965) suggested that the toxic oral dose of microcystin in mice is ~40x larger than the intraperitoneal dose. From the trial, we know that 100 μg/kg is not toxic for mallards by the intraperitoneal route, suggesting that the oral dose would be >4,000μg/kg. A dose of that size would cost about C$2,400 for a single adult mallard.

Another approach to resolve the toxicity of algal blooms for ducks would be to collect and preserve large amounts of suspected toxic algal material from the field, in an area where ducks are dying. The material could be tested for toxicity in mice first. If toxicity is detected using mice, then it could be tested for toxicity in ducks by oral dosing. If it proved to be toxic, the toxin(s) present could then be identified by laboratory analysis. A potential problem with this technique is that a mixture of toxins will likely be present, so it will be difficult to establish which toxins are important.

The toxicity of algae to ducks could also be resolved with laboratory isolations of algal toxins from cultures and further bioassays. A significant advantage of this approach is that experiments can be repeated with changing variables to resolve the details of the pathology. Field trials would be difficult to replicate and may not be effective in determining whether other, unmeasured toxins are influencing results. In addition, field trials cannot resolve complexities such as synergism or bioavailability. Ideally, both field and laboratory approaches should be used.

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