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TYPE C BOTULISM IN PELICANS AND OTHER FISH-EATING BIRDS AT THE SALTON SEA

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Abstract. In 1996, type C avian botulism killed over 10,000 pelicans and nearly 10,000 other fish-eating birds at the Salton Sea in southern California. Although botulism had been previously documented in waterbirds at the Sea, this die-off was unusual in that it involved primarily fish-eating birds. The American White Pelican (*Pelecanus erythrorynchos*) was the species with the greatest mortality in 1996. Since 1996, mortality has recurred every year but losses have declined (<2000 birds/year), with relatively more Brown Pelicans (*P. occidentalis*) than White Pelicans afflicted. In 2000, morbidity and mortality of Brown Pelicans with type C botulism (1311) approached the numbers afflicted in 1996 (2034). In recent years, mortality reached a peak earlier in the summer, July and August, in contrast to 1996 when mortality reached a peak in September. An exotic fish species, tilapia (*Oreochromis mossambicus*), has been implicated as the source of toxin for birds at Salton Sea, but the source of toxin for fish is unknown.

Key Words: avian botulism; *Clostridium botulinum* type C; fish-eating birds; pelicans; Salton Sea; tilapia.

BOTULISMO TIPO C EN PELÍCANOS Y OTRAS AVES PISCÍVORAS EN EL MAR SALTON

Resumen. En 1996, el botulismo aviar tipo C mató a más de 10,000 pelícanos y cerca de 10,000 aves piscívoras en el Mar Salton en el sur de California. Aunque el botulismo se ha documentado previamente en aves acuáticas marinas, esta mortandad fue inusual e involucró principalmente a las aves piscívoras. El Pelícano Blanco (*Pelecanus erythrorynchos*) fue la especie con mayor mortandad en 1996. Desde 1996, la mortandad se ha repetido año con año pero ha disminuido (<2000 aves/año); los Pelícanos Pardos (*P. occidentalis*) fueron relativamente más afectados que los Pelícanos Blancos. En el 2000, la morbidez y mortandad de Pelícanos Pardos con botulismo tipo C (1311) se aproximó al número de los afectados en 1996 (2034). En años recientes, la mortalidad alcanzó el máximo a principios del verano, Julio y Agosto, en contraste a 1996 cuando la mortalidad alcanzó el máximo en Septiembre. Un pez exótico, la Tilapia (*Oreochromis mossambicus*), ha sido implicado como la fuente de toxina para las aves del Mar Salton, sin embargo la fuente de la toxina para los peces se desconoce.

Palabras claves: Aves piscívoras; botulismo aviar; *Clostridium botulinum* tipo C; Mar Salton; pelícanos; Tilapia.

In 1996, nearly 20,000 pelicans and other fish-eating birds at the Salton Sea became sick or died in a large outbreak of type C avian botulism (Friend 2002). The American White Pelican (*Pelecanus erythrorhynchos*) was the species most afflicted, with losses of approximately 9000 birds. Over 2000 endangered California Brown Pelicans (*Pelecanus occidentalis californicus*) were also affected, although many of these (>500) were taken to rehabilitation centers, treated, and ultimately released. Sixty other avian species were found dead during this outbreak, totaling nearly 4500 birds. Among these were Snowy Egrets (*Egretta thula;* N = 779), Ring-Billed Gulls (*Larus delawarensis*; N = 612), Great Egrets (*Ardea alba*; N = 270), Western Sandpipers (*Calidris mauri*; N = 190), Great Blue Herons (*Ardea herodias;* N = 172), Black-Crowned Night-herons (*Nycticorax nycticorax*; N = 169), Eared Grebes (*Podiceps nigricollis*; N = 143), Black-necked Stilts (*Himanoptus mexicanus*; N = 125), and American Avocets (*Recurvirostra americana*; N = 107) (U.S. Fish and Wildlife Service (USFWS), unpubl. data). Over half a million dollars were spent on carcass collection and rehabilitation efforts during this single epizootic (USFWS 1997).

Botulism was first documented at the Salton Sea in 1917, and large-scale outbreaks in waterfowl and shorebirds in the Sea and surrounding wetlands recurred frequently since that time (Friend 2002). However, in the 1990s, botulism outbreaks in waterfowl at the Sea generally declined. The 1996 outbreak was unusual in several respects. Prior to this event, large die-offs of fish-eating birds were not typically associated with type C botulism; most previous reported botulism outbreaks that involved mainly fish-eating birds were caused by type E toxin (Rocke and Friend 1999). Type C botulism mortality in fish-eating birds has generally been documented as an incidental finding related to outbreaks in waterfowl (National Wildlife Health Center (NWHC), unpubl. data). Fish, specifically tilapia (*Oreochromis mossambicus*) were immediately suspected to be the source of toxin for the birds,

although perusal of available literature failed to reveal a previously established association between fish and type C botulism in birds. Tilapia are exotic to the U.S. and were introduced in the Salton Sea and its drains in the 1960s, presumably to control vegetation (Costa-Pierce and Doyle 1997). By the 1970s tilapia were the most abundant fish in the Sea in terms of biomass (Dill and Cordone 1997); the population peak of 1996 was estimated to be approximately 20 million (R. Riedel, pers. comm.).

The 1996 botulism outbreak at the Salton Sea resulted in the largest die-off of pelicans ever reported from any cause. Smaller die-offs of pelicans occurred at the Salton Sea in 1994 (N = 108) and 1995 (N = 10), and several were diagnosed with type C botulism, but until the large outbreak in 1996 the risk of type C botulism in pelicans was not recognized. Here we document losses of pelicans and other fish-eating birds from type C botulism at the Salton Sea from 1994 to 2001 and describe the investigation of the larger outbreak in 1996.

MATERIALS AND METHODS

Investigation of Avian Mortality

Sick and dead birds at the Salton Sea were recovered primarily with airboats and other boats, and occasionally affected birds were found by walking shorelines. Severely moribund birds were euthanized by cervical dislocation, although most of the moribund Brown Pelicans were taken to rehabilitation centers (USFWS, unpubl. data). Blood samples were drawn from selected sick birds by jugular venipuncture, allowed to clot, and centrifuged to collect serum. The serum samples were placed at −20°C and shipped frozen to NWHC for testing. Selected carcasses were submitted to NWHC for necropsy; the remainder was incinerated on-site. During necropsy, a gross examination, including mass and other measurements, a description of wounds and abnormalities, and an evaluation of overall body condition with respect to fat reserves and pectoral muscle development, was conducted on each carcass. Samples of organs were tested for a variety of microbes, parasites, and toxins as indicated by gross findings and field information provided by the submitter.

Tissues for histopathology were fixed in 10% buffered formalin, embedded in paraffin, sectioned for light microscopy, and stained with hematoxylin and eosin for routine examination, Ziehl-Neelsen acid-fast for mycobacteria, and/or Grocott silver for fungi. Bacteria were isolated by inoculation of tissues onto 5% sheep red-blood agar and eosin-methylene blue plates (DIFCO laboratories, Detroit, MI), incubated at 37°C for 72 hr, and then characterized with the API-20E system (Analytab Products, Plainview, NY). Tissues for virus isolation attempts were processed according to Docherty and Slota (1988) and Senne (1989). The presence of botulinum toxin in heart blood or serum was evaluated using either the mouse neutralization test (Quortrup and Sudheimer 1943) or the enzyme-linked immunsorbent assay (Rocke et al. 1998). The remains of fish found in the proventriculus of some carcasses or those regurgitated by sick birds were likewise tested for botulinum toxin.

When exposure to organophosphorus or carbamate agricultural pesticides was suspected, brains were screened for cholinesterase activity. Cholinesterase assays were performed according to Ellman et al. (1961) as later modified by Dieter and Ludke (1975) and Hill and Fleming (1982), including incubation (18 hr at 37°C) and retesting of samples with initially low enzyme activities. Cholinesterase inhibition was calculated by comparison with normal published values (Hill 1988) or control values determined by NWHC (Smith et al. 1995). Liver lead residues were determined according to Boyer (1984).

To determine whether any changes had occurred in temporal patterns of sick and dead pelican recovery from 1994–2001, we summarized available data that had been collected each year by staff of the Sonny Bono Salton Sea National Wildlife Refuge (SBSSNWR) during botulism outbreaks.

Fish Investigations

In mid-August and mid-September of 1996, during the peak mortality in birds, we collected 80 dead tilapia, ten from eight different locations in the Sea and along the shoreline. Only fresh carcasses, with pink to bright red gills, were selected. From a few locations in mid-August and mid-September, we also collected 41 moribund tilapia that were sluggish, exhibited poor fright response, and were easily caught with a dip net. In addition, in mid-September we collected 58 apparently healthy tilapia near the deltas of the Alamo, New, and Whitewater rivers using gill nets or minnow traps with assistance of personnel from the California Department of Fish and Game. All fish collected were immediately placed on ice in the field and either necropsied the same day or frozen as soon as possible for later necropsies. Sampled fish averaged 73 g in mass and most ranged from 12 to 15 cm in total body length.

In the laboratory, fish carcasses were rinsed and intestinal tracts were removed from all those collected and ground with a mortar and pestle with minimal amounts of sterile saline added if necessary. The samples were placed at 4°C overnight for toxin extraction, and then centrifuged at 3000 rpm in a Sorvall RI6000 (Global Medical Instrumentation Inc., Clearwater, MN) to separate fluid from the sediment. The fluid was tested for type C botulinum toxin by ELISA and/or by mouse test. We also tested 30 of the healthy fish captured for the presence of *C. botulinum* spores by inoculating intestinal contents into Cooked Meat Medium (Difco) in an anaerobic hood and incubating for five d at 37°C. Culture supernatants were then tested for the presence of type C botulinum toxin by ELISA. Samples from sick fish, including intestinal contents and fluid from the peritoneal cavity, were also cultured for bacteria by inoculating the material into several tubes of brain heart infusion broth (Difco) with 3% added NaCl and incubating at 20°C and 37°C for 24 hr. Broth cultures were streaked onto thiosulfate-citrate-bile sucrose agar (Bekton Dickson, Sparks, MD); colonies were subcultured on blood agar plates (Bek-

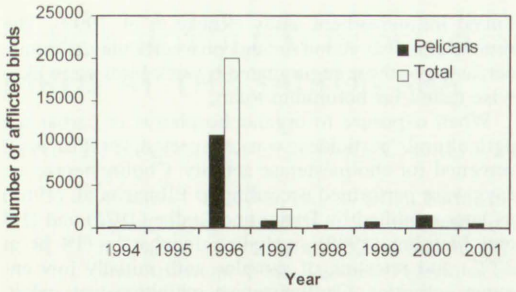

FIGURE 1. Estimated numbers of pelicans and total fish-eating birds (if available) affected by avian botulism at the Salton Sea, 1994–2001.

ton Dickson) for 24 hr incubation and then identified using the API-20E system.

RESULTS

Avian Diagnostics 1994–2001

Every year since 1994, type C botulism has been diagnosed as the cause of death of pelicans submitted to NWHC from the Salton Sea, and numerous pelicans were picked up sick or dead (Fig. 1). During 1996, 22 of 41 carcasses/tissues of fish-eating birds sent from the Salton Sea to the NWHC between 21 August and 18 September tested positive for type C botulinum toxin by mouse test and/or ELISA. Heart blood samples from four of 11 White Pelicans and eight of 13 Brown Pelicans submitted for testing were found to contain type C botulinum toxin. Other species submitted that tested positive for type C botulinum toxin during this time were single individuals of the Great Blue Heron, Black-crowned Night-heron, Snowy Egret, Eared Grebe, Northern Shoveler, and Green-winged Teal, and three individuals of the Great Egret. Fish remains recovered from the Great Blue Heron also tested positive for type C toxin. Thirty-two carcasses tested were negative for type E botulinum toxin. Eight carcasses were tested for the presence of cholinesterase inhibition and were found to have normal levels. Two were tested for lead poisoning and were also negative. None of the birds examined had any significant gross or histologic lesions, and no significant pathogens were found by microbial analyses.

In subsequent years, specimens were sent to the NWHC in late spring and early summer to confirm the onset of outbreaks. Once the diagnosis of type C botulism was confirmed, sick and dead pelicans and other fish-eating birds were documented as having the disease based on observation of clinical signs. These include bilateral paresis or paralysis of leg, neck, and wing muscles, which are manifested in the birds' inability to lift their heads, ambulate, or fly, as well as paralysis of the nictitating membrane (Rocke and Friend 1999). Estimates of botulism mortality by pelican species and year are included in Table 1.

TABLE 1. Pelicans Affected by Botulism at the Salton Sea, 1994–2001[a]

Year	White Pelicans		Brown Pelicans		Total affected
	Sick[b]	Dead	Sick[b]	Dead	
1994	—[c]	89	—	19	108
1995	—	7	—	3	10
1996	300	8539[d]	905	1129	10,873
1997	181	304	143	234	862
1998	24	94	133	121	372
1999	23	54	444	203	724
2000	103	88	994	317	1502
2001	69	45	342	110	566

[a] USFWS, SSNWR unpubl. data.
[b] When possible, sick birds were sent to rehabilitation centers.
[c] Data unavailable.
[d] Number includes birds that were euthanized.

Temporal Patterns of Botulism Outbreaks, 1996–2001

During 1996, the first disease-stricken pelicans were detected on 15 August. It was estimated the outbreak had begun within the prior two wks. Ninety-five percent of the total affected pelicans that year were detected during the months of August and September, when 4234 and 5897 individuals were collected respectively. Collection rates dropped significantly to 537 total birds in October and 16 in November when the outbreak subsided.

The August/September peak in the number of birds afflicted with botulism continued in 1997, 1998, and 1999, when 75%, 87%, and 88%, respectively, of total affected birds were detected in those months, although mortalities were first detected in May 1998 and in July 1999 and continued through November. In 2000 and 2001, botulism outbreaks at the Sea reached a peak in July and August and subsided in September. During 2000, 72% of affected birds were collected in July and August and only 13% in September. Collections of total affected birds during 2001 reached a peak in August at 45%, whereas collections in June, July, and September totaled 14%, 18%, and 15%, respectively. No data were available in SBSSNWR records to compare temporal patterns of mortality in pelicans for 1994 and 1995.

Tilapia Sampling, 1996

Type C botulinum toxin was detected in the gastrointestinal tracts of both sick and dead tilapia at various sites around the Sea. The highest percent of fish with toxin (50–60%) were found

FIGURE 2. Percentage of dead tilapia collected in 1996 that were positive for type C *botulinum* toxin.

dead in or near the deltas of the New, Alamo, and Whitewater rivers (7–38% at other areas; Fig. 2). In mid-August, only five moribund fish were collected (Salton Sea State Recreation Area (SRA) 2, Wister 1, and Bombay Beach 1), but three (60%) of these were found to contain type C botulinum toxin. Despite attempts to culture other bacteria from their intestinal contents, no significant growth was detected. During a collection in mid-September, 36 moribund fish were caught (Salton Sea SRA 11, Bombay Beach 25), but only three (8%) were found to contain type C botulinum toxin. Most of the fish in the second collection had gross external signs of bacterial septicemia, including hemorrhage of the skin and bases of fins and extensive ascites. *Aeromonas* spp., *Pseudomonas* spp., and *Vibrio* spp. were isolated from most of the moribund fish. Of the 58 apparently healthy tilapia tested in mid-September, none were found to contain type C botulinum toxin in their intestinal contents. Two of ten fish collected at the New River were culture positive for *C. botulinum* type C, although none of the 20 fish collected at the Alamo and Whitewater River deltas were culture positive.

DISCUSSION

Prior to the botulism outbreak at the Salton Sea in 1996, type C botulism in fish-eating birds was considered infrequent (Rocke and Friend 1999). Pelicans were diagnosed with the disease in 1994 and 1995 at Salton Sea and occasionally elsewhere in North America, but rarely in numbers exceeding 100, and often fewer than ten (NWHC, unpubl. data). Botulism was not considered a major threat to pelican populations. However, it has been estimated that White Pelicans lost to botulism in 1996 represented nearly 15% of the western population of that species (USFWS 1997). Additional die-offs of this magnitude could be detrimental to this population. Fortunately, since 1996 the die-offs have been considerably smaller, both in the severity and in the number of species afflicted. However, botulism outbreaks vary from year to year, most likely depending on local environmental conditions (Rocke and Friend 1999).

Interestingly, comparative mortality rates in Brown and White pelicans have changed over the period covered by this review. From 1994 to 1997, losses from type C botulism in White Pelicans were greater than in Brown Pelicans. This trend reversed in the last four years (1998–2001), with losses in Brown Pelicans higher than White Pelicans (Table 1). The reason for this trend is unknown. It might reflect differences in feeding behavior between the two pelican species as fish communities in the Sea change, or it might reflect differences in toxin availability as a result of year-to-year variation in environmental conditions. In 1996 large numbers of tilapia were available and pelicans could readily choose between healthy, sick, and dead fish, both species most likely preferring the easily caught sick, yet still live fish. As tilapia populations declined over the last few years (Riedel et al. 2002), pelicans may have been forced to settle for dead prey with increasing frequency, a behavior perhaps more readily adopted by Brown Pelicans. Alternatively, pelican migration and use patterns may also result in differences in populations at risk for each species from botulism each year. These differences may be reflected in the apparent temporal shift in botulism outbreaks at the Sea. From 1996–1999, the peak in numbers of afflicted birds at the Sea occurred during August and September, regardless of the date of onset. In 2000–2001, outbreaks appear to have begun in May and June and reached a peak in July and August, a month or so earlier than they had in previous years. Brown Pelicans may have been the species at greatest risk for botulism intoxication during these past few years, as most White Pelicans tend to arrive at the Sea later in the season (USFWS, unpubl. data). Unfortunately, there are few data available to calculate populations at risk during botulism outbreaks for either species of pelican, and a number of unknown interacting factors may have been responsible for the trends observed. Currently the California Brown Pelican population remains stable (D. Anderson, pers. comm.), and losses from botulism to date have been insignificant in relation to the total population.

Fish have previously not been documented in the literature as a primary source of type C botulinum toxin for birds. However, the presence of type C toxin in freshly dead and sick tilapia is

strong evidence that these fish are the source of toxin for fish-eating birds in the Salton Sea. Unfortunately, it is unknown how tilapia acquire the toxin. One possibility is that fish consume invertebrates or some other food source that contains pre-formed toxin. Laboratory studies have shown that tilapia are sensitive to type C botulinum toxin (Lalitha and Gopakumar 2001; T. Rocke, unpubl. data). Another possible explanation is that tilapia acquire toxin when botulinum spores in their gut germinate and the bacteria proliferate and produce botulinum toxin. Toxic infections such as this occur in animals that are stressed or otherwise compromised from disease or nutritional factors, and as a result, their intestinal environment becomes conducive for spore germination and anaerobic bacterial growth. These types of infections have been documented in human infants (Midura and Arnon 1979), horses (Swerczek 1980), and other animals (Minervin 1967). Bacterial and parasitic infections, high temperatures, and reduced oxygen levels are likely important stressors for tilapia in the Salton Sea (Kuperman and Matey 1999, Riedel et al. 2002; NWHC, unpubl. data). Studies are ongoing to evaluate the role of tilapia in the initiation of outbreaks in fish-eating birds. A greater understanding of the dynamics of this disease may help managers prevent another large outbreak from happening at the Salton Sea and will aid research and management in other parts of the continent where botulism occurs in fish-eating birds.

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