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A RELATIONSHIP BETWEEN AVIAN CARCASSES AND LIVING INVERTEBRATES IN THE EPIZOOTIOLOGY OF AVIAN BOTULISM

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Abstract: A survey of the sources of *Clostridium botulinum* type C toxin possibly utilized as food by aquatic birds in an epizootic area of avian botulism in northern Utah showed that living aquatic and terrestrial invertebrates normally found in close association with dead, decomposing birds commonly carried the toxin. Of 461 samples associated with 21 species of avian carcasses, 198 were toxin-positive. Invertebrate species not normally scavengers of vertebrate tissues were less commonly and less highly toxic, particularly when captured 30 cm or more from a carcass; six of 237 samples of such aquatic invertebrates contained low-level toxin. Of the species tested, blow fly larvae (Calliphoridae) were the most consistently and highly toxic, although others, particularly adult and larval stages of several species of beetles (Coleoptera), contained toxin at levels probably significant in the epizootiology of the disease. An estimated 0.05 to 0.25 g of the most toxic fly larvae or 15 g of the most toxic beetles tested carried a mediam lethal dose for an adult mallard duck. Examination of stomach contents of aquatic birds dead of botulism showed that some had consumed invertebrates.

INTRODUCTION

Since "western duck sickness" was reported to be a form of botulism in 1930,[14,19] one primary research objective has been to identify the naturally occurring media in which *Clostridium botulinum* type C multiplies and produces its toxin in waterfowl marshes. Equally necessary to an understanding of the epizootiology of the disease is evidence that any such toxin-carrying materials are (1) sought out as foods by aquatic birds, (2) ingested incidentally as contaminants of the birds' normal diet, or (3) consumed by another species—an invertebrate, for example—that is in turn consumed by aquatic birds.

Proof of the sources of toxin responsible for most outbreaks of avian botulism is lacking, because the stomachs of birds too sick to escape capture usually contain little recognizable food material. Carcasses of some species of invertebrate animals satisfy the requirements of *C. botulinum* type C as media for growth and toxin production in the laboratory, and there is circumstantial evidence that they may do so under field conditions[3,4,12,14,15,16,21,22,24,35].

Vertebrate carcasses support the production of particularly high levels of botulinum toxins.[4,12,16,22,34,35] Because most of the species of aquatic birds commonly affected with botulism are not carrion feeders, however, it is necessary to postulate a mode of toxin transport from source to bird, if the vertebrate carcass is to be considered important in the epizootiology of the disease. As early as 1909, Hastings[18] suggested that "limberneck" of poultry was the "result of the fowl's eating maggots from dead carcasses," although he apparently did not suspect that botulinum toxins were the cause of the disease. Other investigators confirmed the toxicity of maggots (fly larvae) from bird carcasses for chickens,[3,5,18,35] turkeys,[11] ducks,[20,28,31] and game farm pheasants and quail.[2,3,6,7,24,30] The presence of type C toxin in other terrestrial and

aquatic invertebrates[3,16,17,21,22] has been reported; the consumption of maggots and many other species of invertebrates by wild aquatic birds[1,20,22,32,33,36] is well documented.

The study reported here was designed (1) to confirm earlier reports that blow fly larvae and other living aquatic and terrestrial invertebrates carry *C. botulinum* toxin, (2) to establish the levels of toxin carried by the various invertebrate populations and estimate whether the volumes of invertebrates consumed by aquatic birds might contain toxic or lethal doses, (3) to compare the levels of toxin carried by invertebrates living on or in close proximity to bird carcasses with others not closely associated with carcasses, and (4) to provide support to earlier studies indicating that intoxicated birds had recently consumed various species of invertebrates.

STUDY AREA

The Bear River Migratory Bird Refuge is located 24 km west of Brigham City, Utah, north of Great Salt Lake. About 100 of its 260 km² are marshland created by the impoundment of Bear River water. Dikes divide this area into five units of approximately equal size. South of the main dike are marshes varying in size with the volume of water supplied by the Bear River. The Refuge provides habitat for numerous nesting and migratory birds.

There is a history of acute, epizootic waterfowl disease, presumably botulism, in northern Utah at least as early as 1910,[9] particularly on the delta of the Bear River north of Great Salt Lake. One of the purposes of establishing the Refuge, in fact, was to impound this water and thereby make possible a certain amount of control over its depth and distribution, in the hope of controlling the disease.

The mortality recorded in this general area has ranged from a few hundred birds to hundreds of thousands, generally occurring between mid-July and September. On the Refuge, annual losses are variable and largely unpredictable. For example, about 30,000 and 50,000 birds died in 1967 and 1971, respectively, while the heaviest loss in the intervening years was about 1,600 in 1970, the year of this study.

Collection of invertebrate samples began 20 May 1970, before there was evidence of botulism on the Refuge, and continued through 2 October, at which time losses had subsided. Early in the study, samples were taken from Units 1, 2, 3, and 4, but as the summer advanced, botulism became relatively more prevalent in the northwest portion of Unit 3, and after 17 August, all collections were made there.

MATERIALS AND METHODS

Field Studies

From late May until early July, invertebrate samples were collected from all bird carcasses encountered. As botulism losses increased, however, carcasses could be selected from three types of marsh habitat: (1) various kinds of vegetation, (2) dry or damp shoreline, and (3) water, varying in depth from less than 1 to about 90 cm (in channels). The condition of each carcass, generally related to the time elapsed since death, was classified as (1) maggot-free (very recently dead, and not yet fly-blown; or flyblown but eggs not yet hatched), (2) maggot-infested (larvae in any state of development), or (3) consumed (carcass reduced to feathers, bones, and fragments of dry skin and flesh).

Invertebrates associated with carcasses, collected between 0900 and 1300 hrs, were placed in jars of gelatin-sodium phosphate buffer (GPB)[1] and refrigerated until taken to the laboratory, where they were stored at —17 C. A few fly larvae from each carcass were reared to maturity for identification of species.

[1] GPB of pH 5.0 was used in the collecting jars to compensate for alkalinity of the water; in laboratory procedures, a pH 6.2 buffer was used.

Collections of invertebrates not closely associated with bird carcasses were made with hand-held dip nets or Tyler sieves from an airthrust boat making crisscross transects of the marsh. Specimens were never taken from vertebrate carcasses, but if a dead bird happened to be on the transect line, netting was interrupted 30-60 cm before reaching the carcass and continued as it was passed. Bottom species (such as chironomid larvae and oligochaetes) were collected by straining mud through Tyler sieves. No attempt was made to collect in marginal or fringe areas (water's edge to dry land).

Samples from the nets and sieves were transferred to shallow enamel pans and grossly visible species separated in the field to jars of GPB. From this point on, they were generally handled as described for the species associated with vertebrate carcasses.

Daily minimum - maximum ambient temperatures and water levels for Unit 3 were obtained from the records of the Bear River Refuge.

Laboratory Studies

Preparation of samples

Preliminary to testing for toxicity, invertebrates were thawed, rinsed in GPB, and drained on absorbent towels. A 1.0 g portion (or the entire sample if the quantity was smaller) was ground in a mortar and diluted 1:10 (wt/vol) in GPB. The suspension was centrifuged at low speed for 30 sec to settle larger particles and the supernatent fluid used for the intraperitoneal (ip) mouse toxicity test (MTT) for type C toxin.

Mouse toxicity tests

Invertebrate extracts were screened for mouse toxicity, and the positive samples were titrated for estimation of toxin levels in mouse minimum lethal doses (MLD).

In the screening test, a 0.1 ml protective dose (5 International Units) of type C specific antitoxin (Fort Dodge Laboratories[2]) was given ip to one of a pair of 15-20 g mice, and both were given 0.5 mg ip injections of oxytetracycline hydrochloride (Liquamycin, Pfizer[2]) to minimize bacterial infection. About 30 min later, each of the pair was injected with 0.2 ml of invertebrate extract. Death of the unprotected mouse and survival of the protected one within 5 days was considered to be a positive test for type C toxin.

Toxin levels of all positive samples were measured by titration in mice. The range of toxicity of each positive extract was found by injecting 0.1 ml doses of 10-fold serial dilutions. Because of the large number of samples, only two mice per dilution were used and MLD were only approximated. Two-fold dilutions within the range so established were then tested. The mouse ip MLD of type C toxin per gram of invertebrate was recorded as the denominator of the highest dilution that killed both mice within 5 days.

Since toxemia occurs early in the course of botulism in the species of birds that have been tested, the demonstration of toxin in blood serum has been used extensively as a diagnostic test. The procedure was the same as that described for invertebrate extracts, except that blood serum (taken by cardiopuncture) was substituted for the extract. When an adequate sample was available, 0.5 ml of serum was injected into one or two protected and an equal number of unprotected mice. The volume of serum obtainable from small birds sometimes limited the dose to as little as 0.1 ml per mouse. Quantitative measurments of serum toxin levels were not made.

Examination of digestive tract contents

Sick birds collected for food examinations were killed by breaking the neck. The upper digestive tracts, tied off at

[2] Reference to trade names does not imply endorsement of commercial products by the Federal Government.

the anterior end of the esophagus and just posterior to the ventriculus, were removed in the field and refrigerated until examined grossly and microscopically in the laboratory. All sufficiently intact animal and plant items were classified to the lowest possible taxon.

RESULTS

Carcass-associated Invertebrates

Of a total of 461 invertebrate samples associated with 173 decomposing avian carcasses of 21 species tested for toxin [126 ducks of 10 species; 17 shore birds of 3 species; 2 waders of 2 species; 10 gulls of 2 species; 12 pelicans (*Pelecanus erythrorhychos*); 3 American coots (*Fulica americana*); 2 western grebes (*Oechmophorus occidentalis*); and one yellow-headed blackbird (*Xanthocephalus xanthocephalus*)], 198 (43%) were positive, and the MLD was measured on 171. Titers ranged from less than 50 to 409,600 mouse MLD per g of sample (Table 1).

From 20 May to 15 July, few sick and dead birds were observed in the field, only 17 carcasses suitable for collection being found. Air temperatures at this time ranged from highs of 21-27 C to lows of 5-10 C. Beginning 15 July, a sharp rise in the numbers of sick and dead birds was observed, with concomitant temperature increases to highs of 27-32 C and lows of 10-15 C. After 1 September, temperatures fluctuated, but highs and lows generally dropped about 10 degrees. An increase in the amount of water flowing into the Refuge and a rise in water level accompanied the lower temperatures. The number of sick and dead birds decreased.

Species of fly larvae varied in relative abundance through the summer, *Phaenicia sericata* and *Phormia regina* predominating. *Protoformia terra-novae* and *Lucilia* sp. prefer cool weather and were not encountered after 9 July. No toxin-positive samples of the latter two genera were obtained. Some samples of all other species collected were positive for type C toxin.

The first toxic fly larvae (*P. regina*), obtained 4 June, contained 400 mouse MLD of toxin per g. Toxin levels ranged from 0 to 3,200 MLD until 15 July, when one sample reached a titer of 102,400 MLD—5 days after the first case of botulism (in a California gull, *Larus californicus*) was diagnosed. On 17 July, a second sample of maggots (*P. sericata*) reached the same titer, and six sick birds and 30 fresh carcasses were observed in the immediate area. Sera from five of the sick birds were positive for botulism. From 15 July through 2 October, 92% of the maggot samples collected contained type C toxin. Twenty-one of these, taken from avian carcasses of eight species in all habitat types, reached a level of 100,000 MLD or greater. Through the entire collecting period, titers ranged from less than 50 to 409,600 MLD. The seasonal distribution of type C toxin from fly larvae is presented in Figure 1.

The numbers and types of invertebrates (both aquatic and terrestrial) other than fly larvae varied among collections according to seasonal population fluctuations, weather conditions, time of day, location of carcass, and the agility of the collector.

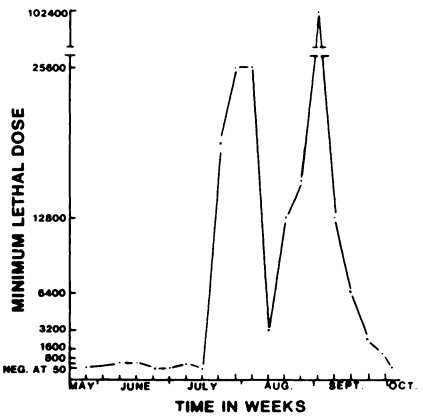

FIGURE 1. Median weekly mouse ip MLD per g of fly larvae samples, 20 May through 2 October 1970.

TABLE 1. Toxicity (mouse ip MLD/g) of invertebrates associated with avian carcasses, Bear River Refuge, 20 May - 2 October 1970.

Invertebrate group	Maggot-free carcasses		Maggot-infested carcasses		Consumed carcasses	
	No. Positive/ Total	MLD Range	No. Positive/ Total	MLD Range	No. Positive/ Total	MLD Range
Calliphoridae and Sarcophagidae, Larvae	NC[1]		120/142	50-409600	NC	
Aquatic: Adults Larvae and Nymphs[3]	0/28		35/167	50-1600	NC	
Terrestrial: Adults and Larvae[4]	4/14	50-400	31/73	50-6400	3/14	50-100
Flying Adults[5]	1/8	50	4/15	50-100	NC	

[1] NC—None collected.

[2] *Protophormia terra-novae, Lucilia* sp., *Lucilia illustrus,* Sarcophagidae sp., *Cynomyopsis cadaverina, Phormia regina, Phaenicia sericata* and *Bufolucilia silvarum.*

[3] Corixidae, adults, nymphs, eggs (waterboatmen); Notonectidae, adults, nymphs, skins (back swimmers); Amphipoda (scuds); Chirinomidae, larvae, eggs (midges); Stratiomyidae, larvae (soldier fly); Tabanidae, larvae (horse fly); Dytiscidae, adults, larvae (diving water beetles); Hydrophilidae, adults (water-scavenger beetles); Zygoptera, nymphs, skins (damselfly); Anisoptera, nymphs (dragonfly); Ephemeroptera, nymphs (mayfly); Ostracoda (seed shrimp); Hirudinea (leech); Mollusca (snails); snail egg masses.

[4] Carabidae (predacious ground beetle); Staphilinidae (rove beetle); Silphidae, adults, larvae (carrion beetle); Dermestidae, adults, larvae (skin or larder beetles); Heteroceridae (variegated mud-loving beetle); Chrysomelidae (leaf beetle); Isopoda (sowbug); Collembola (springtail); Lepidoptera, larvae (moth); Forficulidae (earwig); Araneida (spider); Orthoptera (cricket); Calliphoridae pupae (blow fly).

[5] Tabanidae (deer flies); Calliphoridae (blow flies); Ephydridae (shore flies); Odonata (damselfly); Saldidae (shore bugs).

Thirty-five of 195 aquatic invertebrate samples tested contained type C toxin. Except in snails, the titers did not exceed 400 MLD per gram of invertebrate. Gelatinous snail egg masses were deposited among the feathers of carcasses, and many young were found on them. Fifteen of 37 snail samples tested were positive, their titers ranging from 50 to 1,600 MLD. Four of seven samples of snail egg masses contained toxin at levels of 50 to 400 MLD. One of two samples of damsel fly (Zygoptera) skins and one of notonectid skins, both of which had been shed on carcasses, had titers of 400 and 200 MLD respectively.

Other aquatic species positive for type C toxin included: Corixidae, 1 of 24; Notonectidae, 2 of 12; Amphipoda, 2 of 5; larval Tabanidae, 1 of 1; adult Dytiscidae, 2 of 14; larval Dytiscidae, 1 of 10; nymphs of Zygoptera, 2 of 12; and nymphs of Ephemeroptera, 3 of 17. All toxin-positive aquatic invertebrate samples tested were associated with maggot-infested carcasses, the maggots ranging in titer from 200 to 409,600 MLD, with a median of 51,200 MLD.

Of 101 terrestrial invertebrate samples collected, 38 were positive for type C toxin. These included collections from maggot-free, maggot-infested, and consumed carcasses. Thirty-six of the 38 positive samples were predaceous, carrion, or scavenger Coleoptera adults and larvae; titers ranged from 50 to 6,400 MLD. Thirty-one of these were associated with maggot-infested carcasses; of 22 titrated, the median was 400 MLD. The medium titer of positive samples collected from maggot-free and consumed carcasses, however, was only 50 MLD. The median titer of the maggot samples collected in conjunction with terrestrial invertebrates was 51,200, with a range of 800 to 409,600 MLD.

Terrestrial invertebrate samples containing type C toxin included: Carabidae, 11 of 20; Staphilindae, 2 of 6; adult Silphidae, 4 of 9; larval Silphidae, 5 of 11; adult Dermestidae, 8 of 15; larval Dermestidae, 4 of 7; mixed adult beetles, 2 of 2; Isopoda, 1 of 2; and larval Lepidoptera, 1 of 1.

Flying insects on a carcass or in its immediate vicinity were more difficult to capture. Of 23 samples collected, 5 were positive, but titers did not go above 100 MLD. Toxin-positive species included: Calliphoridae, 4 of 15; Saldidae, 1 of 2.

In all groups of invertebrates tested, many samples induced signs of botulism but no death in unprotected mice, indicating the presence of low levels of toxin.

Invertebrates not associated with carcasses

All invertebrate samples not associated with carcasses were aquatic species. Two hundred thirty-seven samples (10 taxonomic groups) were tested for toxicity (Table 2). Six samples appeared to be positive at the 1:50 dilution in the screening test. These included samples of larval Ephemeroptera, larval Chironomidae, larval Zygoptera, Notonectidae, larval Coleoptera, and Ostracoda. When these samples were titrated, however, none was toxic, even at the 1:50 dilution.

Avian serum toxicity tests

The results of the MTT's on the sera of sick birds, recorded as number of samples positive for type C toxin over the number tested, were: ducks (10 species), 100/128; shore birds (7 species), 4/47; waders (2 species), 12/16; coots (1 species), 7/11; and pelicans (1 species), 0/2.

All birds showed signs of intoxication, ranging from slight leg weakness to prostration, but there was no way of ascertaining whether they were in the incipient or the convalescent stage of paralysis.

Digestive tract contents

The upper digestive tracts of eight 1- to 2-week-old gadwall (*Anas strepera*) ducklings, seven black-necked stilts (*Himantopus mexicanus*), five avocets (*Recurvirostra americana*), one dowitcher (*Limnodromus scolopaceus*), one Wilson's phalarope (*Steganopus tricolor*), and one ibis (*Plegadis falcinellus*), all presumably affected with botulism, were examined. In most cases the contents were scanty and highly fragmented. The

TABLE 2. Toxicity tests on specimens not closely associated with vertebrate carcasses.

Material tested	No. of fresh samples	Positive for toxin
Ephemeroptera (mayfly larvae)	32	1
Chironomidae (midge larvae)	39	1
Zygoptera (damselfly larvae)	27	1
Anisoptera (dragonfly larvae)	4	0
Corixidae (water boatmen)	46	0
Notonectidae (backswimmers)	40	1
Coleoptera (water beetles)	23	1
Microzooplankton (rotifers, microcrustacea, protozoa, others)	4	0
Mollusca (snails)	19	0
Ostracoda (seed shrimp)	3	1
Totals	237	6

gizzard of one black-necked stilt contained three lead shot. Two gadwall ducklings, within the first week of life, contained blow fly larvae fragments and skins containing 1,000 mouse MLD of toxin per g. Invertebrate materials present in the remainder of the birds included corixid fragments, corixid eggs, snails, Coleoptera fragments, a damsel fly larva, chironomid larvae, and a shore fly (Ephydridae).

DISCUSSION

Among the results of this study, three findings are clear: (1) Invertebrates collected from avian carcasses, or in their immediate vicinity, much more commonly contained type C toxin than did those collected at some distance away; in fact, samples of all such species were toxic at some period of the study; (2) blow fly and flesh fly larvae were more commonly found on avian carcasses than were other species of invertebrates, and (3) these larvae more consistently contained toxin, and at higher levels, than did other invertebrates closely associated with carcasses. The median mouse MLD per g of maggots (12,900) was higher than the highest level of toxin found in other invertebrates.

There is no published evidence that the activity of maggots on a decomposing carcass either increases or decreases the potency of type C toxin that may be present. Doyle[12] states that the flesh of a chicken carcass was just as toxic when fed to guinea pigs as were maggots reared on the carcass. The fact that maggots are voracious feeders is the most likely explanation for the high levels of toxin that they sometimes contain, and these levels may provide presumptive evidence of the high toxicity of the carcass.

Maggots usually far outnumbered other species of grossly visible invertebrates that fed upon avian carcasses, and they were present for longer periods of time than were most other species. Live, paralyzed birds are commonly already infested with fly larvae (personal observation). Additional eggs may be deposited at intervals after death of the bird and, as a result, a single carcass may

contain larvae ranging in development from early first instar through late third instar. At summer temperatures, the carcass may become a writhing mass of maggots within 24 hrs and be virtually devoured within 72 hrs.

With the exception of late third instars and those prematurely deprived of food (by being washed off the carcass, for example), maggot size was not related to toxin level. Voiding of the gut contents before pupation probably accounts for the relatively low toxicity of mature larvae and pupae.

California gulls, Franklin's gulls (*Larus pipixcan*), Forster's terns (*Sterna forsteri*), American coots, and turkey vultures (*Cathartes aura*) were seen feeding on maggot-infested bird carcasses. Although shore birds often probed alongside carcasses, neither they nor ducks were observed feeding directly from them. In this and earlier studies,[8,16] ducks are reported to consume maggots readily once they are dislodged from the carcass by wave action or other means. When carcasses on land were physically disturbed, virtually decomposed, or overpopulated with the various species of invertebrates, maggots were observed migrating (presumably to find other suitable media in which to complete their development), thereby making themselves vulnerable to capture by predators. Most species of birds were seen feeding in areas containing many maggot-infested carcasses, but in no case could they be observed closely enough so that the food items could be recognized.

At some time between the ingestion of toxin and the appearance of frank paralysis, birds appear to lose the ability or the inclination to feed. During this interval, which may range from 2 h to a day or more, depending upon the dose of toxin, many kinds of food materials are beyond recognition.[22] However, maggots and other identifiable invertebrates have been found in the stomachs of affected birds in a few cases.[2,22,28,29]

Our examinations of the upper digestive tracts of a limited number of ducklings and shore birds suggested they might contain recognizable food material more commonly than do those of mature ducks, possibly because they are more susceptible to type C toxin and, therefore, become paralyzed and vulnerable to capture before the materials are thoroughly digested.

Although only two gadwall ducklings of 23 birds examined in 1970 contained maggot remains in the upper digestive tract, a larger study carried out in 1972 (unpublished) added to their number. Maggots (2 to 402) were found in three 4-week-old redheads (*Aythya americana*), two 1-week-old gadwalls, and one juvenile California gull.

Because individual birds of the species tested vary greatly in their susceptibility to type C toxin (as do different lots of commercially produced experimental birds), LD_{50} measurements by the oral route have been difficult to reproduce. The range has been from 20,000 to 100,000 mouse ip LD_{50} among groups of adult mallard ducks tested at this station over a period of 20 years (unpublished). Assuming that wild ducks may fall into a similar susceptibility range, an average 0.05 to 0.25 g of maggots containing about 409,600 mouse MLD per g would provide an LD_{50} dose for a duck, as would about 15 g of the most toxic terrestrial beetles. Lee, Vadlamudi, and Hanson[24] found eight third instar blow fly larvae equivalent to a lethal dose for a pheasant. Gunderson[16] experimentally intoxicated ducks with 15 blow fly larvae given by mouth. We have induced intoxication in ducks by force-feeding 0.5 g samples of maggots containing 50,000 mouse MLD of type C toxin (unpublished).

The fluctuation in numbers of invertebrate species other than maggots collected during the course of this study has been observed by others[21,23] and may be accepted as a normal marsh phenomenon. Our results show that invertebrates other than fly larvae feeding on or seeking shelter from an avian carcass may pick up type C toxin. Generally, the higher the titer of the fly larvae from a carcass, the more likely other species of invertebrates from that carcass were to contain toxin.

Predaceous, carrion, or scavenger beetles feed directly on bird carcasses or, in some cases, on maggots and other insects they harbor[8] (personal observation). Though snails are predominantly vegetarian, they occasionally ingest animal material. Such species as *Physa* and *Lymnaea* (present on the Bear River Refuge) are active scavengers.[25]

The importance of the vertebrate carcass is further emphasized by the results of the study of invertebrates not closely associated with carcasses. The presence of toxin in the six samples positive in the screening test could not be confirmed. Had these invertebrates temporarily rested on or near toxic carcasses and acquired toxin only externally, they likely would have lost a large part of it during our subsequent washing and extracting procedures. At least four of the positive samples were collected in areas scattered with waterfowl carcasses. Any toxin retained by these invertebrates, when captured for food by a bird, would add to the total toxin level consumed by that bird. Dead invertebrates also may serve as sources of toxin, either by virtue of having consumed it during life, or because of its production in their tissues after death.

One can only speculate on precisely what happened during the week of July 15 to cause the sharp increase in toxin levels in the marsh (as evidenced by maggot toxicity and bird mortality). Early in the collecting period, the general ecological picture (relatively low temperatures and high water levels and the scarcity of birds showing signs of botulism) suggested that the few carcasses found may have been those of birds dead of other causes. While such carcasses may contain type C toxin (personal experience), the levels appear to be generally lower than those of birds dead of botulism, although we do not yet have statistical support for this opinion.

Increasing temperatures and accompanying lower water levels very likely brought about concentrations of aquatic invertebrates in smaller water areas, and possibly die-offs occurred because of overpopulation and increased salinity. The mass of growth media for *C. botulinum* provided by the invertebrate carcasses and the more favorable incubation temperatures combined to promote toxin production, resulting in the beginning of an epizootic.

From this point on, bird carcasses and the invertebrates feeding upon them undoubtedly contributed a major part of the toxin that perpetuated the outbreak. In our experience, dead invertebrate and plant tissues incubated in the laboratory have never supported toxin production at levels comparable to the high ones observed in this study (unpublished data).

As high levels of toxin became apparent in invertebrates, numbers of sick and dead birds increased accordingly. Toxicity increased with incubation (as evidenced by increased toxicity of the invertebrates), until they were consumed and maggots pupated, at which time toxin levels dropped or became negative. This was the pattern found by Theiler[34] and by Coburn and Quotrup.[10] Except for an unexplained decline in mid-August, median weekly toxin levels of fly larvae remained high until the advent of cooler weather and higher water levels in early September (Fig. 2).

By methods now available, it would not be possible to eliminate invertebrate populations that play a role in the epizootiology of avian botulism, while sparing those important as food for aquatic birds. Their numbers can be reduced, however, by removing the dead birds they utilize as food, a practice long advocated.[16,20,22,27] Although there are no published reports of well-controlled experiments showing that removal of these carcasses influence the course of an outbreak, our data show that doing so before infestation with maggots and other invertebrates has occurred would prevent the development of sources of highly potent toxin in a form readily consumed by many species of aquatic birds.

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