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USE OF SENTINEL MALLARDS FOR EPIZOOTIOLOGIC STUDIES OF AVIAN BOTULISM

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ABSTRACT: Captive-reared mallards (*Anas platyrhynchos*) were used as sentinels to study the epizootiology of avian botulism at the Sacramento National Wildlife Refuge, Willows, California (USA) from 1986 to 1989. Sentinel mallards were wing-clipped, and 40 to 50 birds were confined in 1.6-ha enclosures in 11 selected wetlands (pools). Enclosures were searched intensively three to four times weekly from July through October. Sick and dead wild and sentinel birds were collected, necropsied, and tested for type C botulism toxin. Botulism epizootics occurred in sentinel mallards in 1986, 1987, and 1989, but only a few isolated cases of botulism were detected in 1988. In most epizootics, botulism also was detected simultaneously in wild birds using the same pool outside the enclosure. Epizootics in sentinels were initiated and perpetuated in the absence of vertebrate carcasses. A sex-specific trend in the probability of intoxication was detected, with males contracting botulism at a higher rate than females. Daily mortality rates of sentinels during botulism epizootics ranged from 0.0006 to 0.0600, with a mean of 0.0190. These rates would result in the daily loss of 0.6 to 60 birds per thousand at risk. The use of sentinel birds provided an effective means of gathering site-specific epizootiologic data.

Key words: Avian botulism, *Clostridium botulinum* type C, sentinels, mallards, *Anas platyrhynchos*, mortality rates.

INTRODUCTION

Quantitative research on avian diseases in free-flying populations is limited by our inability to accurately determine several important epizootiologic parameters: the index case, the first case of mortality in an epizootic; morbidity and mortality rates, the number of sick or dead animals per number at risk; and the site of exposure to the disease-causing agent. This information is required to assess the significance of a disease event in a population and to compare epizootics from different sites or time periods. This knowledge also would be helpful in developing and evaluating effective methods for disease prevention or control.

Use of sentinel birds in disease research circumvents some of these problems and provides a means for gathering site-specific epizootiologic information in a controlled situation. Sentinel birds, with no known disease exposure, can be restricted to a study site by wing clipping, confinement in cages or pens, or by other means. Because their activities are confined to the study site, the occurrence of an infectious or toxic disease in sentinel birds must result from exposure to a pathogenic agent at that site. Identification of the exposure site facilitates epizootiologic study of the relationship between exposure and site-specific ecological and microbiological factors. Captive avian sentinels have been used successfully in natural ecosystems to study leucocytozoonosis in waterfowl (Khan and Fallis, 1968; Desser et al., 1978), avian malaria (*Plasmodium relictum*) in Hawaiian forest birds (Warner, 1968), avian pox and malaria infections in wild turkeys (*Meleagris gallopavo*) (Forrester, 1991), and equine encephalitis virus infections in bobwhite quail (*Colinus virginianus*) (Williams et al., 1971). In these studies, sentinels were used only to detect the presence of the pathogen; no attempts were made to determine rates of morbidity or mortality from a particular pathogen.

We used captive-reared mallards (*Anas platyrhynchos*) as sentinels to study ecological conditions associated with type C avian botulism epizootics in the Central Valley of California (USA) from 1986 through 1989. Avian botulism, a serious disease of wild waterfowl throughout the world, is caused by ingestion of toxin pro-

duced by the bacterium *Clostridium botulinum*. Spores of *C. botulinum* type C are commonly found in wetlands, but, unfortunately, spore density is not a good predictor of the risk of a botulism outbreak in birds (Sandler et al., 1993). In our study, we used sentinel mallards as an indicator of the availability of botulism toxin and also to approximate rates of botulism mortality in waterfowl. In this paper, we describe our methods for maintaining and monitoring large numbers of sentinel mallards in wetlands, discuss the use of wild mallards versus captive-reared mallards for sentinels, report differences in the probability of contracting botulism between male and female sentinels, and compare rates of sentinel mortality to previously published estimates for wild waterfowl.

MATERIALS AND METHODS

Our study was conducted at the Sacramento National Wildlife Refuge (SNWR) near Willows, California (39°20′N, 122°20′W); SNWR comprises 2,995 ha of intensively managed wetlands (pools). This site has a documented history of botulism since 1958, with epizootics typically occurring during August through October (J. G. Mensik, pers. comm.). We selected 11 pools for study (Fig. 1), ranging in size from 4 to 75 ha. The selected pools included seasonally flooded (from late August through April) and permanently flooded (year-round) wetlands. Previous losses from botulism in these pools were variable (low to high) (J. G. Mensik, pers. comm.).

Pools were drained at the beginning of the study (April to May 1986), and a 1.6-ha enclosure was built in each to confine sentinel birds. The enclosures were constructed with 2 m high, 19 × 32 mm mesh, plastic game-bird netting (Centoflex M, Internet, Inc., Minneapolis, Minnesota, USA) fastened by hog rings to 9-gauge steel support wires attached to 2.4 m steel T-posts erected every 6 m. Corners were braced with 2.4-m wooden fence posts placed in gravel-packed holes. We constructed a gate and a funnel trap for capturing sentinel birds in one corner of each enclosure and raised small dirt islands (9 to 30 m²) for roosting and loafing. After flood-

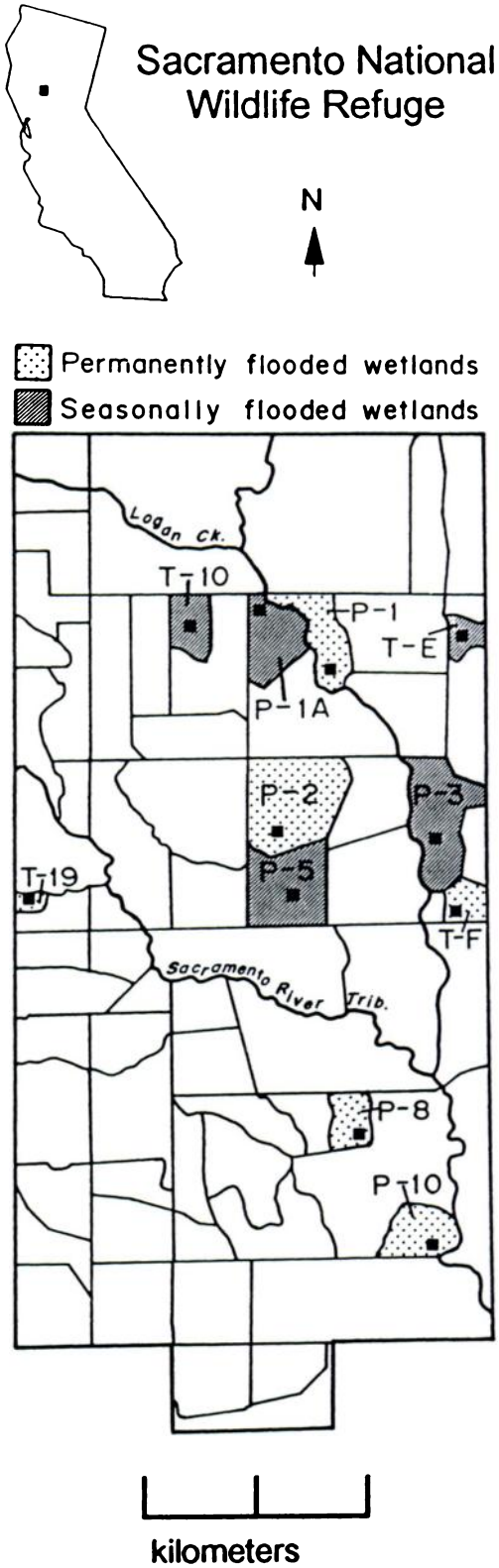

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FIGURE 1. Wetland pools selected for botulism study at Sacramento National Wildlife Refuge, Willows, California. Site of 1.6-ha sentinel enclosures are marked with a black box.

ing of the pools, water depth in completed enclosures was generally 30 to 60 cm, but ranged to 120 cm in the deepest location. Vegetative cover varied among enclosures, but dominant plant species included hardstem bulrush (*Scirpus acutus*), cattail (*Typha* spp.), swamp timothy (*Heleochloa schoenoides*), smartweed (*Polygonum* spp.), and cocklebur (*Xanthium strumarium*). Wild birds had access to the enclosures at all times.

Captive-reared mallards (*Anas platyrhynchos*; Wild Wings of Oneka, Hugo, Minnesota) of mixed sex and age were obtained for use as sentinels. All birds were pinioned (at 1 day of age) or wing clipped to prevent flight and were marked with a numbered leg band and yellow plastic patagial tag to identify individuals in the field. Forty to 50 captive-reared mallard sentinels were placed in enclosures in permanently flooded pools in June or July and maintained through October of each year from 1986 to 1989. Flooding of seasonal pools generally began in August, and 40 to 50 sentinels were maintained and monitored in these pools during September and October of each year. Because we had no prior knowledge of sex-specific differences in the rates of botulism intoxication, no attempt was made to equalize the numbers of male and female sentinels in each enclosure.

To compare botulism mortality rates between wild and captive-reared birds, we captured free-ranging male mallards in baited funnel traps and used them as sentinels in 1987. These wild mallard sentinels were wing-clipped, marked with leg bands and patagial tags; 20 birds were placed in each of two enclosures with captive-reared sentinels. Although all sentinels had access to natural foods in enclosures, we periodically provided supplemental scratch and waste grains (mostly rice) and grit.

Scavenging invertebrates, particularly fly larva or maggots that feed on decaying carcasses, are one source of botulism toxin for waterfowl (Duncan and Jensen, 1976). In this study, however, we were specifically interested in botulism epizootics that were not the result of a carcass-maggot cycle. We attempted to prevent this cycle of botulism in our sentinel enclosures by finding and removing any sick and dead birds before they became maggot-infested. All enclosures were searched diligently on foot or by canoe three to four times each week and daily during epizootics. Retrieving dogs also assisted in locating carcasses. Healthy sentinel birds were counted during each search and trapped periodically for an accurate inventory. Sick, dead, and missing sentinels (both wild and captive-reared mallard sentinels) were replaced with healthy birds to keep the number of bird-exposure-days constant.

All sick birds found within enclosures were taken to the laboratory. A blood sample was withdrawn from the jugular vein and tested for type C botulism toxin using the mouse neutralization test (Quortrup and Sudheimer, 1943). Sick sentinels then were euthanized by cervical dislocation and necropsied. Free-ranging wild birds with signs of botulism were injected intraperitoneally with antitoxin and placed in a recovery pen; if they survived, they were released. All intact, fresh carcasses found within enclosures were necropsied. Blood collected from the heart of each carcass was tested for botulism toxin. If warranted by necropsy observations, tissues were collected, frozen at −20 C and later analyzed for lead as described by Rocke and Samuel (1991). Birds with gross pathological signs of lead exposure and which had liver lead concentrations ≥2.0 ppm (wet weight) were considered to be lead-poisoned. Scavenged carcasses also were examined, and tissues or bones were collected if possible. In addition, many carcasses of wild birds found in our study pools but outside the enclosures were necropsied. Predation was diagnosed as a cause of death based on the presence of mortal wounds such as punctures caused by tooth or claws; with accompanying hemorrhage, as well as tracks and other signs of predators. In all of these cases, carcasses were fresh. These carcasses also were tested for botulism.

McNemar's test was used to determine if the occurrence (presence or absence) of botulism in captive-reared sentinel birds was consistent with the occurrence of botulism in free-ranging wild birds in the same pool (Zar, 1984). We considered a botulism epizootic to have occurred in wild birds within a specific pool, with birds having ingested toxin within the pool, if >20 sick or dead wild birds were found there, and botulism was confirmed in at least two of these.

Botulism mortality rates for sentinel mallards in enclosures with epizootics were determined by the method of Heisey and Fuller (1985) and included all birds that contracted botulism regardless if they were found sick or dead. Mean daily mortality rates were calculated to compare botulism losses in sentinel birds with previously published estimates for wild birds. Mean weekly mortality rates were used to depict patterns of botulism intoxication in sentinels in relation to the number of wild bird carcasses picked up by refuge personnel.

To compare botulism losses between different sentinel cohorts, we calculated cumulative probabilities of contracting botulism (Reed and Rocke, 1992) using the staggered entry Kaplan-Meier survival estimator (Kaplan and Meier, 1958; Pollock et al., 1989) that allows for new animals to be added during the study. To approximate the number of birds at risk of con-

tracting botulism, missing sentinels were counted as half a bird between the last date they were observed and the date they were known to be missing (Harris et al., 1950; Crowley and Breslow, 1984). A chi-square log-rank test (Pollock et al., 1989) was used to compare cumulative probabilities of contracting botulism between male and female captive-reared sentinels (of the same age group) and between wild and captive-reared sentinels (males only) held in the same enclosure. The chi-squared statistic was converted to a Z-statistic with the appropriate sign (Crowley and Breslow, 1984) and compared to a table of critical values for a two-sided distribution. Overall trends in the rate of botulism between sexes were analyzed by pooling the Z-statistic from each enclosure to produce a composite Z-statistic (Anderson and Burnham, 1976; Reed and Rocke, 1992).

RESULTS

Type C avian botulism was the leading cause of morbidity and mortality (38%) among 382 sick or dead captive-reared sentinels found in our enclosures and examined over the 4-yr duration of the study. Botulism also was confirmed in 261 of the 321 free-ranging dabbling ducks that were found sick or dead in or near enclosures during the same time period, and in 10 wild mallards used as sentinels in 1987.

Eight botulism epizootics occurred in sentinel mallards in wetland enclosures in 1986, 1987 and 1989 (Table 1). In all but one of these, botulism epizootics also were detected and confirmed simultaneously in free-ranging wild birds in the same pool outside the enclosure. In 1988, two cases of botulism were also detected in T-F (Fig. 1), but they were widely separated (68 days), and botulism was not detected in wild birds in any SNWR wetland that year. In two pools (P-IA in 1987 and P-3 in 1989, Fig. 1), botulism epizootics were confirmed in wild birds, but sentinels in enclosures in the same pools had no morbidity or mortality. Using McNemar's test, no difference ($Z = 0$; $P = 0.5$) was detected between the occurrence of botulism epizootics in enclosed sentinels and wild birds using the same pool.

Mean daily rates of botulism mortality in sentinel birds were calculated for the duration of an epizootic within a pool, for the duration of the season within a pool, and for the duration of the season for all pools, including those without epizootics (Table 1). The daily rate of botulism mortality during an epizootic was highest (0.03 to 0.06) in pools where mortality was low (≤ 4 birds) and of short duration (≤ 2 days), but on a seasonal basis, the daily rate of mortality in these pools was generally lower than average (<0.0030). The highest daily mortality rate for a season was detected in P-2 in 1986 (0.0138). Of the four years, 1986 had the highest daily rate of botulism mortality in sentinels (0.0017) and 1988 had the lowest (0.0001). The mean daily rate of mortality during epizootics in wild mallard sentinels used in P-2 and P-8 (Fig. 1) in 1987 was 0.0216 (95% confidence interval: 0.0000 to 0.0428) and 0.0294 (95% confidence interval: 0.0258 to 0.0331), respectively.

In two pools (P-2 and P-8), botulism occurred consistently in three of the four years of the study (Fig. 2). In 1986, weekly rates of botulism mortality in sentinels in both P-2 and P-8 peaked during early September, although it was much higher in P-2 (0.68) than in P-8 (0.05). Likewise, a greater number of sick or dead wild birds was found in P-2 ($n = 627$) than P-8 ($n = 115$); most carcasses were found during the week of 15 September 1986. During the summer of 1987, botulism occurred sporadically in P-2, and losses in both sentinels ($n = 8$) and wild birds ($n = 40$) were much lower than the previous year. In P-8, three peaks in weekly rates of sentinel mortality occurred in 1987, one in July (0.04), a second in late August (0.10), and a third in late September (0.13). The latter two peaks corresponded well to the numbers of wild bird carcasses found by refuge personnel. In 1989, botulism occurred in three sentinels in the enclosure in P-2, but no dead wild birds were found in the rest of P-2. Mortality in P-8 was sporadic in 1989, but peaks in weekly mortality in August and September coincided with epizootics in wild birds.

TABLE 1. Rates of botulism mortality in sentinel mallards in wetland enclosures at the Sacramento National Wildlife Refuge, Willows, California in 1986 through 1989.

Year	Pool	Number botulism cases[a]	Length of epizootic (days)	Mean daily botulism rates in sentinels: During epizootic	During season[b]	All pools combined for each year[c]
1986	P-2	88 (+)[d]	63	0.0257 (0.0203–0.0311)[e]	0.0138 (0.0109–0.0167)	
	P-8	7 (+)	49	0.0021 (0.0005–0.0038)	0.0011 (0.0003–0.0019)	0.0017 (0.0013–0.0021)
1987	P-2	8 (+)	103	0.0018 (0.0005–0.0031)	0.0015 (0.0004–0.0025)	
	P-8	16 (+)	100	0.0042 (0.0021–0.0063)	0.0034 (0.0017–0.0050)	
	P-5	4 (+)	2	0.0412 (0.0000–0.0808)	0.0026 (0.0000–0.0051)	
	P-10	3 (+)	1	0.0600 (0.0000–0.1248)	0.0006 (0.0000–0.0012)	0.0008 (0.0006–0.0010)
1988	T-F	2 (−)	68	0.0006 (0.0000–0.0015)	0.0004 (0.0002–0.0010)	0.0001 (0.0000–0.0002)
1989	P-2	3 (−)	2	0.0319 (0.0000–0.0673)	0.0006 (0.0000–0.0012)	
	P-8	14 (+)	90	0.0040 (0.0019–0.0062)	0.0030 (0.0014–0.0046)	0.0006 (0.0003–0.0008)

[a] Number of captive-reared sentinel birds sick or dead from botulism.
[b] July through October.
[c] Including all pools, even those without botulism epizootics.
[d] (+) = Concurrent botulism epizootic detected and confirmed in wild birds outside the enclosure; (−) = no epizootic outside enclosure.
[e] 95% confidence interval.

In most pools there was no difference in mortality rates among male and female captive-reared sentinels (Table 2). In P-2 in 1986 and P-8 in 1989, however, males contracted botulism at a higher rate than females ($P \leq 0.10$); and in all but one of the remaining seven epizootics, a general trend in this direction was evident. Combining results from all pools with botulism epizootics and all years, the composite Z-statistic indicated an overall sex-specific trend in the probability of mortality (Composite-Z = 2.11; $P < 0.05$), with males contracting botulism at a higher rate than females.

We also compared rates of botulism intoxication between wild and captive-reared mallard sentinels in P-2 and P-8 in 1987. Because only male wild mallards were used in the study, only male captive-reared sentinels were used for comparison. The cumulative rate of contracting botulism was significantly higher ($P = 0.07$) in wild mallard sentinels than captive-reared sentinels in P-2 (0.26 vs. 0.10), and suggestive, but not significant, in P-8 (0.51 vs. 0.33; $P = 0.16$). A significant trend was evident in the combined test (Composite-Z = 2.25; $P < 0.05$) with wild-mallard sentinels contracting botulism at a higher rate than captive-reared sentinels.

After botulism, the second most frequent cause of illness or death among captive-reared sentinels was lead poisoning (19%) from the ingestion of lead pellets. Most lead poisoning cases (48) occurred in sentinels in one pool (P-8), which had a history of game bird hunting in the fall and winter. Lead poisoning also occurred (n = 23 cases) in a pool closed to hunting (T-19), but adjacent to a previously hunted area (Fig. 1).

Predation was the third most frequent cause of death in captive-reared sentinels

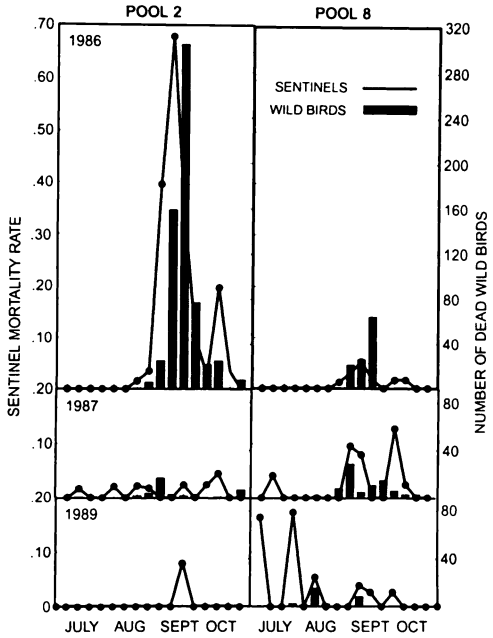

FIGURE 2. Weekly rates of botulism mortality in sentinel mallards and numbers of dead wild birds picked up per week in two pools (P-2 and P-8) at the Sacramento National Wildlife Refuge, Willows, California, in 1986, 1987, and 1989. No epizootics occurred in 1988.

(16%). However, 51 of the 62 predator kills occurred over a 2-day period in one enclosure when a dike failed, leaving >50% of the sentinel enclosure on dry ground and the remainder with very shallow water. Sentinels were apparently unable to evade raccoons under these conditions, and all but one was killed. Predation ceased when the dike was repaired and the pool was flooded again. The remaining 11 known predator kills occurred sporadically over the 4-yr study and were caused primarily by avian predators.

DISCUSSION

Because of the inherent difficulty of finding and counting all affected animals in epizootics and determining the population-at-risk, few investigators have attempted to evaluate seasonal rates of disease mortality in waterfowl populations or rates during specific epizootics. Using data collected during 87 botulism epizootics between 1979 and 1988, Samuel (1992) estimated a daily probability of botulism mortality of 0.0005 for mid-continental mallards during the breeding and post-

TABLE 2. Cumulative probability of botulism mortality in male and female sentinel mallards in wetland enclosures at the Sacramento National Wildlife Refuge, Willows, California in 1986 through 1989. Juvenile mallards were used as sentinels in 1986–88; adults were used in 1989.

Pool	Year	Cumulative probability of mortality[a]		Z[b]
		Male	Female	
P-2	1986	0.85 (50, 3,141)[c]	0.74 (38, 3,446)	1.892[d]
	1987	0.21 (5, 3,342)	0.13 (3, 3,290)	0.646
	1989	0.13 (1, 844)	0.05 (2, 4,222)	0.813
P-8	1986	0.07 (3, 4,499)	0.14 (4, 2,468)	−0.949
	1987	0.40 (11, 3,313)	0.27 (5, 2,212)	0.715
	1989	0.50 (5, 790)	0.26 (9, 3,890)	1.741[d]
T-F	1988	0.05 (1, 2,651)	0.04 (1, 2,874)	0.091
P-5	1987	0.08 (2, 898)	0.08 (2, 898)	0.011
P-10	1987	0.14 (3, 2,195)	0 (0, 3,989)	1.367
Composite Z				2.109[e]

[a] Probability of intoxication determined with the Kaplan-Meir survival estimate.
[b] Z-statistics were calculated by comparing male and female sentinels in each enclosure.
[c] The first number in parentheses indicates the number of sentinel birds that contracted botulism; the second indicates the number of bird-exposure days.
[d] Indicates Z-statistic significant at $P < 0.10$.
[e] Indicates Z-statistic significant at $P < 0.05$.

breeding seasons (April through September). Samuel recognized two opposing biases in this estimation. Because wild bird carcasses are difficult to find and many sick or dead birds are consumed by predators or scavengers, mortality during the botulism epizootics was probably underestimated. In contrast, the estimated daily rate of botulism mortality was applied to the entire midcontinental population of mallards, but, at any given time, only a portion of the population was actually at risk. This bias is likely to overestimate botulism mortality. As a first approximation, Samuel (1992) assumed these biases approximately counterbalanced each other.

In our study, sentinel birds provided a well-defined population-at-risk that was intensively monitored. Subsequently, the occurrence of illness or death was more easily documented, resulting in less-biased estimates of cause-specific mortality. Using the same methods as Samuel (1992), we estimated that the average daily mortality rate from botulism in captive-reared sentinel mallards at SNWR over the entire season of study (July through October) ranged from 0.0001 to 0.0017, with a mean of 0.0008 over the 4-yr duration of the study. This rate, which includes data from all enclosures with or without botulism epizootics, was slightly higher than Samuel's (1992) estimate of 0.0005 for midcontinental mallards, even though our study was conducted during years when botulism losses in waterfowl in the Central Valley were considered low.

Daily mortality rates during botulism epizootics (the time interval between the first and last botulism mortality in sentinels) ranged from 0.0006 to 0.0600, with a mean of 0.019 in captive-reared sentinels and 0.025 in wild-mallard sentinels. Thus, the mean daily loss from botulism was close to 20 birds per thousand at risk. During a typical fall (September, October) with a mallard population of 25,000 or more at SNWR (J. G. Mensik, pers. comm.), a mortality rate of 0.019 would result in the death of nearly 500 mallards per day. Comparable rates of loss in other waterfowl species that frequently contract botulism are unknown, although differential mortality is commonly observed among waterfowl species.

Botulism epizootics in sentinel birds coincided with mortality in free-ranging wild birds in the same pool in all but a few cases. Unlike our sentinel birds, free-ranging wild birds could easily move from pool to pool, and it was possible for wild birds to ingest toxin in one pool and die or become sick in adjacent pools. Even so, based on our results, we believe that the probability of botulism in sentinels and wild birds using the same pool was equivalent. In 1986, the epizootic pattern of botulism in wild birds based on carcass pickup was similar to weekly mortality rates of sentinel mallards in pools with epizootics, with a lag of about 1 wk in peak mortality of wild birds. However, trends in botulism mortality in sentinels and wild birds did not coincide as well in 1987 and 1989. The absence of botulism in wild birds during early peaks of mortality in sentinels in July of these years may have been due to the limited population of wild birds at risk during this time of the year at SNWR. Also, enclosures with sentinels were monitored on a daily basis during botulism epizootics, whereas surveillance for wild bird mortality by refuge personnel was less frequent and less systematic.

We observed that the wild mallards we captured and used as sentinels in 1987 contracted botulism at a higher rate than captive-reared mallard sentinels. Wild mallards may be more susceptible to botulism toxin or, because of their feeding habits, more likely to ingest toxin-laden food items than captive-reared birds. Therefore, they might be preferred to captive-reared sentinels in future studies. However, wild-mallard sentinels were more difficult to observe and capture than captive-reared sentinels, especially in pools where the water was >100 cm deep. As a result, wild-mallard sentinels in early stages of botulism may have been missed or overlooked.

In addition, wild mallard sentinels were more inclined to escape through small holes in the enclosure or by diving under the netting. The number of missing birds per bird-exposure-day for wild-mallard sentinels (32/1097) was considerably higher than that for male captive-reared sentinels (1/2318) in the same enclosures during the same time interval. Thus, the population-at-risk for wild mallard sentinels was not as well-defined as that for captive-reared sentinels. We found that captive-reared mallards were more useful sentinels than wild mallards because they were less inclined to escape and easier to trap and inventory.

Male captive-reared sentinels became sick or died from botulism at a higher rate than females. These results are similar to Reed and Rocke's (1992) study conducted concurrently at SNWR in which male captive-reared mallards had a higher probability of botulism intoxication than females, particularly in adult birds (Reed and Rocke, 1992). Differential rates of intoxication may have resulted from sex-specific preferences in food habits or physiologic differences between sexes in the absorption of botulism toxin through the gut lining. In future studies on waterfowl disease, the possibility of differential sex susceptibility should be considered. Also, because estimates of relative risk are difficult to obtain with wild birds, sentinel studies could be used to evaluate sex- and age-specific rates of other mortality factors in waterfowl.

Maggot-infested carcasses are a known source of botulism toxin for waterfowl (Duncan and Jensen, 1976); birds that have died from any cause can initiate and perpetuate botulism through a carcass-maggot cycle (Reed and Rocke, 1992). In our study, however, botulism epizootics occurred and continued in sentinels even in the absence of carcasses. Because enclosures were searched intensively to remove sick or dead birds before they became maggot-infested, it is unlikely that carcasses were the primary source of botulism toxin for sentinels. Also, since most botulism cases in sentinels coincided with epizootics in wild birds in the same pool outside enclosures, we believe that the source of toxin was not focal, but widespread throughout the pool. Unfortunately, toxic food items were not identified.

In past studies on botulism epizootics in wild birds (Jensen and Allen, 1960; Duncan and Jensen, 1976), the site of exposure could not be determined with certainty, and the population-at-risk was not known. In contrast, in our botulism study, the use of sentinel birds with a known and constant population-at-risk assured knowledge of the exposure site and permitted the calculation of cause-specific mortality rates. The sentinel approach may be an effective method for studying other waterfowl diseases for which knowledge of the site of exposure or population-at-risk is necessary. Examples include avian cholera and toxicoses from environmental contaminants. The location and numbers of wild bird carcasses may not accurately reflect either the site of exposure to a disease-causing agent or the magnitude and significance of an epizootic. We have demonstrated that sentinels can be effectively maintained and monitored for disease-causing agents in natural wetlands.

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