The Ecology of Avian Botulism at the Salton Sea, California

A Thesis
Presented to the
Faculty of
California State Polytechnic University, Pomona

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biological Sciences

by
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1975

Acknowledgements

I would like to express my appreciation to Dr. L.J. Szijj, Dr. R.L. Barlet, Dr. E.K. Mercer, and Dr. G.R. Stewart for their invaluable assistance and encouragement during this study.

Special thanks are extended to Donald Tiller, Assistant Manager, Salton Sea National Wildlife Refuge, for supplying information regarding waterfowl population numbers.

I wish to thank the Bureau of Sportfisheries and Wildlife (BSFW) and the California Department of Fish and Game for their cooperation during this study.

I am indebted to Dr. Wayne Jensen, Bear River Refuge, Brigham City, Utah for kindly supplying specific antiserum without which testing would have been impossible.

Abstract

Botulism results from the ingestion of toxin produced by the bacterium Clostridium botulinum, of which there are six immunologically distinct types; these may be designated as A through F. Observations of waterfowl in and around the southern end of the Salton Sea indicate their close association with regions where botulism outbreaks occur. Samples of aquatic invertebrates, collected from waters bordering the Salton Sea, and liver taken from birds affected with botulism proved to be toxic when tested in a mouse protection test. Samples of mud, water, and vegetation proved to be non-toxic.

This study has shown that conditions favoring the growth of botulism bacteria may be present throughout the warmer months of the year. It is postulated that outbreaks occur when organic matter containing concentrations of bacteria or toxin are ingested by waterbirds. Usually only small numbers of waterbirds appear to be affected. Numerous fatalities occur only when large concentrations of waterbirds congregate in regions which favor growth of botulism bacteria and release of botulism toxin.

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Chapter I. Introduction

Botulism is caused by the ingestion of toxin produced by the bacterium Clostridium botulinum, an anaerobic, gram-positive bacillus which produces heat resistant spores (2). There are six specific types of botulism bacteria; these can be distinguished immunologically and are designated A through F. Human botulism is most often caused by types A,B, E, and F while botulism associated with birds is caused by types C and D and in some cases E (2) (19) (20).

Botulism toxin is one of the most powerful poisons known. However, it is heat liable and is quickly inactivated at 100 C within 10 minutes. Crystalized toxin has been found to have a molecular weight of 900,000 and is composed of 19 amino acids (20) (25). When ingested the toxin is absorbed by the stomach lining. Paralysis, due to inhibition of acetylecholine at cholinergic nerve endings, becomes evident as more and more of the toxin is absorbed. Death is the result of respiratory failure.

Sources of Toxin

Human botulism is usually associated with the ingestion of low acid home canned vegetables, fruits, and meat products. Home cannned and preserved foods have accounted for most outbreaks of human botulism since 1910, a small number of cases are ascribed to commericaly preserved foods. The sources of many outbreaks remain unknown.(34).

Avian botulism is thought to be associated with the ingestion of botulism toxin while feeding (1) (19) (22). Numerous theories have been proposed attempting to explain how toxin originates in items consumed by birds. Some researchers believe that toxin may be produced in vegetation as botulism bacteria grow (17) (10). Others hold that animal materials are necessary for the growth of bacteria and subsequent production and release of botulism toxin (10) (19) (20) (21).

Research

In the summer of 1910 a disatrous death toll among waterfowl in the western United States was observed (24). The malady responsible for the waterfowl deaths was given the name Western Duck Sickness. In years following the initial observed outbreak, large numbers of waterfowl continued to sucumb to the disease; most died during the summer months (12). In 1912 it was reported that 30,000 dead birds were picked up on the Weber River flats west of Ogden, Utah. On the delta of the Great Salt Lake 44,000 birds were gathered and burned between August 22 and September of that same year (24).

Attempts were undertaken to discover the cause of the massive waterfowl die-offs. Theories of what was responsible for the cause ranged from poisoning by the ingestion of chemical substances to the inhalation of sulfur fumes by those waterfowl affected (37) (38). It was not until 1930 that Hobamier (17) (18) established the presence of Clostridium botulinum type C toxin in the tissues of sick birds and identified the toxin as the causative agent responsible for waterfowl deaths. Some seven years

prior to this discovery toxin produced by type C bacteria had been found to cause "limberneck" in domestic chickens (24). Boutlism research has concentrated primarily upon the cycle of toxin production. Although there is some consensus of opinion as to the sources of the ingested toxin a great deal of conjecture concerning the exact conditions under which toxin is produced remains. Two theories concerning origin have emerged during the last sixty years. The first was proposed by Quortrup and Holt(30) in 1940 and was refered to as the sludge bed theory. The major tenets of the theory are as follows:

1. Clostridium botulinum bacteria exist in the mud and soil of lakes, marshes, and ponds.
2. Under certain conditions large quantities of decaying organic matter are available to the bacteria.
3. Dissolved oxygen in these areas becomes deminished as decomposition occurs, temperatures and pH are high.
4. Under these conditions the bacteria grow and toxin is produced.

A second theory, the microenvironment theory, was developed by Bell and his co-workers in 1955 (4). This theory emphysizes the importance of animal materials in bactrial growth and toxin production. The theory is as follows:

1. Clostridium botulinum type C spores germination, reproduction, and toxin production takes place in small discrete particles such as invertebrate carcasses.
2. These particles contain all the essentials for bacterial metabolism, and in that respect are independent of ambient medium.

3. The toxin ingested by waterbirds is probably in the bacteria which reside in the particles, rather than in the form of soluble, freely diffused toxin.

The microenvironment concept is the most widely accepted explanation for avian botulism outbreaks (22). Botulism toxin has been demonstrated in invertebrates, both dead and alive, aquatic and terrestrial (10) (20). Some researchers believe the disease can be contracted only by the ingestion of infected invertebrates. Invertebrates are believed to serve as incubators and food packets for disease causing bacteria. It is generally held that dead invertebrates are more toxic than those which are living. However, live invertebrates which are carrion feeders, such as the blow fly (Phaenicia sericata), may contain substantial quantities of botulism toxin (23).

Conditions Necessary for the Occurance of Avian Botulism

Botulism bacteria require specific conditions to grow and produce toxin. These conditions include proper pH, temperature , absence of oxygen and proper nutrient sources. Laboratory tests by Meker, Bell and Hoyer (28) indicate the optimum pH for all stages of metabolism occur with a rather narrow range on the acid side of neutrality (Table 1).with the greatest toxin production at pH 5.7. However, the pH of the ambient medium may not be critical if the bacterium is contained within particulate animal matter, such as an invertebrate carcass whose pH is closer to the optimum for bacterial growth.

There is disagreement as to the optimum temperature required for growth of bacteria in situ. The generally accepted temperature resulting in maximum vegetative growth in situ is 37 C (10). However, Hunter (20) believes that 25 C produces maximum vegetative

Table 1. Metabloisl of *Clostridium botulinum* type C in relation to pH* (28)

Activity	pH Range	Optimum**
Spore Germination	5.9-8.0	6.2-7.3
Growth in Vegetative Form	Not determined but probably broader than Germination	6.6
Inhibition of Growth	5.0-5.2	
Greatest Bacterial Population	6.1-6.3	6.3
Lowest Bacteial Population	5.7-5.8	
Sporulation	5.8-6.3	5.8-6.1
Toxin Production	5.7-7.0	5.7-6.2

* Data derived under conditions of controlled pH.
** Optimum level judged by amount of toxin produced.

growth in natural situations. Experiments by Quortrup and Sudiheimer (30) (31) indicating growth characteristics of botulism bacteria at varied temperatures are listed in Table 2. While maximum growth rate of bacterial populations are attained most rapidly at 37 C this rate persisted for only six days at which time it began to decline. At lower temperatures the time required to reach maximum growth rate may take longer, but when reached continues for as long as 19 days.

Although Clostridium botulinum requires anaerobic situations for growth in situ it can also be cultivated in the laboratory using a medium containing a reducing substance which prevents the formation of peroxides (20). Under natural conditions there is little to prevent vegetative growth of botulism bacteria provided adequate temperature and food are available. Anaerobic conditions can be brought about by aerobic bacteria which utilize available oxygen and then die. Areas most favoring growth of botulism causing organisms are low in oxygen content. When aerobic bacteria inhabit such areas and organic material is made available a suitable environment for botulism bacteria is soon created. If one accepts the microenvironment concept then external factors governing oxygen concentrations may be of little importance (4) (20).

Botulism bacteria require a protein rich medium in order to grow and produce toxin. Early researchers made reference to vegetation as a possible medium for botulism production (9) (30). More recent evidence indicates that while vegetation may serve as a food source for the small invertebrates in which toxin is developed animal matter is the most important food source for the bacterium (19) (27). Invertebrates are now considered to be the main source

Table 2. Optimum Temperatures of Growth of *Clostridium botulinum* (10)

Temperature	Toxin Developed	Maximum Growth Developed	Maximum Growth Persisted
37 C	2 days	3 days	6 days
30 C	2 days	4 days	13 days
25 C	3 days	8 days	16 days
20 C	6 days	20 days	19 days

of toxin production leading to avian botulism (9) (13) (21) (22) (23) (31).

Topography of Outbreak Areas

Areas in which botulism is likely to occur have a distinct topography (21) (22) (30). Suitable habitats for bacterial growth and toxin production are usually found along the finges of bodies of alkaline inland waters. Mudflats with shallow converings (2-3 inches) of water are ideal for possible botulism outbreaks (33). Agricultural lands which have been flooded by rain or irrigation overflow serve as another habitat in which botulism may occur. Any area that attracts large numbers of waterfowl and is subject to large and rapid changes in water level is a possible botulism site (17) (19) (22) (29). Such areas usually contain large numbers of invertebrates which may serve as an easy and abundant food source for waterfowl. Changes in water level may kill invertebrates making them even more readily available for hungery birds and at the same time produce micro-environments that promote the growth and reproduction of the botulissm causing organisms. Invertebrates may be concentrated by wind and wave action in flooded areas. Where there is a sudden drop in water level, the resulting dead invertebrates are much easier to obtain as food than when alive.

Objectives of the Study

Avian botulism has been studied in many areas of the Western United States, however, no studies have been undertaken at the Salton Sea. This study attempted to pinpoint the habitat, origin, and cycle of avian botulism at the Salton Sea. It was hoped that investigations would reveal the following information:

1. Those species of waterbirds most affected by avian botulism at the Salton Sea.
2. Those areas of the Salton Sea where botulism outbreaks most commonly occur.
3. The ecological relationships existing between affected avian species and the outbreak areas.

Chapter II. Description of Study Area

Initially the entire southern end of the Salton Sea was to be included in this study. However, the immensity of the area and the time restraints made such a study impossible. As a result areas populated by relatively large numbers of seasonal waterbirds were chosen. Another criterion used in selecting areas for study was the appearance of the region: if it appeared likely to be an area ecologically compatible with botulism production it was included.

Most of the work was concentrated in two large areas. Both were located along the southern end of the Salton Sea and included portions of a State and Federal waterfowl refuge (Fig. 1). The first study area extended from the mouth of the New River northward to Red Hill (Fig. 2). The second extended from Red Hill Marina northward to the Y16 section of the Imperial Waterfowl Management Area. Wister Unit (Fig.3).

The study was conducted along brackish waters bordering the Sea and in some cases in the Sea itself. Most of the areas were covered by very shallow water (3"-8") and were not subject to rapid changes in water level although seasonal fluctuations did raised water levels three feet or more. This is particularly true of the region designated by the California Department of Fish and Game as Y16. Changes in water levels were caused by irrigation run-off, precipitation and evaporation.

Shore vegetation in the area consisted of Tamarix sp., Atriplex sp., Allenrolfea occidentalis, and and a number of other plants

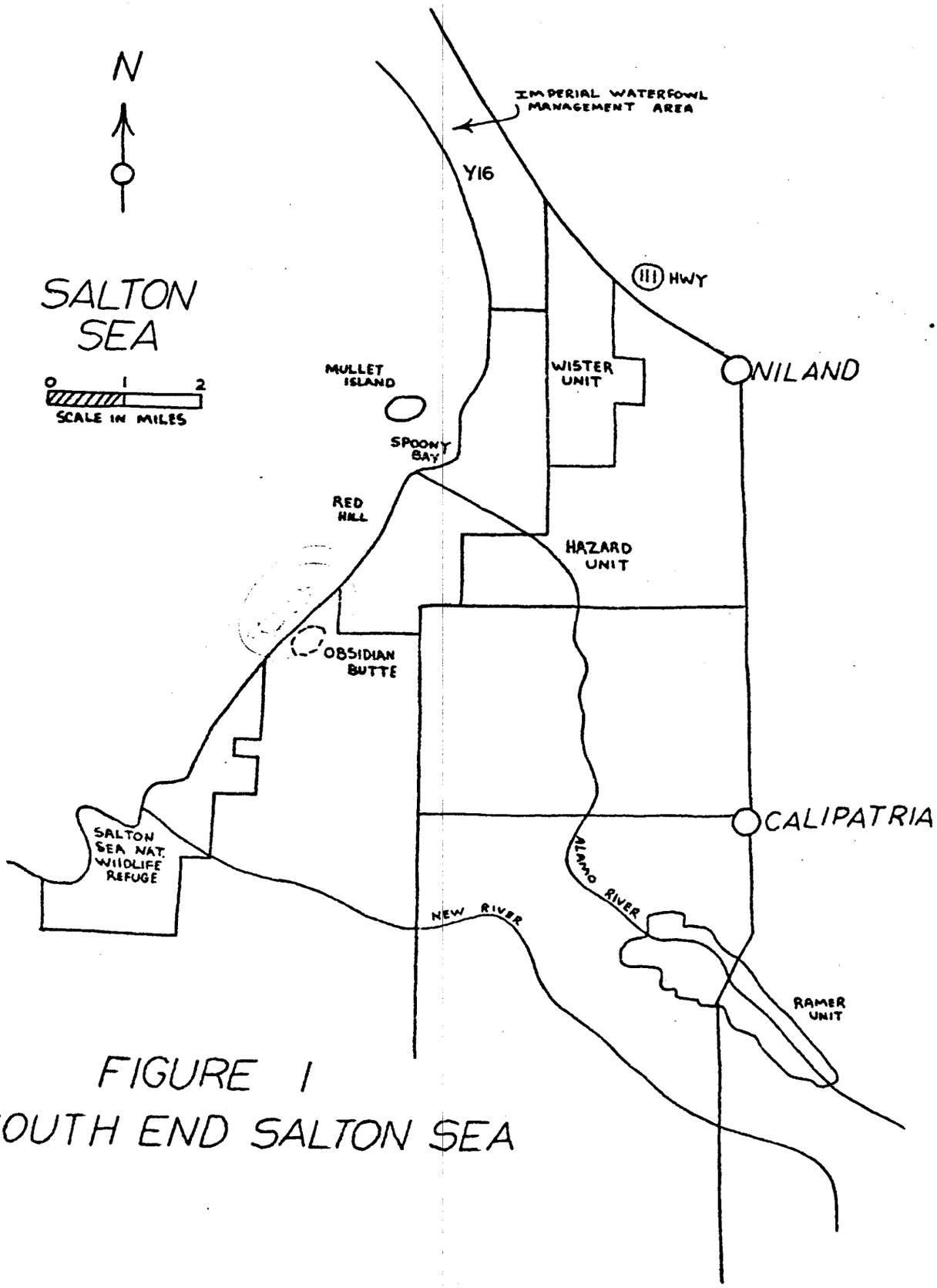


FIGURE 1
SOUTH END SALTON SEA

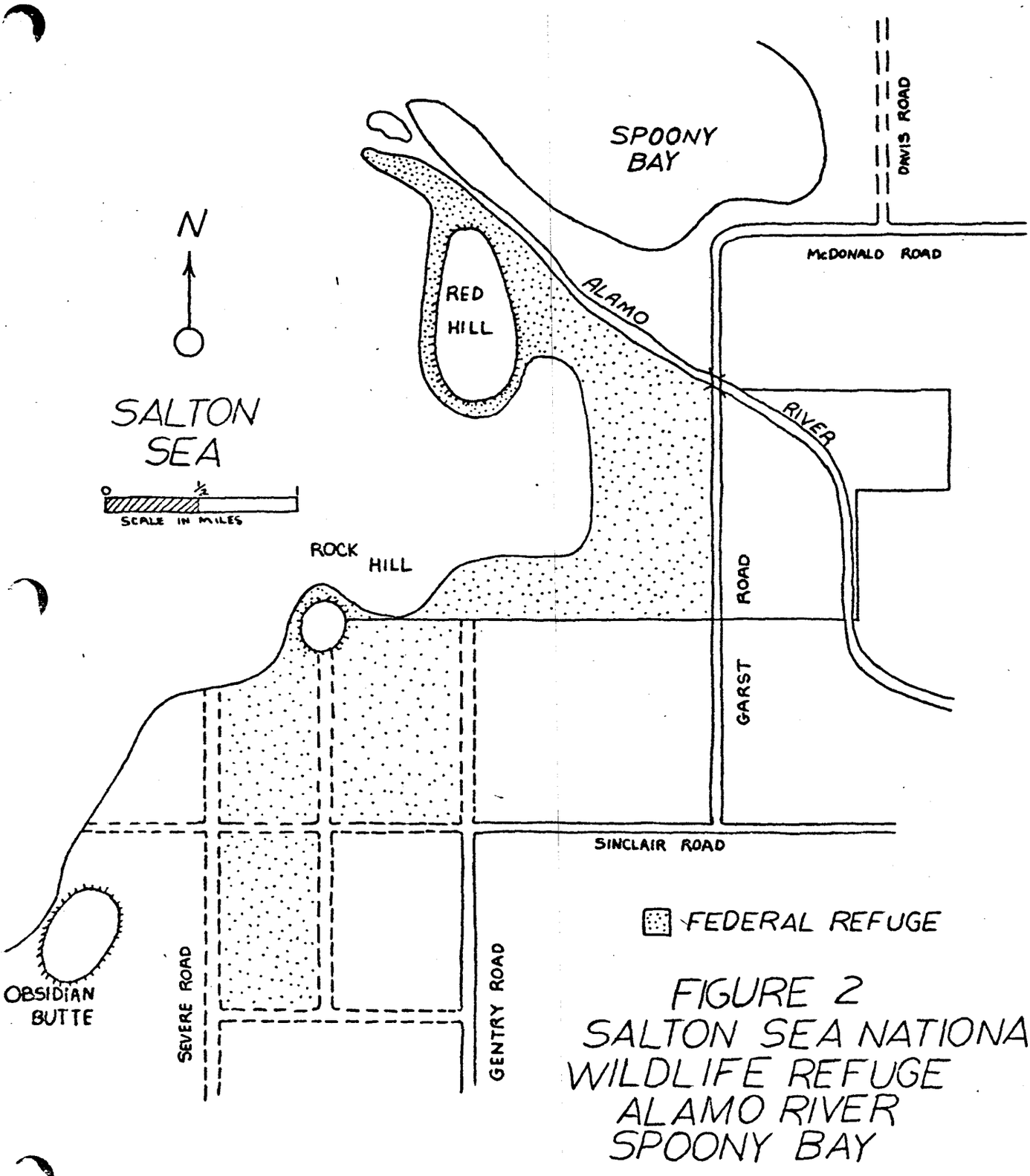


FIGURE 2
SALTON SEA NATIONAL WILDLIFE REFUGE
ALAMO RIVER
SPOONY BAY

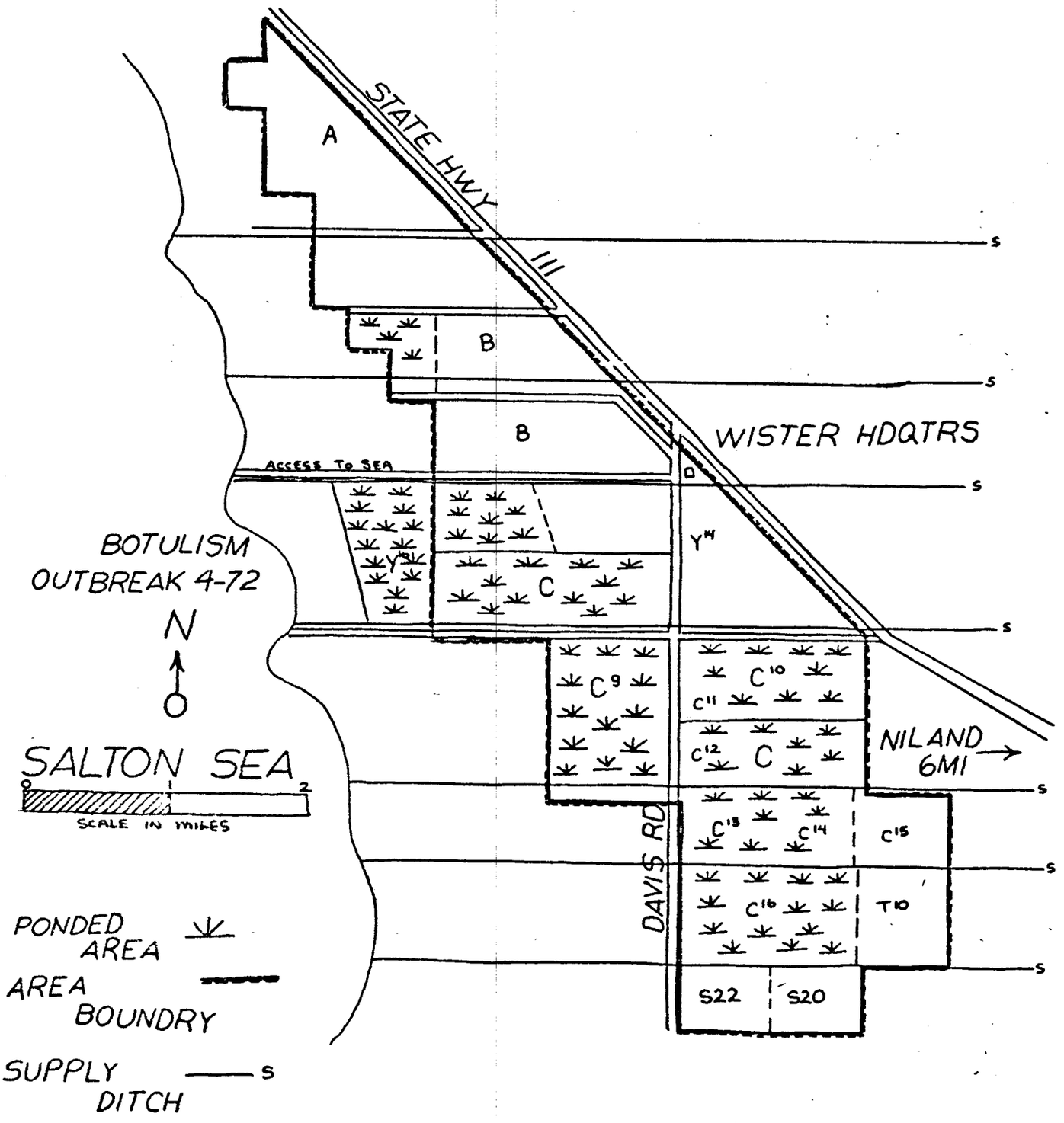


FIGURE 3
IMPERIAL WATERFOWL MGT
AREA

adapted to alkaline environments. Frequently, there were fallen trees and dead brush protruding from the shallows. The region between the New River and Red Hill was bordered partially by abandoned farm land dotted with the remains of old buildings and rusting machinery. Some portions included masses of Cattails (Typha latifolia)and Cottonwood Trees (Populus fremonti).

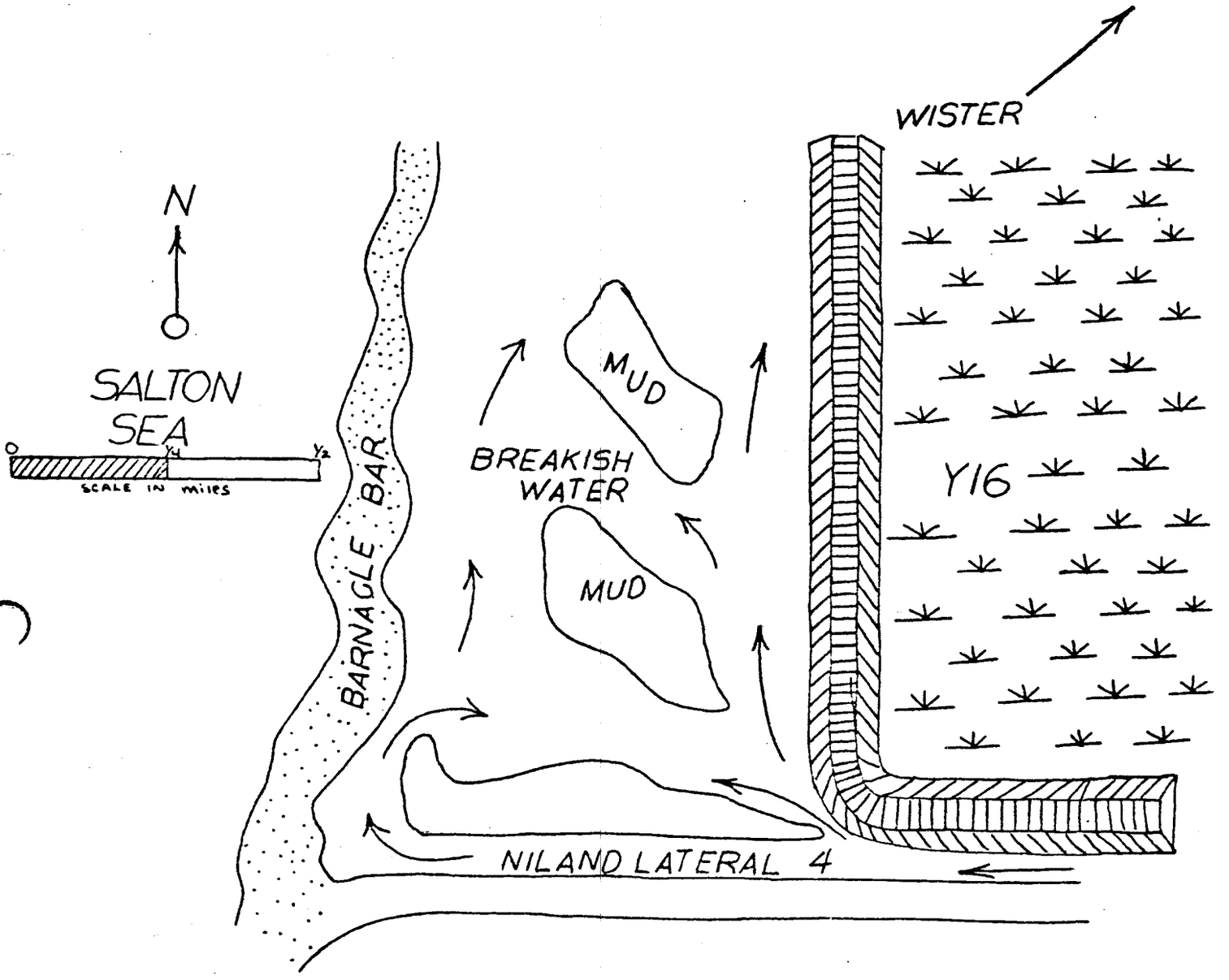
Drainage

Numerous canals drain into the southern portion of the Salton Sea. These carry run-off water from surrounding agricultural regions. The New River constantly dumps silt laden water into the Sea, and this has resulted in a shallow, foul smelling, debris strewn delta several miles wide. Between the New River and Red Hill, a series of lateral canals carry water into the Sea. Each forms it own shallows and mud flats. Near Red Hill is a very shallow area similar to that found at the mouth of the New River.

The Alamo River drains into the Salton Sea by way of the Red Hill Marina. Northeast of the marina the river pushes water into an area locally known as Spoony Bay (Fig. 2). Spoony Bay is a shallow region with large, open mud falts. Additional water is added to the bay by a drainage canal running parallel to McDonald Road. Spoony Bay attracts large numbers of waterbirds because of its nearness to agricultural areas and other food sources. The water is brackish and polluted as evidenced by numerous dead fish, birds and assorted debris littering the shallow regions of the bay.

Between Red Hill Marina and Y16 of the Imperial Waterfowl Management Area, three canals empty into the Sea. Each is paralleled by a road allowing easy access during the dry portions of the year. Nialand lateral canal #4 enters the Sea at the southern boundry

of Y16. The main study area extended form this point to the northern boundry of the section. Extending morthward form lateral #4 is an extensive expanse of shallow, brackish water about a mile and a half long. A road running parallel to the Sea separates fresh, ponded water form these shallows. Brackish shallows extend from the road westward approximately three-quarters of a mile to a long, low barnacle bar that hold back the Sea (Fig. 4). Water depth in the shallows, measured in 1972, ranged from 3" to 18" in the spring to 3' in the winter months.



ROAD-DIKE

POND

FIGURE 4
Y16 REGION-IMPERIAL WATERFOWL MANAGEMENT AREA

Chapter III. Methods and Materials

Field Observations

Observations of areas were carried out on a somewhat irregular basis before botulism outbreaks were noted. When sick birds began to appear observations were made more regularly, usually on a weekly schedule. Personnel from both the Bureau of Sport Fisheries and Wildlife and California Department of Fish and Game were helpful in makeing observations in my absence. Field observations were most irregular from October through January of 1971-1972 and 1972-1973 because of the hunting season. During these times observations were restricted, as movement in protected areas would alarm birds making them more vulnerable to hunting.

Types and numbers of waterfowl and shorebirds were noted during field observations. However, because it was almost impossible to make a count of all birds in the areas under study, waterfouwl counts made by the Bureau of Sport Fisheries and Wildlife (Salton Sea National Wildlife Refuge) were used as extimates of bird populations present.

Sampling of Suspected Toxin Producing Sources

Samples of water, mud, and vegetation were collected prior to botulism outbreaks. Sampling continued during outbreak periods as well. Water temperatures were measured using a standard centigrade thermometer. Oxygen content of waters in some study areas was measured using an oxygen analyzer (YST Model 51). The pH was measured using a pocket pH Meter (Beckman Model 180).

During botulism outbreaks dead and sick birds were collected

for testing and observation. Mortality rates among individual species were noted as were those regions showing greatest numbers of mortalities and morbidity.

Collection, Handling, and Testing of Sample Materials

1. Mud, Water, and Vegetation Sampling

Samples of mud, water and vegetation were collected by wading through study areas. Samples were placed in small containers and transported to the laboratory where they were either tested for toxin content or frozen for testing at a later date. Usually no more than 12-18 hours elapsed before samples were tested or frozen. Materials were tested without incubation or were incubated at 37C for various periods of time before testing. Samples to be incubated prior to testing were placed in thioglycolate (Difco) or Cooked Meat media (Difco) which have been found to meet the unique metabolic requirements for optimum growth and reproduction of botulism causing organisms.

Samples which had not been frozen or incubated were homogenized using a Warring blender or by rapid stirring with a spoon. A small portion of the homogenate was removed and filtered through a 0.45 micron Millipore@ filter. Samples containing large particles were clarified prior to filtration by centrifugation. Filtered samples were injected into mice which were observed for evidence of toxin induced symptoms. In some instances samples were administered orally. The time between administration of sample and appearance of symptoms was recorded as was the time required for mice to succumb to the effects of the toxin.

2. Testing of Avian Blood Samples

A few samples of avian blood were collected for testing. Blood was taken from morbid birds by cardiac puncture. Five to ten cc of blood were collected in citrated tubes to prevent clotting. Samples were kept cool in a closed container until they could be returned to the laboratory. In the laboratory each sample was centrifuged and the serum seperated form the packed cells. One cc aliquots of serum were tested by intraperitoneal injection into mice.

3. Mouse Protection Test

A mouse protection test was used in testing the toxicity of samples collected during this study. It provided an excellent method of testing suspected toxin containing samples without elaborate preparations. The test used was a modification of that described by Sencer (34). Two mice of about equal size were selected for each sample tested. Specific antiserum was used to protect one of the mice. A supply of Clostridium botulinum type C antiserum (prepared in horses) was graciously supplied by Dr. Wayne Jensen, Fish and Wildlife Service, Brigham City, Utah. Mice were protected by injecting 0.2 cc of specific antiserum intraperitoneally. Twenty-four hours following introduction of antiserum, the protected and unprotected mice were injected in the same manner with 0.2 cc of a suspected toxin containing sample. The mice were observed closely for signs of toxic effect. Symptoms of toxin activity included ruffling of fur, dyspnea, paralysis of hind-quarters, and muscular twitching. Display of symptoms and death of the unprotected mouse within twenty-four hours was regarded as evidence that the sample

tested contained botulism toxin. In some instances, when test animals were in short supply, or large numbers of samples were to be tested, a single unprotected mouse was used. Display of symptoms and death of the single mouse necessitated retesting the samples in the manner described above.

4. Collecting and Testing of Aquatic Invertebrates

Invertebrates were collected in three ways. First, a fine mesh nylon net was used to sweep the shallow waters of study areas. Invertebrates collected in this manner were placed in containers which were sealed. The contents were tested at a later date. Secondly, invertebrates were trapped using aquatic insect traps similar to those described by Espinosa and Clark (11). A third method sued to collect dead invertebrates was to simply wade along the shoreline of shallow areas and scoop up by hand masses of invertebrates that had died and were concentrated by wind and water action. Invertebrates were identified by using a binocular scope. The species identified are listed in Table 3.

Several grams of invertebrates were placed in a mortar and graound to a smooth suspension. One cc of 0.02 molar phosphate buffer, pH 7.1, was added for each gram of material ground. This ground material was then tested in one of three ways:

1. Ground invertebrates were placed in tubes containing no nutrient media and incubated before testing for toxin activity in mice.
2. Ground invertebrates were centrifuged, the supernate removed and filtered through a .45 micron Millipore@ filter, and then injected intraperitoneally into mice to test for toxin activity.

Table 3. Aquatic Invertebrates Collected in Botulism Study Areas

Polychaete Worms - *Neanthes succinea*

Branchipods

Copepods

Amphipods - Scuds

Cladocerans - Water Fleas

Insects - Family Corixidae - Water Boatmen - *Corisella* sp.

3. Five cc of ground invertebrate suspension was given orally to mice to test for toxin activity.

Invertebrates prepared in 1 above were tested for toxin content by administering to mice intraperitoneally and orally after incubation.

Samples of invertebrates were also tested using domestic pigeons. Invertebrate samples were prepared as above and incubated for two weeks at 37 C before being given orally to pigeons. Dosage was from 2 to 5 cc of incubated suspension per bird.

5. Terrestrial Invertebrates

Maggots were the only terrestrial invertebrates collected and tested. This was due to the low concentration of other terrestrial invertebrate types in study areas, and because maggots have been implicated as toxin sources in other outbreaks of botulism (10, 19, 23). Maggots were removed from the carcasses of waterfowl and shorebirds for testing. In some cases maggots were macerated in the same manner as were aquatic invertebrates. Maggots were tested for toxin either by injection or oral administration to mice.

6. Testing Waterbird Tissues for Toxin

Dead birds were collected from study areas and taken to the laboratory. Liver and breast tissue were removed and 5 cc of 0.02 molar phosphate buffer, pH 7.1, were added for each gram of tissue. Samples were placed in a Waring blender and ground to a smooth suspension and then centrifuged to remove large particles. Samples not tested immediately after preparation were frozen for testing at a later date. Incubated samples were not centrifuged but were placed in a 37 C incubator for periods of from 2 to 6 weeks before testing.

Tissue samples were tested in mice either orally or intraperitoneal injection. Those samples tested orally were not filtered while those

injected were filtered through a 0.45 micron Millipore@ filter. Five cc was the normal oral dosage while 0.2 cc was the amount usually given intraperitoneally.

Following the method of Shaw and Simpson (35) several birds were placed in plastic bags and sealed. The sealed bags were incubated at 37 C for four days. At the end of this time the liver and a portion of the breast of the incubated birds were removed, ground with 5 cc of 0.02 molar phosphate buffer (pH 7.1) per gram of tissue, centrifuged, and filtered through a 0.45 micron Millipore@ filter. The filtrate was tested by injecting 0.2 cc intraperitoneally into protected and unprotected mice. A small sample of liver from each carcass was frozen for later testing while another was placed in 0.02 molar phosphate buffer, 7.1, (5cc/gram tissue) and incubated anaerobically for an additional period before testing. Livers from non-affected birds were used as controls. Tissues from these birds were treated in an identical manner to those taken from birds suffering from botulism.

7. Additional Testing

Eighteen waterfowl and whorebirds suffering from avian botulism were collected and kept for observation. All were placed in shaded areas, given fresh water, and food. Some were given specific antiserum. Observations of affected birds continued until they either recovered or died. Tissues of dead birds were removed and tested for tosin.

Chapter IV. Results

Botulism Outbreaks in the Study Areas

Serious outbreaks of avian botulism occured in two areas during this study. The first was noted about March 25, 1972 and continued into the first weeks of July. The second was discovered on October 28, 1972 and continued into December. Although sick birds could be found in small numbers along most of the southern shoreline during the first outbreak the largest losses were in the Y16 section of the Imperial Wildlife Area - Wister Unit. For this reason most of the observation and sampling was done in this area. The second outbreak was more isolated than the first. Almost all affected birds were found along the Alamo River delta and adjacent Spoony Bay.

Sampling of outbreak areas was carried out before, during, and after botulism occurances. Conditions existing prior to the spring outbreak were not favorable to the growth of botulinum bacteria. Temperature and pH measurements indicated conditions to be somewhat less than favorable for optimum vegetative growth of *Clostridium botulinum* type C (Table 4).

Conditions of Botulism Occurance at the Salton Sea

Initial surveys of study areas showed that, while conditions during warmer months favored growth of botulism bacteria, no toxin or toxic source could be demonstrated in the samples collected. However, during the period between outbreaks it was possible to find sick and dead birds along the margins of the Sea. No definite data

Table 4. Temperature and pH Measurements of Study Areas Taken Before Botulism Outbreak of March 25, 1972

Temperature Range for Growth of Botulism Bacteria (20)	25C - 30C
pH Range for Growth of Botulism Bacteria (20)	5.9 - 8.0

Region of Sample	Temperature	pH
Obsidian Bute 10-2-71	23C	8.5
Wister Y16 10-22-71	22C	8.8
Wister Y16 10-22-71	24C	8.3
Spoony Bay 11-29-71	15C	6.5
Obsidian Bute 1-5-72	10C	6.2
Spoony Bay 1-5-72	18C	6.7
Obsidian Bute 1-15-72	10C	6.2
Obsidian Bute 1-15-72	12C	5.0
Wister Y16	20C	6.5

was available to indicate that botulism toxin was responsible. Perhaps some of the birds represented the normal mortality among waterbirds present. Some of the sick birds observed showed symptoms of avian botulism, and dead waterbirds were often found in a pose similar to those which had died during botulism outbreaks. In many instances the feathers surrounding the vent was incrusted with a green feces.

On March 18, 1972 a sick male Shoveler (Spatula clypeata) was discovered along the mud flats bordering the New River. The bird exhibited signs of avian botulism, that is, inability to move, nasal secretions, paralysis of the nictitating membrane, and the presence of green fecal material around its vent. There were no other sick or freshly dead waterbirds in the area. On March 25, 1972 a call was received from the California Department of Fish and Game which stated that an outbreak of avina botulism was in progress at the Imperial Wildlife Area. The greatest concentration of sick and dead birds was to be found in the Y16 section of the area. The outbreak was still greatly in evidence on April 1 although steps had been taken by the California Department of Fish and Game to remove dead and sick birds. At the Wister Unit, headquarters for the Imperial Wildlife Area, survivors of the outbreak were placed in wire pens and given freshwater and food. The birds present included species of waterfowl and shorebirds; many were near death or had already died. A summary of birds present is presented in Table 5.

Observations of the Y16 section indicted large numbers of sick and birds had not been removed. Along the banks bordering Y16 were the carcasses of many waterfowl and shorebirds. All appeared to have been there only a short while; dead birds were not bloated, nor

Talbe 5. Birds Collected by California Department of Fish and Game During Spring Botulism Outbreak 4-1-72

Shoveler - Spatula clypeata

Ruddy Duck - Oxyura jamaicensis

Bufflehead - Bucephala albeola

Widgeon - Mareca americana

Green-Winged Teal - Anas carolinensis

Surf Scoter - Melanitta perspicillata

Eared Grebe - Podiceps caspicus

American Avocet - Recurvirostra americana

Black-Necked Stilt - Himantopus mexicanus

California Gull - Larus californicus

were maggots in evidence. My movements through the area alarmed many waterbirds which were suffering from apparent botulism. These birds were unable to fly and could escape only by moving across the water using their wings as oars. The birds manifested classic symptoms of the first stages of botulism toxin action on the nervous system (27). Other birds, slightly more affected, tried to escape by diving beneath the water in which case they soon surfaced because they lacked ability to control their actions. Most of the birds exhibited symptoms of the later stages of botulism intoxication. Such individuals could not move, nor did they make any effort to do so when prodded. Many had sought refuge from sun and predators beneath vegetation, only to die a short time later. The majority of birds found dead were in a sitting position, their heads were lowered to their breasts and their eyes were closed. In almost all cases the vent of affected birds was surrounded by masses of greenish-white fecal material.

Results of Toxin Assays from Affected Birds

Fourteen waterbirds suffering from what was believed to be avian botulism were collected for observation and testing (Table 6). Livers from eight of these birds were removed and tested for toxin. Breast tissue in some cases was also tested. Five liver samples proved to be toxic while none of the breast tissues taken showed evidence to toxin. A summary of the tests of these and other liver samples is in Table 7. Specific information regarding tissue tests is in Table 8. Blood samples taken from six species of birds affected by apparent botulism failed to show evidence of toxin in a mouse protection test.(Table 9). Samples of water, mud, and algae collected

Table 6. Birds Collected for Observation and Testing During April, 1972 Botulism Outbreak

Species	Treatment	Results
Shoveler-Male (Spatula clypeata)	Water Orally Antiserum-2ml/IP	Recovered and Released
Shoveler-Male (Spatula clypeata)	Water Orally	Same as above
Ruddy Duck-Female (Oxyura jamicensis)	Water Orally Antiserum-2ml/IP	Died
Ruddy Duck-Female (Oxyura jamicensis)	Water Orally	Died
Cinnamon Teal-Male (Anas cyanoptera)	Water-Orally	Recovered and Released
Eared Grebe-Male (Podiceps caspicus)	Water-Orally Antiserum-2ml/IP	Died
Eared Grebe-Male (Podiceps caspicus)	Water Orally	Died
Eared Grebe-Female (Podiceps caspicus)	Water Orally	Died
Blk. Nkd. Stilt (Himantopus mexicanus)	Water Orally Antiserum-2mls/IP	Died
Dowitcher (Limnodromus spp.)	Water Orally Antiserum-1ml/IP	Died
Dowitcher (Limnodromus spp.)	Water Orally	Died
Semipalmated Sandpiper (Ereunetes pusillus)	Water Orally	Died

Table 6. (Contd)

Avocet-Male (*Recurvirostra americana*)	Water Orally Antiserum-2ml/IP	Died
Avocet-Female (*Recurvirostra americana*)	Water Orally	Died

Table 7. Summary of Toxic Liver Tissue Tests

Species of Birds from Which Toxic Livers were Removed	Species of Birds Whose Livers Were Used as Controls
Killdeer - Charadrius vociferus Liver collected 4-72	Pintail - Anas acuta Liver collected 10-72
Cinnamon Teal Anas cyanoptera Liver collected 4-72	Pintail - Anas acuta Liver collected 10-72
Ruddy Duck - Oxyura jamaicensis Liver collected 4-72	Shoveler - Spatula clypeata Liver collected 10-72
Avocet - Recurvirostra americana Liver collected 4-72	Shoveler - Spatula clypeata Liver collected 10-72
Avocet - Recurvirostra americana Liver collected 5-72	Red Head - Aythya americana Liver collected 10-72
Avocet - Recurvirostra americana Liver collected 10-72	Ruddy Duck - Oxyura jamaicensis Liver collected 10-72
Blk. Nke. Stilt - Himantopus mexicanus Liver collected 4-72	Grn. Winged Teal Anas carolinensis Liver collected 10-72

Table 8. Tissue Samples Tested for Botulism Toxin Content

Area of Collection	Material Tested	Testing Method	Results and Comments
Wister YI6 4-I-72	Killdeer *Charadrius vociferus* Liver	Incubation in buffer* 2wks/37C Dosage IP .2ml/mouse	Toxic-Death after 2Ihrs,
Wister YI6 4-I-72	Cinnamon Teal *Anas cyanoptera* Liver	same as above	Toxic-Death after 24hrs.
Wister YI6 4-I-72	same as above Breast	same as above	Not Toxic
Wister YI6 4-I-72	Ruddy Duck *Oxyura jamaicensis* Liver	same as above except dosage increased-.4ml	Toxic-Death after 9.25hr.
Wister YI6 4-I-72	same as above	same as above	Not Toxic
Wister YI6 4-I-72	Avocet *Recurvirostra americana* Liver	Buffer* added 5ml/gm tissue Tested IP .2ml/mouse	Not Toxic
Wister YI6 4-I-72	same as above	Incubated 2wks/37C Tested IP .2ml/mouse	Toxic-Death at 20hr.
Wister YI6 4-I5-72	Ruddy Duck *Oxyura jamaicensis* Liver	same as above	Not Toxic
Wister YI6 5-25-72	Avocet *Recurvirostra americana* Liver	same as above	Toxic- Death at 24hr.
Wister YI6 I0-I4-72	Avocet *Recurvirostra americana* Liver	same as above	Toxic-Death after I2hr.
Wister YI6 4-I8-72	Blk. Nkd. Stilt *Himantopus mexicanus* Liver	same as above	Toxic-Death after I2hr,

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Table 8 . (Contd)

Wister YI6 4-I8-72	Eared Grebe Podiceps caspicus Liver	same as above	Not Toxic
East Salton Sea I0-20-72	Pintail (Control) Anas acuta Liver	same as above	Not Toxic
Wister YI6 I0-20-72	Pintail (Control) Anas acuta Liver	same as above	Not Toxic
Eastern Salton Sea I0-20-72	Shoveler (Control) Spatula clypeata Liver	same as above	Not Toxic
East Salton Sea I0-20-72	Shoveler (Control) Spatula clypeata Liver	same as above	Not Toxic
East Salton Sea I0-20-72	Red Head (Control) Aythya americana Liver	same as above	Not Toxic
Wister YI6 I0-20-72	Ruddy Duck (Control) Oxyura jamaicensis Liver	same as above	Not Toxic
Wister YI6 I0-20-72	Green Winged Teal (Control) Anas carolinensis Liver	same as above	Not Toxic

* Buffer - .02 molar phosphate pH 7.I

Talbe 9. Testing of Avian Blood Samples Taken During Botulism Outbreaks

Area of Collection	Species	Amount Tested	Results
Wister YI6 4-I9-72	Blk. Nkd. Stilt* Himantopus mexicanus	Iml/Ip	Not Toxic
Wister YI6 4-I9-72	Eared Grebe * Podiceps caspicus	Iml/Ip	Not Toxic
Obsidian Bute 4-I9-72	Ruddy Duck * Oxyura jamaicensis	Iml/Ip	Not Toxic
Hospital Pen Salton Sea National Wildlife Refuge II-I7-72	California Gull Larus californicus	Iml/Ip	Not Toxic
Hospital Pen Salton Sea National Wildlife Refuge II-I7-72	Ruddy Duck Oxyura jamaicensis	Iml/Ip	Not Toxic
Hospital Pen Salton Sea National Wildlife Refuge II-I7-72	Green Winged Teal Anas carolinensis	Iml/Ip	Not Toxic
Alamo River II-I7-72	Coot Fulica americana	Iml/Ip	Not Toxic
Alamo River II-I7-72	Green Winged Teal Anas carolinensis	Iml/Ip	Not Toxic

* Indicates birds that died.

from areas often having conditions suitable for the growth of Clostridium botulinimu proved to be non-toxic (Table 10).

Invertebrate Populations During the Spring Botulism Outbreak

The Y16 section contained large populations of invertebrates during the spring outbreak. The most numerous invertebrates present were the Water Boatman (Corisella sp.) and the Pile Worm (Neanthes succinea). Along the banks bordering the Y16 section were masses of dead and decaying invertebrates washed up by water and wind. The shallows swarmed with thousands of invertebrates. Samples of both living and dead invertebrates proved to be toxic to mice. Invertebrate samples were also toxic to pigeons (Columba livia) (Table 11).

Maggots collected from decaying waterbirds did not cause symptoms or death when tested in mice (Table 12).

Conditions During the Winter Botulism Outbreak

During the botulism outbreak that occured in the months of October through December, infected birds first appeared along the edges of the Alamo River delta and on the mudflats near the edges of Spoony Bay. Waterbird mortalities in the other areas were minor.

Samples of mud and water collected and tested at this time did not indicate the presence of botulism toxin. Blood samples from birds collected during the outbreak by the California Department of Fish and Game proved to be positive for toxin when tested in mice. This is contrary to the results obtained in this study when testing blood samples collected from the same area a few days later: all samples proved to be negative when tested in mice.

Species of Birds Affected by Botulism

Observations of study areas during the first outbreak showed

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Table IO. Mud, Algae, and Water Samples Tested for Botulism Toxin

Area of Collection	Material Tested	Testing Method	Results
Obsidian Bute I0-2-7I	Water	pH-Temperature Oxygen content	pH-8.5 Temp-23C Oxygen-I5ppm
Obsidian Bute I0-2-7I	Water	same as above	pH-8.7 Temp-25C Oxygen-I0ppm
Wister YI6 I0-22-7I	Water	same as above	pH-7.8 Temp-22C Oxygen-I0ppm
Wister YI6 Shallows I0-22-7I	Water	same as above	pH-8.8 Temp-22C Oxygen-8ppm
Wister YI6 Shallows I0-22-7I	Water	same as above	pH-8.3 Temp-24C Oxygen-I0ppm
Spoony Bay Shallows II-29-7I	Water	same as above	pH-6.5 Temp-I5C
Spoony Bay Shallows II-29-7I	Water	same as above	pH-6.0 Temp-23C
Obsidian Bute I-5-72	Water	same as above	pH-6.2 Temp-I0C Oxygen-40ppm
Obsidian Bute I-5-72	Water	same as above	pH-6.0 Temp-I8C Oxygen-30ppm
Obsidian Bute same as above	Water	2ml/orally mouse protection test	Not Toxic
Spoony Bay I-5-72	Water	pH-Temperature Oxygen content	pH-6.7 Temp-I8C Oxygen-30ppm

Table IO. (Contd)

Spoony Bay same as above	Water	Incubated in thioglycolate 2wks/37C 2ml/mouse dosage-Oral	Not Toxic
Obsidian Bute I-I5-72	Water	pH-Temperature	pH-6,2 Temp-IOC
Obsidian Bute I-I5-72 8''Deep	Water	same as above	pH-5.0 Temp-I2C
Obsidian Bute I-I5-72	Water	same as above	pH-6.6 Temp-I4C
Obsidian Bute Observation Point 4-I5-72	Water	same aa above	pH-7.7 Temp-I8C
Obsidian Bute Same as above	Water	2ml/mouse dosage-Oral	Not Toxic
Wister YI6 4-I5-72	Water	same as above	Not Toxic
Wister YI6 same as above	Water	pH-Temperature	pH-8.I Temp-22C
Wister YI6 4-I8-72	Water	same as above	pH-7.4 Temp-7.4
Wister YI6 same as above	Water	Incubated in thioglycolate 2wks/37C 2ml/mouse dosage-Oral	Not Toxic
Wister YI6 4-28-72	Water	pH-Temperature	pH-7.6 Temp-27C
Wister YI6 same as above	Water	2ml/mouse dosage-Oral	Not Toxic
Wister YI6 7-I5-72	Water	pH-Temperature	pH-7.4 Temp-27C

Table IO. (Contd)

Wister YI6 8-I6-72	Water	same as above	pH-7.5 Temp-36C
Wister YI6 same as above	Water	2ml/mouse dosage-Oral	Not Toxic
Wister YI6 same as above	Water	Incubated in thioglycolate 2wks/37C 2ml/mouse dosage-Oral	Not Toxic
Wister YI6 I0-26-7I	Mud and Algae	Slurried and given to mice 2ml/mouse dosage-Oral	Not Toxic
Obsidian Bute I-I5-72	Mud	Incubated in thioglycolate 2wks/37C 2ml/mouse dosage-Oral	Not Toxic
Obsidian Bute same as above	Mud	.4ml/mouse after above incubation	Not Toxic
Spoony Bay I-I5-72	Algae	Slurried and given to mice 2ml/mouse dosage-Oral	Not Toxic
Spoony Bay same as above	Algae	same as above	Not Toxic
Obsidian Bute I-I5-72	Algae	same as above	Not Toxic
Obsidian Bute I-I5-72	Mud	same as above	Not Toxic
Obsidian Bute 3-3-72	Mud	3gm placed in thioglycolate incubated 2wks/37C 2ml/mouse dosage-Oral	Not Toxic

Table IO. (Contd)

Obsidian Bute same as above	Mud	Slurried and given to mice 2ml/mouse dosage-Oral	Not Toxic
Wister YI6 4-I8-72	Mud	same as above	Not Toxic
Wister YI6 8-I6-72	Mud	5gm placed in thioglycolate incubated 2wks/37C 2ml/mouse dosage-Oral	Not Toxic
Spoony Bay IO-2I-72	Mud	same as above	Not Toxic
Spoony Bay same as above	Algae	same as above	Not Toxic
Alamo River Spoony Bay II-I7-72	Mud	Incubated in thioglycolate 2wks/37C .2ml/mouse dosage-IP	Not Toxic
Wister YI6 II-I7-72	Mud	same as above	Not Toxic

Table 11. Testing of Aquatic Invertebrates for Botulism Toxin Using Domestic Pigeons (Columba livia)

Pigeon no.	Protection Given	Sample	Symptoms	Results
I-Control	None	None	None	None
2-Protected	2ml antitoxin IP 24hr before testing of sample	2ml macerated aquatic invertebrates Incubated 2wks/37C before test dosage-Oral	None	Normal
3-Protected	same as above	5ml macerated aquatic invertebrates Incubated 2wks/37C before test dosage-Oral	None	Normal
4-Unprotected	None	same as above	First symptoms at 4hr. Sitting still Later symptoms - Prostration, green feces, nasal secretions, paralysis of the nictitating membrane.	Death 46hr. after sample was given
5-Unprotected	None	2ml macerated aquatic Invertebrates Incubated 2wks/37C before test dosage-Oral	First symptoms at 12hr. Sitting still, eyes opening and closing slowly.	Death 59hr. after sample was given

Table I2. Maggots Collected and Tested for Botulism Toxin

Area of Collection	Testing Method	Results and Comments
Spoony Bay Dead Shoveler II-26-7I	Incubation in thioglycolate 2wks/37C dosage-Oral 2ml mascerated maggots/mouse	No symptoms Not Toxic
Spoony Bay same as above	Incubated in water from same area. 2wks/37C dosage-Oral 2ml mascerated maggots/mouse	No symptoms Not Toxic
Spoony Bay same as above	Material from above centrifuged and injected IP into mice. .2ml/mouse	No symptoms Not Toxic
Spoony Bay same as above	Mascerated maggots given orally to mice 2ml/mouse	No symptoms Not Toxic
Spoony Bay Dead Shoveler II-26-7I	Slurry made from 20 maggots given orally 2ml/mouse	No symptoms Not Toxic
Wister YI6 Coot 3-9-72	I2 maggots placed in thioglycolate incubated 2wks/37C given orally 2ml/mouse	No symptoms Not Toxic
Wister YI6 same as above	Material from above centrifuged and injected IP into mice. .2ml/mouse	No symptoms Not Toxic
Wister YI6 Ruddy Duck 8-22-72	procedure same as above	No symptoms Not Toxic
Spoony Bay Pintail II-I7-72	I5 maggots placed in thioglycolate incubated 2wks/37C given orally 2ml/mouse	No symptoms Not Toxic
Spoony Bay same as above	I5 maggots mascerated and fed to mice 2ml/mice	No symptoms Not Toxic

a wide variety of bird species to be affected (Table 13). During the first weeks it appeared that the majority of birds found sick or dead were waterfowl. As the outbreak progressed fewer affected waterfowl were found but many shorebirds appeared to be suffering from botulism. Affected shorebirds continued to dominate the list of affected birds for approximately two weeks at which time there was a decline in their morbidity and mortality. It was then noted that larger shorebirds and gulls were most numerous among the sick and dead resulting form botulism. Several Snowy Egrets (Leuopheys thula) were found suffering from botulism. Two others were found dead and were assumed to have died as the result of botulism. During the final weeks of the first outbreak, a Common Merganser (Mergus merganser) and three California Gulls (Larus californicus) exhibited symptoms of botulism poisoning.

The second outbreak produced more fatalities among waterfowl than among shorebirds. Observations and counts by the Bureau of Sportfisheries and Wildlife indicated the largest number of affected birds were found among diving species of waterfowl. Ruddy Ducks (Oxyura jamaicensis) were the most numerous of the affected species. Ruddy Ducks were also the most numerous of all waterfowl in the area during most of the year. Their populations were exceeded in numbers only in the winter months, December through January, by Pintails (Anas acuta). The estimated numbers of waterfowl present during this study are given in Table 14. Figures were compiled from information supplied by the Bureau of Sportfisheries and Wildlife. Because Ruddy Duck numbers were always large, it does not seem surprising to find them hardest hit during botulism outbreaks. There is, however, a rather puzzling

Table I3. Birds Affected by Avian Botulism
at the Salton Sea, California
October, I97I through December, I972

Species	Affected Spring - 72	Affected Winter - 72
Shoveler-Spatula clypeata	*	*
Pintail-Anas acuta	*	*
Green Winged Teal-Anas carolinensis	*	*
Widgeon-Mareca americana	*	*
Ruddy Duck-Oxyura jamaicnesis	*	*
Coot- Fulica americana	*	*
Avocet- Recurvirostra americana	*	*
Sandpiper- Eriola sp.	*	*
Dowitcher-Limnodromus sp.	*	*
Western Grebe- Aechmorphorus occandatalis	*	*
Eared Grebe-Podiceps caspicus	*	*
Calif. Gull-Larus californicus	*	*
Snowy Egret-Lecuophoxy thula	*	*
Surf Scoter-Melanitta pericillata	*	
Cinnamon Teal-Anas cyanoptera	*	
Bufflehead-Bucephala albeola	*	
Blk. Nkd. Stilt-Himantopus mexicanus	*	
Killdeer-Charadrius vociferus	*	
Sora-Porzana carolina	*	

Table I3. (Contd)

Species	Affected Spring - 72	Affected Winter - 72
Canvasback-Aythya valisineria		*
Lesser Scaup-Aythya affinis		*
Mallard-Anas platyrhynchos		*
Bonaparte's Gull-Larus philidelphia		*
Blk. Bellied Plover-Squatarola squatarola		*

Table I4. Waterfowl Population Numbers from the Salton Sea
October, I97I - February, I973

Species	I0-7I	II-7I	I2-7I	I-72	2-72	3-72	4-72	5-72	6-72
Ruddy Duck *Oxyura jamaicensis*	II,450	3,753	I4,650	I6,309	28,820	7,056	I,6I4	N.C	4I5
Shoveler *Spatula Clypeata*	I,520	I,920	4,843	7,200	5,575	I,550	250	N.C	0
Grn.Winged Teal *Anas carolinensis*	I,960	I,950	3,060	4,250	3,425	950	25	N.C	5
Cinnamon Teal *Anas cyanoptera*	6I0	I52	I09	I00	200	500	4I3	N.C	I00
Pintail *Anas acuta*	I,975	I,945	3,000	4,000	I45,000	45	40	N.C	65
Widgeon *Mareca americana*	2I0	845	3,I37	4,200	3,550	325	0	N.C	0
Canvasback *Aythya valisineria*	I00	75	500	600	700	0	0	N.C	0
Scaup *Aythya affinis*	I00	200	I00	600	600	50	50	N.C	0

Table I4. (Contd)

Species	7-72	8-72	9-72	I0-72	II-72	I2-72	I-73	2-73	3-73
Ruddy Duck *Oxyura jamaicensis*	425	800	6,8I0	22,606	2I,020	20,I75	I9,400	28,600	38,650
Shoveler *Spatula clypeata*	0	0.	I,840	3,860	6,000	6,500	6,675	7,525	3,850
Grn. Winged Teal *Anas carolinensis*	235	800	I,840	2,080	2,025	3,I00	2,700	4,I00	I,900
Cinnamon Teal *Anas cyanoptera*	235	800	I,840	52I	5I2	425	450	450	825
Pintail *Anas acuta*	65	0	4,000	6,600	7,562	9,500	7,500	49,600	87,325
Widgeon *Mareca americana*	0	0	I5	8,000	5,I32	5,500	6,400	7,950	2,300
Canvasback *Aythya valisineria*	0	0	0	45	550	900	925	I00	5
Scaup *Aythya affinis*	0	0	0	250	400	725	525	I75	75

situation envolving another species of diving duck found at the Salton Sea. Canvasback Duck (Aythya valisineria) populations averaged about 337 individuals during the period from October 15 through December 10, 1972. Approximately 150 of these waterfowl were found to have contracted avian botulism, of which 125 died. During this same period, Pintail populations averaged 7,450 individuals, yet only 850 were found to be affected by botulism. This indicates that some factor is present which must account for the higher mortality rate among diving species of waterfowl. This will be discussed further in the next chapter.

Shorebird mortalities during the winter outbreak were overshadowed by those of waterfowl. Since efforts were concentrated largely on observing and analyzing botulism as it occured among waterfowl, it is possible that a number of affected shorebirds were overlooked. However, the numbers of shorebirds present at the Salton Sea during the winter months are fewer than in spring which may also account for fewer shorebirds being affected by botulism at this time.

Chapter V. Discussion

The southern end of the Salton Sea has long been recognized as an area in which a high incidence of avina botulism occurs. Hobmaier (17) (18) commented that the south Salton Sea was one of the two major outbreak areas in 1931; the other was near Colusa, California. He noted that botulism persisted through October at the Salton Sea while in Colusa it terminated around the end of September. It was speculated that botulism persisted longer at the Salton Sea because of higher prevailing temperatures.

The southern Salton Sea offers an ideal habitat for the growth of the botulism causing organism. The combination of relatively high temperatures, shallow alkaline waters, and abundant decaying organic materials provides favorable conditions for the propagation of the organism. Many sections of the Salton Sea are inaccessible and therefore control measures used in other outbreak areas cannot be applied. With these favorable conditions existing one would expect toxin containing sources could be easily found. However, this was not the case. There has been difficulty in locating toxic sources in other potential botulism producing areas, and the Salton Sea is no exception. Many researchers, among them Hunter (20) and Jensen (22), have implicated invertebrates as major sources of botulism infection, yet they cannot state with absolute certainty that invertebrates represent the only source.

Kalmbach (24) in previous investigations demonstrated the presence of botulism toxin in grain which had been submerged in waters of marshy areas. Quortrup and Holt (30) also demonstrated that vegetation

could serve as a substrate upon which these bacteria could grow and produce toxin. In spite of the evidence which confirms vegetation to be a suitable growth substrate for botulism bacteria, there is greater evidence which shows its importance to be minor when compared to other substrates. Even if fegetation were to act as a substrate for the production of large concentrations of botulism toxin, it is difficult to imagine how it might be ingested by waterbirds. Most waterbirds do not consume decaying vegetation. If toxin were produced and then subsequently released into the waters of outbreak areas where it might be ingested by birds along with food or water, there would still be little possibility of ingesting sufficient quantities of toxin to cause botulism morbidity. Hunter (20) in numerous tests failed to demonstrate the presence of botulism toxin in water taken form outbreak areas. Jensen (21) reports that in one outbreak area toxin was found in water. However, the concentration was such that approximately 160 liters of water would have to be ingested by one bird before ill effects would result. Needless to say, toxin distributed in water cannot be held responsible for causing botulism outbreaks. Toxin could not be demonstrated in water samples taken form areas of the Salton Sea during this study. The absence of toxin in vegetation tested is consistent with recent findings, and while vegetation may serve as a possible substrate for growth of botulism bacteria in situ, it cannot be considered of consequence in causing botulism outbreaks.

Since botulism toxin is most probabley produced at low levels in vegetation and is diluted to nonlethal levels in water, only items which can contain concentrations of toxin must be responsible for botulism outbreaks. Invertebrates are considered to be the

primary items in which botulism is produced and transmitted to waterbirds. In effect they are small containers in which anaerobic conditions may exist allowing botulism bacteria to grow and produce toxin. In this study aquatic invertebrates were shown to be toxic. The concentration of terrestrial invertebrates, with the exception of maggots, was not great in the areas studied at the Salton Sea. It seems unlikely that these would serve as a significant source of botulism infection. The absence of toxin in maggots taken from carcasses of dead waterbirds is contrary to results obtained in other studies. Hunter (19) (20) maintains that maggots are to be considered as a main conveyance of botulism toxin in California. While Jensen and his associates (23) support this thesis to some degree, they do not believe maggots to be the only source of toxin.

If maggots had been shown to be toxic in this study, it is doubtful they would have been important in spreading botulism. Bell (4) has stated that while maggots may develop in and on the carcasses of dead waterfowl, other waterfowl seem to loathe to pick maggots from them. However, if maggots are removed from the carcass, by wind, water or other forces, they may be eaten. While observing the spring botulism outbreak, it was noted that maggots were present, but at no time were waterfowl seen to feed on them. Shorebirds were seen feeding near carcasses of dead fish and birds and it is quite possible that they may have consumed maggots. Only during the winter outbreak were carcasses seen in water near large concentrations of waterfowl and none of these showed evidence of maggots. Species ofwaterfowl most affected by botulism confined their activities to areas of deep water and the chance of them encountering maggots seems rather remote.

Detection of Botulism in Waterbirds

The recognition of avina botulism is subjective if definitive testing is not performed. In order to diagnose an outbreak a number of items must be considered. When looking at an area where botulism is thought to be occuring, consideration of topography, climatic conditions, and the condition of waterbirds in the area is essential. Because testing of suspected toxin sources can take days or even weeks, it is important to recognize the symptoms of avian botulism. Symptoms may sometimes be confused with tose of Newcastles Disease and Avian Cholers. There are, however, two distinct symptoms which enable one to distinguish botulism from these two diseases. First, botulism is the only avian disease known in which there is paralysis of the nictitating membrane (10). Second, in the later stages, paralysis of the neck muscles results in the inability of infected birds to raise their heads. This symptom may commonly be reffered to as "limberneck" and is also seen in domestic fowl affected by botulism.

Blood samples taken from infected waterbirds at the proper stage of intoxication are useful in disease diagnosis. However, if birds have ingested sublethal doses of toxin, or if they are on the way to recovery, toxin levels in the blood may be so slight that they cannot be detected (8). Personnel from the California Department of Fish and Game took samples from birds affected with botuoism during the winter outbreak. These samples proved to be positive for toxin when tested in a mouse protection test. Three samples of blood collected two days later form birds in the same area proved to be negative for toxin when tested in the same manner

by the author. It is believed that negative results occurred because of the lowered toxin titer in the blood. Blood samples collected during the spring outbreak by the author also proved negative when tested for toxin. Whether or not these birds had neutralized the toxin in their bodies and were suffering from hunger and dehydration rather than botulism poisoning is unknown. All birds from which blood was taken during the spring outbreak died. It appears in many instances that botulism may serve to weaken a bird and not actually kill it. Once the bird is weakened, it is unable to move about, secure food and water, or escape predators. A bird could conceivabley show no indications of intoxication, be near death, and still have been brought to this state by botulism poisoning.

Spring - 1972 Botulism Outbreak

This outbreak came suddenly and persisted for a period of about four months. Waterbird losses were minimal, probably due to the low number of birds residing in outbreak areas at this time of year. The reason for the greatest waterbird losses at the Y16 section of the Imperial Waterfowl Area can be linked to the abundant and easily accessible aquatic invertebrates present. Because Y16 is within a state game refuge, it was impossible to obtain living waterbirds of examination and analysis when they were not affected by botulism. Therefore, specific food items consumed by waterbird species could not be determined. Consequently, observations of feeding waterbirds were necessary to attempt to discover what each species was eating. By carefully stalking snf observing birds, it could be seen that many were feeding on small objects in the water and along the shoreline. It can

be assumed that at least part of the food consumed consisted of aquatic invertebrates. Shovelers were seen to feed by moving in tight circles stirring up the mud and water with their feet and straining materials through their sieve-like bills. There seems no possible way in which a bird feeding in this manner could select food items. This is evidence that at least this species of waterfowl did ingest aquatic invertebrates. During the spring outbreak a number of waterbirds were collected and their digestive tracts analyzed. However, little was revealed regarding food preferences of bird species affected. Distinct, identifiable food items were few. Hunter (20) states that the ideal time for collection of specimens for examination of gizzard contents is within an hour or so from the time a bird ingests an item. Unfortunately, one of the first things that a bird suffering from botulism does is to stop eating. By the time the bird is in a condition which enables capture, the ingested soft food items are unidentifiable.

Jensen (23) found that two types of aquatic insects, backswimmers and waterboatmen, had the ability to kill unprotected mice but not mice which had been previously protected with botulism antitoxin. Extracts which could kill mice were made from both living and dead insects.

I believe that the most important factor responsible for the outbreak of spring botulism was the ingestion of aquatic insects (Corisella sp.) and polychaete worms (Neanthes succinea) which had died as a result of natural occurrances. Invertebrates were present in large concentrations at the time of the spring outbreak and wind and water action killed many thousands daily. These were beached by water and wind where they subsequently died. Further action by

water and wind swept them back into the water where they mixed with food items upon which birds were feeding. In some cases dead invertebrates were probably consumed as they lay exposed on the shore. In the interval between death and ingestion, botulism spores and bacteria within the intestinal tract of the invertebrates had ample opportunity to grow and produce toxin. It is conceivable that invertebrates need not to have had large amounts of toxin in them, but simply contain an actively growing, toxin producing bacterial population. There are those who believe most botulism toxin is liberated upon lysis of botulism (5) (7). Boroff and his associates (6) have shown that as much as 90% of the toxin responsible for botulism is liberated during cellular destruction. Jensen (personal communication) reports while he cannot say that no toxin is released before lysis of cells occurrs, he does believe that lysis is responsible for a large part of the soluble toxin that appears in a culture as it develops. Further, the toxicity of Clostridium botulinum cell suspensions for mice increases markedly after cells are reptured by ultrasonic waves, probably because the toxin is more readily absorbed when released. It can be speculated that when invertebrate carcasses containing botulism bacteria are ingested, their cell walls may be destroyed along with the softer body parts by enzymes of the intestinal tract. Toxin liberated in this manner could act as does freely ingested toxin and still not be destroyed by the proteolytic enzymes which are responsible for the destruction of bacterial cell walls. The resistance of botulism toxin to proteolytic enzymes explains how it is able to retain its

toxicity even after being exposed to the bertebrate gastrointestinal tract (5).

Winter 1972 Outbreak

This second more severe outbreak of avian botulism occurred when physical conditions were least favorable for the growth of botulism bacteria. Temperatures had dropped and aquatic invertebrate populations were at low levels. Waterfowl populations were much larger than they had been in spring due to fall migration. The migratory movement of waterfowl had caused large concentrations of birds within a relatively small area. Hunting pressure further crowded birds together. The Alamo River had flooded a week prior to the appearance of the first cases of avian botulism. Fields adjacent to the river and part of Spoony Bay were also flooded. Steps were taken by the Bureau of Sport Fisheries and Wildlife and the California Department of Fish and Game to control the outbreak. Dead birds were collected and destroued by burning. Those which were sick were placed in a recovery pen located at the Salton Sea National Wildlife Refuge headquarters. The outbreak lasted approximately two months; during this time an estimated 5,000 birds died of botulism. Thes figures were compiled by the Bureau of Sport Fisheries and Wildlife and are listed in Table 15.

Sources of botulism toxin which caused this outbreak remain a mystery. It was theorized by Brian Hunter, Wildlife Pathologist, California Department of Fish and Game, who had visited the area during the early stages of the outbreak, that the main source of waterbird infection was caused by the ingestion of terrestrial invertebrates killed by the flooding. However, this speculation was made after little observation and, to my knowledge, no testing

Table I5. Birds Affected by Botulism
During the Winter Outbreak
I972

Species Affected	Number of Dead Resulting from Botulism	Number of Sick Resulting from Botulism
Ruddy Duck - Oxyura jamaicensis	2,200	400
Shoveler - Spatula clypeata	800	50
Pintail - Anas acuta	800	50
Greenwinged Teal - Anas carolinensis	500	75
Canvasback - Aythya valisineria	I25	25
Lesser Scaup - Aythya affinis	I5	I0
Mallard - Anas platyrhynchos	5	0
American Coot - Fulica americana	250	I50
Sandpiper - Ereunetes sp.	75	I5
American Avocet - Recurvirostra americana	I30	25
Dowitcher - Limnodromus sp.	25	I0
Black-Bellied Plover - Squatarola squatarola	25	30
California Gull - Larus californicus	I0	I0
Eared Crebe - Podiceps caspicus	I00	75
Western Grebe - Aechmorphorus occidentalis	I	0
Snowy Egret - Leucophoyx thula	3	2

Note: Figures compiled by Bureau of Sports Fisheries and Wildlife, Salton Sea National Wildlife Refuge.

of suspected sources. Personal observations do not agree with those conclusions made by Mr. Hunter. No evidence of dead invertebrates of any kind were found in waters of the area. At the time of this outbreak, invertebrate populations, both aquatic and terrestrial, were at very low levels. Species of waterfowl most affected carried out the majority of their activities in waters where the likelihood of encountering drowned invertebrates, if present and overlooked, would have been remote. Maggots were not in evidence, but may have been present in some secluded portions of the outbreak area. There is the possibility that sick or wounded birds seeking refuge in covered areas died and remained undetected during clean-up operations. These could have then served as a substrate upon which maggots nigh grow. Maggots produced in such areas would not be easily available to foraging waterfowl.

Two other possible toxin sources responsible for the winter outbreak are:

1. Toxin buried in the vegetation and mud of outbreak areas liberated by flooding.
2. Dead animal materials containing toxin, or actively growing bacteria buried in the substrate and liberated by flooding.

In the first instance it is unlikely that botulism toxin could have remained undetected in the outbreak areas. Testing of numerous samples for toxicity always proved to be negative. Flooding surely would have diluted toxin to nonlethal concentrations. Sampling of outbreak areas never revealed concentrations of dead animal materials buried in bottom sediments. Removal and distribution of these materials, had they been present, to where they might be ingested by waterfowl is highly unlikely.

Feeding Habits of Waterbirds and Waterfowl Populations

No direct realtionship between feeding habits of waterbird species and their susceptability to botulism was shown by this study. This is the result of restirctions placed on the molestation of birds in the areas studied. It was impossible to collect birds for analysis of food items consumed at any other time than in the fall hunting season. Even at this tile, shorebirds could not be collected. Despite this disadvantage, there are a number of inferences that can be made. First, some diseased waterbirds are present throughout the year. Avian botulism is most commonly caused by the ingestion of toxin contained in some food source, Therefore, it seems highly probable that toxin contining sources are present thoughout the year. Second, if a bird consumes more animal material than vegetation, the chances of it becoming infected with botulism toxin are greater than those of a bird feeding strictly upon vegetation. Ruddy Ducks (Oxyura jamaicensis) and Shoveler Ducks (Spatula clypeata) were two species of waterfowl which suffered high mortalities during botulism outbreaks. Both species include large quantities of animal materials in their diets. Animal materials consumed include aquatic gastropods, polychaete worms, and adult and larval insects (3) (9) (26). In both botulism outbreaks, species whose diets included large concentrations of animal materials suffered greater proportional losses than did species which fed primarily on vegetation.

When botulism was first noted in the spring there appeared to be a high rate of infection among waterfowl. Later waterfowl losses decreased and small shorebirds were most affected by botulism. The outbreak terminated after large shorebirds and gulls became affected.

It appears as if this cycle is due to individual species feeding habits, or perhaps the changing from one food source to another as spring became summer.

A third inference concerns the susceptibility of avian species to botulism. Studies indicated that migrating birds may be more susceptible to botulism than those residing in areas where botulism normally occurs (10). In regions of high alkalinity, such as the Salton Sea, there may be an impairment of the salt gland. This impairment, coupled with sublethal doses of botulism toxin, may result in the death of migrating birds (10) (35). There is also the possibility that birds residing in an outbreak area could develop a degree of immunity not found in newly arriving birds. Analysis of these two factors in regard to botulism susceptability were not undertaken in this study.

At the beginning of the spring, 1972 outbreak, small numbers of waterbirds were present in the area. Data supplied by the Bureau of Sport Fisheries and Wildlife indicated that the majority of waterfowl had migrated. Though no data are available to indicate numbers of shorebirds present, observations indicated a decrease in shorebird numbers during the hotter months of the year. Waterfowl populations were at their lowest levels in June, 1972. This was near the time when botulism ceased to be a serious problem. Analysis of data indicated a relationship between waterfowl populations and average monthly temperatures (Table 16). Figure 5 compares average monthly temperatures taken at the nearest monitoring station (El Centro, California) with monthly waterfowl populations recorded from the Salton Sea National Wildlife Refuge. At the highest average

Table I6. Waterfowl Populations and Average Monthly Temperature Readings*

Month	Number of Waterfowl Present All Species	Averate Monthly Temperatures Degrees C
I0-7I	2I,025	20.6
II-7I	I4,065	I5.8
I2-7I	32,499	I0.3
I-72	40,059	I0.4
2-72	I9I,995	I5.8
3-72	I3,75I	20.9
4-72	3,842	20.9
5-72	N.C.	25.0
6-72	860	3I.4
7-72	I,I80	34.0
8-72	2,250	32.0
9-72	24,375	28.8
I0-72	49,309	22,5
II-72	49,25I	I5.4
I2-72	5I,200	II.5
I-73	48,625	I0.8
2-73	I07,660	I4.5
3-73	I0,605	I4.7
4-73	6,535	I9.5

* Waterfowl counts were obtained from Bureau of Sport Fisheries and Wildlife, Salton Sea National Wildlife Refuge.
Temperature readings were obtained form Climatological Data for California. Temperatures from El Centro, California

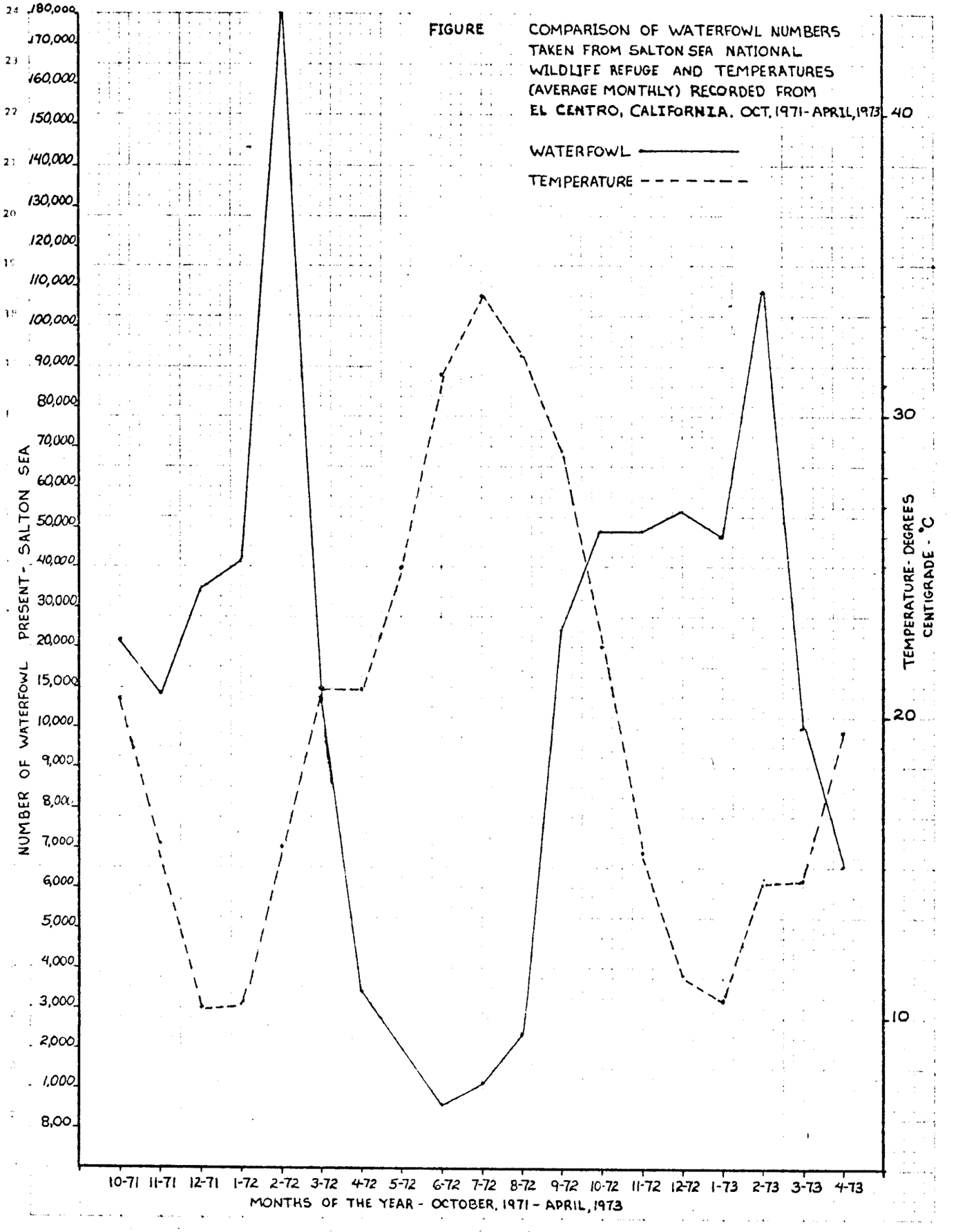
FIGURE
COMPARISON OF WATERFOWL NUMBERS TAKEN FROM SALTON SEA NATIONAL WILDLIFE REFUGE AND TEMPERATURES (AVERAGE MONTHLY) RECORDED FROM EL CENTRO, CALIFORNIA. OCT. 1971 - APRIL, 1973
WATERFOWL
TEMPERATURE
NUMBER OF WATERFOWL PRESENT - SALTON SEA
180,000
170,000
160,000
150,000
140,000
130,000
120,000
110,000
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
15,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
800
TEMPERATURE - DEGREES CENTIGRADE - °C
40
30
20
10
10-71 11-71 12-71 1-72 2-72 3-72 4-72 5-72 6-72 7-72 8-72 9-72 10-72 11-72 12-72 1-73 2-73 3-73 4-73
MONTHS OF THE YEAR - OCTOBER, 1971 - APRIL, 1973

monthly temperature the lowest numbers of waterfowl were present, while periods of lowest temperatures coincide with maximum waterfowl populations. It is significant that the spring outbreak came at a time when populations of waterfowl were declining. Botulism ceased to be of consequence when waterbird populations reached their lowest levels. Although remaining waterbirds were few, conditions favoring bacterial growth and toxin production remained adequate. This was due largely to the increasing aquatic invertebrate population and increasing temperatures. As temperatures continued to rise, conditions became less favorable for bacterial growth. At the same time fewer, and fewer birds remained to ingest contaminated items. This combination of events led to the end of the spring outbreak.

Waterfowl populations began to increase about the month of August, 1972. Temperatures were beginning to decline and it is believed botulism did not become serious because of the low numbers of waterbirds present in outbreak areas. As October approached, monthly temperatures became more suited for botulism development. On October 28, 1972 the winter outbreak began resulting in the death of thousands of birds. The outbreak was ended by diligent clean-up efforts by the Bureau of Sports Fisheries and Wildlife and the California Department of Fish and Game. Hunting pressure may also have served to help scare waterfowl from outbreak areas thereby reducing mortalities. Perhaps if hunting had been allowed at an earlier date there would have been minimal botulism losses.

Additional Studies and Reccomendations

The question of how botulism originates and infects waterbirds at the Salton Sea is still not completely answered. Much more time and study are necessary before a thorough understanding of the ecology of avian botulism is achieved.

A great deal of information remains to be gathered concerning food and feeding preferences of waterbirds. Collection of various waterbirds throughout the year is necessary to establish prefered food items and feeding areas. Periodic examination of materials ingested, along with the testing of similar items collected from feeding areas, could lead to the discovery of additional toxin sources. Such studies might also aid in determining the susceptibility of areas to outbreaks of avian botulism. This would be adventageous in that it would permit control measures to be taken to prevent outbreaks before they occurred. Such investigations would require obtaining special permission from state and federal suthorities.

Observations of waterbirds played an important part in the present study. Future studies should include extensive observations of waterbirds throughout the year. A small, shallow draft boat for making observations in inaccessible areas would aid in collecting additional information on waterbird habits. Observations should concentrate upon methods of feeding used by waterbird species, areas in which they feed, and upon movements from area to area. Investigations of this nature would be difficult for one person to undertake. Two to four people could best perform these observations.

Measurements of oxygen, pH and other physical factors have been of little value in helping to pinpoint outbreak areas. It would be best to identify the ecological associations present within

a given area and then include such measurements rather than randomly sampling a large number of areas.

Further work needs to be done in the laboratory to determine more precisely the conditions under which Clostridium botulinum grows and produces toxin in situ. At present there are discrepancies in the literature concerning optimum conditions required for maximum growth, maximum toxin production and method of intoxication.

Any future study attempting to encompass all the items mentioned would be a tremendous undertaking for one person to accomplish in a short period of time. Numerous researchers have worked may years to accumulate the information now known about avian botulism. The only way in which new, significant information can be found is by indepth, concentrated observation and testing.

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