Study of the toxic potentials of duck botulism in artificially flooded areas

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A STUDY OF THE TOXIC POTENTIALS OF DUCK BOTULISM

IN ARTIFICIALLY FLOODED AREAS

by

DANIEL A. POOLE

B.S., Montana State University, 1950

Presented in partial fulfillment
of the requirements for the degree of
Master of Science in Wildlife Technology

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1952
This thesis has been approved by the Board of Examiners in partial fulfillment of the requirements for the degree of Master of Science in Wildlife Technology.

Chairman of the Board of Examiners

Dean of the Graduate School

Date Apr. 28 1952
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D. A. P.
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INTRODUCTION

The history of waterfowl mortality attributed to botulism poisoning is well documented (Kalmbach and Gunderson, 1934, and Kalmbach, 1938). Kalmbach and Gunderson (1934) state, "It would seem, therefore, from the review of pertinent literature, that the earliest records of true duck sickness that may be pointed to with reasonable assurance are those mentioned by Wetmore for the early nineties at Great Salt Lake." Severe waterfowl mortality, again in the Great Salt Lake area, in the years 1910-1912 led to the initial investigation, in 1914, of the enigmatic malady. Research during the period from 1914 to 1930 resulted in refutation of many theories promulgated as being either individually or correlatedly responsible for the observed syndrome in naturally afflicted waterfowl. Some of the disproven theories relative to the cause of this duck sickness were bacterial infection, dietary deficiency, gases, industrial wastes, lead poisoning, parasites, selenium poisoning, toxic algae and toxic salts.

Kalmbach (1930) successfully produced the syndrome of botulism in experimental ducks following the feeding of incubated livers of naturally afflicted ducks. Giltner and Couch (1930) first isolated the bacterium Clostridium botulinum, type C, from the tissues of dead ducks and from mud collected in duck sickness areas. Kalmbach and Gunderson (1934) demonstrated that the toxic products of the bacteria could produce duck sickness in the field.

Subsequent research has disclosed much information concerning the ecological conditions under which the bacteria develop and elaborate
toxin in sufficient concentration to cause heavy mortality among waterfowl. The implications of some of the more recent research findings form the basic hypothesis for this paper and are reviewed in the following sections.
HISTORICAL REVIEW

The Organism

Five principal strains of *Clostridium botulinum* are known and designated as types A, B, C, D, and E. Types A and B are involved in human botulism. Type C causes duck sickness in wild fowl, forage poisoning in livestock, and mortality among domestic poultry. Type D is a causative factor of *lamsiekte* among livestock in South Africa. Type E has been isolated from spoiled fish in Russia. These bacteria are culturally similar but are differentiated by the individual specificity of their toxins (Zinsser and Bayne-Jones, 1939).

*Clostridium botulinum*, type C, is a rod shaped, spore forming bacterium about 4 to 6 microns in length and 0.9 to 1.2 microns in width. The organisms occur singly or in short chains. They are slightly motile and possess from four to eight peritrichious flagella. The organism may persist for an indefinite period in spore form and then suddenly become active in favorable growth conditions. The oval spores are usually situated near the end of the bacillus. The type C spores are less resistant to heat than the spores of types A and B (Zinsser and Bayne-Jones, 1939). Conditions suitable for organism growth and multiplication are media of alkaline nature, the absence of oxygen, and an optimum temperature (Kalmbach and Gunderson, 1934). In situations suitable for growth the organisms may multiply rapidly and secrete an exotoxin which, if ingested in sufficient quantity, will cause the syndrome of botulism observed in afflicted wild ducks. The organisms are saprophytic and can grow in media of both animal and vegetable origin (Zinsser and Bayne-
The general pH growth range of *Clostridium botulinum,* type C, is near neutrality (Bell, personal communication). The optimum laboratory culturing temperature is near 37 degrees C. (Bell, personal communication). Gunderson (1932) believes alkalinity may be influential upon the natural growth of the organism in two ways, either by direct stimulation of the bacterium or by inhibition of the growth of organisms which may be competitive with *Clostridium botulinum.*

**The Toxin**

The excretory product of *Clostridium botulinum* is a powerful toxin which acts in extremely small amounts. Batson (1940) has been able to kill pigeons with an injection of one-one millionth of a cubic centimeter of toxin. Coburn (1942) demonstrated two fractions, A and B, in dehydrated toxin. Fraction A is thermolabile, non-antigenic, not neutralized by type C antitoxin, stable at room temperature, resistant to bacterial action, and has no specific antibody. It is a neurotoxin which acts without delay but is destroyed by strong alkali. Fraction B cannot withstand bacterial action and is toxic by injection only.

The toxin is destroyed by heating at 80 degrees C. for 30 minutes (Kalmbach and Gunderson, 1934). Results from experiments on destruction of toxin by direct exposure to sunlight and air are not in accord. The ingestion of toxin in an alkaline solution is said to facilitate its absorption (Gunderson, 1932). The toxin can withstand the acidity and alkalinity of the gastrointestinal tract (Zinsser and Bayne-Jones, 1939). Ambache (1948) working with the toxin of *Clostridium botulinum,* type A, suggests that the toxin is capable of inducing a change in capillary permeability which allows its passage or that it is
broken down to a smaller diffusible molecule which is still neurotoxic.

Gunderson (1932) suggests the possibility that the presence of toxin renders the intestinal wall more permeable to passage of the type C organism.

Species of Waterfowl Afflicted and Character of the Syndrome

Kalmbach (1935) lists a total of 69 species, in 21 families, of North American wild birds known to have contracted botulism under natural conditions. The puddling duck or probing shorebird seem to be most likely to ingest the toxin. The species of ducks and geese found in nature to have exhibited the syndrome associated with botulism are:

| White Fronted Goose       | Anser albifrons       |
| Canada Goose              | Branta canadensis     |
| Common Mallard            | Anas platyrhynchos    |
| Common Black Duck         | Anas rubripes         |
| Gadwall                    | Anas strepera         |
| American Pintail          | Anas acuta            |
| Green Winged Teal          | Anas carolinensis     |
| Blue Winged Teal           | Anas discors          |
| Cinnamon Teal              | Anas cyanoptera       |
| Shoveler                   | Spatula clypeata      |
| Baldpate                   | Mareca americana      |
| Redhead                    | Aythya americana      |
| Ring Necked Duck           | Aythya collaris       |
| Canvasback                 | Aythya valessinaria   |
| Lesser Scaup Duck          | Aythya affinis        |
| American Goldeneye         | Bucephala clangula    |
| Bufflehead                 | Bucephala albeola     |
| Ruddy Duck                 | Oxyura jamaicensis    |
| Red Breasted Merganser     | Mergus serrator       |

Pimie (1935) has added the mute swan, Cygnus olor.

Kalmbach and Gunderson (1934) did not find migrant ducks more susceptible than resident waterfowl to intoxication. Williams (1934) found that generally the Pintail and Green Winged Teal and less constantly, the Mallard, undergo a mortality loss proportionate to their representation in an outbreak area. But the Cinnamon Teal, Shoveler and Baldpate have a mortality loss greater than their proportionate numbers.
Hammond (1950) could find nothing in the data of the sex ratios of ducks contracting botulism in North Dakota to support a premise that the excess males in populations of wild ducks resulted from an excessive female loss to botulism. He believes mortality of the sexes to be related more to botulism conditions existing during their respective periods of moult.

The syndrome of botulinum intoxication in wild ducks has been well described by Kalmbach and Gunderson (1934). The general clinical picture is one of muscular weakness brought about by impairment of the nervous system. In wild birds it is manifest by their inability to fly, then to walk, paralysis of the neck muscles and nictitating membrane of the eye and inhibition of pulmonary action. The body temperature is subnormal. A flow of greenish diarrhoea stains the vent feathers and in some instances the anus is occluded by the hardening of a white renal discharge. Quortrup and Sudheimer (1934) found paralysis of the nictitating membrane in 73 per cent of the botulistic ducks examined.

The following observations were recorded from a Mallard duck that received a sublethal dose of toxin (Kalmbach and Gunderson, 1934): the bird refused to eat and drink following ingestion of the toxin and prior to displaying evidence of sickness.

2h hours: flight poor, unsteady on feet, hesitant walk.

30 hours: may be unable to fly, sculls itself over water by wings and feet, strong tendency to squat, may be able to stand. The wings are no longer held in a concise folded position. Sometimes the primaries touch the ground. The head is supported on the crook of the neck but may, at times, fall off and rest on the ground. The duck can swim after loss of ability to fly and stand and may attempt to dive to
elude capture. This effort shows further debility, for the bird cannot
get under the surface of the water.

24 to 48 hours: dypnea sets in during this period. The nici-
tating membrane of the eye may exhibit paralysis. A flow of greenish
diarrhoea stains the plumage around the vent and the hardening of a
whitish renal flow may completely occlude the anus. An excessive lach-
rymal discharge may solidify over the eyeball and impair the vision.

48 to 60 hours: the duck exhibits peak debility during this
period. The bird may be prostrate with the neck outstretched, wings
relaxed, feet beneath it or extending backward, a slow rate of respira-
tion and sub normal temperature. Death may follow shortly. A greater
dose of toxin will result in a more rapid sequence of events.

The gizzards of afflicted waterfowl contain only gravel or hard
parts of undigested food. The alimentary tract contains little or no
food. The small intestine is shrunken and firm and the cloaca may be
distended because of blocking of the vent by solidified renal matter.
A congestion of the meninges of the posterior half of the brain has
been noted.

A duck may exhibit signs of recovery on about the third or
fourth day following ingestion of asublethal dose of toxin. The bird
will move, hold neck erect, preen and drink large quantities of water
which may cause the bird to choke slightly thus revealing probable im-
pairment of the muscles of deglutition. On the fifth and sixth days
the bird may walk, take food, stretch and flap wings and may, in a day
or two more, take wing.

Some birds retain evidence of sickness by paralysis of aleg
or wing. Such birds usually become emaciated, fail to take care of
their plumage and seldom undergo complete recovery. Most intoxicated ducks are "in good flesh" because of the relatively short period of sickness prior to death. Only those birds experiencing prolonged sickness or repeated contact show emaciation.

Quortrup and Jensen (1944) state that flies are probably largely responsible for the mucopurulent discharges common in natural cases of botulism. Flies, crawling unhampered over the eyeballs of the paralyzed ducks, appear to feed on the lachrymal discharges. Streptococci, proteus and coliform organisms have been cultured from the eye discharges.

Kalmbach and Gunderson (1943) suspect that sublethal cases predominate in the field and the focal points of toxin are localized. A relatively small proportion of waterfowl frequenting a toxic area become ill. Approximately 65 to 70 per cent of afflicted ducks rescued in the field and placed in dry enclosures with fresh water and shade have a complete recovery. Banding experiments revealed that hospitalized ducks have about three times the chance of surviving botulism intoxication as those birds banded and left in the field (Williams and Jensen, 1943).

Factors contributory to mortality in intoxicated waterfowl are exposure, drowning, predation, starvation and dehydration.

**Botulism Season**

Western duck sickness occurs throughout its range in greatest intensity and frequency during the period from August 15th to September 15th. Little precipitation, excessive evaporation, low water, waterfowl concentration, water stagnation and the wane of many forms of life are coincident with the usual botulism season (Quortrup and Sudheimer, 1942).
Range and Distribution

The area in which outbreaks repeatedly occur conforms roughly to the range of alkaline soils throughout western United States (Kalmbach, 1935). Within this area outbreaks of duck sickness occur year after year in varied intensity. The range extends from southern Alberta and Saskatchewan south to Mexico, and from the Dakotas, Minnesota and Nebraska southwesterly to Texas and westerly to California and southern Oregon. Gunderson (1932) found the range of repeated botulism outbreaks to lie within areas of recent volcanic activity. The lava deposits are largely basalt and contribute greatly to the alkalinity of the soil wherein the botulinum organism is found.

Some observers express the opinion that botulism outbreaks are becoming more widespread throughout the general western range of the causative organism. Land use, irrigation practices, and agricultural procedures are considered to be factors pertinent to the spread. Others believe that the outbreaks are not now more widespread than in former days. They contend that the increased reports on botulism outbreaks correspond to a greater degree of settlement of the west and the increased number of trained waterfowl observers afield.

Some epizootics among waterfowl have occurred in regions well outside the usual range of western duck sickness. These sporadic outbreaks are characterized by the small number of waterfowl afflicted and the variety of outbreak conditions. Pirnie (1935) notes definite record of fatalities to swans at the W. K. Kellogg Sanctuary and elsewhere in the State of Michigan. Austin and Austin (1931) gave an account of finding sick turnstones and sanderlings in Massachusetts which exhibited the typical symptoms of botulism. The birds had fed upon the decaying
carcasses of beached blackfish which harbored adult, larval and pupal forms of blowflies. In 1941, four cases of duck mortality were noted in New York State in which the afflicted birds exhibited the botulism syndrome (Cheatum, 1950). The incubated liver of a black duck which was found paralyzed near Dyke, Virginia, yielded the Clostridium botulinum, type C, organism (Kalmbach and Gunderson, 1934). Zimmerman (1946) recorded an outbreak of botulism in Wisconsin.

**Mortality**

The following mortality figures represent severe waterfowl loss that has elicited attention over the past years. Proof of the causative agent is lacking in many instances, but there exists a similarity of syndrome to known botulism cases (Kalmbach and Gunderson, 1934, Kalmbach, 1938, and Hervey et al., 1948). Waterfowl mortality, which involves relatively few ducks, occurs each year in many areas. The yearly impact of this loss on waterfowl populations is not excessive, but the cumulative impact may be great.

1912- "Thousands" of birds died in King and Kern Counties in California. "Untold thousands" of birds died around El Fros, Saskatchewan, Canada. "About half a million" ducks died around the mouth of the Bear River and Willard Spur areas in Utah.

1913- 30,000 birds died on Weber River Flats, Utah.

1942- 462 birds buried at Bear River, Utah, between August 22nd and September 21st.

1913- 46,723 ducks buried at Bear River, Utah, between September 7th and 26th. This figure was believed to constitute less than 20 per cent of the mortality in the area.
40,000 birds died at Buena Vista and Tulare Lake Basins in California.

1925- 25,000 to 50,000 birds died at Tulelake, California.

100,000 birds died at Lake Malheur, Oregon.

1929-100,000 to 300,000 birds estimated dead in the vicinity of Great Salt Lake, Utah.

1932- 250,000 birds died in Bear River Bay area, Utah.

1933- 800 dead birds per lineal mile along the New and Alamo Rivers in the Imperial Valley, California.

15,000 to 20,000 shore birds and ducks died at Stobart and Namaka Lakes, Alberta, Canada.

1936- 50,000 waterfowl died in the Great Salt Lake Valley, Utah. More than 13,000 birds died at Upper Des Lacs Lake, North Dakota.

16,369 sick and dead ducks collected at Bear River, Utah.

1937- 11,735 sick and dead ducks collected at Bear River, Utah.

1938- 33,378 birds buried at Medicine Lake, Montana.

More than 10,000 birds succumbed at Fox Lake, Montana.

1939- 11,635 birds buried at Medicine Lake, Montana.

20,201 sick and dead ducks collected at Bear River, Utah.

1940- 26,750 sick and dead ducks collected at Bear River, Utah.

1942- 23,351 sick and dead ducks collected at Bear River, Utah.

1945- 17,631 sick and dead ducks collected at Bear River, Utah.

1945- 50,000 dead ducks in South Bay area in mid-September near Bear River, Utah.
Distribution of the Organism in the Field

The bacterium, Clostridium botulinum, type C, has been demonstrated in many samples which were collected in the field. Giltner and Couch (1930) and Gunderson (1932) cultured the organism from samples of mud and the livers which were removed from sick ducks. Kalmbach and Gunderson (1931) found the organism in masses of living and dead Lemma, copepods, snails, sarcophagid fly larvae, dead and living hydrophilid larvae, decaying tubers of beyonnet grass, and strands of filamentous algae. The organism was also found in barley, wheat, and mixed grain which were submerged for a time in water of an outbreak area. Coburn and Quorstrup (1939) found that a 15 day decomposition of the green algae, Cladophora, supported a growth of the organism with good toxin production. Quorstrup and Holt (1941) believed that practically all forms of vegetative matter in process of decay are important media in the field for Clostridium botulinum, type C. Quorstrup and Sudheimer (1942a) isolated the organism from duck intestines and fish. Gunderson (1932) isolated the botulinum organism from 45.8 per cent of 96 samples which were collected in an outbreak area. He isolated the organism from only 6.3 per cent of 110 samples collected in a non-disease area. Positive cultures were obtained from 17 of 27 livers from sick ducks in areas of sickness, whereas only 1 of 34 livers from ducks in non-sickness areas were positive for the organism.

Distribution of Toxin in the Field

Kalmbach and Gunderson (1931) found the preformed toxin in duck sickness areas. Dead bird carcasses, larvae of sarcophagid flies, mud beneath a dead duck, water over grain strewn on a mud flat, and water from shallow pools were found to contain the preformed toxin. They
demonstrated toxin in mixed masses of insects, copepods, snails, and \textit{Lemna} incubated in the laboratory. Gunderson (1932) demonstrated free toxin in 22 of 76 sample materials collected in the field.

Duck carcasses were placed over a three inch layer of soil in pans and the underlying soil mixed, at intervals, with equal volumes of water. This mixture was fed to normal ducks. The presence of toxin in the soil was demonstrated for a period of 25 days (Quortrup and Sudheimer, 1942c).

Quortrup (1934b) took mud cores for toxicity tests in mice from four general types of marsh areas. The sites sampled were mud under deep water, mud under shallow water, recently dried mud along the water's edge (3 days exposure), and thoroughly dried mud (over 3 days exposure). The mud samples were extracted with distilled water and the inoculum injected into white mice. Nearly 15 per cent of the total number of samples injected were found to be toxic. Approximately 60 per cent of the mud samples from the zone of recently dried mud were toxic. The author believes that the percentages expressed are high. Quortrup usually subjected experimental mice to large volumes of inoculum. The 15 per cent figure includes the many toxic samples found in the zone of recently dried mud and serves to convey an erroneous impression relative to the field distribution of toxin. The toxicity results should be expressed individually by general sample sites for purpose of comparison. This would amplify the toxicity of the zone of recently dried mud.

Jensen et al. (1944) tested the relationship between the aerobic bacterial populations, toxicity, and moisture in the soil at selected field stations. It was found that the toxicity of the mud samples varied inversely with the aerobic bacterial populations and directly with moisture.
Quortrup and Sudheimer (1942b) previously suggested a symbiotic relationship between *Clostridium botulinum*, type C, and *Pseudomonas aeruginosa*, an oxygen-consuming aerobe found conducive to production of botulinum toxin of high titer in mixed cultures.

Ostracods, found swarming from July to September at the Bear River Migratory Bird Refuge, were artificially silted over with mud and subjected to toxicity tests. These sites all became toxic and some remained so for 15 days (Williams and Nelson, 1941). Maggots which floated free of duck carcasses were taken readily by experimental ducks and were found to be highly toxic (Harvey et al., 1947).

**Laboratory Findings on Toxin**

Frozen toxin was found to undergo no loss of titer (Quortrup and Holt, 1940). Coburn and Quortrup (1938) exposed toxin to sunlight for nearly 45 hours without deleterious effect. Large doses of filtered toxin were placed in isolated potholes, in moist soil, and in dry soil. The workers were unable to detect the presence of toxin at any site after 24 hours (Quortrup and Sudheimer, 1942c). Grain was mixed with a toxic culture, air dried, and fed to waterfowl. Toxicity persisted up to 90 days (Quortrup, 1943a). A dried liver from an intoxicated duck was stored in a refrigerator for 13 months. It was then immersed in water and permitted to soak for an hour without stirring. The water was found to be toxic after an interval of one hour (Quortrup and Sudheimer, 1942d). Researchers have been unable to produce toxin of sufficient titer in cultured duck feces to kill experimental ducks, but have been able to kill mice (Quortrup and Jensen, 1944). Bags of liver, dehydrated in an ice-box, were inoculated with *Clostridium botulinum*, type C, and placed in duplicate positions in soil, water, and air. The bags were collected
once a week and extracted for toxicity tests. Toxin was produced most consistently in wet organic soil sites (Hervey et al., 1945).

Quortrup and Sudheimer (1942a) do not believe that preformed toxin exists in nature for any appreciable length of time except in those cases in which it might be frozen, dried, or possibly under conditions free of competitive bacterial action.

Quortrup and Sudheimer (1943) demonstrated the presence of botulinus toxin in the blood stream of intoxicated ducks. Toxin was detected as late as 68 hours following ingestion.

Conditions Correlated with Outbreaks

Evaporation and precipitation. -- In general the duck sickness season corresponds with low rainfall and excessive evaporation throughout its range. Evaporation leads to concentration of salts. This may result in alkalinity or salinity deleterious to toxin production (Gunderson, 1932). Quortrup (1940) believes evaporation should be expressed solely in terms of lowering water levels as no correlation exists between evaporation and botulism outbreaks. Coburn and Quortrup (1938) observe that the rain curve in excess of 0.2 inch and sickness outbreaks are closely correlated. Thus rainfall may actually bring about conditions more suitable for toxin production. Scrutiny of precipitation data since 1937 does not reveal any continuous, direct or compelling correlation between rainfall and sickness (Quortrup and Sudheimer, 1942f). Kalbach (personal communication) states, "locally and periodically precipitation has been associated with the end as well as the beginning of outbreaks".

Temperature. -- There appears to be no correlation between high temperatures and duck sickness (Quortrup, 1940). The field temperatures coincident with outbreaks are generally much cooler than optimum laboratory
culturing temperatures. In 1942 a total of 23,354 dead and sick ducks were collected at the Bear River Migratory Bird Refuge. Quortrup (1940) made the following temperature observations throughout the summer:

- **Pre-botulism season:** July 1st to August 15th, mean air temperature 23.5 degrees C.
- **Botulism season:** August 15th to September 15th, mean air temperature 20.9 degrees C.
- **Post-botulism season:** September 15th to October 15th, mean air temperature 16.1 degrees C.

*Clostridium botulinum*, type C, has a wide range of temperature tolerance, wider perhaps than the range of normally competitive bacteria. It may be that cooler temperatures indirectly favor the growth of the botulinus organism and result in excessive toxin production (Quortrup and Sudheimer, 1942f).

**Abundance of birds.**—There appears to be a relationship between peak bird populations and sickness. But large numbers of birds have been present on the Bear River Migratory Bird Refuge at the same time that no botulism outbreaks have occurred. Therefore bird numbers alone are not a determining factor (Quortrup, 1940).

**Wind.**—Hervey et al. (1945) notes that wind action without prior stabilization of the shore line does not appear conducive to sickness. Quortrup (1940) observed an association between wind direction and outbreak occurrence.

**Chemical factors.**—Quortrup and Sudheimer (1942a) list several chemical analyses made in a botulism area. The analyses included pH, dissolved oxygen, carbonates, bicarbonates, chlorides, and total salts.
Only pH and dissolved oxygen were considered to be conducive to toxin production. Quortrup and Holt (1941) suggested a direct relationship between deficiency of oxygen and presence of toxin in field tested materials.

Water level and water movement.—Batson (1940) lists many botulism outbreaks in South Dakota which followed refilling of dry or nearly dry lake basins. The inundation of the terrestrial vegetation in these lake basins was said to have resulted in a degree of anaerobiosis favorable to the production of toxin. In some cases mortality closely followed the refilling of lake basins while in other instances mortality did not occur until the following year. The highest mortality persisted in the latter instances. No differentiation was made between mortality which occurred the same season after refilling the dry and the nearly dry lake beds. The species of ducks afflicted were predominately those in the shallow water feeders. Batson was able to intoxicate experimental ducks by confining them in a pen embracing an area of shallow water, mud shore, and dry grassy land. The depths of the lakes involved are not given, but the habits of the species of ducks afflicted are known. Batson attributed the shore line concentration of sick and dead ducks to their effort to reach dry land. However, he successfully conducted intoxication experiments along the shore. It is possible that the botulism reported by Batson was more intimately associated with the shore line than he originally had supposed.

Coburn and Quortrup (1938) found that the greatest bacterial activity occurs during that period of time which was required for the top two or three inches of silt to evaporate to dryness. The drying areas entrapped many forms of potential media.
Water levels, moving constantly up or down result in no apparent disease. However, intoxication does occur when the level is stabilized for several days and then raised over the drying mud flat (Jensen, 1943). Serious sickness occurred when the water was at its lowest level and a storm drove the water over the exposed mud flats (Quorstrup and Sudheimer, 1942f). Hervey et al. (1946), working at the Bear River Migratory Bird Refuge, manipulated the water levels in refuge Units 3 and 4 to test the effect of such action on the production of botulinum toxin. The total number of sick ducks found daily along the dikes in each unit was used as a measure of toxicity in the unit. In late July the water level in each unit was raised and then lowered to expose the previously inundated bottom. The shore line of Unit 4 was stabilized against the mud bottom whereas the Unit 3 water level underwent a lowering of one-half inch each day from evaporation. A south wind in August forced the water over the mud flats in both units. The total number of sick ducks collected along the dikes in Unit 4 increased from 8 to 281 and in Unit 3 from 18 to 61. The duck population on Unit 3 was 200,000 and on Unit 4 was 100,000. From August 7th to the 30th there were five times as many duck losses in Unit 4 regardless of the greater waterfowl usage of Unit 3.

Botulism investigators have used caged ducks to test various areas for toxin production in conjunction with water level experiments. The cages permitted the ducks to come into contact with all of their immediate environment. Both wild and domestic species of ducks have been used in the experiments. Botulism has been demonstrated in every instance of reflooding of the recently dried mud flat (Quorstrup, 1943b).

Williams (1940) placed caged ducks on the mud flat in front of
the deepening water in Unit 3 of the Bear River Migratory Bird Refuge on September 17th. All of the cages were inundated by September 18th. A check on September 20th revealed:

- Cage 1: 1 dead, 1 appeared normal
- Cage 2: 3 dead, 2
- Cage 3: 2 dead, 3
- Cage 4: all ducks appeared normal
- Cage 5: 1 dead, 2
- Cage 6: 3 dead, 2
- Cage 7: 2 dead, 3
- Cage 8: 2 dead, 2
- Cage 9: all ducks appeared normal
- Cage 10: 1 dead, 3

Another check on September 23rd revealed two more dead birds in Cage 10.

This information supports the theory of the toxicity of a reflooded mud flat and strongly suggests that the toxin occurs at small foci, for not all of the birds in any one cage died. It may be that the points of toxin production are not homogeneously distributed throughout the entire drying mud flat. However, the most important conclusion to be drawn from the information is that sickness can be produced following flooding of a drying mud flat.

Williams (1950) has recorded the following notes on water level variation and occurrence of botulism during a series of experiments with caged ducks. On August 31st he placed eight pintail ducks in each of four wire pens located as follows:

- Pen 1: on an area of fringe mud and water
- Pen 2: on an area of fringe mud and water
- Pen 3: in water two inches deep
- Pen 4: in water two inches deep.

On September 2nd the ducks were observed to be in good health. Strong winds on September 4th caused inundation of Pens 1 and 2. On September 5th, six dead ducks were found in Pen 1, and four dead and two sick ducks in Pen 2. The data reveal that the mud fringe had been drying
for a minimum of four days. During the period from September 6th to September 9th the water level receded beyond Pens 3 and 4 thereby placing all the pens on a drying mud flat. Strong winds on September 9th brought about inundation of all four pens. One of the two ducks which remained in Pen 1 from September 5th was dead. Pen 2 was not in operation. All of the 16 ducks in Pens 3 and 4 were dead. Pens 3 and 4 had been on a drying mud flat for a minimum of one day to a maximum of three days prior to flooding on September 9th. This observation supports the contention that recently exposed mud may be toxic when reflooded, but it is contrary to the theory that water stabilization is a necessary part of the reflooding procedure. Stabilization of water levels prior to outbreaks which followed reflooding has often been noted in the field. However, stabilization is apparently not necessary for production of toxin. It may be that a stabilization period contributes either to a greater distribution of toxin or to production of toxin of higher titer, or to both.

Sperry (1947) presents a summary of factors found pertinent to botulism:

1. That botulism rarely kills many ducks on an impoundment that has its shore line against steep banks.

2. That duck sickness is directly related to the production of toxin in shallow alkaline impoundments with an exposed, nearly flat, lake bottom as shore line.

3. That an abundance of water coming into and going out of a lake has little beneficial effect on sickness trends if extensive shore lines remain on the flat terrain of an exposed lake bottom.
4. That water can safely be put over a thoroughly dried lake bottom provided the flooding does not create stable shore lines during the process.

5. That the theory, linking major botulism outbreaks with changes in water levels and movements of shore lines, should be given a severe test. This theory, in brief, is that stabilization of a shore line on a gently sloping old lake bottom is so favorable to toxin production that the fringe area from the waters' edge to dry land is a serious danger zone whenever shallow inundation makes it a feeding ground for ducks."

The most serious waterfowl loss to botulism in any one season occurs in definite peaks with a much smaller mortality interspersed between the peaks. The work on botulism done by the U.S. Fish and Wildlife Service suggests a correlation between the peaking of outbreaks and the flooding of the damp mud margins of the shallow alkaline lake basins. The outbreaks may reach epidemic proportions quickly and are followed by a gradual decline in the number of additional waterfowl afflicted. Some workers consider the small number of sick ducks which appear between peaks as a carryover from the preceding outbreak. Many potential sources of toxin have been demonstrated in the field. It is conceivable that one or more of these sources may, at times, serve to intoxicate waterfowl.

Toxin production in the field is manifest by the appearance of sick birds. An incubation period of several hours precedes visible evidence of intoxication. The overall conditions contributory to the production of toxin may have changed during the incubation period.
BOTULISM STUDY AT THE BEAR RIVER MIGRATORY BIRD REFUGE DURING THE SUMMER OF 1951

The analysis of literature on botulism suggests a relationship of production of type C toxin and inundation of damp mud margins of alkaline lakes in certain regions of western North America. There exists a need to study in greater detail the toxic potentials of the re-flooded mud flat.

During the summer of 1951 a number of flooding experiments were conducted on small areas of the mud flat at the Bear River Migratory Bird Refuge in Utah. Botulism has occurred regularly at this refuge for many years. The conditions observed in conjunction with natural outbreaks in past years were simulated in an attempt to produce toxic conditions. It was hoped that a degree of success could be attained in producing toxic conditions. The ability to produce toxic areas at will would facilitate studies of the ecology of the bacterium, *Clostridium botulinum*, type C, the phenomenon of toxin production, the food of ducks in toxic areas, and methods of control of outbreaks of botulism.
MATERIALS

Pekin Ducks

The use of caged Pekin ducks in botulism work has been satisfactory. These domestic birds are more easily handled than wild ducks, which, when confined in a cage, spend much time seeking escape. The caged Pekin duck devotes much more time to seeking food. A duck in search of food and drink may be expected to contact the toxin if it occurs within the limits of the cage.

The ducks were banded to provide individual identification, and were denied food for 24 hours prior to exposure. No food other than that which occurred naturally within the cages was available to the ducks during the exposure period, usually an interval of 24 hours. Sick ducks were to be tested by antitoxin injection and ultimately discarded. All ducks not visibly sick were held several days for observation prior to re-exposure (Plate 1).

Laboratory Mice

White mice were injected with water samples which were collected periodically from the experimental sites. Bell (personal communication) has found white mice to be one hundred thousand times more susceptible to intraperitoneal injections of toxin than a Pekin duck which was given the toxin orally. Quortrup (1946) found penicillin bacteriostatic for Clostridium botulinum, type C, and other gram positive organisms. Streptomycin is bacteriostatic for many gram negative organisms. Neither penicillin nor streptomycin have a deleterious effect upon botulinus toxin.
All mice were given intraperitoneal injections of 0.1 cc of penicillin (25 units) and 0.1 cc of streptomycin (25 units) not less than one hour before receiving the water sample injections. This was done to minimize mouse mortality from causes other than botulinum intoxication. The mice to be used as controls were given additional intraperitoneal injections of 0.1 cc of polyvalent antitoxin (5 units each of types A, B, and C) at this time.

The penicillin was obtained in crystalline form from the Lederle Laboratories Division, American Cyanamid Company, New York, New York. The streptomycin was obtained in crystalline form from the Upjohn Company, Kalamazoo, Michigan. The antitoxin was purchased from the Jensen-Saltsbury Laboratories, Kansas City, Missouri.

The penicillin and streptomycin bases were each diluted with distilled water to 250 units per cubic centimeter and were refrigerated in stoppered flasks at 4 degrees C. Fresh solutions were prepared every two weeks. The antitoxin was also kept under refrigeration.

Water Samples

A syringe was used to collect the water samples from just above the bottom. The samples were put into stoppered vials and taken into the laboratory. Each sample was centrifuged at approximately 2000 rpm for 10 minutes prior to injection of the supernatant fluid into mice.

A fresh syringe and hypodermic needle was used for injecting each water sample. The syringes and needles were boiled in water for 15 minutes before use. One protected mouse and one unprotected mouse were each given intraperitoneal injections of 0.1 cc of the water sample and caged for a three day observation period. The interval between water sample collection and water sample injection was usually about two hours.
Two experiments were conducted to test for possible loss of toxicity in water samples collected in the field. Two ampules of dehydrated toxin of known toxicity to mice were taken into the field and mixed with known volumes of mud and water. These mixtures received similar treatment both in time and preparation as did the water samples. Dilutions to 50 M.L.D. (mouse-intraperitoneal) of one mixture and to 25 M.L.D. of the other proved toxic to mice. No variation in toxicity was noted between centrifuged and non-centrifuged samples. This work indicates that there was no important loss of titer in the procedure used for the collection and preparation of water samples.
EXPERIMENTAL PROCEDURES

Movable Watertight Rings

The rings were used in an attempt to create toxic conditions by flooding small portions of drying mud flat. Two sizes of rings were used. The larger rings were made from 20 gauge sheet metal and were eight feet in diameter and 20 inches high. The smaller rings were made by removing the ends from No. 10 tin cans (6 inches in diameter).

The development of mud flat sites for ring experiments was subject to the overall refuge water conditions. The unprecedented volume of water available for refuge use in 1951 limited development of suitable expanses of drying mud. The usual pattern of refuge water is one of declining water levels as the summer progresses. The receding water exposes the previously inundated mud bottom. A variation in dryness of the exposed mud exists in relation to the time interval since the mud was last flooded.

It was the author's intention to mark the water levels in several areas each day in order to establish the different exposure zones of drying mud. Ring experiments could then be conducted in several of the zones in an attempt to delimit time intervals in relation to toxin production after flooding. As a result of the great influx of water into the refuge, only isolated areas of exposed mud flat appeared for short intervals throughout the study period. Movement of the large rings to many of these areas was extremely laborious and, as a consequence, small rings were used in most of the subsequent experimentation.
Notes were kept on the placement of rings upon the various exposure zones prior to flooding. The distance to the water table was measured at each ring site. A soil core, 12 inches square and 6 inches deep was taken in each of the five large rings flooded on July 17th. The core was taken in two layers, each three inches deep. A soil core sample of this weight and bulk was impractical. A one inch diameter soil core was taken to the aforementioned depths at subsequent ring experiment sites. Each moist soil sample was mixed thoroughly and the water was extracted by filtration for toxicity tests in mice. The dry soil samples were mixed with a small volume of distilled water to facilitate extraction for toxicity tests. The organic content of the soil was determined for each sample taken on July 17th by the hydrogen peroxide-wet oxidation method (Wright, 1939). Organic debris was present upon a ring site surface in only one instance.

A standard procedure was followed in flooding and testing for toxin at each ring site. The large rings were flooded by use of a hose which was connected to an irrigation pump (Plate 2). Water was poured from a dipper into the small rings. An effort was made to avoid disruption of the mud surface upon addition of the water. The depths, to which the rings were flooded, varied. The rings were pushed deep into the mud to minimize water seepage loss.

A sample of the water which was used for flooding was taken for toxicity tests. The experiments were generally terminated at the end of a 24 hour period. The flooding was done usually in the afternoon and water samples were taken for toxicity tests at approximate intervals of 1, 19, and 24 hours thereafter. A few rings were flooded in the morning and the water-sampling intervals were approximately 1, 8, and 24 hours
thereafter. In a few instances an experiment was conducted an additional
2½ hours and water samples were taken from 43 to 48 hours after the in-
itial flooding.

Large rings.—A circular wire pen was erected adjacent to each ring
site (Plate 2). One Pekin duck was confined in each ring and wire pen.
The use of a wire pen permitted the testing of the naturally occurring
conditions near each ring site during the experimental period. If the
duck in the ring became intoxicated and the duck at the natural site did
not, one could suspect that the presence of toxin was a result of the
conditions experimentally established. However, if toxicity occurred
solely at the natural site, one could use the conditions observed at
the natural site as a basis for additional ring experimentation.

A total of 11 large ring experiments were conducted. Twenty
soil extract samples and 32 water samples were tested in mice for
toxicity. Thirty Pekin ducks were exposed to experimental conditions
during the study period. Toxin production was not detected at any time
during the experimental periods. Table I summarizes the large ring
flooding data.

Small rings.—No measurements of water depth were taken following
initial flooding. It was necessary to add water to several rings prior
to termination of the experimental periods. In these instances the
samples were taken at least five minutes after adding the water. Some
experiments were conducted in duplicate. One ring enclosed a portion of
the natural environment (Plate 3). Organic material was added to an ad-
jacent ring in an attempt to stimulate the botulinum organism. The solid
media were worked into the top one-half inch of mud prior to flooding.
Liquid media were poured over the mud surface 15 minutes prior to flooding.
In some instances the mud and water were stirred thoroughly before taking a water sample. This was done to put toxin, if adsorbed to soil particles, into solution.

A total of 64 small ring experiments were conducted. Eighty soil extract samples and 202 water samples were tested for toxicity. Toxin production was not detected at any time during the experimental periods. Table II summarizes the small ring flooding data.

**Diked Water Areas**

The mud-flat-site for construction of the diked water areas was chosen by Fish and Wildlife Service personnel who were familiar with distribution of botulism outbreaks at the refuge. The impoundments are in the northeast corner of refuge Unit 4, west of the P-line borrow pit channel and four hundred yards south of the Whistler three-way water diversion gate (Figure 1). The mud flat has been built up by deposition of silt from water of the influent channel and by action of the refuge unit water. The mud surface slopes down from east to west.

An impoundment, subdivided into five equal units 30 feet wide by 100 feet long, was constructed perpendicular to the refuge unit shoreline. The impoundment walls consisted of two parallel rows of shiplap lumber which were driven on end into the mud. The space between the walls was filled with mud to make a watertight barrier. A catwalk was built upon the walls and on stakes in the center of each unit. The mud surface within the units was not disturbed during the construction period (Figure 2 and Plate 4).

The water for experimental use was pumped from the channel of the borrow pit into two storage tanks having a combined capacity of 1,250 gallons (Plate 5). A gravity flow pipe system transferred the
# TABLE I

## SUMMARY OF LARGE RING FLOOING DATA

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>Organic Content of soil, per cent</th>
<th>Days Mud Exposed</th>
<th>Water Table Depth, Inches</th>
<th>Flooding Depth, Inches</th>
<th>Time Flooded</th>
<th>Water Sample Intervals, to Mice</th>
<th>Toxicity</th>
<th>Remarks</th>
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<td>14.49</td>
<td>3.42</td>
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<td>2</td>
<td>11.10</td>
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<tr>
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<td>7.20</td>
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</tr>
<tr>
<td>5</td>
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<td>5.89</td>
<td>1 plus</td>
<td>1</td>
<td>3</td>
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<td>ducks used for 48 hrs.</td>
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<tr>
<td></td>
<td>23</td>
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<td>0</td>
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<td>48 hr. period</td>
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<td></td>
</tr>
</tbody>
</table>

*: Ring placed in 2" of water and seven-eighths of the mud bottom was exposed to the air by pumping. After three days of exposure, trypticase soy broth was poured liberally over the mud and water. A duck was confined in the ring and water added to flood the exposed mud. The first water sample was taken one hour following flooding. Additional samples were taken daily for a period of four days.
### TABLE II (Cont.)

<table>
<thead>
<tr>
<th>Site</th>
<th>Days Mud Exposed</th>
<th>Days Water Table Depth, Inches</th>
<th>Water Table Depth, Inches</th>
<th>Flooding Time</th>
<th>Time Flooded</th>
<th>Water Sample Intervals, Hours</th>
<th>Media Added</th>
<th>Stirring Depth, Inches</th>
<th>Toxicity to Mice</th>
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<tr>
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<td>1/2</td>
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</tr>
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<td>1/2</td>
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<td>1/2</td>
<td>2:15PM</td>
<td>1,19,2h</td>
<td>liver infusion</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:10PM</td>
<td>1,19,2h</td>
<td>corn meal</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>13</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:00PM</td>
<td>1,19,2h</td>
<td>Kracke's</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:00PM</td>
<td>1,19,2h</td>
<td>Kracke's</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:00PM</td>
<td>1,19,2h</td>
<td>Kracke's</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>23</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:15PM</td>
<td>1,19,2h</td>
<td>liver infusion</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:15PM</td>
<td>1,19,2h</td>
<td>trypticase soy</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>unknown</td>
<td>1/2</td>
<td>1/2</td>
<td>2:15PM</td>
<td>1,19,2h</td>
<td>trypticase soy</td>
<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
</tbody>
</table>
water from the tanks into the units. Individual valve controls in each unit permitted regulation of water flow. Pipe extensions from the valves projected beneath the water surfaces into wooden kegs sunk into the bottoms of the units. This minimized aeration of water which was added to the units and disturbance of the mud by its flow (Plate 6).

**Soil Samples**

A soil core, 12 inches square and six inches deep, was taken from each unit on July 2nd. The core was divided into two layers each of which was three inches thick (Figure 2). The moist samples were stirred thoroughly and the water was extracted from a portion to be used for toxicity tests in mice. The dry soil samples were mixed with a small volume of distilled water and extracted for toxicity tests. The organic content of each sample was determined by the hydrogen peroxide wet oxidation method (Wright, 1939). Table III presents the results of the soil organic content analysis.

**Table III**

<table>
<thead>
<tr>
<th>Unit Number</th>
<th>Depth of Sample</th>
<th>Organic Content in Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&quot;-3&quot;</td>
<td>3&quot;-6&quot;</td>
</tr>
<tr>
<td>1</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
Figure 1. Bear River Migratory Bird Refuge

- Water level gauges
- Experimental diked water areas
Figure 2. Liked water areas

Legend:
- Vegetation
- Soil cores
Bacteria Samples

On August 6th, 10 soil samples were collected in sterile vials from the top two and three-eighths inches of mud in each diked area (Figure 2). The samples were sent to the Rocky Mountain Laboratory, Hamilton, Montana, for determination of the presence of Clostridium botulinum, type C. The soil samples from Units 1, 2, and 3 have been tested. The samples from Units 4 and 5 have not been cultured. The soil was cultured in trypticase soy broth plus fresh guinea pig liver. Toxin was demonstrated and confirmed by antitoxin in 10 of the 30 samples tested. The samples found positive for the organism were:

Unit 1 - samples 1, 3 and 6.
Unit 2 - samples 5, 8, and 9.
Unit 3 - samples 1, 2, 5, and 7.

The presence of the organism signifies only that toxicity could occur if the conditions for growth and toxin production prevailed.

Invertebrate Collections

Samples of macroscopic animal life which occurred in the soil and water of each unit was collected and preserved for identification. The collection procedure entailed random searching through water and soil samples until no new forms were observed. The larger forms were preserved in 70 per cent ethyl alcohol and the smaller forms in 10 per cent formalin. The main collection dates were August 19th, 23rd, and 24th. Some specimens were collected upon observation throughout the period of study.

The invertebrates which were collected and identified are:
Pulmonata

**Physa sp.**

**Gyraulus** sp.

Oligochaeta: Tubificidae

Rhynchobdellida:

Glossiphonidae

Crustacea

Cladocera

Amphipoda

Podocarpa

Odonata

Zygoptera - juveniles

Ephemerida - juveniles

Hemiptera:

Corixidae - juveniles and adults

Notonectidae - juveniles and adults

Hesperomeliidae - juveniles and adults

Diptera:

Tipulidae - juveniles

Culicidae - juveniles

Chironomidae - juveniles and adults

Dolichopodidae - adults

Ephydridae - juveniles

Coleoptera:

Dytiscidae - juveniles and adults

Staphylinidae - adults

Hydrophilidae - juveniles and adults

Parnidae - juveniles and adults

Araneae

Hydrachnidae

**Vegetation.**

Specimens of plants in each unit were collected for identification. Data on abundance of terrestrial and emergent plant species were obtained
ducks utilized much of the aquatic vegetation and made determination of abundance of plant species impractical. Four grid stations were used along each of both sides of each impoundment. The distance from the dry end of the unit to the furthest growth of emergent vegetation in the wet end of the unit was measured. This distance was divided by four and a grid count made at the mid-point of each quarter. The data were taken on October 3rd. The material, presented in tabular form after Oates (1949), is merely indicative of vegetative cover in the units.

The vegetation present in each unit is summarized in Tables IV, V, VI, VII, and VIII.

### TABLE IV

PLANTS PRESENT IN UNIT I

<table>
<thead>
<tr>
<th>Species</th>
<th>Quadrat Number</th>
<th>Total Quaadrats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Individual Plants)</td>
<td>(Total Individuals)</td>
</tr>
<tr>
<td>Polygogon monspeliensis</td>
<td>1 5</td>
<td>2</td>
</tr>
<tr>
<td>Scirpus paludosus</td>
<td>(1) (1)</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td>1 2 4 5 6 7 8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(14) (6) (4) (21) (17) (13) (8)</td>
<td>(63)</td>
</tr>
</tbody>
</table>

*Other plants present in unit but not recorded in grids are Chenopodium sp., Distichlis stricta, Potamogeton pectinatus, Salicornia rubra, and Zannichellia palustris.

### TABLE V

PLANTS PRESENT IN UNIT 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Quadrat Number</th>
<th>Total Quaadrats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Individual Plants)</td>
<td>(Total Individuals)</td>
</tr>
<tr>
<td>Scirpus paludosus</td>
<td>1 2 3 4 5 6 7 8 8</td>
<td>8</td>
</tr>
</tbody>
</table>

*Other plants present in unit but not recorded in grids are Distichlis stricta, Lemma minor, Polygogon monspeliensis, Potamogeton pectinatus, Zannichellia palustris.
### TABLE VI

**PLANTS PRESENT IN UNIT 3**

<table>
<thead>
<tr>
<th>Species</th>
<th>Quadrat Number (Individual Plants)</th>
<th>Total Quadrats (Total Individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypogon monspeliensis</td>
<td>5 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Scirpus paludosus</td>
<td>1 2 3 4 5 6 7 8 (42)(37)(21)(12) (9)(39)(31)(18)</td>
<td>8 (209)</td>
</tr>
</tbody>
</table>

*Other plants present in unit but not recorded in grids are Distichlis stricta, Lemna minor, Potamogeton pectinatus, Salicornia rubra, Zannichellia palustris.*

### TABLE VII

**PLANTS PRESENT IN UNIT 4**

<table>
<thead>
<tr>
<th>Species</th>
<th>Quadrat Number (Individual Plants)</th>
<th>Total Quadrats (Total Individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distichlis stricta</td>
<td>5 (6)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Scirpus paludosus</td>
<td>1 2 3 4 6 7 8 (11)(18)(11)(42)(15)(10)(37)</td>
<td>7 (1lb)</td>
</tr>
</tbody>
</table>

*Other plants present in unit but not recorded in grids are Lemna minor, Polypogon monspeliensis, Potamogeton pectinatus, Salicornia rubra, Zannichellia palustris.*
## TABLE VIII

PLANTS PRESENT IN UNIT 5a

<table>
<thead>
<tr>
<th>Species</th>
<th>Quadrat Number (Individual Plants)</th>
<th>Total Quadrats (Total Individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Distichlis stricta</em></td>
<td>1 2 5 6 (9) (2)(29)(10)</td>
<td>4 (50)</td>
</tr>
<tr>
<td><em>Eleocharis palustris</em></td>
<td>3 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td><em>Levena minor</em></td>
<td>7 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td><em>Polypogon monspeliensis</em></td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Sagittaria cuneata</em></td>
<td>7 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Scirpus paludosus</em></td>
<td>1 2 3 4 5 6 7 8 (15)(25) (8)(18)(15)(28)(22) (5)</td>
<td>8 (136)</td>
</tr>
</tbody>
</table>

*Other plants present in unit but not recorded in grids are Potamogeton pectinatus, Salicornia rubra, Scirpus acutus, Typha latifolia, Zamioculcas palastris.*

**Water Temperature**

Maximum and minimum daily water temperatures were recorded at a position which was two inches below the surface of water in Unit 4 (Figure 2). The instrument was protected by a shadeboard. A Taylor, Six's Maximum and Minimum Self Registering Thermometer was used. The temperatures recorded are presented according to Quotrup (1940) to permit comparison with his observations. The water temperature data are as follows:

**Pre-botulism season: July 12th to August 15th**

- mean minimum temperature 17.8 degrees C.
- mean maximum temperature 29.3 degrees C.
- average temperature 23.5 degrees C.
- high temperature for the period 35.0 degrees C.
- low temperature for the period 12.2 degrees C.
Botulism season: August 16th to September 15th
mean minimum temperature 12.5 degrees C.
mean maximum temperature 27.6 degrees C.
average temperature 20.0 degrees C.
high temperature for the period 35.0 degrees C.
low temperature for the period 6.11 degrees C.

Post-botulism season: September 16th to September 27th
mean minimum temperature 10.0 degrees C.
mean maximum temperature 25.0 degrees C.
average temperature 17.5 degrees C.
high temperature for the period 29.4 degrees C.
low temperature for the period 3.89 degrees C.

Operation of Diked Water Areas

Two of the five diked units were originally designated as water manipulation areas. Water manipulation consists of alternate periods of flooding and drying mud flats. The three remaining units were used for investigations of stabilized water level-mud surface relationships. In time this plan revealed duplication of effort and an additional unit was converted to a water manipulation area. This information is presented in a later part of this section.

In a letter dated August 2nd, Mr. E. R. Kalmbach, Biologist, U. S. Fish and Wildlife Service, recommended a period of water stabilization from seven to fourteen days prior to flooding the drying mud flat, the optimum period being from ten to fourteen days. The flooding should be done slowly until a critical depth of one-half inch of water is present over the former shore line.
The work of Quortrup (1943b) pertaining to toxicity of certain zones of drying mud was also checked in some of the flooding experiments.

Each unit was completely inundated on July 2nd. On July 9th the water levels were adjusted to conform to the schedule of unit operation. A water level gauge was set near each valve control to facilitate accurate maintenance of the desired water depths. Evaporation loss was offset by flow of water from the storage tanks. The storage water was tested daily for toxicity in mice and also prior to each flooding experiment. None of the 77 water samples which were tested from the storage water were toxic to mice. Rain on August 4th and 5th made it necessary to pump water out of units 2, 3, and 4.

Caged ducks were placed in each unit to test for toxin production (Plates 4, 6, and 7). The cages were seven feet square. One duck was confined in each cage. The number of caged ducks per unit varied with the schedule of unit operation. Fresh ducks were used each day. The cage positions were changed daily. Water samples, for toxicity tests in mice, were taken daily at each wet cage position. The sampling interval was shortened during flooding experiments.

Unit 1.—This unit was established initially to stimulate a mud flat dotted with drying puddles. The puddle basins were formed by pounding the damp mud surface with sand bags prior to flooding the unit on July 2nd. On July 9th the water level was lowered to create the drying puddle area. Periodic addition of water into the unit maintained the experimental condition. The gradient within the unit did not permit complete utilization of the unit in the desired manner. The eastern one-half of the unit was used as a puddle dotted mud flat. The remaining one-half was covered with water to a maximum depth of two and three-quarters inches.
One caged duck was used in each of the two sections from July 9th to August 24th. The original plan was abandoned on August 25th.

The water level was lowered to locate the new shore line on the previously inundated mud surface. The reason for this action is given in a later part of this section. Water up to two inches deep covered one-third of the unit. Two general cage positions were used during water level stabilization periods. One cage was placed in the water section and the other upon the drying mud, well back from the edge of the water. The water level was stabilized until September 10th.

Water was put into the unit at 11:55 PM on September 10th. Ten caged ducks were placed in the area at 3:50 PM. The first water samples were taken from flooded cages at 4:00 PM (Figure 3). The water in the storage tanks was permitted to drain slowly into the unit until the former shore line was under one inch of water. Additional water samples were taken 19 hours after initiation of the experiment. The experiment was terminated on September 12th.

The water loss from evaporation between September 12th and 20th was slight. The water was pumped down to stabilization level on September 20th and maintained until September 24th. Water was put slowly into the unit at 10:00 AM on September 24th. Twelve caged ducks were placed in the area at 3:00 PM. The first water samples were taken from flooded cages at 3:25 PM (Figure 4). The water in the storage tanks was permitted to drain into the unit until the former shore line was under one inch of water. Additional water samples were taken at intervals of 24 and 30 hours after initiation of the experiment. The experiment was terminated on September 26th.

A total of 128 water samples was tested for toxicity in mice, and
Legend:
--- Stabilized shore 1
~ Extent of Model
□ Cape shift

Liner samples
△ 1 hour
○ 12 hour

Figure 3. Unit 1, September 10th.
Figure 4. Unit 1, September 24th.

Legend:
--- Stabilized area
~ Extent of flooding
◻ Cape drift

Water samples:
△ 5 hour
○ 24 hour
□ 30 hour
161 Pekin ducks were exposed during the study period. Toxin production was not detected in the unit.

**Unit 2.**—This unit was established as a water manipulation area. On July 9th the water levels were adjusted to place the shore line on a previously inundated mud surface. The western one-third of the unit was covered with water to a depth of two inches. Two general cage positions were used during the water level stabilization periods. One cage was placed in the water section and the other upon the drying mud well back from the stabilized shore line.

Water was put into the unit at 3:33 PM on July 15th. Four caged ducks were placed at intervals along the length of the impoundment. The first water samples were taken from flooded cages at 4:30 PM (Figure 5). The former shore line at this time was under three inches of water. Additional water samples were taken at intervals of 19 and 43 hours after initiation of the experiment. The experiment was terminated on July 18th.

The water level was restabilized on July 20th. The maximum interval of mud exposure to the air was four days. Flooding began on 9:30 AM on July 20th. Three caged ducks were placed in the unit. The first water samples were taken at 10:30 AM (Figure 6). The flow of water was cut off at 11:00 AM, at which time the water was three inches deep over the former shore line. Additional water samples were taken at intervals of 52, 2h, and 48 hours after initiation of the experiment. The experiment was terminated on July 23rd.

The position of the declining water level was marked each day until it reached experimental level on July 27th. This procedure defined the zones of mud exposed each day. On July 27th at 2:25 PM five cages were placed in the various zones and the unit flooded until the former shore
Legend:

- Stabilized shore
- Extent of flooding
- Water sample

- 1 hour
- 10 hour
- 43 hour

Figure 5. Unit 2, July 15th.
Figure 6. Unit 2, July 20th.
line was under one inch of water. The first water samples were taken at 3:25 PM from flooded cages (Figure 7). Additional water samples were taken at intervals of 19, 43, and 77 hours after initiation of the experiment. The experiment was terminated on July 31st.

The water levels were raised by rainfall on August 4th and 5th, and were restabilized on the latter date. The excessive amount of water used to offset daily evaporation on August 17th disrupted the stabilization period. The experimental level was re-established on August 21st.

Water was put into the unit at 10:00 AM on September 3rd. Seven caged ducks were put into the unit at 11:00 AM. The first water samples were taken from flooded cages at 12:30 PM (Figure 8 and Plate 7). An additional water sample was taken 19 hours after initiation of the experiment. The experiment was terminated on September 5th.

The water level evaporated down to the stabilization level on September 15th and was maintained until September 26th. On this date water was put into the unit at 10:00 AM. At 11:05 AM two cages were placed in the water which was one-eighth to one-quarter inch deep over the former shore line. Ten cages were placed in the unit at 2:30 PM. The first water samples were taken at 11:00 AM (Figure 9). Additional water samples were taken at intervals of $\frac{3}{2}$, 2h, and 30 hours after initiation of the experiment. The experiment was terminated on September 28th.

A total of 160 water samples was tested for toxicity in mice, and 210 Pekin ducks were exposed during the study period. Toxin production was not detected in the unit.

Unit 3.—This unit was established in order to test the toxicity of a continuously stabilized shore line. One-half of the unit was covered
Figure 7. Unit 2, July 17th.
Figure 8. Unit 2, September 3rd.
Legend:
- Stabilized shore 1
- Extent of flooding
- Cage shift

Water samples:
△ 1 hour
○ 5½ hour
□ 24 hour
▽ 30 hour

Figure 9. Unit 2, September 26th.
with water to a depth of three inches. Two caged ducks were used in the unit. One cage was placed upon the stabilized edge and the other was located in the water. The cages were moved daily to new sampling areas, a fresh duck inserted, and water samples taken.

Rainfall on August 4th and 5th raised the water level. The stabilization program was begun anew on August 6th.

A total of 16 water samples was tested for toxicity in mice, and 156 Pekin ducks were exposed during the study period. Toxin production was not detected in the unit.

Unit 4—This unit was established as a water manipulation area. On July 9th the water levels were adjusted to place the shore line on a previously inundated mud surface. Two-thirds of the unit was covered with water to a depth of four inches. Two general cage positions were used during water stabilization periods. One cage was placed in the water and the other was upon the drying mud, well back from the shore line.

At 9:30 AM on July 20th water was put into the unit. Three caged ducks were placed at intervals along the length of the impoundment. The first water samples were taken at 11:00 AM (Figure 10). The former shore line was under one inch of water. Additional water samples were taken at intervals of 6\frac{1}{2}, 2\frac{1}{2}, and 48 hours after initiation of the experiment. The experiment was terminated on July 23rd.

Rain on August 4th and 5th disrupted the water level stabilization period and the levels were restabilized on August 5th.

At 2:00 PM on August 20th water was put into the unit. The water was one-half inch deep over the former shore line at 4:00 PM. The water was shut off and six caged ducks were placed in the unit. The first water samples were taken at intervals of 18 and 24 hours after initiation.
Figure 10. Unit 4, July 20th.

Legend:
--- Stabilized shore
- Extent of flooding
□ Cage shift

Water samples
Δ 1 hour
○ 6½ hour
□ 24 hour
▽ 48 hour
of the experiment (Figure 11). The experiment was terminated on August 22nd.

The water level was stabilized on August 21st. At 9:10 AM on September 12th water was put into the unit. The water was one and one-half inches deep over the former shore line at 11:30 AM. Ten duck cages were placed in the unit and the first water samples were taken (Figure 12). Additional water samples were taken at intervals of 5 and 24 hours following initiation of the experiment. The experiment was terminated on September 14th.

A total of 133 water samples was tested for toxicity in mice, and 196 Pekin ducks were exposed during the study period. Toxin production was not detected in the unit.

Unit 5.—This unit was originally established to simulate a marsh area covered with three to five inches of water. One caged duck was confined in this unit. The cage position was changed daily, a fresh duck inserted and a water sample taken. On August 25th the initial plan was abandoned and the water level was lowered to create a puddle dotted mud flat in the western portion of the unit. The reason for this action is given in a later part of this section.

One duck was confined in a cage moved daily about the puddle dotted area. A fresh duck was used each day and a water sample was taken. This procedure was followed until termination of the study.

A total of 81 water samples was tested for toxicity in mice, and 76 Pekin ducks were exposed during the study. Toxin production was not detected in this unit.

Site 6.—This test position was located adjacent to the impoundments in the natural refuge Unit 1 area. Two cage positions were used. One cage
Figure 11. Unit 4, August 1984.

Legend:

- Stabilized shore

- Extent of flooding

- Cage shift

Water samples

\(\Delta\) 2 hour

\(\circ\) 18 hour

\(\square\) 24 hour
Legend:
--- Stabilized shore 1
— Extent of flooding
□ Cage shift

Water samples
△ 1 hour
○ 5 hour
□ 24 hour

Figure 12. Unit 4, September 12th.
was placed upon the dry mud flat and the other in water which varied from two to four inches in depth. The cages were moved daily, a fresh duck inserted and a water sample taken from the wet cage site. The dry cage was flooded by rising refuge waters on July 20th, August 1st, 8th, and 24th, September 19th and 20th.

A total of 84 water samples was atoxic to mice, and 153 Pekin ducks were exposed during the study period. A Pekin duck placed in a cage in two inches of water on August 26th was found dead on August 29th. Injections of the blood serum of this duck killed only mice unprotected with botulinus antitoxin. The liver was removed aseptically and cultured in trypsinase soy broth at 37 degrees C. Toxin was demonstrated and confirmed by antitoxin tests. Washings of the contents of the ventriculus were atoxic to mice.

A duplication in the use of the impounded units became apparent August 25th. This led to the abandonment of the experimental plans initially set for the operation of the units. Unit 5 was originally established to test for toxin production in water three to five inches in depth. The duck cages positioned in the water in Units 1, 2, 3, and Site 6 served as checks for toxin production in water of similar depth. Further, it was felt that more flooding experiments should be conducted. A revision of plans was made and Unit 5 was accordingly drained and converted to a puddle dotted mud flat, thereby concentrating many forms of animal life in the shallow basins. The water level in Unit 1 was lowered and a shore line stabilization and flooding program instituted.

Table IX summarizes the flooding experiments conducted in the diked units.
<table>
<thead>
<tr>
<th>Unit</th>
<th>Date</th>
<th>No. Days</th>
<th>No. Caged Ducks</th>
<th>Dates of Water Sample Collection</th>
<th>Firstname</th>
<th>Lastname</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sept. 20</td>
<td>16</td>
<td>20</td>
<td>1 5 24 30</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>July 15</td>
<td>4</td>
<td>4</td>
<td>1 19 24</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>July 20</td>
<td>6</td>
<td>6</td>
<td>1 19 24 30</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>Sept. 26</td>
<td>12</td>
<td>12</td>
<td>1 19 24</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>5</td>
<td>Aug. 20</td>
<td>18</td>
<td>18</td>
<td>1 19 24</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>
The second time was used as a control. One-quarter inch of water was added to the culture containing the lenticular toad, and culture media of lower and typhaceous separations were brought at 27 degrees C. The toad was demantled and cultured in triplicate for 90 days. 

The toad was not demantled in any of the experiments. The culture was not demantled in any of the experiments. 

Two small roots were set up in duplicate situations.

In the Rocky Mountain Laboratory, it was determined that the presence of the botulism toxin was detected by commercial methods. These were sent to the Rocky Mountain Laboratory to determine the presence of botulism by commercial methods. These were sent to the Rocky Mountain Laboratory to determine the presence of botulism by commercial methods.

Experiment 1: Portion of 12 non-sterile duck livers were taken from a number of healthy, growing animals.

The direction.

Limited experimentation of this nature.

Results obtained for investigation were other than for the main program of the study. The method of the study was intended to find answers to questions which arose during the course of the study. These experiments were conducted during the study period. They
manner was toxic to mice. Water samples were taken from both rings at intervals of 1, 19, 24, and 72 hours following initiation of the experiment. Only the one hour water sample from the ring containing the culture was toxic to mice.

Experiment 3.—On September 13th, thirty cubic centimeters of trypti-case soy broth were poured over the soil surface of the ring which contained the culture material. This was done in an attempt to stimulate the botulinum organisms known to be in the soil. One-half inch of water was added to the ring. Toxin was not demonstrated in soil cores taken prior to flooding nor in water samples which were taken afterward at intervals of 1, 19, and 24 hours.

**Toxin**

The solutions of suspected toxic material were prepared for injection into mice in the usual manner. Any deviations from this procedure are noted in the text.

Experiment 1.—On August 10th an ampule of dehydrated toxin of a titer of 100,000 M.L.D. (mouse-intraperitoneal) was taken into the field and mixed with 50 cc. of mud and water. The resulting suspension had a maximum titer of 2,000 M.L.D. This material was initially used in checking for titer loss in field collected samples as previously described. The mixture was placed in a stoppered flask and refrigerated at 4 degrees C. A number of experiments were conducted to test the stability of the toxin in the septic mixture. The inoculum was taken either from the clear supernatant liquid overlying the sedimented soil particles or the solution was thoroughly mixed prior to taking the sample. The results of the experiment are presented in Table X.
TABLE I
SUMMARY OF EXPERIMENT 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Source</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 12</td>
<td>0.1 cc of a dilution (20 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.025 cc (50 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.1 cc (200 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td>Aug. 13</td>
<td>0.025 cc (50 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.1 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td>Aug. 14</td>
<td>0.1 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>2 of 2 mice</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (300 M.L.D.)</td>
<td>mixed solution</td>
<td>2 of 2 mice</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (400 M.L.D.)</td>
<td>mixed solution</td>
<td>2 of 2 mice</td>
</tr>
<tr>
<td>Aug. 22</td>
<td>0.05 cc (100 M.L.D.)</td>
<td>mixed solution</td>
<td>1 of 2 mice</td>
</tr>
<tr>
<td></td>
<td>0.1 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (300 M.L.D.)</td>
<td>mixed solution</td>
<td>2 of 2 mice</td>
</tr>
<tr>
<td>Aug. 28</td>
<td>0.1 cc (200 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (300 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (400 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.1 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (300 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (400 M.L.D.)</td>
<td>mixed solution</td>
<td>1 of 2 mice</td>
</tr>
<tr>
<td>Sept. 11</td>
<td>0.2 cc (400 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.25 cc (500 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.3 cc (600 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.4 cc (800 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (400 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.25 cc (500 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.3 cc (600 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.4 cc (800 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
</tbody>
</table>

Experiment 2.—On August 22nd an ampule of dehydrated toxin of a titer of 500,000 M.L.D. (mouse-intraperitoneal) was taken into the field and mixed with 50 cc of mud and water. The resulting solution had a maximum titer of 5,000 M.L.D. This material was initially used in checking for titer loss in samples which were collected in the field as previously described. The mixture was put in a stoppered flask and placed in mud in the field to a depth level with the surface of the mixture. The results of the experiment are presented in Table XII.
TABLE XI

SUMMARY OF EXPERIMENT II

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Source</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 24</td>
<td>0.25 cc of a dilution (25 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.25 cc of a dilution (50 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.02 cc (100 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.04 cc (200 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.125 cc of a dilution (25 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.25 cc of a dilution (50 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.02 cc (100 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.04 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td>Aug. 27</td>
<td>0.02 cc (200 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.06 cc (100 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.08 cc (200 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.02 cc (200 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.06 cc (300 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.08 cc (400 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td>Sept. 1</td>
<td>0.1 cc (500 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (750 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (1000 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.1 cc (500 M.L.D.)</td>
<td>mixed solution</td>
<td>unprotected</td>
</tr>
<tr>
<td></td>
<td>0.15 cc (750 M.L.D.)</td>
<td>mixed solution</td>
<td>unprotected</td>
</tr>
<tr>
<td>Sept. 6</td>
<td>0.2 cc (1000 M.L.D.)</td>
<td>clear solution</td>
<td>l of 1 mouse</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (1250 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.3 cc (1500 M.L.D.)</td>
<td>clear solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.2 cc (1000 M.L.D.)</td>
<td>mixed solution</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.25 cc (1250 M.L.D.)</td>
<td>mixed solution</td>
<td>l of 1 mouse</td>
</tr>
<tr>
<td></td>
<td>0.3 cc (1500 M.L.D.)</td>
<td>mixed solution</td>
<td>l of 1 mouse</td>
</tr>
</tbody>
</table>

The results of both Experiment 1 and Experiment 2 indicate that
toxin may have affinity for soil particles, and may be rarely distributed
throughout undisturbed water. The results also show that the rate of
loss of toxicity in the toxin sample stored in the mud was greater
than in the refrigerated mixture.

Experiment 3.—At 1:30 PM on August 23rd an ampule of dehydrated
toxin of a titer of 500,000 M.L.D. (mouse-intraperitoneal) was mixed with
50 cc of water. The mixture was poured in approximately equal portions
over the mud surface within two small rings. A control ring was located
adjacent to the test rings. At 11:00 AM on August 24th a film of water
was poured over the mud in each ring. Samples taken five minutes later were atoxic to mice. The mud surface in each ring was stirred to a depth of one inch and an additional sample taken. These samples were atoxic to mice.

On August 25th a soil core was taken to a depth of one inch from each ring. The soil extract was injected into mice. One test ring soil extract was toxic.

All similar samples taken on August 26th were atoxic. This work indicates a possibility of rapid loss of toxicity in field planted toxin samples. This observation agrees with the work of Quortrup and Sudheimer (1942).

**Experiment 4.**—On August 23rd a number of small rings were placed on a mud flat at 11:45 AM and various organic materials added to the mud within each ring. The liquid media were injected randomly into the mud to a depth of one inch. The solid media were worked into the top one-half inch of soil surface. In some instances the mud surface was stirred prior to taking water samples for toxicity tests. The first samples were taken at 4:30 PM. Table XII summarizes the results of the experiment.
TABLE XII

SUMMARY OF EXPERIMENT 4

<table>
<thead>
<tr>
<th>Site</th>
<th>Medics</th>
<th>8/23</th>
<th>8/24</th>
<th>8/25</th>
<th>8/26</th>
<th>8/27</th>
<th>8/28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated mud</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 trypticase soy broth</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 control</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 trypticase soy broth</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 control</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moist mud</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 corn meal</td>
<td>atoxic</td>
<td></td>
<td>atoxic</td>
<td></td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td>2 control</td>
<td>atoxic*</td>
<td></td>
<td>atoxic</td>
<td></td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td>3 corn meal</td>
<td>atoxic*</td>
<td></td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td>4 control</td>
<td>atoxic</td>
<td></td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td>5 trypticase soy broth</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 trypticase soy broth</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 liver</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 liver</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet pool, 1/4 inch deep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 trypticase soy broth</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 trypticase soy broth</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 corn meal</td>
<td>atoxic*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 corn meal</td>
<td>atoxic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#mud stirred to a depth of one inch five minutes before taking water sample

Experiment 5.—On August 23rd a number of small rings were placed in duplicate at various sites and trypticase soy broth poured over the mud surface within one of each pair of rings. In some instances water samples were taken both before and after stirring the mud within the ring. Table XIII summarizes the results of the experiment.
### TABLE XIII

**SUMMARY OF EXPERIMENT 5**

<table>
<thead>
<tr>
<th>Site</th>
<th>Ring</th>
<th>8/23</th>
<th>8/24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud under water one inch deep</td>
<td>Control</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stirred</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>not stirred</td>
<td>atoxic</td>
<td>no sample</td>
</tr>
<tr>
<td>Mud under water one-eighth inch deep</td>
<td>Control</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stirred</td>
<td>atoxic</td>
<td>no sample</td>
</tr>
<tr>
<td></td>
<td>not stirred</td>
<td>atoxic</td>
<td></td>
</tr>
<tr>
<td>Moist mud</td>
<td>Control</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stirred</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>not stirred</td>
<td>atoxic</td>
<td>no sample</td>
</tr>
<tr>
<td>Dry mud</td>
<td>Control</td>
<td>atoxic</td>
<td>atoxic</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stirred</td>
<td>atoxic</td>
<td>no sample</td>
</tr>
<tr>
<td></td>
<td>not stirred</td>
<td>atoxic</td>
<td></td>
</tr>
</tbody>
</table>

**Experiment 6.**—Miscellaneous samples were taken on September 3rd from an area of duck sickness south of the Bear River Migratory Bird Refuge for toxicity tests in mice (Plate 8). The samples tested are as follows:

- Water under dead duck: toxic (1 of 1 mice dead)
- Water from isolated pool: atoxic
- Stirred mud in pool: atoxic
- Mud at edge of isolated pool: atoxic
- Liquid around Ostracods: atoxic
- Gloeochiria balls soaked in water: atoxic
- Gloeochiria balls, macerated: atoxic
LIMITED OBSERVATION OF NATURAL OUTBREAKS

The material discussed in this section is presented in Figure 13. In most instances the data were taken from refuge records. Weather data were obtained from standard Weather Bureau instruments located at refuge headquarters. The refuge is nearly 100 square miles in area. Therefore, it is not assumed that the data presented in the accompanying figure are representative of conditions over the entire refuge area; at best, they may be used as an indicator of general conditions.

The observation of natural outbreaks was a secondary objective of the study program. The gathering of sick and dead ducks entails much time and requires special equipment and many man hours of labor. The fulfillment of the primary objective of the program restricted participation in assaying the extent and distribution of botulism on the refuge.

Wind Movement

The wind recording apparatus was calibrated to register the number of miles of wind movement for each 24 hour period. This information may not reliably indicate the probable influence of the force of wind upon water levels. A slight breeze of constant velocity may, over a 24 hour period, register a total mileage comparable to that of a strong wind over a much shorter period of time. Only the latter wind, or one of similar nature, may cause inundation of the damp mud margins of the shallow refuge lakes. The direction of the wind is also an important factor. A wind from the south is potentially able to flood the greatest area of exposed mud. This is discussed in the consideration of the water level fluctuation. Wind direction-recording equipment was not available at
Figure 11. Natural Outbreak Data
the refuge. Persons familiar with the refuge agree that nearly all the summer winds blow from a southerly direction.

**Water Level Fluctuation**

The refuge contains five shallow, diked lakes designated by unit number (Figure 1). Each unit or lake is approximately 5,000 acres in area. The water level of each unit can generally be controlled. The remaining refuge acreage consists of expanses of shallow water and mud flat. The demands of agricultural interests for water for irrigation from the Bear River generally determines the volume of water available for refuge use. Thus, the water supply may vary both seasonally and annually.

The refuge is built upon the delta of the Bear River. The land gradient slopes down to the south and west. The gradient is approximately 12 inches to the mile. The units occupy the northern portion of the refuge lands. They are confined on the south by a main dike which extends roughly in an east-west direction. Spur dikes extending from the main dike separate the various units. The water level in each unit may be regulated by addition or removal of water. The volume of water which is removed from the units governs the extent of exposed mud areas in the refuge lands south of the main dike.

When the water levels of the units decline, expanses of drying mud flat are exposed at the northern end of each unit. A strong south wind can force the lake water to flow back over the mud. On some occasions the wind has driven water across the mud for distances in excess of one-half mile. Sperry (1947) found this condition to be conducive to botulism outbreaks.

Water level gauges are located at the south end of each unit near the junctions of the spur and main dikes (Figure 1). The units are deepest
near the main dike. In periods of calm or north wind the water is at its highest level at this end. However, a south wind will force the water toward the shallow end of the unit. In this situation a lower water level is registered on the gauges. It may take several days of calm weather before the water level will regain equilibrium.

The water level gauge reading in any one unit is not a true measure of actual water level fluctuation for the entire unit. The reading is governed by the position of the gauge and the wind direction. Reference A on Figure 13 charts, at 12 hour intervals, the water level fluctuations registered on a self-recording, portable water level instrument (Plate 9) which was located near the experimental impoundments in refuge Unit 1. The unit water level gauge was located approximately one and one-half miles south of this instrument (Figure 1). Reference is made to the 12 hour reading of the portable instrument between September 20th and 21st. The Unit 1 gauge indicated a rise of water level to 1205.0 feet which should mean a decline in the water level at the northern end of the unit. However, the portable instrument registered 0.2 of a foot rise in water level for the same period. This observation will be discussed more fully in the section on duck mortality from botulism.

Precipitation

It is impossible with the few data given on the incidence of sick ducks to attempt correlation of rainfall and botulism outbreaks during the 1951 season.

Air Temperature

The temperature data are presented after Quortrup (1940) to permit comparison with his observations. The air temperatures recorded
throughout the summer are:

Pre-botulism season: July 1st to August 15th
mean minimum temperature 15.4 degrees C.
mean maximum temperature 32.1 degrees C.
average temperature 23.7 degrees C.
high temperature for period 37.8 degrees C.
low temperature for period 10.0 degrees C.

Botulism season: August 16th to September 15th
mean minimum temperature 12.4 degrees C.
mean maximum temperature 28.4 degrees C.
average temperature 20.4 degrees C.
high temperature for period 33.9 degrees C.
low temperature for period 5 degrees C.

Post-botulism season: September 16th to October 3rd
mean minimum temperature 7.9 degrees C.
mean maximum temperature 23.4 degrees C.
average temperature 15.6 degrees C.
high temperature for period 28.9 degrees C.
low temperature for period 3.8 degrees C.

The air temperatures are generally much lower than the optimum laboratory culturing temperature (37 degrees C.) for the botulinus organism. On July 18th the warmest air temperature (37.8 degrees C.) was recorded for the summer period. No duck sickness was noted on the refuge at this time. There appears to be no direct correlation between high temperatures and duck sickness.
Incidence of Botulism

It has been previously indicated that the censusing of sick and dead ducks was beyond the scope of the present investigation. The refuge superintendent, Mr. V. T. Wilson, kindly furnished the following data which was compiled from the botulism outbreaks. A total of 14,365 dead ducks and 1,273 sick ducks were collected by refuge personnel during the summer and early fall of 1951. The sick ducks were recorded by species, date, and general area of collection. Only the species and total number of dead ducks in each general area were recorded. The latter data are of no use in charting outbreaks. The distribution by time of sick ducks is presented in Figure 13.

The sick and dead ducks were collected when the refuge superintendent had men available for the work. As a consequence, the data which were recorded are not a compilation of continuous and equal effort. It is worth noting that in most instances collection crews were sent into the field shortly after occurrence of water level fluctuations which are generally associated with botulism outbreaks.

Reference B (Figure 13) is an addition of dead ducks collected by the writer to the sick ducks reported on this date. Both the dead and sick birds were found a short distance west of the experimental impoundments. The collection of dead birds was not complete. This area could be observed easily during routine work. No sick ducks were in evidence on July 25th. A strong south wind blew for about one hour on the evening of July 25th. The sick and dead birds were found the next morning. Approximately 90 per cent of the total number of birds observed were dead.

On Figure 13 a general lack of synchrony exists between the
occurrence of sick ducks and water level fluctuations. The more graphic examples of this are in Unit 3 on August 29th and September 12th and in Unit 4 on September 12th. Two important factors should be considered; one, the relative position of each water level gauge, and two, the very long north shore line of each unit. A large number of sick ducks were collected in Unit 3 on August 29th and 30th. The water level curve shows a deepening of water at the south end of the unit which should cause exposure of mud at the north end. This observation is contradictory to the basic hypothesis of the study. However, the water gauge in Unit 2 registers a decline of 0.2 of a foot in water for the same period. It appears that a southwest wind caused the water decline in Unit 2. This same wind would force water into the vicinity of the gauge in Unit 3. It would also flood mud areas in the northwest portion of the unit. Therefore, it can be seen that gauge readings for any one unit may not be a true indication of water movement over the whole unit. This observation on unit water level readings and probable wind direction can be quite successfully applied to each conspicuous disagreement between the number of sick birds collected and the unit water level curve. This fact further substantiates the original premise on the development of botulism outbreaks.

In some instances (Figure 13) the sharp decline of water levels at the water level gauges apparently is not followed by an outbreak of botulism. Some reasons for this incongruity between the basic hypothesis of the study program and the refuge records of the sick and dead ducks which were collected may be summarized as follows:

1. The collection of sick ducks did not immediately follow development of toxic conditions.
2. The majority of the birds which were collected were dead. This may be attributed to: one, the production of toxin of high titer; two, a widespread production of toxin which favored its ingestion by birds; three, the feeding activities of the birds on the area increased their chances of repeatedly contacting toxin; four, the interval between toxin ingestion and bird collection was favorable to increased mortality; five, the interval between toxin ingestion and bird collection was favorable to recovery of many birds which otherwise may have been recorded as sick ducks. As previously indicated, the data on dead birds are compiled by species and area of collection. No dates of collection are given.

3. Very few waterfowl utilized the area at the time of flooding.

4. The wind did not push the water in the direction of a mud flat.
DISCUSSION

Duck sickness did occur among waterfowl at the Bear River Migratory Bird Refuge in 1951. Refuge records show that 1,273 sick and 11,365 dead ducks were collected from July 12th through September 26th. Therefore, conditions favorable to toxin production must have existed in the field. Some experienced waterfowl workers may point out that the number of afflicted birds was not excessive, the outbreaks appeared to be of very short duration, and toxicity, therefore, was not generally prevalent throughout the refuge. This observation may be correct. However, the fact remains that botulism did occur naturally whereas attempted duplication of the more obvious natural conditions failed to produce toxicity.

Fish and Wildlife Service personnel have made many observations of botulism outbreaks following flooding of drying mud flats. Williams (1940), Quortrup (1943b), and Sperry (1947) present data which substantiate the observations.

The writer's limited observation of duck sickness in the field indicates a probable correlation of wind, water level movement, and outbreaks of botulism. This is in keeping with previous observations. The instances of toxicity adjacent to the experimental units have been discussed. The major numbers of sick and dead ducks are distributed along the shore and in shallow offshore water. Williams (1943) states that flareups in sickness do definitely follow wind action and that the afflicted birds do not represent ducks that have drifted ashore.

The negative character of the results from this study program does not conform with observations of natural outbreaks. However, data from outbreaks this past summer do agree with the primary hypothesis. The flooding dates of some diked units and rings coincides closely with
the appearance of sick and dead ducks in the field, but toxicity was not detected in the experimental areas. The technique of collection, preparation, and testing suspected toxic water samples appears to be sound. Additional experiments indicated the likelihood of toxin being associated with mud. The water samples were taken at the bottom and invariably included some mud. The Pekin ducks liberally sifted through the mud and the stirring of mud in the small ring experiments should have brought pre-formed toxin into solution. If conditions conducive to botulism toxin production were duplicated in the experimental areas, the methods used for its detection should have been satisfactory.

We have, governed by the necessity of the situation and in an attempt to gain knowledge of definite character, relied wholly upon the procedure of flooding portions of an exposed mud flat. While conducting a review of literature on duck botulism it became evident that some workers were speculating about toxin production and the source of toxin to waterfowl. Conversation with persons who were interested in botulism disclosed many divergent ideas relative to outbreaks of botulism. Much imaginative thought has been given to the problem. However, since many of the opinions have little basis in controlled experiments, not much could be gained by describing them here.

The experimentation consisted of an attempt to create toxicity in small diked areas by simulating, as closely as possible, the gross conditions which were set forth by investigators of botulism. The negative results of the study are evident. There remains, in the final analysis of this study, certain obvious discrepancies between our experimental procedures and the nature of conditions commonly associated with natural outbreaks of botulism. In the following discussion the writer will
attempt to analyze these discrepancies.

There has developed over the years a consideration of various factors in relationship to botulism outbreaks. They are evaporation and precipitation, temperature, the presence of birds, chemical factors, wind, water level movement, and the botulinus organism.

The experimental and natural sites had similar exposure to evaporation and precipitation, temperature, presence of birds, and the botulinus organism. The organism has been demonstrated in many materials and places on the refuge. It seems to have an ubiquitous distribution. Evaporation, precipitation, and temperature should be similar since each site had equal exposure. As previously discussed, none of these three factors have been directly linked with botulism outbreaks. Evaporation is thought of solely in terms of declining water levels although the action may influence the concentration of salts in the water. General precipitation apparently has no direct nor constant correlation with duck sickness. Its relation to toxin production is probably more accurately expressed in terms of raising water levels. The usual summer temperature range appears to have no stimulative or inhibitory influence on toxin production. Both domestic and wild ducks utilized some of the experimental areas. Laboratory work has indicated the contaminating influence of flocks of ducks has little relation to toxicity. The variable abundance of ducks using an area may be viewed as a differential in degree of sampling of various areas. Thus the difference in the number of waterfowl afflicted in any area may be proportional to the total number using the area.

Chemical factors, wind, and water level movement may not have been precisely duplicated in all of the rings and diked units. Some investigators
believe only pH and dissolved oxygen of the water are worthy of further investigation. (Quortrup and Sudheimer, 1942a).

The water for flooding ringed areas was taken directly from the lake water. The water used in the diked units was taken from a nearby borrow pit. However, in neither area, ring or diked unit, was toxin detected.

The walls of the diked units and rings were barriers to the wind and prevented disturbance of the water. Outside of the experimental units strong winds caused waves which tore at the aquatic vegetation and cast it ashore. The waters were roiled with silt and debris. This physical action of wind driven water could not be duplicated in the experimental areas. The stirring prior to water sample collection in the small rings was done to put toxin into solution. The mixing of mud and water by the ducks did not make toxin available as neither ducks nor the water samples indicated it. Only preformed toxin would be demonstrated in these instances.

Another difference in the action of wind, stirring, and the activity of experimental ducks is the manner and duration of the physical action brought about by strong winds. The organic content of mud varies widely, as shown in Table III. The decomposition of the organic debris reduces the supply of dissolved oxygen. The wave action puts silt and organic debris into suspension. A preponderance of oxidizable material in the water may decrease the dissolved oxygen to a point favorable for elaboration of toxin. The abatement of wind and wave action is followed by a settling of the suspended materials. Photosynthesis by the aquatic plants would replenish the dissolved oxygen content of the water. Conversely, if the toxin is preformed at scattered foci, the buffeting
wave action may put it into solution and or dislodge the foci which are ingested by waterfowl.

The natural water level movement has not been accurately duplicated in the diked units. We have spoken of shore line stabilization in natural lakes in a hypothetical sense. It is doubtful if the shore line remains absolutely stable in the large refuge water areas. Reference A on Figure 13 reveals a dynamic water level condition at the shallow end of refuge Unit 4. This constant fluctuation would cause alternate flooding and exposure of the mud just above the waters' edge and may in some way contribute to toxicity. Thus the period of stabilization and subsequent toxicity may be more directly related to the overall period of wetting and drying rather than to the absolute maintenance of a stable shore line. Further, the work of Williams (1940) demonstrated that a period of water level stabilization is not mandatory prior to an outbreak of botulism.

It is stressed that no definite knowledge exists of the manner of toxin production in the field. Complete information on the modes of toxin availability to waterfowl is lacking. Research and observation have shown botulism outbreaks to closely follow flooding of exposed mud. The research program conducted this past summer dealt solely with an attempt to create toxicity in small areas by simulation of gross conditions believed to be contributory to outbreaks in the field. The results of this work do not agree with those of previous findings on toxicity of reflooded mud flats.
SUMMARY

1. A review of literature on botulism in waterfowl suggests a relationship of many outbreaks of botulism and inundation of the damp margins of shallow alkaline lakes.

2. During the summer of 1951, 109 flooding experiments were conducted on small areas of mud flat at the Bear River Migratory Bird Refuge in Utah. An attempt was made to simulate the conditions which have been observed in conjunction with natural outbreaks in past years.

3. The experiments consisted of controlled flooding of diked units 30 feet wide by 100 feet long, and movable watertight rings. Some rings were eight feet in diameter and others were six inches in diameter. Pekin ducks were exposed on diked areas and in the large rings. Periodic samplings of water and soil extracts from all flooding experiments were injected into mice for toxicity tests.

4. A total of 11 large ring experiments were conducted. Twenty soil extract samples and 32 water samples were tested for toxicity in mice. Thirty Pekin ducks were exposed to experimental conditions during the study period. Toxicity was not detected in the large rings.

5. A total of 64 small ring experiments were conducted. Eighty soil extract samples and 202 water samples were tested for toxicity in mice. Toxicity was not detected in the small rings.
6. The occurrence of boron intercalation greatly contributed with floodling
within or be absorbed to the soil particles.

7. These results were found to either confirm
recorded and first ordered samples with the test of the terpene
fraction test results. A rapid decrease in toxicity was found in both
oxygen and the toxin. The toxin was more toxic in the soil in
membrane experiments were conducted with the type C bloom.

They were not detected in the diked water.

The water samples were injected into mice for toxicity tests. Toxicity
concentrated and 799 ducks were exposed to experimental conditions and
recorded for toxicity tests. Twenty diked experiments were
continued in cages in the water. Periodic water samples were taken
the remainder of the diked water were used for flooding experiments and

6. Three of the five diked units were used for flooding experiments and
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PLATE 1. Holding pens for Pekin ducks. A series of five small enclosures in the foreground were used for exposed duck observation pens. The ducks were confined in the dry cage at the right prior to use. (U.S. Fish and Wildlife Service photo by E. R. Kalmbach)

PLATE 2. Large watertight rings. Experimental ring at right, natural site at left. The irrigation pump delivered water from channel in foreground to the ring. (U.S. Fish and Wildlife Service photo by E. R. Kalmbach)

PLATE 5. Water storage tanks. The water used for maintaining experimental conditions in the diked units was pumped from the channel into the tanks. The pipe delivered the water to the diked units. (U.S. Fish and Wildlife Service photo by E. R. Kalmbach)
PLATE 4. Mixed water areas. Workers are changing the Pekin ducks and the cage positions. Pipeline in center extends from the water storage tanks to the west wall of the impoundments. (U.S. Fish and Wildlife Service photo by E.R. Kalmbach)
PLATE 6. Water control valves at west end of the experimental units. (U.S. Fish and Wildlife Service photo by E. R. Kalmbach)

PLATE 7. Pekin duck cages. Photo taken on September 3rd during a flooding experiment in Unit 2. (U.S. Fish and Wildlife Service photo by E. R. Kalmbach)

PLATE 9. Portable water level gauge