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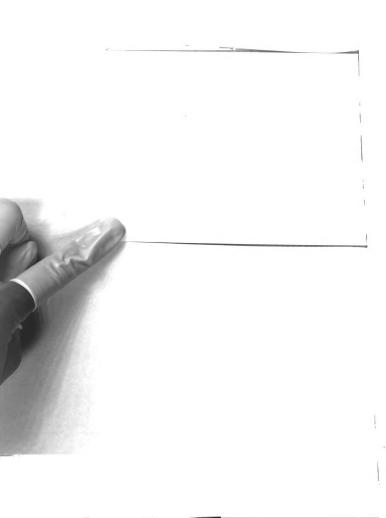
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# THE ROLE OF WATERFOWL IN THE DISPERSAL OF ALGAE

By

HAROLD EUGENE SCHLICHTING, JR.

#### A THESIS

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

### DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

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Approved QW Present

#### ABSTRACT

Ideas expressed by most ecologists concerning the dispersal of micro-organisms by waterfowl have been largely based on assumptions or upon data from a few field collections. A series of controlled experiments was conducted at the W. K. Kellogg Bird Sanctuary on Wintergreen Lake, Kalamazoo County, Michigan, to determine the possible role played by waterfowl.

Ducks were trapped and, in certain phases of the experiment, some were washed in a detergent. They were then placed in a water pen in Wintergreen Lake for periods of time varying from 15 minutes to 24 hours. After removal from the water pen, the ducks were exposed to the air for periods ranging from 15 minutes to 32 hours, either in an air cage or by being hung on a clothesline in a harness. A second series of experiments was conducted in which the ducks were placed in a mud pen. The ducks were restrained in a holding funnel while plastic boots filled with boiled pondwater were tied around their feet to remove any organisms present.

Micro-organisms obtained in the boot washings were cultured in soil-water medium. Washings from the bills and feathers, the Contents from the gullets, and faecal material of some of the birds,

and also washings of field-collected birds were cultured. All cultures were examined microscopically to determine the presence or absence of organisms. In some instances examinations were also made of the uncultured material.

Controls were maintained by (1) sampling water taken from the water pen, while the ducks were there, to determine the microorganisms present, (2) exposing the ducks and boiled pondwater to the air for the same period of time, and (3) observing cultures of unexposed boiled pondwater as used in the washings and uninoculated culture medium to determine the presence or absence of microorganisms. Environmental data such as humidity, wind velocity, air temperature, and sky conditions during the period of investigation were recorded.

One hundred and six waterfowl, representing seventeen species, were washed with boiled pondwater. Forty-one birds were used for the field data, whereas 23 ducks were used in the controlled experiments in 1955 and 42 in 1956. Viable organisms found on the waterfowl were 87 species from the feet, 26 from the feathers, 25 from the bills, 14 from the gullet, and 8 from the faecal material.

The modes of dispersal as well as the nature of the aquatic environment determine what organisms are to be found in a given environment. Although often not considered, these modes are

also important in explaining the distribution of aquatic microorganisms throughout the world.

#### PREFACE

#### A Truism:

Hardly any branch of natural history has been so neglected as that which treats of the various modes by which the different classes of organisms have become dispersed over the surface of the globe.

Alfred R. Wallace (1893-1913)

Quotation from Kew (1893, p. v of Preface)

The primary objective in undertaking the following research
was to demonstrate under what conditions algae might be dispersed
by waterfowl and to study previously unknown factors through controlled experiments. This study was conducted in the hope that it
might stimulate interest in problems of dispersal of micro-organisms
and that it might be the basis for future research in this field to give us
more insight into methods by which organisms are dispersed.

No man is an island, entire of itself; every man is a piece of the continent, a part of the main. [Merton, 1957, p. 21]

This seems especially true in ecological research. One cannot isolate himself from the work and ideas of others if he is to contribute to the advancement of knowledge. It is therefore with great respect and gratitude that those who have aided in advancing this research are acknowledged.

Dr. Gilbert M. Smith of Stanford University was the first to arouse my interest in the dispersal of algae, and were it not for his encouragement the study would not have been initiated.

I am also deeply indebted to Dr. G. W. Prescott of the Department of Botany and Plant Pathology and Dr. T. W. Porter of the Department of Zoology, Michigan State University, for their encouragement, understanding, and guidance. I also wish to thank Drs.

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#### CHAPTER I

#### INTRODUCTION

#### Origin of the Problem

While studying at the University of Michigan Biological Station in 1953, the writer became interested in the role waterfowl are assumed to play in the dispersal of algae and protozoa.

The following quotations from Dr. G. M. Smith's text, The Freshwater Algae of the United States, called my attention to the fact that there was very little experimental evidence to support the generalizations made by various ecological investigators in respect to algal dispersal.

Smith (1933, p. 11, 12) states:

All discussions of the means by which alga [sic] are dis-Persed have been based upon general observations rather than a detailed study, and it is not definitely known whether algae are transported in a vegetative or in a resting stage.

The importance of zygotes and resting cells has been greatly overemphasized in discussions on dispersal; it is very probable that dissemination of vegetative cells is of far greater importance than that of resting cells. Streams assist in the dispersal of algae, but the two major agencies transporting algae from one locality to another are birds and the wind. Those [Persons] who argue for transportation by birds hold that most of the algae are carried in half-dried mud adhering to the bird's

feet, but lodging of algae among the bird's feathers may be fully as important a factor. Transfer of plankton algae is brought about by migratory aquatic birds moving from one body of water to another.

After realizing that a problem existed, the literature was searched to learn what investigations concerning methods of dispersal had been conducted. When it became clear that very little evidence was at hand, a plan of research was devised by which it could be learned whether waterfowl do play an influential role in the dispersal of freshwater algae. Attempts were made to develop several schemes which would show empirically whether wind, flooding, rain run-off, water currents, floating objects, birds, insects, fish, reptiles, amphibians, mammals, and man were all involved in the dispersal of aquatic organisms. However, this thesis concerns only the role of waterfowl in the dispersal of algae.

#### Related Problems of Dispersal

Various agencies have been credited with dispersing microorganisms throughout the world.

## Physical agencies

#### Wind. --

There have been recorded in all periods of historic time, however, showers of one kind or another of animals and plants

or their products-showers of hay, of grain, of manna, of blood, of fishes, or frogs, and even of rats. [McAtee, 1917, p. 217]

Many researchers such as Gislen (1940 and 1948), Messikommer (1943), Hudson (1889), Beger (1927), and Huber-Pestalozzi (1937) have stressed the importance of air currents in the dispersal of micro-organisms.

Gislen (1940, p. 22), Hudson (1889, p. 173), and Pennak (1953, p. 15) have indicated that the ability of an organism to form a light spore or cyst will probably also explain the wide distribution of that species and its being easily dispersed by wind currents.

Pady (1957, p. 351) reported that fungal spores were present in the air throughout the year, but were seasonal in their distribution, with peaks in July and August, and occurred in low concentration during the winter. The intensity of the wind was also directly related to its spore-load. He stated that additional work is necessary to determine spore-loads at different intervals during the day. This work should be concerned mainly with variations in temperature and humidity and their effects on the number and kinds of air-borne fungal spores.

Gislen (1948, pp. 124-125), in the summary of his work, states in part:

Small organisms have considerable possibilities of distribution by convection air currents and winds at moderate altitudes. Examples are given of such distribution over great distances. But as the animals are often strictly specialized

ecologically (herbivores, parasites, etc.) they have particular difficulties to overcome in their new surroundings.

Numbers of micro-organisms are constantly being driven up into the air to return again to earth in rain showers or downward air currents.

Micro-organisms are very resistant to unfavorable factors met with in the air-sea. Some may be distributed through the air in an anabiotic stage. Being often hermaphrodite or parthenogenetic, many of them can give rise to progeny from a single individual which happens to arrive in suitable surroundings. Their resistance to low temperature, low barometric pressure and drought is superior to that of all other organisms. Nevertheless, in comparison to larger forms, they are very sensitive to radiations, especially ultra-violet, which seem to check their distribution more than that of larger forms.

Meier (1933, p. 380) adds, as far as some green algae are concerned, upon a 6-minute to 18-hour exposure to ultra-violet that:

In the regions of ultra-violet beyond 3022 A. the approximate limit of ultra-violet irradiation in nature, the green algal cells were killed.

Wave lengths longer than 3022 A., that is, wave lengths of 3130, 3341, and 3650 A., had no appreciable lethal effect on the algae.

Gislen (1948, p. 125) asserts that:

No geographical borders or barriers exist for microforms. They are often cosmopolitan, or else regionally distributed around the whole globe in certain climatic belts.

Under favorable conditions, especially in humid air, the harmful influence of radiation is diminished, and microforms may be transported alive by winds over greater distances than in clear and dry weather.

However, Hyman (1940, p. 71) makes the following statement:

The cysts of Protozoa occur attached to grasses, and Other objects, in the soil, etc., and may be disseminated by Various agents but do not float about in the air to any extent.

In Puschkarew's experiments, air inoculation of sterile cultures resulted in only 13 species, chiefly small amoebas and flagellates and one ciliate (Colpoda).

Frequently, sterile cultures exposed to the air fail to develope any Protozoa.

The ability of Protozoa to encyst and survive the effects of drying for long periods of time has been discussed by many (Hyman, 1942, p. 71; Gislen, 1940, p. 21; Kudo, 1946, pp. 147-149; Pennak, 1953, p. 15; and Galbraith and Taylor, 1950, p. 938). The formation of a spore or cyst which can withstand desiccation favors but does not guarantee a wide distribution of particular species. As will be seen later, my results upon exposing sterile pondwater to the air for various periods of time were very similar to those of Puschkarew.

Talling (1951, pp. 160-161) states:

Dispersal of small viable resting stages in wind-borne dust is frequently postulated but difficult to detect (c. f. Gislen, 1943). The exposed and drying mud on the margins of ponds would readily contribute to such aerial dust, as several authors have pointed out (c. f. Pettersson, 1940). An empirical approach to the problem is possible from observations of the entry of small aquatic organisms into sterilized cultures or infusions left exposed to the air. Its frequent rapidity led several algological Workers (e. g. Eddy, 1925; Pettersson, 1940; Messikommer, 1943) to emphasise [sic] the importance of wind dispersal for fresh-water algae. However, the total number of species obtained was small, as in the earlier experiments of Puschkarew (1913) on Protozoa, in terrestrial habitats such as soil. Probably more appropriate to conclude, as Puschkarew did, that such culture experiments have not established the importance of Wind in the dispersal of the aquatic micro-fauna and flora. . . . In general, although many individual examples of the dispersal of small aquatic organisms by wind have been established, the Over-all significance of such dispersal is still not clear.

Flooding and rain run-off. The spread of algal species is aided also by flooding and rain run-off. Poretskii (1926, p. 798) states: ''Normal phytoplankton organisms of the Nevka introduced by the flood into the pond rapidly decreased and had entirely disappeared in 40 days.'' This would indicate that although new organisms are brought into an area by flooding, it still does not guarantee their establishment.

Water currents and floating objects. -- Water currents and floating objects also carry algae from one location to another in a given body of water but are relatively unimportant in populating a separate body of water.

## Biological agencies

Birds.--Ridley (1930, p. 489), Taylor (1954, pp. 569-572),

Savile (1956, p. 441), and others have shown that birds can transport

the seeds of plants, externally or internally, relatively great distances

in a viable condition. In fact it has been pointed out by Krefting and

Roe (1949, pp. 271-286) and Ridley (1930, p. 489) that seeds passing

through the digestive tract of a bird may be in better condition to

germinate than if they had not been eaten.

Insects.--Probably the greatest proponent of algal dispersal by insects is W. Migula (1888). In studying the scrapings from different body parts of water beetles, Migula (1888, p. 516) recorded species of the following genera: Anabaena, Characium, Synedra, Oscillatoria, Scenedesmus, Navicula, Protococcus, Cosmocladium, Aphanochaete, Chlamydomonas, Cocconies, Palmella(?), Penium, Chroococcus, Hapalosiphon, Fragilaria, Encyonema, and Meridion. He concludes that the role of aquatic insects in the dispersal of algae is more important than either that of water birds or the air currents.

Kew (1893, pp. 62-63, 67) states that aquatic insects may also play a role in the dispersal of mollusks and fish:

the opinion that the larger aquatic insects-especially the Cytiscides-might without doubt be the means of conveying fish-spawn from one piece of water to another and Mr. Wallace in like manner, discussing the means of dispersal of fishes, observes that water-beetles flying from one pond to another ''may occasionally carry eggs.''

Irénée-Marie (1938, pp. 32, 35) reported finding members of the genus Closterium in the claws of a large Dytisid. He listed desmids found on the body of a dragonfly (Libellula sp.) and a beetle.

Messikommer (1943, pp. 315-316) also credited dragonflies with dispersing micro-organisms from one body of water to another in

encysted forms and spores, also as vegetative cells, if the distance was not too great.

It may well be that sterile culture media exposed to the air may be contaminated by micro-organisms carried by insects rather than from the air currents. Yet in the literature Beger (1927, p. 393) and others attributed organisms found in these exposed sterile media as being air-borne and probably in the encysted stage.

A study is being conducted at Cornell University, New York, by Mr. Bassett Maguire, Jr., to advance our knowledge of the role of aquatic insects, especially Diptera, in the dispersal of microorganisms.

Fish.--Dispersal of higher aquatic plants by fish has been discussed by Ridley (1930, pp. 516-518); the role of fish in the dispersal of algae has been mentioned by Irénée-Marie (1938, p. 31), Lefèvre (1940, pp. 347-349), Velasquez (1939, pp. 386, 389, 403), Tiffany (1927, p. 303), and others.

The algae can be carried within the digestive tract and then defecated in a viable condition when the fish reaches a new location, or carried externally on the body, especially along the edge of the scales.

Tiffany (1927, p. 33) remarked in respect to the species and varieties of algae found in an identifiable condition in the digestive tract of a young gizzard shad:

The species and varieties of algae identified from this young gizzard shad numbered 57, distributed in the following groups: 11 Myxophyceae, 3 Euglenidae, 1 Phaeophyceae [Dinobryon setularia], 2 Heterokontae, 13 Bacillariae, and 27 Chlorophyceae.

Velasquez (1939, p. 403) removed the contents from various sections of the digestive tract of the gizzard shad (Dorosoma cepedianum), cultured these under sterile conditions, and found:

The species and varieties of viable algae are practically the same throughout the alimentary canal. It may therefore be concluded that whatever species are quickly destroyed and digested are quite immediately effected near the mouth end of the alimentary canal. In this connection, the large number of common genera that were not recovered at all in culture is significant. No specimens or only an insignificant number even turned up in culture of any of the following groups always present in ordinary freshwater habitats: (a) Bacillarie, (b) Volvocaceae group, (c) Dinobryon of the Heterokontae, and (d) filamentous green algae.

There survived 30 species and varieties of Chlorophyceae:
12 species and varieties of Myxophyceae; 4 species of Bacillariae,
2 species of Heterokontae; and 1 species of Euglenophyceae. The
Order Chlorococcales (Chlorophyceae) had the greatest number of
Viable algae. It seemed that cell wall modifications or rather
special secretions resisted the digestive fluids of the fish.

Faecal material from three fresh-water fish (Cyprinus carpio, Gardonus rustilus, and Brama brama) was cultured by Lèfevre (1940, pp. 733-739) and the most common algal groups were: Protococcales, Flagellates, Cyonophyceae, Dinoflagellates, and Volvocales. The

desmids and many diatoms seem particularly susceptible to destruction by intestinal secretions.

Irénée-Marie (1938, p. 31) states that fish can carry countless desmids cemented to their scales which may then become detached by the fish brushing aquatic vegetation.

Reptiles.--Most of the work on the role of reptiles in the dispersal of algae has been carried out on turtles (Edgren, Edgren, and Tiffany, 1953, pp. 733-739). Ridley (1930, p. 515) does mention the role that lizards and tortoises play in the dispersal of higher plants but does not discuss the algae. Vinyard (1953, pp. 63-64) mentioned that:

... Certain aquatic or semi-aquatic animals are exceptionally good algal habitats in themselves. The dearth of information on the species of algae occurring on such substrates, as well as on the identity of such animals bearing algal growths, has made it apparent that much useful information might be obtained on the nature of these plant-animal relationships.

In his study of algal growths on some turtles in Oklahoma he listed the species of algae found on the various turtle species. Neill and Allen (1954, p. 583) stated that ''. . . as a turtle moves from pond to pond, it may disseminate the epicolous alga."

Painted turtles (Chrysemys picta) have been observed by the author near the W. K. Kellogg Gull Lake Biological Station of Michigan State University traveling overland between ponds carrying an

obvious algal growth upon their backs as well as various species of leeches adhering to the edge of their carapaces. The turtles probably are also important in the dispersal of algae from one body of water to another.

Amphibians.--Irénée-Marie (1938, p. 32) has considered the possibility of frogs dispersing algae. Of the twenty-five frogs examined, only one did not carry algae externally. From the body of one small frog he obtained 326 desmids representing nine different genera. Migula (1888, p. 517) asserts that water beetles and frogs play an influential part in the dispersal of algae and that frogs may carry more kinds of microscopic plants and animals than do water beetles. Salamanders may also play a role in dispersing micro-organisms.

Mammals.--Irénée-Marie (1938, pp. 31-32) trapped a mink

(Mustela vision, probably) and removed from its fur 169 desmids,

sixteen species in nine genera, and some unclassified cells. The

genus Closterium was especially abundant.

Therefore it seems probable that mammals may contribute to the transport of algae and protozoa as well as fish eggs, snail eggs, et cetera, from one body of water to another, but there is no direct evidence to support this assumption.

### **Man.--**Talling (1951, p. 160) states:

Man himself has been an agent in the dispersal of fresh water organisms. In addition to accidental transportations, the vexed question of artificial transplantations belongs here. . . . Also, even the energetic field naturalist may be unconsciously responsible for extending the range of a species, as he empties the residue of his collections of the day into some convenient pond or stream.

Man has had a great influence upon the distribution of various species of higher plants and animals through modification of the environment and introduction of new species. Probably a long time must elapse before we shall have measured the total effect man exerts on the environment. Although we do know that the introduction of even one species of higher plant or animal into a new area can have great effects upon the ecology of that area, the effects of a newly introduced protozoan or algal species upon a given plankton population has not as yet been studied.

Eddy (1925, p. 143) states:

and Euglena, are pioneers of the initial stage. Seeding conditions for these forms are generally much better than for the filamentous types. From the early appearance of Flagellates and Diatoms in sterile cultures and initial stages, it is very evident that the encysted forms of these species are very widely and readily dispersed. The higher types of filamentous algae are either not so readily dispersed, or do not possess so wide a range of adaptability and require more favorable conditions than offered by the initial stages.

As suggested by Dr. K. L. Osterud (1955), the study of the initial bacterial, protozoan, or algal invaders of a pond and their modification of the aquatic environment would be well worth investigating.

For an additional review of the various problems in the dispersal of micro-organisms. The Elements of Chance in Pond Populations. by J. F. Talling (1951), is suggested.

#### CHAPTER II

#### HISTORY OF THE PROBLEM

An exhaustive search of the literature reveals only a few references to the problem of dispersal of micro-organisms by birds.

Charles Darwin was probably the first investigator to conduct any type of controlled experiment to demonstrate that waterfowl may be important in the transport of aquatic organisms.

Darwin (1859, pp. 302, 304) states:

When ducks suddenly emerge from a pond covered with duck-weed, I have twice seen these little plants adhering to their backs; and it has happened to me, in removing a little duck-weed from one aquarium to another, that I have unintentionally stocked the one with fresh-water shells from the other. But another agency is perhaps more effectual; I suspended the feet of a duck in an aquarium, where many ova of fresh-water shells were hatching; and I found that numbers of the extremely minute and just-hatched shells crawled on the feet, clung to them so firmly that when taken out of the water they could not be jarred off, though at a somewhat more advanced age they would voluntarily drop off. These just-hatched molluscs [sic], though aquatic in their nature, survived on the duck's feet, in damp air, from twelve to twenty-hours; and in this length of time a duck or heron might fly at least six or seven hundred miles, and if blown across the sea to an oceanic island, or to any other distant point, would be sure to alight on a pool or rivulet.

We should not forget the probability of many fresh-water forms having formerly ranged continuously over immense areas, and then having become extinct at intermediate points. But the

wide distribution of fresh-water plants and of the lower animals, whether retaining the same identical form or in some degree modified, apparently depends in the main part on the wide dispersal of their seeds and eggs by animals, more especially by fresh-water birds, which have great powers of flight, and naturally travel from one piece of water to another.

According to Talling (1951, p. 159), Darwin ''performed the simple experiment of dipping a severed duck's foot in an aquarium to test the viabilities of aquatic organisms left stranded on the feet; a repetition of this experiment in nature would be of interest.''

Following Charles Darwin, the first person to do actual research to discover whether algae may be carried externally on waterfowl was Jules de Guerne. His work, Sur les dissemination des organismes d'eau douce par les Palmipèdes, published in 1888, is certainly the first major contribution to the study of waterfowl in the dispersal of micro-organisms. Although he conducted the original research, he is seldom quoted directly in the literature, and then only briefly.

Reference had been made to De Guerne's work by Zacharias (1888, p. 369), Huber-Pestalozzi (1938, p. 72), and a few others, but most English-writing ecologists have preferred to quote German workers (i.e., Zacharias, 1888; Zschokke, 1900; and Beger, 1927).

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According to Talling (1951, p. 159), Darwin ''performed the simple experiment of dipping a severed duck's foot in an aquarium to test the viabilities of aquatic organisms left stranded on the feet; a repetition of this experiment in nature would be of interest.''

Following Charles Darwin, the first person to do actual research to discover whether algae may be carried externally on waterfowl was Jules de Guerne. His work, Sur les dissemination des organismes d'eau douce par les Palmipèdes, published in 1888, is certainly the first major contribution to the study of waterfowl in the dispersal of micro-organisms. Although he conducted the original research, he is seldom quoted directly in the literature, and then only briefly.

Reference had been made to De Guerne's work by Zacharias (1888, p. 369), Huber-Pestalozzi (1938, p. 72), and a few others, but most English-writing ecologists have preferred to quote German workers (i.e., Zacharias, 1888; Zschokke, 1900; and Beger, 1927).

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Because De Guerne's work has a direct bearing on this research, I have included my translation of the original French publication in the Appendix.

Thus, in tracing the history of the problem, one may regress from Smith (1933) to Beger (1927) to Zacharias (1888) to De Guerne (1888) and finally to Darwin (1859). Between 1850 and 1875, Sir Charles Lyell, A. R. Wallace, Charles Darwin, and their associates probably exchanged ideas freely. This group undoubtedly should receive the credit for having laid the foundation for much of our present knowledge of dispersal and distribution of plants and animals.

Kew (1893) in The Dispersal of Shells gave many indications as to how various mollusks may be dispersed throughout the world. He is probably most frequently quoted by English ecologists.

Various workers have reported finding snail eggs, pieces of higher aquatic plants, mollusks, insects, et cetera, adhering to the feathers, feet, and bills of waterfowl. Darwin (1859) observed Lemna adhering to the feathers, and young fresh-water snails adhering to the feet of ducks. Later, in 1878, he reported a clam (Unio complenatus) attached to the toe of a blue-winged teal (Querquedula discors) shot in Massachusetts. Zacharias (1888, p. 368) stated that Humbert found winter eggs of crustacea on the feathers of wild ducks, and Kew (1893, pp. 47, 52) reported that a

mallard was shot 'in the Sahara desert a hundred miles from water'' with mollusk eggs 'attached by the glutinous coating to one of the feet.'' He also stated that pieces of aquatic plants may adhere to the body of waterfowl when they leave a body of water. Reid (1892, p. 278) stated that the nest of the stickleback fish is attached to water plants and would likely be transported with the plants when they become attached to waterfowl.

... Molluscs might possibly be carried in the crops of birds considerable distances, and others be distributed and established in new districts or on islands, as the living shells might be ejected from the crops, or the birds might be killed by birds of prey [or hunters] and the contents of the stomach dislodged and scattered. [Kew, 1893, p. 161]

I picked a living bark beetle out of the feathers of an owl knocked down in flight in the highlands of Fiji. Owls have been seen at sea 1,000 miles from the nearest land. [Zimmerman, 1948, p. 54]

Saville (1956, p. 441) stated that <u>Lemna</u> and <u>Spirodela</u> were probably spread largely by adhering to the body feathers and feet of waterfowl as they rise from ponds in which these plants are growing.

There is no wish on the part of the author to imply that these early reports are untrue. But a more detailed account of these observations discussed in the literature would eliminate much misinterpretation in some ecological reports. One is inclined to be critical of another's work but sometimes it is more difficult to produce something better.

Also the author had observed <u>Lemna minor</u> and filamentous green algae adhering to the feathers and feet of ducks when they were first removed from the water. After the ducks had been hanging in the air 10 minutes, the <u>Lemna minor</u> and algae were not found (Chapter VI).

We will do well to keep in mind Darwin's remark, "How ignorant we are with respect to the many curious means of occasional transport." [Zimmerman, 1948, p. 62]

Ström (1924, p. 141) asserted that the flights and migrations of wading and swimming birds are of great importance in the distribution of organisms, perhaps especially for local habitats, and that much research remains to be done concerning the possibilities and means of the distribution of algae.

Some of this much-needed work, done recently by Irénée-Marie (1938) and Messikommer (1948) has aided us greatly in obtaining a clearer idea of the role which waterfowl play in the transport of algae.

In Flor Desmidiale de la Region de Montreal (1938, pp. 32, 33) Fr. Irénée-Marie described the washing of the feet of a Blue Heron in filtered water after observing its flight between peat bogs. The following desmids were recorded:

Genus	Number Observed	Number of Species
Closterium	31	3
Penium	5	. 1
Pleurotaenium	5	2
Triploceras	32	2
Staurastrum	18	3
Spondylosium	2 filaments, 17 cells	1
Netrium	9	1
Cosmarium	9	2

A total of 126 cells of 15 species representing eight different genera were found. Irénée-Marie had declared a belief that many specimens remained on the walls of the vessel containing the washings.

He also killed ducks before they could land in the water of the peat bog. The washings of the plumage of one duck yielded 517 desmids representing 31 species and 13 genera.

In Algennachweis in Entenexkrementen (1948, pp. 23-24)

Messikommer reported his findings from the direct microscopic examination of fresh faecal material from ducks. He found fragments of higher plants, empty diatom frustules of Synedra, Cocconeis,

Fragilaria, Cymbella, Epithemia, Navicula, and Gomphonema, an

unclassified living ciliate, epidermal cells of sedges, fragments of different filamentous green algae consisting of two species of Oedogonium and Microspora quadrata and often Tribonema vulgare.

Staurastrum cingulum, Scenedesmus ecornis, Spirogyra sp., moss leaves, vessels of higher plants, and pieces of insects also were observed.

Klingle (1940, p. 191) used sterile water to wash the feet of sixteen Spotted Sandpipers (Actitus macularia) which he shot on Inaqua Island in the West Indies. He found eleven small seeds, two species of desmids, microscopic green algae, and a number of unclassified amoeba-like organisms. There may have been others like Klingle who have washed birds in the field making brief comments about their observations.

## CHAPTER III

# HUNTERS' DATA SHEETS AND FIELD DATA

A preliminary investigation concerning the problem of algal dispersal by waterfowl was begun in the fall of 1953. The objectives of this initial investigation were to determine (1) what micro-organisms are carried externally on waterfowl, and (2) in what stage they are carried; i.e., vegetative, encysted, or in a spore stage.

By use of the hungers' data sheet (Appendix A) which was a form that the hunters were requested to fill out, the following information was secured: (1) name of the bird shot; (2) sex of the bird; (3) Position of the bird when shot; i.e., coming into, leaving, or swimming in the body of water; (4) name and location of the body of water; (5) date; (6) time of day the bird was shot; (7) name and address of the hunter; and (8) additional remarks.

The heads and feet of the ducks were removed by the hunters and placed in a new number 5 paper bag along with the corresponding hunters' data sheet. Later washings were made from the bills and feet using boiled pondwater.

#### Methods

The glassware used in this research, after being cleaned, was boiled in tap-water for 30 minutes and allowed to dry on fresh paper toweling. Distilled water was boiled 20 to 30 minutes in 250-ml. Erlenmeyer flasks, capped with cotton plugs, and allowed to cool to room temperature. The sterile water was used for the sterile washings of the birds' feet and bills.

The medium for culturing algae and protozoa consisted of a clay-loam soil moderately rich in humus which was obtained from a university farm (Southworth). The soil was boiled in distilled water for 30 minutes, allowed to stand for periods of time varying from three hours to two days, and again boiled for 30 minutes. It was then poured into sterile fingerbowls, covered with sterilized glass plates, and allowed to cool. Later the medium was inoculated with centrifuged material from the bird washings. With each preparation of medium there were at least two controls to which no foreign matter was added.

The feet and bills of the birds were washed by swirling in the boiled distilled water for three minutes, although some specimens were allowed to soak overnight. On a few occasions, the washing periods were even longer. These washings were made

from one hour to as long as three months after the shooting of the bird. The birds had been stored in the Zoology Department cooler at approximately 40 degrees Fahrenheit. Approximately one-third of the washing was centrifuged and studied immediately after washing, another third was placed in culture media, and the remaining third was preserved in 10-percent formalin solution.

Samples of four to twelve drops of medium were removed from the surface, the middle, and the bottom of each fingerbowl.

These samples, examined microscopically, were considered to contain representatives of the algal and protozoan inhabitants in the culture.

#### Field Collections

# Hunters' data sheet

The waterfowl were collected from various hunters in different localities. Six common snipe (Capella gallinago) were shot by Dr. A. D. Geis at about 9:00 a.m. on October 15, 1953.

They were flushed from the mud flats along the edge of Crooked Lake (Barry County, Michigan). The snipe were kept at air temperature until 3:00 p.m. Then they were placed in the Zoology Department's cooler at 40 degrees Fahrenheit. They remained in the

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cooler until 4:30 p.m., October 16, 1953; then washings were made of their feet and bills.

The feet of three snipe were washed for approximately two minutes in boiled distilled water. A sterilized scalpel was placed between the toes of the birds and shaken vigorously for several seconds to remove any material which might have lodged there. A second beaker was used to wash the feet of the remaining three birds. The bills of four birds were washed in a third beaker.

After being centrifuged, approximately the upper three-fourths of the liquid was decanted.

In Table I are listed the other birds that were collected.

The Organisms observed microscopically are recorded in Table II.

# Seattle, Washington

On April 25, 1955, at 9:30 a.m. a Mallard duck (Anas platyrhynchos) was taken from Lake Washington at Seward Park.

The excess water was shaken from its feet. After the bird had been held in the air for five minutes, each foot was placed in a small jar containing boiled pondwater and shaken vigorously. The washings were centrifuged at low speed and examined at 11:30 a.m.

A filament of Oscillatoria sp., a few unclassified small (3µ) green unicells, debris, and pieces of diatom frustules were observed.

TABLE I

BIRDS EXAMINED IN THE HUNTERS' DATA
SHEET STUDY, FALL, 1953

Bird No.	Quan- tity of Birds Shot	Name	Body of Water	Period of Time between Shooting and Washings
1	1	Ruddy Duck (Oxyura jamaicensis)	Lake Lansing	4 days
2	2	Wood Duck (Aix sponsa)	Rose Lake	5 days
3	1	Black Duck (Anas rubripes)	Rose Lake	8 days
4	1	Redhead (Aythya americana)	Lake Lansing	2 hours
5	2	Coot (Fulica americana)	Lake Lansing	2 hours
6	1	Canada Goose (Branta canadensis)	Wintergreen Lake	3 days
7	1	Blue Goose (Chen caerulescens)	Saginaw Bay Marshes	22 days
8	1	Green-winged Teal (Anas carolinensis)	Saginaw Bay Marshes	22 days
9	3	Black Duck (Anas rubripes)	Saginaw Bay Marshes	22 days
10	6	Black Duck (Anas rubripes)	Macks Creek, Stanwood	13-24 days

TABLE I (Continued)

Bird No.	Quan- tity of Birds Shot	Name	Body of Water	Period of Time between Shooting and Washings
11	2	Buffle-Head Duck (Bucephala albeola)	Lake Lansing	12 hours
12	1	Ruddy Duck (Oxyura jamaicensis)	Lake Lansing	12 hours
13	3	Coot (Fulica americana)	Lake Lansing	12 hours
14	1	Canada Goose (Branta canadensis)	Wintergreen Lake	26 hours
15	6	Common Snipe (Capella gallinago)	Crooked Lake	31 hours

TABLE II

ORGANISMS OBSERVED ON MICROSCOPIC EXAMINATION OF WASHINGS FROM THE BIRDS IN THE HUNTERS' DATA SHEET STUDY, FALL, 1953

Bird No.	Portion of Bird Washed	Preparation of Material	Organisms Observed
1	Bill, Feet	Cent. Wash.a	No living algae or protozoa
2	Bill, Feet	Cent. Wash.	No living algae or protozoa
3	Bill, Feet	Cent. Wash.	No living algae or protozoa
4	Feet	Cent. Wash.	Euglena gracilis, fungal spores
		Cult. Media <sup>b</sup>	Nostoc verrucosum, Oscillatoria limnetica
5	Bill, Feet	Cent. Wash.	Oscillatoria amphibia
		Cult. Media	Oscillatoria amphibia
6	Feet	Cent. Wash.	Navicula sp., unclassified ciliates
7	Bill, Feet	Cent. Wash.	Colpidium sp., fungal spores
8	Bill, Feet	Cent. Wash.	No living algae or protozoa
9	Feet	Cent. Wash.	No living algae or protozoa
		Cult. Media	No living algae or protozoa
	Bill	Cent. Wash.	Geminella minor
		Cult. Media	Geminella minor
			la.

a Centrifuged Washing.

<sup>&</sup>lt;sup>b</sup>Culture Media.

TABLE II (Continued)

Bird No.	Portion of Bird Washed	Preparation of Material	Organisms Observed
10	Feet	Cent. Wash.	Peranema sp., Navicula sp., Kirchneriella subsolitaria
	Bill	Cent. Wash.	Navicula sp., Peranema sp., Collembola
11	Feet	Cent. Wash.	Scenedesmus abundans, S. arcuatus, Gloeocystis gigas, Pinnularia sp.
		Cult. Media	Gloeocystis vesiculosa, G. gigas, Aphanocapsa elachista, Oscilla- toria Agardhii
	Bill	Cent. Wash.	Oscillatoria limnetica, Gloeo- cystis sp., Gloeocapsa aerugi- nosa
12	Feet	Cent. Wash.	Oscillatoria limnetica, O. Agardhii, Scenedesmus arcuatus
		Cult. Media	Oscillatoria sp.
13	Feet	Cent. Wash.	Scenedesmus arcuatus, Gloeo- cystis vesiculosa, Sphaerella lacustris
		Cult. Media	Nostoc sp., Oscillatoria sp.
	Bill	Cent. Wash.	Pediastrum Boryanum, Aphanocapsa elachista, Oscillatoria amphibia
14	Feet	Cent. Wash.	Gloeocystis vesiculosa, Palmella mucosa

TABLE II (Continued)

Bird No.	Portion of Bird Washed	Preparation of Material	Organisms Observed
14	Feet	Cult. Media	Colpoda steini
	Bill	Cent. Wash.	Sphaerocystis Schroeteri
		Cult. Media	Protococcus viridis, Trochiscia granulata, Sphaerocystis Schroeteri
15	Feet	Cent. Wash. and Cult. Media	Frontonia sp., two unclassified amoebae, Navicula sp., Phacus spp., Geminella sp.
	Bill	Cult. Media	Nostoc sp.

The washings were placed in the Zoology Department cooler until May 2. Upon examination of the washings May 2, the following were seen: Ulothrix sp., Oscillatoria sp., Monas sp., and Chilomonas sp. On May 11 Ulothrix sp., Oscillatoria sp., Monas sp., an amoeba, fungal spores, and unclassified cysts were present. The culture was found to contain the same organisms on May 16 with the addition of Euglena sp. and an unclassified holotrich.

One-half of the April 25 washings were added to a sterile hay infusion and the other half to a wheat culture medium on May 2. On May 19 the hay infusion contained Vorticella sp., Monas sp., Colpoda sp., bacteria, and a Hartmonella-like amoeba. Colpoda sp., Paramecium bursaria, Vorticella sp., and unclassified cysts were present on May 27 and 28. The wheat culture contained Vorticella sp., Monas sp., Ulothrix sp., and Holotrich: Pithothorax sp. On May 30 the wheat culture contained Colpoda sp., Ulothrix sp., Oscillatoria sp., Bodo sp., Monas sp., and Plagiocampa sp., and the hay infusion contained only bacteria and Paramecium bursaria.

# Pine River, Michigan

On November 7, 1955, at 10:30 a.m., a Goldeneye Duck

(Glaucinetta clangula americana) was flushed from the main stream

of the Pine River between St. Louis and Porter, Michigan. The

bird was in the air an estimated 30 to 45 seconds before being shot down; it fell into the grass on a high bank (8 to 10 feet) about 20 feet from the river. The feet and bill were washed immediately by being shaken vigorously for two minutes in vials of sterile pondwater.

At 3:50 p.m. of the same day the washings were returned to Michigan State University and planted in culture flasks of soil-water media.

Examination of the material on November 26 and December 9, 1955, showed the following organisms: Feet washings: Gyrosigma sp., Gomphonema sp., Navicula sp., Cyclotella sp., Gloeocystis sp., Nannochloris bacillaris, Chlamydomonas Cienknowskii, Scenedesmus longus, S. armatus, Ulothrix sp., and Cladophora sp. Bill washings: Ulothrix sp., Euglena sp., Gomphonema sp., and Oscillatoria sp.

A Gadwall Duck (Anas strepera) was flushed from an arm of the Pine River at 11:00 a.m. Duckweed (Lemna minor) was abundant in the area, although the duck may not have been in it. The bird was in the air 30 to 45 seconds before being shot. It fell in the grass about 50 yards from the river. Washings of the feet were made immediately. These washings were planted in culture flasks at 3:50 p.m. The feet appeared perfectly clean. Even under the toe nails, no dirt particles were visible. Examination of the culture showed Nannochloris bacillaris, Scenedesmus spp., Scenedesmus

abundans, Ulothrix sp., Mougeotia sp., Spirogyra sp., Cyclotella sp.,
Navicula sp., Gomphonema sp., Gyrosigma sp., and Oscillatoria sp.

Samples were also planted from the upper portion of the gullet of each bird. The gullet of the Goldeneye contained two seeds of Potomogeton sp. which germinated, and a filament of Spirogyra sp. Also present were Gloeocystis sp., Ankistrodesmus convolutus, Mougeotia sp., Gyrosigma sp., Navicula sp., Cyclotella sp., and protozoan: Monas-like flagellates. The gullet of the Gadwall showed Euglena sp., Navicula sp., Gyrosigma sp., Spirogyra sp., Lepocinclis sp., Chromulina sp., Nannochloris sp., and rotifer: Bdelloidea.

# St. Joseph River, Michigan

A Mallard drake (Anas platryhynchos) was shot by Dr. M. D. Pirnie on November 13, 1955, in the St. Joseph River rice beds. Excess water was shaken from the duck and it was placed in a clean plastic bag for protection from contamination. The bird was kept in the Zoology Department cooler until November 16; then the feet and the bill were washed and the cultures made.

On December 3 the following forms were noted in the culture media. Feet washings: Scenedesmus spp., S. quadricauda, Nannochloris bacillaris, Ankistrodesmus spp., Chlorella vulgaris,

Synedra sp., Cyclotella sp., and Navicula sp. On December 9

Kirchneriella sp. was found in small numbers in addition to the above organisms. Bill washings: Navicula sp., Valkamphia-like amoebae, and two species of unclassified flagellates. At this time there were large numbers of protozoa but no green or blue-green algae.

## Port Sanilac, Michigan

On July 13, 1957, a Spotted Sandpiper (Actitis macularia) was shot while walking in moist sand and debris on the shore of Lake Huron approximately one and one-half miles south of Port Sanilac, Michigan. The feet were washed immediately by being placed and shaken in a vial of boiled well-water for three minutes. A saturated solution of mercuric chloride was added to the vial in sufficient quantity to double the original volume of well-water. This solution remained standing for three minutes to allow organisms to settle out and then the upper half of the solution was decanted. An equal volume of 6-3-1 (Transeau's) solution was added to the remaining portion containing the organisms.

A microscopic examination on August 1, 1957, showed the following organisms to be present: Quadricula closterioides, Pediastrum Boryanum, filaments of Rhizoclonium sp. with Characium sp. attached, Phormidium sp., Synedra sp., Fragilaria sp. Tabellaria sp.

frustule, pieces of a diatom frustule (Amphora sp.), and epidermal hairs of higher plants.

Another Spotted Sandpiper was shot on dry wood chips 20 yards from the shore of Lake Huron. Upon microscopic examination only pieces of insect exoskeleton and an Alternaria-like fungal spore were found in the washings.

An Eastern Belted Kingfisher (Megoceryle alcyon) was shot out of the air; it fell into moist sand a few feet from the lake.

Fragilaria sp. frustules, Navicula sp., Synedra sp. frustule, pieces of insect exoskeleton, epidermal cells of higher plants, a few unclassified cysts or spores, and debris were found in the washings.

A Purple Martin (Progue subis subis) shot under the same conditions yielded only debris and a few unclassified spherical cells (Cyanophyta)  $2\mu$  in size upon microscopic examination.

On July 15 two Ring-billed Gulls (Larus delawarensis) were shot. One fell in dry grass on a high bank about 30 feet above the level of the lake. Its feet were washed in a vial of boiled well-water. A microscopic examination on August 1 revealed one cell of Gloeocystis gigas, a broken Ostracod valve, pieces of insect antennae, unclassified spherical cysts or spores, 3 µ in diameter, and debris.

In the culture of the bill washings of the gulls, bacteria, an unclassified fungal mycelium, and cysts or spores were observed.

About 2 ml. of the liquid contents of the upper part of the gullet were also cultured. Peranema-like flagellates and a brown fungal spore were seen.

About 3 ml. of faecal material were obtained from the bird and cultured. This culture on August 1 showed a Navicula sp. frustule, pieces of insect, bacteria, and debris.

The second gull fell in dry sand on the shore. Four to six drops of the settled washings revealed only sand grains, hairs, bacteria, pieces of insect antennae, and debris.

The gullets were packed with earthworms, indicating that these birds had not been in the water for some time prior to the shooting. Gulls are frequently seen feeding in farm fields one-half mile from the lake shore.

#### CHAPTER IV

#### CONTROLLED EXPERIMENT, 1955

Controlled experiments were devised to demonstrate and evaluate the role of waterfowl in the dispersal of algae. This method has many advantages over the interpretation of data gathered from waterfowl shot in the field by hunters. Important factors not available from the Hunters' Data Sheet were: (1) where the bird was just prior to being shot, (2) how long the bird had been in the air before being shot, (3) where the duck fell after being shot, and (4) what microscopic organisms occur in these environments.

### Description of Wintergreen Lake

The controlled experiments were conducted on Wintergreen

Lake at the W. K. Kellogg Bird Sanctuary in section 8, Ross Township, Kalamazoo County, Michigan (Figure 1).

Fetteroff (1952, pp. 4, 7) stated that Wintergreen Lake has an area of 39.33 acres, has a mean depth of 7.56 feet, and a volume of 297.16 acre feet, the maximum depth being 21.33 feet. Although

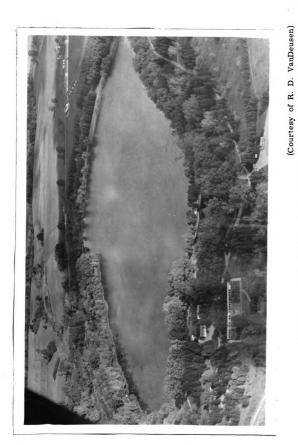


Figure 1. Aerial View of Wintergreen Lake

there are no permanent feeder streams, the lake has a drainage area of 530 acres.

#### He continued:

At the south end of the adjoining swamp area, there is an outlet which empties into Gull Lake, a half mile distant. Presumably, springs located on the north and northeast shore keep the lake at a fairly constant level.

Wintergreen Lake lies in the Kalamazoo-Mississianawa morainic system outwash plain. This plain is characterized by numerous lakes in the morainic basins and in the pits in the outwash plain. Wintergreen Lake is one of many small pit lakes in the vicinity.

Bottom deposits are variable. The south and west shore is [generally] pulpy peat to a depth of three feet, where marl becomes intermixed with it. Marl is predominant to a depth of about twelve feet in all other parts of the lake except the east and northeast shore. These shores are exposed to wave and wind action and are sandy to a depth of 2.5 feet where marl again becomes predominant. Beyond the twelve-feet depth the bottom is of a fine organic ooze.

The area in which the research was conducted was in shallow water on the west side of the lake. The substratum was sandy and covered with silt. Because of the activity of the ducks, there was very little silt within the water pen. Matted algae floated freely nearby during the month of July and in the first two weeks of August. A dense bed of lily pads was growing three feet beyond the deeper end of the water pen. There was no wave action during the period of investigation.

#### Materials

A laboratory was available in the boathouse at Wintergreen Lake where the experiments were conducted. Carboys of tap-water and distilled water were brought in for water supply. A culture rack was placed at the northeast entrance of the boathouse to hold the flasks after inoculation. From approximately 6:30 a.m. until 9:30 a.m. each day, the flasks received direct sunlight and the remainder of the day, diffused light. During the three weeks in which the experiments were conducted, the air temperature in the boathouse fluctuated from 25 degrees to 30 degrees Centigrade.

Glassware (slides, pipettes, and cover slips) was washed in a detergent solution, composed of one-fourth cup of Tide to nine cups of tap-water. They were then boiled in distilled water for one hour on two consecutive days and allowed to cool to room temperature.

Plastic boot squares described below were washed with the detergent solution and rinsed well with sterile distilled water before use.

Solution prepared in the same manner was also used to scrub the air cages and holding funnel before and after each experiment.

The culture medium was prepared by placing 72 grams of sandy loam soil and 100 ml. of distilled water in 250-ml. Erlenmeyer

flasks. These were plugged with cotton. The flasks were then autoclaved at 15 to 20 lbs. pressure and at 100 to 120 degrees

Centigrade for one hour on each of two consecutive days.

Funnel traps, constructed from perma-netting over wooden frames, were used to capture the ducks. The traps were approximately 12 feet long, four feet high, and five feet wide. The entrances to them were funnel-like in shape; i.e., the inner portion of the opening was smaller than the outer portion (Figure 2).

An enclosed pen of perma-netting was constructed in the water where the depth was 10 to 15 inches. The dimensions of the pen were: length, 14 feet; width, 5-1/2 feet; and height, 6-1/4 feet. It was used during the experiment to retain the ducks within a given environment. As the study progressed, I learned that the dabbler ducks required a resting place at night. To provide this, an iron pipe two inches in diameter and three feet long was placed across one corner of the water pen about two inches below the surface of the water (Figure 3).

Two cages were constructed for holding the ducks in the air.

One cage was made from an orange crate, the other from an apple box two feet long, one foot wide, and one foot high. Chicken wire with two-inch mesh was placed across the bottom of the air cages to protect the ducks' feet from faecal contamination. The cages



Figure 2. Funnel Trap



Figure 3. Water Pen

were elevated 43 inches above the ground. They were placed between two trees which were 30 feet apart and provided shade except between 10:00 a.m. and 3:00 p.m. (Figures 4 and 5).

Another piece of apparatus constructed was a metal holding funnel with two five-inch slots cut in the underside. The sharp edges of the slots were covered with tape to prevent injury to the ducks' legs. This funnel was used to hold the ducks and restrain their legs while work was being done on their feet (Figure 6).

Clean plastic squares were cut from a clear plastic tablecloth. These were cupped, filled with 5 ml. of boiled distilled water, and were then tied securely with string around the legs of the ducks to form boots.

# Controls

Three types of controls were used during this period.

- (1) Cultures were planted with lake water from the water pen.
- (2) Boiled pondwater from open fingerbowls was cultured after standing on the air cages. (3) One autoclaved flask of the soilwater media was kept free of inoculation and examined microscopically.

The controlled experiment consisted of the following:



Figure 4
Air Cage



Figure 5. Duck in Air Cage



Figure 6. Duck with Plastic Boots in Funnel

- 1. The ducks were captured in the funnel traps which had been placed in shallow water along the shore of Wintergreen Lake.

  Corn was used as bait to entice ducks to enter the trap.
- 2. According to the phase of the experiment being conducted, the ducks were washed for three to five minutes in a pan of the detergent solution at that time. They were then placed in the water pen.
- 3. While the ducks were in the water pen for varying periods of time, a check of the lakewater in the pen was made to determine the major organisms present. This check was accomplished by culturing 10 ml. of lakewater from the water pen (Table IV), and also by recording the organisms found on both the upper and lower surfaces of glass slides which had been exposed to the lakewater inside the water pen.
- 4. After removal from the water pen, the ducks were placed in the air cage for various lengths of time.
- 5. The ducks were placed in the metal holding funnel and the plastic boots were tied about their feet to obtain the desired washings. The boots were squeezed several times to impose an agitator-type motion upon the ducks' feet to wash off any organisms present.

6. The washing from one foot was centrifuged at low speed for five minutes. All but I ml. at the bottom was decanted. The remainder was shaken and three drops of it were removed for microscopic examination in the fresh state.

The washing from the other foot was poured into flasks of autoclaved soil-water medium which were placed in the culture rack in the boathouse.

- 7. The pH of the culture flasks before inoculation was 6.1 as obtained with a Beckman pH meter. At the conclusion of the culture examination the pH varied from 5.5 to 6.7. Flask 31 (Table III) to which Sphagnum had been added gave a pH reading of 4.8
- 8. The culture flasks were kept in the Botany Department greenhouse at Michigan State University between October 18, 1955, and January 25, 1956. The air temperature during this period ranged from 67 degrees to 76 degrees Fahrenheit.
- 9. Following completion of the culture examination, approximately 5 ml. of the cultured material were added to vials containing an equal amount of 6-3-1 solution. These were preserved for a permanent reference of the organisms cultured during the 1955-1956 period.
- 10. Microscopic examinations of the cultures were made three times during the eight months after inoculation. The method

of examination was to mix by swirling the liquid portion of the flasks and pipette out 5 ml. into a small beaker. This liquid was mixed again and three drops were withdrawn for microscopic study. Five transects were counted of each drop under high dry objective (430x) and two to five transects under low magnification. The organisms observed were classified and recorded (Table III).

Anabaena sp., Asterococcus sp., Chlamydomonas sp., Chlorella sp.,

Chlorococcum sp., Gloeocystis gigas, Palmella sp., Chroococcus sp.,

Gloeocapsa sp., Oscillatoria sp., protozoa: Anisonema-like flagellate,
and an unclassified flagellate, fungal hyphae: Alternaria sp., and a

fern prothallus.

TABLE III

DATA FROM CONTROLLED EXPERIMENTS WITH
CLASSIFICATION OF ORGANISMS, 1955

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
1		Aug. 4, 1955	4	0	3 hrs. 15"	15''	
2		Aug. 4, 1955	3	0	3 hrs. 28''	30''	
3		Aug. 4, 1955	(	Culture			
4		Aug. 6, 1955	0	0	3 hrs.	3811	

TABLE III (Continued)

Planted Culture Examinations						
I	II	III				
August 25, 1955  Unclassified spherical blue-green cell  Scenedesmus sp.  Aphanocapsa sp.	November 18, 1955  Chlamydomonas sp. Chlorococcum sp. Chlorella sp.					
August 25, 1955  Protococcus-like spores	November 19, 1955  Chlorococcum sp.  Encysted Chlamydomonas sp.					
August 25, 1955  No living cells observed	November 19, 1955  Fungal mycelium  unclassified					
August 25, 1955 Unclassified dark brown spore	November 19, 1955  Nannochloris bacil- laris					

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
5		Aug. 6, 1955		4 hr. 2 Air Sa			
6		Aug. 8, 1955	5	0	25''	30''	
7	Re- wash	Aug. 8, 1955	5	0	30''	3 hrs. 5''	

TABLE III (Continued)

## Planted Culture Examinations

I	II	Ш	
August 25, 1955	November 19, 1955  Nannochloris bacil-	April 7, 1956  Nannochloris bacil-	
Unclassified yellow- green flagellate	laris	laris Chlorella sp. Gloeocapsa sp. Colorless flagel- lates	
August 23, 1955	November 19, 1955	April 7, 1956	
Only bacteria observed	Protococcus viridis	Oedogonium sp. Protococcus viridis Nannochloris bacil- laris Gloeocystis gigas	
August 25, 1955	November 19, 1955	April 14, 1956	
Arachnochloris-like  cell  Rhizoclonium fon-  tanum  Unclassified blue- green cell	Rhizoclonium fon- tanum	Rhizoclonium fon- tanum	

TABLE III (Continued)

		<u> </u>					
Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
9		Aug. 15, 1955	0	0	15"	l hr.	Arachnochloris sp. Epidermal cells from the duck's feet
10		Aug. 15, 1955	0	0	15"	l hr.	Aphanocapsa sp. Oval yellow- green cell 17μ by 20μ
11		Aug. 15, 1955		2 hr. 3 Air Sa			

TABLE III (Continued)

Planted Culture Examinations						
I	II	III				
August 26, 1955  Nothing observed	December 7, 1955  Sphaerocystis Schroeteri Gloeocystis gigas Tetraedron minimum Bacteria					
August 26, 1955  Nothing observed	Protococcus viridis Sphaerocystis Schroteri Tetraedron minimum Oedogonium sp. Unclassified green unicell Bacteria					
August 26, 1955  Unclassified fungal spore	December 7, 1955  Chroococcus sp.  Gloeocystis gigas	April 14, 1956  Oscillatoria sp. Gloeocystis gigas				

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
12		Aug. 16, 1955	Fresh Lake Water 10 ml. of Tide Solution added to flask after inoculation				Aphanocapsa sp.  Microcystis sp.  Euglena sp.  Oscillatoria sp.
13		Aug. 16, 1955	5		e Expos		Oscillatoria sp. Aphanocapsa sp. Microcystis sp.

TABLE III (Continued)

Planted Culture Examinations						
I	II	III				
August 26, 1955  Nannochloris bacil- laris	Chlamydomonas globosa Scenedesmus quadricauda Oscillatoria lim- netica Euglena proxima Navicula sp. Amoeba Holotricha ciliates					
Scenedesmus armatus Chlamydomonas sp. Spirulina sp. Oscillatoria sp. Diatoms  August 24, 1955  Arachnochloris-like cell	Scenedesmus spp. (2) Scenedesmus armatus Gloeocystis gigas Oedogonium sp. Phacotus lenticularis Ankistrodesmus convolutus Navicula sp. Phacus orbicularis P. acuminata P. pyrum Lepocinclis acuta Amoeba verrucosa Rotifer Euchlanis	April 14, 1956  Encysted Chlamydodomonas sp. Oscillatoria spp. (2) Anabaena sp. Navicula sp. Monas-like flagellates Frontonia-like cilliate				

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
14		Aug. 16, 1955		Faecal	Sample		
15		Aug. 17, 1955	5	30''	15"	30''	Diatom frus- tules Dead brownish- green fila- ments

TABLE III (Continued)

Planted Culture Examinations						
I	II	III				
August 26, 1955	December 8, 1955	April 14, 1956				
Piece of Elodea leaf Unclassified algal spores Unclassified proto- zoa Bacteria	Chlamydomonas sp. Arthrospira sp. Spirulina sp. Phormidium sp. Oscillatoria spp. (2) Small phytoflagellates Unclassified protozoan cysts	Gloeocystis gigas Spirogyra sp. Oscillatoria spp. (2) Spirulina sp. Paramecium bur- saria				
August 27, 1955  Debris Only	Scenedesmus sp. Ankistrodesmus convolutus Chlamydomonas sp. Encysted green spheres Tetrahedron mini- mum Phormidium sp. Navicula sp.					

TABLE III (Continued)

			<del>,</del>	<b></b>		<del>, , , , , , , , , , , , , , , , , , , </del>	
Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
16		Aug. 17, 1955	5	30''	15''	5''	Fungal spore
17		Aug. 17, 1955	5	hr. Aii	r Sampl	e	
18		Aug. 18, 1955	5	30''	30''	30''	

TABLE III (Continued)

Planted Culture Examinations						
I	II	III				
August 27, 1955 Debris only	Chlamydomonas sp. Scenedesmus arcuatus Gloeocapsa sp. Ankistrodesmus convolutus Unclassified phytoflagellates					
August 27, 1955 Debris Only	December 8, 1955  Fungal spore (Alternaria sp.)	·				
August 27, 1955 Debris Only	Scenedesmus sp. Carteria multifilis Franceia sp. Chlamydomonas sp. (palmella stage) Ankistrodesmus convolutus					

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
19		Aug. 18, 1955		3 hr. 4 Air Sa			
20	l (bill)	Aug. 22, 1955	0	l day	l hr.	30''	Blood cells from leg injury
21	l (feet)	Aug. 22, 1955	0	1 day	l hr.	511	Arachnochloris- like cells

TABLE III (Continued)

# Planted Culture Examinations

I	II	III
October 12, 1955 Chlorococcum sp.	December 30, 1955  Sphaerocystis sp.	April 15, 1956 Oscillatoria sp.
Sphae rocystis sp.	Asterococcus sp. Chlorococcum sp. Palmella sp. Chrysocapsa sp.	Chlamydomonas sp. Anisonema-like flagellate
October 14, 1955	December 31, 1955	
Aphanothece sp.	Nannochloris sp.	
November 11, 1955	December 31, 1955	April 20, 1956
Scenedesmus bijuga Chlamydomonas sp. Oscillatoria angustissima Navicula sp. Euglena sp.	Scenedesmus abun- dans Ankistordesmus convulutus Oscillatoria angus- tissima Phormidium sp.	Scenedesmus bijuga Scenedesmus abun- dans Mougeotia sp. Oscillatoria sp. Navicula sp.

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
22	2 (feet)	Aug. 22, 1955	5	l hr.	30''	30''	Feet: only debris Bill: Arachno- chloris-like cells
23	2 (feet)	Aug. 22, 1955	0	l day	2 hr.	30''	Encysted green unicells, blood cells, and epidermal cells from the duck's feet
24	2 (bill)	Aug. 22, 1955	0	l day	2 hr.	5''	

TABLE III (Continued)

## Planted Culture Examinations I II III November 9, 1955 December 31, 1955 Scenedesmus abun-Scenedesmus quaddans ricauda Scenedesmus quad-Scenedesmus abunricauda dans Chlamydomonas Ankistrodesmus globosa convolutus Chlamydo monas Chlamydomonas sp. pseudopertyi Tetraedron sp. Chlamydomonas sp. Phormidium sp. Chlamydomonas Navicula sp. mucicola Ankistrodesmus sp. Tetraedron sp. Navicula sp. Euglena minuta November 9, 1955 December 31, 1955 Debris only Phormidium foveolatum Microcystis sp. Plectonema nostocorum Phormidium sp. November 5, 1955 December 31, 1955 Fungal mycelium Fungal mycelium

	; ;



TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
25	4	Aug. 22, 1955	0	4 hrs.	30''	30''	Epidermal cells from the duck's feet Unclassified spores
26		Aug. 22, 1955		Air Sa	ample		
27	6	Aug. 23, 1955	5	24 hrs.	30''	25''	No algal cells

TABLE III (Continued)

Planted Culture Examinations							
I	II	III					
Chlamydomonas sp. Scenedesmus quadricauda Ankistrodesmus sp. Oscillatoria limnetica Navicula sp. Zoomastigidina	December 31, 1955  Scenedesmus abundans Scenedesmus quadricauda Chlamydomonas spp. Chlamydomonas globosa Phacotus-like cell Ankistrodesmus sp. Tetraedron sp. Oscillatoria sp. Navicula sp. Monas-like flagellates						
November 5, 1955  Nannochloris bacil- laris	January 6, 1956  Nannochloris bacil- laris Chlorella sp.	April 20, 1956  Fern prothallus  Chlorella sp.  Anisonema-like cell					
November 5, 1955  Ankistrodesmus convolutus Scenedesmus bijuga	January 6, 1956  Ankistrodesmus convolutus Scenedesmus bijuga Protococcus sp.						

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
28 (s) <sup>a</sup>	5 (died)	Aug. 23, 1955	5	13 hrs.	3011	30''	Cosmarium sp. Arachnochloris- like cell Navicula sp. Euglena sp. Misc. diatoms and spores
31 (s)	1	Aug. 24, 1955	5	24 hrs.	l hr.	30''	Arachnochloris- like cell
32 (s)	7 (died)	Aug. 24, 1955	5	19 hrs.	2 hrs.	30''	Arachnochloris- like cell Unclassified blue-green unicell
33 (s)	2nd 3	Aug. 25, 1955	5	19 hrs.	4 hrs.	25''	Debris only (boot fell off during wash- ing)

a(s) a lump (3 to 4 grams) of Sphagnum peat was added to the culture flask.

TABLE III (Continued)

Planted Culture Examinations							
I	II	III					
November 4 and 9, 1955 Ankistrodesmus sp. Fungal spores Chlamydomonas sp. Chrysidella sp.	January 6, 1956  Scenedesmus bijuga Ankistrodesmus convolutus Chromulina sp. Chlamydomonas sp.						
October 28, 1955  Tetradesmus wisconsinense	January 6, 1956  Tetradesmus wis- consinense	April 25, 1956  T. wisconsinense Chlorella sp. Phormidium sp. Zooflagellate					
October 28, 1955  Ankistrodesmus convolutus	January 6, 1956  Ankistrodesmus  convolutus						
October 28, 1955  Bacteria, Fungal spore Unclassified bluegreen unicell	January 6, 1956 Nothing observed						

TABLE III (Continued)

						·	
Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
34		Aug. 24, 1955		Air Sa	ample		
35 (s)	6	Aug. 26, 1955	5	26 hrs.	24 hrs.	30''	Debris only
36 (s)	8	Aug. 26, 1955	5	33 hrs.	16 hrs.	30"	Gray spores
37 (s)	2nd 2	Aug. 26, 1955	5	24 hrs.	16 hrs.	30''	Blood cells from foot injury
38		Aug. 26, 1955	30 hr. Air Sample				

TABLE III (Continued)

Planted Culture Examinations							
I	II	III					
October 28, 1955  No living cells observed	January 6, 1956 Nothing observed						
October 27, 1955  Fungi Spores Scenedesmus sp.	January 6, 1956  Nannochloris bacil- laris  Monas-like flagel- lates						
October 27, 1955 Fungi Bacteria	January 6, 1956 Fungi						
October 21, 1955  Fungal spore Alternaria sp.	January 6, 1956  Nannochloris-like cell Anabaena (young filament)						
October 18, 1955  Anabaena sp. (young)	January 6, 1956 Anabaena sp.						

TABLE III (Continued)

Flask No.	Duck No.	Date: Cul- ture Plant	Tide Wash Time in Min.	Time in H <sub>2</sub> O Pen	Time in Air Cage	Boot Wash Time	Uncultured Washing Examination
39		Aug. 26, 1955	Sam	ple of ]		ater	

TABLE III (Continued)

### Planted Culture Examinations

October 14 and 15, 1955 Scenedesmus armatus Scenedesmus abundans Ankistrodesmus Convolutus Oedogonium sp. Oscillatoria sp. Fragilaria sp. Navicula sp. Paranema sp. Cladoceran  II  January 6, 1956  April 25, 1956  Scenedesmus bijuga Ankistrodesmus Convolutus Chlorella sp. Mougeotia sp. Tetraedron sp. Chlamydomonas sp. (encysted) Nannochloris bacillaris Gloeocapsa sp. Oscillatoria imnetica Oscillatoria sp. Fungal spore Alternaria sp. Fragilaria sp. Monas-like flagellates		· · · · · · · · · · · · · · · · · · ·	
Palmella-like   Cells   Scenedesmus bijuga   Ankistrodesmus   Convolutus   Convol	I	II	III
	Scenedesmus armatus Scenedesmus abundans Ankistrodesmus convolutus Oedogonium sp. Oscillatoria sp. Fragilaria sp. Navicula sp. Paranema sp.	Palmella-like cells Scenedesmus arma- tus Scenedesmus lim- netica Misc. diatoms Ankistrodesmus convolutus Closterium gracile	Scenedesmus bijuga Ankistrodesmus convolutus Chlorella sp. Mougeotia sp. Tetraedron sp. Chlamydomonas sp. (encysted) Nannochloris bacillaris Protococcus viridis Gloeocapsa sp. Oscillatoria limnetica Oscillatoria spp. (2) Navicula sp. Fungal spore Alternaria sp. Fragilaria sp. Monas-like flagel-

TABLE IV

ORGANISMS IN WATER PEN, 1955

Date	Samples	Organisms Found
August 24	10 ml. Lake Water Cultured	Gloeocapsa sp. Gloeocystis sp. Oscillatoria sp. Spirulina sp. Diatoms
August 27	Planted Slides	Green algae:  Arachnochloris sp.  Gloeocystis sp.  Blue-green algae: Chroococcus sp. Others: Arcella sp. Cladoceran Entosiphon sp. Glenodinium sp. Gomphonema sp. Monas-like flagellates Peranema sp. Synura sp. Rotifers Diatoms Spores Snails Other ciliate protozoa

### CHAPTER V

### CONTROLLED EXPERIMENT, 1956

#### Modifications

In 1956 the research plans were modified in order to obtain more precise data in respect to waterfowl dispersal of algae. The general procedure as outlined in Chapter IV was continued with the following modifications.

- 1. The media was prepared by placing 24 grams of loam soil into a flask and then adding 25 ml. of water from Wintergreen Lake. Pinches (approximately one-sixteenth of a teaspoon) of CaCO<sub>3</sub> and starch were added to another set of flasks before the addition of the soil. The flasks were then autoclaved for one hour at 15 pounds pressure on each of three consecutive days.
- 2. The sizes of the Erlenmeyer culture flasks were reduced from the 150 or 200 ml. to 50 ml.
- 3. A 1.625 solution of Roccal (active ingredient, alkyl  $C_8H_{17}$  to  $C_{18}H_{37}$  dimethyl benzyl ammonium chlorides-10%, Sterwin Chemicals, Inc., New York) was used for the storage of clean slides and coverslips.



Figure 7. Wintergreen Lake Showing Filamentous Algal Mat and Lily Pads with Boathouse in Background



Figure 8. Ducks in Water Pen

- 4. Each flask was flamed before and after the cotton plugs were removed. Pipettes, slides, and coverslips were also flamed prior to use even though they had been cleaned and stored in Roccal solution.
- 5. After removal from the water pen, the ducks were harnessed and hung upon a clothesline, instead of being placed in the air cages (Figure 9). At the suggestion of Mr. R. D. VanDeusen (1956) an elastic band was placed over the ducks' eyes to reduce their activity while they were hanging on the clothesline. This blind did not materially affect the activity of the ducks in these experiments, and was discontinued.
- 6. Clean cheesecloth netting was placed around the ducks when they hung in the air for long periods of time (15 hours or over). Netting was also placed over most of the fingerbowls of boiled pondwater which were exposed to the outside air about five feet above ground to reduce the possibility of micro-organism contamination by insects (Figure 10). This may be an important factor depending on the specific conditions under which the research is carried out. The results did not seem to indicate that contamination by insects had occurred.
- 7. Following the suggestion of Dr. W. E. Wade (1956) the boot-wash time was reduced from 30 minutes to 15 minutes. There



Figure 9. Ducks in Harnesses and Fingerbowl Hanging on Clothesline



Figure 10. Ducks and Fingerbowl Covered with Cheesecloth Netting Hanging on Clothesline

was no essential difference in the number or type of organisms found when one foot of a bird was boot-washed for 30 minutes and the other for 15 minutes (Figures 11 and 12).

- 8. The culture flasks, after being inoculated with the boot washings, were stored in an area where they received indirect light throughout the day (Figure 13) as advised by Dr. E. G. Pringsheim (1956).
- 9. The temperature of the culture flasks was checked by placing a clean centigrade thermometer in a culture flask plugged with cotton. In this way the temperature of the culture medium was recorded as well as the air temperature where the cultures were stored. The water temperature of the culture flask ranged from 14 degrees to 26 degrees Centigrade, while the air temperature fluctuated from 9 degrees to 34.5 degrees Centigrade.
- 10. The pH of the flasks was checked with a glass electrode Beckman pH meter. Two flasks with CaCO<sub>3</sub> had a pH of 7.3 and 7.4. After swirling, the reading was 8.3. The pH of the flasks with plain soil was 6.2. After swirling, the pH was 6.8.
- 11. Microscopic examinations were made of some of the uncultured washings. In Table V are listed the organisms found.
- 12. The culture flasks were sampled in the following manner.

  After the flasks were swirled, a sterile pipette was used to collect

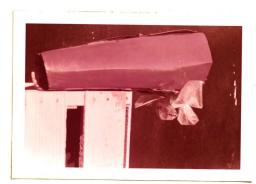


Figure 11. Duck with Plastic Boots in Funnel, Side View



Figure 12. Duck with Plastic Boots in Funnel, Rear View

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			· · · · · · · · · · · · · · · · · · ·



Figure 13. Culture Flasks

medium along the side of the flask from the surface down to the soil. Two drops were taken from the upper, middle, and lower portions of the pipette for sampling. These drops were studied microscopically by examining three or more transects under low power and three or more transects under high power. An additional examination was made of any visible growth in the flask. The organisms present are listed in Tables VI and VII.

- 13. In addition to the water pen series, another set of experiments was conducted whereby the ducks were placed in a pen situated in the mud and organic debris along the lake shore (Table VIII and Figure 14).
- 14. Another improvement over the 1955 procedure was the recording of the environmental conditions. Both relative humidity and air temperature were checked while the experimental ducks were in the air. Wind velocity readings were obtained from the Kellogg Forest Station about three miles from the research area (Table XV).

#### Control Culture Flasks

Ten culture flasks containing sterile soil-water medium were examined microscopically along with the experimental culture flasks.

They served as controls to determine if any contamination of the flasks had occurred either from the soil-water medium or during



Figure 14. Mud Pen

the examination period. Some of the flasks showed bacterial and/or fungal contamination during the eight-month period. However, no algal or protozoan contaminations were observed.

Fingerbowls of boiled pondwater were exposed to the air for various periods of time and cultured in soil-water medium. The following organisms were observed living in the cultures (based on 96 examinations of 32 culture flasks).

Green algae: Chlamydomonas sp., Chlorella ellipsoidea,

C. vulgaris, Euglena sp., Gloeocystis gigas, G. vesiculosa, G. sp.,

Nannochloris bacillaris, Oedogonium sp., Pleodornia californica,

Protococcus sp., Rhizoclonium sp., Scenedesmus abundans, S. bijuga,

S. quadricauda, Sphaerocystis sp., Ulothrix sp., and Vaucheria sp.

Blue-green algae: Chrococcus sp., Gloeocapsa sp., Nostoc sp., Oscillatoria lacustris, O. Minima, O. sp., Pelogloea bacillifera, Phormidium tenue, and P. sp.

Protozoa: Colpoda sp., unclassified amoeba, and an unclassified flagellate.

Higher plants: Elodea sp., Fern prothalia, and Utricularia sp.

Table IX lists the organisms observed microscopically in the control Cultures.

Another control study was culturing a 10-ml. sample of the boiled pondwater as used for washing the bills, feathers, and feet of the ducks. Examination on April 14, 1957, revealed no living organisms.

#### Winter Data

In November of 1956 eight feather samples were obtained from four Mallard ducks. After being in the water pen for one hour on November 2, two ducks were held in the air for ten minutes. Lower breast and undertail covert feathers were removed with sterile forceps. They were placed in vials half-filled with boiled pondwater. The vials were capped, shaken, and left standing for three hours. Cultures were planted with the feather washings on November 2, 1956. Two examinations were made of these cultures. The organisms present are recorded in Table X.

Ten ml. of lakewater were taken from the water pen and planted in soil-water medium on November 2, 1956. A study was made of the uncultured lakewater showing Oedogonium sp., Rhizo-clonium sp., Anabaena subcylindrica, Microcystis aeruginosa, Navicula sp., Synedra sp., and a copepod nauplius to be present.

The first microscopic examinations of the cultures were made on November 16, 1956, and the following organisms were observed:

Nannochloris bacillaris, Oocystis gigas, Scenedesmus quadricauda,

Microcystis aeruginosa, Microspora sp., Oscillatoria spp. (2), Na
vicula sp., and unclassified diatoms. The protozoa present were

Uronema sp., Stylonichia-like ciliate, and an unclassified flagellate.

On April 6, 1957, the second examinations were made and these organisms were present: Ankistrodesmus falcatus, Chlamydomonas sp., Oedogonium sp., Pediastrum Boryanum, Scenedesmus bijuga, Sphaerocystis Schroeteri, Tetraedron minimum, Oscillatoria spp. (3), Navicula sp., and Phacus pleuronectes. Other organisms seen were protozoa: Oxytricha sp., and an unclassified ciliate; nematode: Rhabdolaimus-like; an unclassified rotifer, and bacteria.

The remainder of the feather washings were made on November 23, 1956. These washings are described and the organisms are recorded on Table XI.

Lakewater directly from the water pen was also examined on November 23, 1956. Microcystis aeruginosa, Navicula sp., Oedogonium sp., and a copepod nauplius were seen.

TABLE V

CLASSIFICATION OF ORGANISMS OBSERVED IN UNCULTURED WASHINGS, 1956

Flask No.	Organisms Observed
36 and 37 <sup>a</sup>	Chlamydomonas sp. (encysted) Synedra sp. Oocystis sp. Cosmarium sp.
41 and 42	Occystis Borgei Oscillatoria sp.
43 and 44	Fragilaria sp. Unclassified cysts or spores
45 and 46	Fungal spore: Alternaria sp.
48 and 49	Debris only
52 and $53$	Fungal spore: Alternaria sp.
55 and 56	Bacteria (faecal contamination)
57 and 58 <sup>b</sup>	Rhizoclonium sp. (faecal contamination) Fungal spore: Alternaria sp.
61 and 62	Fungal spore: Alternaria sp. Unclassified spores or cysts (faecal contamination)

<sup>&</sup>lt;sup>a</sup>Centrifuged for five minutes.

Note:  $C_{16}$ , an uncultured air sample, showed only debris.

<sup>&</sup>lt;sup>b</sup>Centrifuged for ten minutes.

DATA FROM CONTROLLED EXPERIMENTS (WATER PEN)
WITH CLASSIFICATION OF ORGANISMS, 1956

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
1	July 12	CaCO <sub>3</sub> <sup>a</sup>	Tide		30	30
2	July 12	plain b	Tide		30	30
3	July 12	CaCO <sub>3</sub>		45 m	inute Air S	Sample
4	July 12	plain		45 m	inute Air S	Sample
5	July 13	plain	Roccal 1:1250		30	30

<sup>&</sup>lt;sup>a</sup>Pinches of CaCO<sub>3</sub> and starch added.

b<sub>No</sub> material added to the soil-water medium.

TABLE VI (Continued)

·	Planted Culture Examinati	ons	
I	II	III	
July 18, 1956 Bacteria	September 25, 1956 Bacteria	December 27, 1956  Protococcus viridis Scenedesmus abundans Bacteria	
July 18, 1956 Bacteria	September 25, 1956 Bacteria	December 27, 1956 Gloeocystis gigas Protococcus viridis	
July 19, 1956 September 25, Bacteria Bacteria		December 27, 1956 Bacteria	
July 19, 1956 Bacteria	September 25, 1956  Vaucheria sp.	December 28, 1956 Bacteria	
July 20, 1956 Bacteria Protozoa: unclas- sified ciliates	September 25, 1956 Bacteria	December 28, 1956 Bacteria	

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
6	July 13	plain	Roccal 1:1250		30	30
7	July 13			1 h	our Air Sa	ample

TABLE VI (Continued)

<b>1</b> 1	Franced Curture Examinations				
I	II	III			
July 20, 1956	September 25, 1956	December 28, 1956			
Protozoa: Monas socialis Bodo-like ciliates Bacteria	Ankistrodesmus spp. (2) Carteria sp. Chlamydomonas sp. Closteriopsis-like cell Scenedesmus quad- ricauda Oscillatoria sp. Unclassified diatom	A. Braunii A. convolutus A. falcatus Chlamydomonas globosa Chlorella vulgaris Scenedesmus bijuga S. quadricauda Oscillatoria limnetica Unclassified diatom			
July 26, 1956	September 27, 1956	December 31, 1956			
Bacteria Fungal hyphae	Bacteria	Chlorella vulgaris Nannochloris bacil- laris Oscillatoria minima Euglena sp. Fungal hyphae: Alternaria sp. Bacteria			

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
8	July 13	plain		1 h	our Air Sa	imple
9	July 17	CaCO <sub>3</sub>	Roccal 1:625		30	30
10	July 17	plain	Roccal 1:625		30	30
11	July 17	CaCO <sub>3</sub>		45 mi	nute Air S	Sample

TABLE VI (Continued)

F	Planted Culture Examinations					
I	II	III				
July 26, 1956	September 27, 1956	January 2, 1957				
Bacteria	Chlorella vulgaris Bacteria	Chlorella vulgaris  Nannochloris bacil-  laris  Pleodorina califor-  nica  Euglena sp.  Bacteria				
July 26, 1956	September 27, 1956	January 2, 1957				
Bacteria	Bacteria	Chlorella vulgaris Euglena sp. Nannochloris bacil- laris Bacteria				
July 26, 1956	September 27, 1956	January 3, 1957				
Bacteria Fungal hyphae	Chlorella vulgaris Nannochloris bacil- laris	Chlorella vulgaris Bacteria				
July 26, 1956	September 27, 1956	January 3, 1957				
Fungal hyphae	Unclassified blue- green cell	Euglena sp. Fungal hyphae: Alternaria sp. Bacteria				

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
12	July 17	plain		45 m	inute Air	Sample
13	July 19	CaCO <sub>3</sub>		30	5	15
14	July 19	plain		30	5	15
15	July 19	CaCO <sub>3</sub>	Roccal 1:625		15	15

TABLE VI (Continued)

I	II	III
July 12, 1956	September 29, 1956	January 3, 1957
Fungal hyphae	Fungal spore: Alternaria sp. Bacteria	Bacteria
Contaminated		
July 31, 1956  Microcystis aeru- ginosa Bacteria	September 29, 1956  Chlorella vulgaris  Navicula sp.	January 8, 1957  Euglena sp.  Fungal hyphae  Bacteria
July 31, 1956 Nothing observed	September 29, 1956  Plectonema-like filament	January 10, 1957  Euglena sp. Oscillatoria sp. Bacteria

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute <b>W</b> ash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
16	July 19	plain	Roccal 1:625		15	15
17	July 19	plain	Roccal 1:625		15	Bill wash- ing
18	July 20	CaCO <sub>3</sub>				15

TABLE VI (Continued)

I	II	III	
July 31, 1956 Bacteria	September 29, 1956  Chlorella vulgaris  Nannochloris bacil- laris Rhabdoderma irreg- ulare Scenesdesmus quad- ricauda	January 10, 1957  Chlamydomonas sp. Nannochloris bacillaris Scenedesmus abundans S. quadricauda Lyngbya sp. Microcystis aeruginosa Oscillatoria sp. Euglena sp.	
July 31, 1956 Bacteria	September 29, 1956  Chlorella vulgaris Oscillatoria lim- netica Bacteria	January 12, 1957  Oscillatoria spp. (2) Bacteria	
July 31, 1956 Bacteria	September 29, 1956 Bacteria	January 12, 1957  Chlamydomonas sp.  Euglena sp.  Bacteria	

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
19	July 20	CaCO <sub>3</sub>				30
20	July 20	plain				15

TABLE VI (Continued)

I	II	III		
July 31, 1956 Bacteria	September 29, 1956  Spirulina sp. Bacteria	January 12, 1957  Oscillatoria lim- netica		
August 1, 1956  Protozoa: Monas sp. Holotrich ciliates Bacteria	Ankistrodesmus sp. Gloeocystis sp. Nannochloris bacillaris Scenedesmus bijuga Tetraedron minimum Unclassified phytoflagellate Arthrospira Gomontiana Chroococcus dispersus Oscillatoria limnetica Euglena minuta Protozoa: unclassified Holotricha	Chlamydomonas spp. (2) Chlorella sp. Gloeocystis gigas Nannochloris bacillaris Tetradesmus sp. Arthrospira Jenneri Microcystis sp. Oscillatoria limnetica Oscillatoria sp. Euglena sp. Navicula sp. Synedra sp. Bacteria		

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in <b>M</b> in.	Boot Wash in Min.
21	July 21	plain				30
22	July 21	CaCO <sub>3</sub>	Tide	15	30	15

TABLE VI (Continued)

I	II	III		
August 1, 1956  Euglena sp. Protozoa: unclassified ciliates	Ankistrodesmus sp. Gloeocystis sp. Nannochloris bacil- laris Scenedesmus bijuga S. quadricauda Tetraedron minimum Unclassified phyto- flagellates Oscillatoria lim- netica Euglena spp. (2) Diatoms (sp.)	Ankistrodesmus sp. Chlorella ellipsoidea Chlorella vulgaris Dactylococcopsis acicularis Scenedesmus di- morphus Scenedesmus sp. Tetraedron minimun Oscillatoria lim- netica Oscillatoria sp. Euglena minuta Euglena sp. Diatoms (3 spp.) Bacteria		
August 1, 1956 Bacteria	October 2, 1956 Bacteria	January 14, 1957 Bacteria Fungal hyphae Unclassified spore		

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
23	July 21	plain	Tide	15	30	15
24	July 23	CaCO <sub>3</sub>	Tide	30	30	15
25	July 23	plain	Tide	30	30	15

TABLE VI (Continued)

I	11	III
August 3, 1956	October 2, 1956	January 15, 1957
Aphanothece cas- tagnei Pelogloea bacillifera Bacteria Protozoa: unclas- sified flagellate	Arthrospira Gomo- tiana Euglena sp. (en- cysted) Oscillatoria spp. (2) Diatom Unclassified phyto- flagellate Bacteria	Arthrospira Jen- neri Euglena sp. Oscillatoria spp. (2) Diatoms (2) Protozoa: unclas- sified flagellate
August 3, 1956	October 2, 1956	January 18, 1957
Aphanothece cas- tagnei	Microcystis-like cell Bacteria	Bacteria
August 4, 1956	October 2, 1956	January 18, 1957
Debris	Oscillatoria spp. (2) Diatoms Bacteria	Euglena sp. Navicula sp. Phormidium tenue Bacteria

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
26	July 23	plain	Tide	30	30	Bill wash- ing
27	July 23	CaCO <sub>3</sub>	Tide	l hr.	30	15
28	July 23	plain	Tide	l hr.	30	15
29	July 23	CaCO <sub>3</sub>	Tide	2 hrs.	30	15

TABLE VI (Continued)

I	II	III	
August 9, 1956	October 2, 1956	January 18, 1957	
Gonium sociale Fungal hyphae Protozoa: unclassified ciliate Unclassified flagellate	Chlorella vulgaris  Nannochloris bacil-  laris Chroococcus dis-  persus Oscillatoria spp. (2)  Euglena sp. (en-  cysted) Diatoms Protozoa: Monas- like flagellate Bacteria	Chlamydomonas sp. Chlorella vulgaris Oscillatoria lim- netica Phormidium tenue Navicula sp. Synedra sp.	
August 9, 1956	October 3, 1956	February 2, 1957	
Bacteria	Bacteria	Oscillatoria lim- netica Bacteria	
August 14, 1956	October 2, 1956	February 2, 1957	
Fungal spore: Alternaria sp. Bacteria	Protozoa: unclas- sified flagellate Unclassified ciliate Bacteria	Oscillatoria acutis- sima O. limnetica Bacteria	
August 14, 1956	October 4, 1956	February 2, 1957	
Aphanothece sp.	Bacteria	Bacteria	

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
30	July 23	plain	Tide	2 hrs.	30	15
31	July 23	CaCO <sub>3</sub>	Tide	2 hrs.	30 Bill Washi	15

TABLE VI (Continued)

I	II	III		
August 14, 1956	October 4, 1956	February 2, 1957		
Arachnochloris sp.  Navicula sp.  Fungal spore:  Alternaria sp.	Chlorella vulgaris  Scenedesmus quad- ricauda Unclassified phyto- flagellate Euglena sp. Diatoms Bacteria	Chlorella ellipsoide Scenedesmus abun- dans Oscillatoria lim- netica Euglena sp. Navicula sp.		
August 14, 1956  Chlorella vulgaris Gloeocystis gigas Nannochloris bacillaris Scenedesmus abundans S. quadricauda	Chlorella vulgaris Chlorella sp. Gloeocystis gigas Kirchneriella sp. Scenedesmus bijuga S. opoliensis Unclassified phyto-	Ankistrodesmus sp.  Oscillatoria lim- netica Oscillatoria sp.  Euglena sp. Navicula sp. Diatom		
Scenedesmus sp. Oscillatoria spp. (2) Euglena minuta Navicula spp. (2) Protozoa: unclassified flagellates Bacteria Fungal hyphae	flagellate Oscillatoria lim- netica O. sp. Euglena sp. Diatoms Protozoa: unclas- sified Holotricha	Bacteria		

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
32	July 24	CaCO <sub>3</sub>	Tide	4 hrs.	30	15
33	July 24	plain	Tide	8 hrs.	30	15
34	July 24	CaCO <sub>3</sub>	Tide	8 hrs.	30	15
35	July 24	plain	Tide	8 hrs.	30	15

TABLE VI (Continued)

I	II	III
August 15, 1956 Bacteria	October 5, 1956 Bacteria	February 7, 1957  Oscillatoria lim- netica Bacteria
August 15, 1956  Fungal spore: Alternaria sp. Bacteria	October 5, 1956  Chlorella vulgaris Oscillatoria sp. Bacteria	February 16, 1957  Chlamydomonas sp. Chlorella vulgaris  Euglena sp. Bacteria
August 15, 1956 Bacteria Protozoa: unclas- sified unicell	October 5, 1956 Bacteria	February 16, 1957  Anabaena sp. Lyngbya limnetica Oscillatoria sub- brevis
August 15, 1956 Bacteria Protozoa: unclas- sified unicell	October 5, 1956  Navicula sp. Bacteria	February 16, 1957  Lyngbya attenuata Oscillatoria sp. Fungal spore

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
36	July 25	CaCO <sub>3</sub>	Tide	16 hrs.	30	15
37	July 25	plain	Tide	16 hrs.	30	15

TABLE VI (Continued)

Planted Culture Examinations					
I	II	III			
August 15, 1956  Protozoa: Oikomo-	October 5, 1957	February 16, 1957			
Protozoa: Oikomo- nas termo Bacteria: Spirillum sp.	Chlamydomonas sp.  Phacus sp.  Euglena sp.  Navicula sp.  Protozoa: Monas  sp.  Peranema sp.  Bacteria	Chlamydomonas globosa Oscillatoria lim- netica Navicula sp. Protozoa: Monas- like flagellate			
Ace gust 15, 1956	October 6, 1956	February 17, 1957			
Fuz mgal hyphae Pr-≪tozoa: Holotrich ≪iliate	Ankistrodesmus sp. Chlorella vulgaris Nannochloris bacil- laris Tetraedron minimum Euglena sp. Phacus sp. Navicula sp. Protozoa: Monas sp.	Ankistrodesmus convulutus Chlamydomonas sp. Aphanocapsa sp. Oscillatoria sp. Phormidium sp. Euglena sp. Phacus sp. Navicula sp. Protozoa: Monas sp. Oikomonas sp. Bacteria			

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TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Tim <b>e</b> in Water Pen	Air Time in Min.	Boot Wash in M <b>i</b> n.
38	July 25	plain	Tide	16 hrs.	30	Bill wash- ing
39	July 25	CaCO <sub>3</sub>	Tide	24 hrs.	30	15
40	July 25	plain	Tide	24 hrs.	30	15

TABLE VI (Continued)

Planted Culture Examinations				
I	II	Ш		
August 16, 1956	October 6, 1956	February 17, 1957		
Oscillatoria lim- netica Fungal hyphae	Nannochloris bacil- laris Scenedesmus sp. Unclassified phyto- flagellate Oscillatoria sp.	Scenedesmus bijuga Tetraedron minimum Oscillatoria sp. Phormidium muci- cola Euglena minuta		
August 16, 1956	October 6, 1956	February 21, 1957		
Fungal hyphae Bacteria	Gloeocystis gigas Oscillatoria spp. (2) Bacteria	Chlamydomonas sp. Scenedesmus bijuga Lyngbya limnetica Oscillatoria angusta O. subbrevis		
August 16, 1956	October 6, 1956	February 21, 1957		
Chromulina sp. Cryptoglena pigra	Euglena sp. (encysted)  Navicula sp. Scenedesmus quadricauda Unclassified phytoflagellate Protozoa: Monas sp. Unclassified ciliate	Chlorella vulgaris Nannochloris bacil- laris Protococcus sp. Rhabdoderma sp. Scenedesmus bijuga S. quadricauda Oscillatoria lim- netica Euglena sp. Navicula sp. Protozoa: Monas sp.		

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
41	Aug. 2	CaCO <sub>3</sub>	None <sup>a</sup>	1 hr.	15	15
42	Aug. 2	plain		l hr.	15	15
43	Aug. 2	CaCO <sub>3</sub>		l hr.	30	15

<sup>&</sup>lt;sup>a</sup>Detergent wash discontinued for remaining washings.

TABLE VI (Continued)

I	II	III
August 17, 1956	October 6, 1956	February 22, 1957
Arachnochloris sp. Unclassified spore	Aphanotheca nidu- lans Bacteria	Chlamydomonas sp. Euglena sp. Oscillatoria lim- netica Bacteria
August 17, 1956	October 9, 1956	February 25, 1957
Fungal hyphae Protozoa: Unclas- sified flagellate	Chlorella sp.  Euglena sp.  Diatoms (2 sp.)  Unclassified phyto- flagellate	Gloeocystis gigas Nannochloris bacil- laris Oedogonium sp. Oscillatoria lim- netica Euglena sp. Navicula sp.
August 18, 1956	October 9, 1956	February 27, 1957
Aphanothece nidu- lans Bacteria	Bacteria Protozoa: unclas- sified flagellate	Microcystis incerta Oocystis Borgei

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
44	Aug. 2	plain		l hr.	30	15
45	Aug. 2	CaCO <sub>3</sub>		l hr.	l hr.	15
46	Aug. 2	plain		l hr.	l hr.	15

TABLE VI (Continued)

I	II	111
August 18, 1956	October 9, 1956	February 28, 1957
Fungal spore Bacteria	Chlorella sp. Chroococcus minu- tus	Chroococcus sp. Gomphonema sp. Oscillatoria limnetica Oscillatoria sp. Bacteria
August 18, 1956	October 9, 1956	March 4, 1957
Bacteria	Bacteria	Oscillatoria sub- brevis Bacteria
August 18, 1956	October 11, 1956	March 4, 1957
Aphanothece nidu- lans Bacteria	Euglena sp.  Microcystis aerugi- nosa Oscillatoria tenuis Bacteria	Oscillatoria sp. Tetraedron minimum

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
47	Aug. 2	plain		l hr.	l hr. Bill <b>Was</b> hi	15
48	Aug. 2	CaCO <sub>3</sub>		l hr.	2 hrs.	15
49	Aug. 2	plain		l hr.	2 hrs.	15
50	Aug. 8	CaCO <sub>3</sub>		l hr.	4 hrs.	15

TABLE VI (Continued)

	Yend	
I	II	III
August 18, 1956	October 11, 1956	March 4, 1957
Apahanocapsa elachista Aphanothece nidu- lans Oscillatoria sp. Rhabdoderma sp.	Chlorella sp. Aphanocapsa sp. Arthrospira sp. Oscillatoria sp. Euglena sp. Protozoa: unclassified flagellate	Euglena sp. Gloeocystis sp. Oscillatoria lim- netica
August 19, 1956	October 11, 1956	March 5, 1957
Gloeothece linearis Bacteria	Chlorella vulgaris Euglena sp. Scenedesmus bijuga Scenedesmus sp.	Euglena sp. Scenedesmus bijuga Bacteria
August 19, 1956	October 12, 1956	March 6, 1957
Bacteria	Bacteria	Chlamydomonas sp. Bacteria
August 20, 1956	October 12, 1956	March 6, 1957
Bacte ria	Arachnochloris-like cell Bacteria	Oscillatoria sp. O. subbrevis

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
51	Aug. 8	plain		l hr.	4 hrs.	15
52	Aug. 8	CaCO <sub>3</sub>		l hr.	8 hrs.	15
53	Aug. 3	CaCO <sub>3</sub>		l hr.	8 hrs.	15
54	Aug. 3	plain		l hr.	8 hrs. Bill <b>W</b> ashi	15

TABLE VI (Continued)

I	II	III
August 20, 1956	October 12, 1956	March 27, 1957
Bacteria	Bacteria	Oscillatoria sp. Bacteria Unclassified spores
August 19, 1956	October 16, 1956	March 27, 1957
Bacteria	Bacteria	Oscillatoria sub- brevis Bacteria
August 19, 1956	October 16, 1956	March 27, 1957
Bacteria	Bacteria	Chlamydomonas sp. Chlorella vulgaris Nannochloris bacil- laris Oscillatoria lim- netica Bacteria
August 20, 1956	October 16, 1956	March 28, 1957
Bacte <b>r</b> ia	Navicula sp. Bacteria	Euglena sp. Navicula sp. Oscillatoria acutis- sima Bacteria

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TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
55	Aug. 3	CaCO <sub>3</sub>		l hr.	12 hrs.	15
56	Aug. 3	plain		l hr.	12 hrs.	15
57 <sup>a</sup>	Aug. 4	CaCO <sub>3</sub>		l hr.	16 hrs.	15
58	Aug. 4	plain		l hr.	16 hrs.	15
59	Aug. 6	CaCO <sub>3</sub>		l hr.	24 hrs.	15
60	Aug. 6	plain		l hr.	24 hrs.	15

<sup>&</sup>lt;sup>a</sup>Washings planted in the remaining cultures in this table were obtained from ducks which had been enclosed in cheesecloth netting while exposed to the air.

TABLE VI (Continued)

Planted Culture Examinations					
I	II	III			
August 20, 1956	October 16, 1956	March 29, 1957			
Bacteria	Bacteria	Bacteria			
August 20, 1956	October 16, 1956	March 29, 1957			
Bacteria	Bacteria	Bacteria			
August 20, 1956	October 16, 1956	March 29, 1957			
Bacteria	Bacteria	Bacteria			
August 20, 1956	October 16, 1956	March 29, 1957			
Bacteria	Bacteria	Bacteria			
August 20, 1956	October 16, 1956	March 29, 1957			
Bacteria	Bacteria	Bacteria			
August 20, 1956	October 16, 1956	March 29, 1957			
Debris	Bacteria	Bacteria			

TABLE VI (Continued)

Flask No.	Date Planted	Soil- Water Medium	Five Minute Wash	Time in Water Pen	Air Time in Min.	Boot Wash in Min.
61	Aug. 15	CaCO <sub>3</sub>		l hr.	32 hrs.	15
62	Aug. 15	plain		l hr.	32 hrs.	15

Note: Numbers 63, 64, and 65 were not used.

TABLE VI (Continued)

I	II	III
September 10, 1956 Bacteria	October 16, 1956 Bacteria	March 29, 1957 Bacteria
September 10, 1956 Bacteria Unclassified spore	October 16, 1956 Bacteria	March 29, 1957 Bacteria

TABLE VII

DATA FROM CONTROLLED EXPERIMENTS (MISCELLANEOUS)
WITH CLASSIFICATION OF ORGANISMS, 1956

Flask No.	Date Planted	Soil- Water Medium	Time in Water Pen	Time in Air	Boot Wash in Min.
A <sup>a</sup>	Aug. 6	CaCO <sub>3</sub>	l hr.	15 hrs.	15
В	Aug. 6	plain	l hr.	15 hrs.	15
С	Aug. 8	CaCO <sub>3</sub>	l hr.	24 hrs.	15 (contami- nated; duck fell on ground)

 $<sup>^{\</sup>rm a}$ Washings planted in these cultures except F and G were obtained from ducks which had been enclosed in cheesecloth netting while exposed to air.

TABLE VII (Continued)

Planted Culture Examinations					
I	Ш				
August 25, 1956	October 27, 1956	April 3, 1957			
Bacteria	Bacteria	Fungal hyphae Bacteria			
August 25, 1956	October 27, 1956	April 3, 1957			
Bacteria	Ankistrodesmus sp.  Nannochloris bacil-  laris  Oscillatoria tenuis  Synechococcus aeru- ginosus	Anabaena affinis Ankistrodesmus convolutus Chlorella ellip- soidea Chroococcus lim- neticus			
August 25, 1956	October 27, 1956	April 5, 1957			
Bacteria	Bacteria	Bacteria			

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TABLE VII (Continued)

Flask No.	Date Planted	Soil- Water Medium	Time in Water Pen	Time in Air	Boot Wash in Min.
D	Aug. 8	plain	l hr.	24 hrs.	15 (contami- nated)
E	Aug. 8	plain	l hr.		15 washing minated)
F	Aug. 9	CaCO <sub>3</sub>	Hutchins goose	5 min.	3

TABLE VII (Continued)

I	II	III
August 25, 1956	October 30, 1956	April 5, 1957
Bacteria	Cylindrospermum sp. Euglena sp.	Anabaena affinis Chlamydomonas sp. (encysted) Euglena sp. Protococcus sp. Bacteria
September 11, 1956	October 30, 1956	April 5, 1957
Navicula sp. Bacteria Protozoa: unclas- sified flagellates	Euglena sp.  Navicula sp.  Oscillatoria sp.  Scenedesmus bijuga	Navicula sp. Oscillatoria sp. Phormidium muci- cola
September 11, 1956	October 30, 1956	April 5, 1957
Fungal hyphae Protozoa: Scyto- monas-like flagellate; un- classified flagel- late; unclassified Heliozoan	Oscillatoria sp.  Phacus sp.  Diatom  Protozoa: unclassified flagellates (2 spp.)	Chlamydomonas globosa Euglena gracilis Navicula sp. Oscillatoria spp. (3)

TABLE VII (Continued)

Flask No.	Date Planted	Soil- Water Medium	Time in Water Pen	Time in Air	Boot Wash in Min.
G	Aug. 9	plain	Hutchins goose	5 min.	3
Н	Aug. 15	CaCO <sub>3</sub>	l hr.	24 hrs.	15
I	Aug. 15	plain	l hr.	24 hrs.	15
J	Aug. 15	plain		Faeca	l Sample

TABLE VII (Continued)

I	II	III
Oscillatoria sp.  Navicula sp.  Scenedesmus sp.  Protozoa: Monas  sp.; unclassified flagellate Rotifer: Philodina  sp.	October 30, 1956  Oscillatoria sp. Palmodictyon sp. Diatom	April 5, 1957  Navicula sp. Oscillatoria tenuis O. subbrevis
September 11, 1956  Bacteria: Spiril- lum sp.	November 1, 1956 Bacteria	April 5, 1957 Bacteria
September 11, 1956 Bacteria	November 1, 1956  Anabaena sp. Oscillatoria sp.	April 5, 1957  Anabaena affinis  Nostoc sp.?  Oscillatoria sp.
September 11, 1956  Debris	November 1, 1956 Bacteria	April 5, 1957 No check made

Faecal San

TABLE VII (Continued)

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Flask No.	Date Planted	Soil- Water Medium	Time in Water Pen	Time in Air	Boot Wash in Min.
К	Aug. 15	CaCO <sub>3</sub>	Faecal Sample		
M <sup>b</sup>	Sept. 29	plain	3 hour Air Sample		
N <sup>b</sup>	Dec. 13	plain	26 hour Air Sample		

bFlask exposed to air where culture examinations were carried out.

TABLE VII (Continued)

Planted Culture Examinations				
I	II	III		
tember 11, 1956  cillatoria sp. classified spores	November 1, 1956  Oscillatoria sp. Bacteria Protozoa: unclassified flagellate	April 5, 1957 No check made		
ecember 27, 1956 Bacteria	April 14, 1957  Nothing observed			
December 27, 1956  Fungal hyphae  Bacteria	April 14, 1957 Fungal hyphae			

DATA FROM CONTROLLED EXPERIMENTS (MUD PEN)
WITH CLASSIFICATION OF ORGANISMS, 1956

Flask	Date Planted	Soil- Water Medium	Time in Mud	Time in Air	Boot Wash in <b>M</b> in.
66	Aug. 15	CaCO <sub>3</sub>	Pen l hr.	l hr.	15
67	Aug. 15	plain	l hr.	1 hr.	15
68	Aug. 15	CaCO <sub>3</sub>	l hr.	2 hrs.	15

# TABLE VIII (Continued)

I	II	III
Protozoa: Scyto- monas-like flag- ellate Bacteria	October 19, 1956  Euglena sp. Protozoa: Monas sp.; unclassified ciliate; unclassified flagellate Bacteria	March 29, 1957  Anabaena sp.  Euglena sp.  Oscillatoria tere- briformis
eptember 7, 1956  luglena sp. Bacteria Protozoa: unclassified flagellates (4 spp.)	October 19, 1956  Euglena sp. Oscillatoria sp. Protozoa: Monas sp.; unclassified flagellates (2 spp.)	March 30, 1957  Chlamydomonas sp.  Euglena sp.  Oscillatoria limnatica
eptember 7, 1956  Protozoa: unclas- sified flagellate Bacteria		March 30, 1957  Anabaena sp. Arthrospira sp. Oscillatoria sp. Phormidium sp.

TABLE VIII (Continued)

Flask No.	Date Planted	Soil- Water Medium	Time in Mud Pen	Time in Air	Boot Wash in Min.
69	Aug. 15	plain	l hr.	2 hrs.	15
70	Aug. 15	plain	l hr.	2 hrs. Bill W	15 Vashing
71	Aug. 17	CaCO <sub>3</sub>	l hr.	4 hrs.	15

TABLE VIII (Continued)

I	II	III	
rotozoa: Monas sp.; Amoeba radiosa acteria	October 19, 1956  Chlorella sp. Nannochloris-like cell Protococcus sp. Hyalotheca-like cell Oscillatoria sp. Euglena sp. Protozoa: unclassified flagellate	March 30, 1957  Chlamydomonas sp. (encysted) Oscillatoria sp. Protozoa: Stylonychia-like ciliate Bacteria	
Protozoa: unclas- sified flagellate Bacteria: Spirillum sp.; other species	October 26, 1956  Chlamydomonas sp. Chlorella vulgaris Scenedesmus sp. Oscillatoria sp. Fragilaria sp. Navicula sp. Pleurosigma sp. Bacteria	March 30, 1957  Chlamydomonas sp. (encysted) Oscillatoria sub- brevis Oscillatoria sp. Euglena sp. Diplonesis sp. Navicula sp. Protozoa: unclassified ciliate	
September 8, 1956 Bacteria	October 26, 1956 Bacteria	March 30, 1957  Anabaena affinis  Microcystis aeru- ginosa Oscillatoria spp. (2)	

TABLE VIII (Continued)

Flask No.	Date Planted	Soil- Water Medium	Time in Mud Pen	Time in Air	Boot Wash in Min.
72	Aug. 17	plain	l hr.	4 hrs.	15
73	Aug. 17	CaCO <sub>3</sub>	l hr.	8 hrs.	15
74	Aug. 17	plain	l hr.	8 hrs.	15

TABLE VIII (Continued)

Planted Culture Examinations				
I	II	III		
otozoa: Proto- monad; Valkam- phia-like amoeba; unclassified Holotricha	October 27, 1956  Chlamydomonas sp. Ulothrix sp. Unclassified phyto- flagellate Oscillatoria spp. (3)  Euglena sp. Protozoa: Valkem- phia-like amoeba; unclassified ciliate	March 30, 1957  Chlamydomonas sp. Oedogonium sp. Gloeocapsa sp. Oscillatoria spp. (2) Protozoa: unclassified Holotricha		
ptember 8, 1956 olpoda sp. acteria	October 27, 1956  Chroococcus minor Protozoa: Pera- nema-like flagellate Bacteria	April 1, 1957  Anabaena affinis  Bacteria		
eptember 8, 1956 rotozoa: unclas- sified flagellate Bacteria	ntozoa: unclas- sified flagellate  Nannochloris bacil- laris			

TABLE VIII (Continued)

Flask No.	Date Planted	Soil- Water Medium	Time in Mud Pen	Time in Air	Boot Wash in <b>M</b> in.
75	Aug. 17	plain	l hr.	8 hrs. Bill W	15 ashing
76 <sup>a</sup>	Aug. 19	CaCO <sub>3</sub>	l hr.	16 hrs.	15
77	Aug. 19	plain	l hr.	16 hrs.	15

aWashing planted in the remaining cultures obtained from ducks which had been enclosed in cheesecloth netting while exposed to the air.

TABLE VIII (Continued)

I	II	III	
September 8, 1956 Debris	October 27, 1956  Chlorella vulgaris Protococcus sp. Scenedesmus abundans Euglena sp.	April 1, 1957  Chlamydomonas sp. Scenedesmus quad- ricauda Oscillatoria sp. Euglena sp. Protozoa: unclas- sified ciliate	
September 8, 1956 Bacteria Protozoa: unclas- sified flagellate	October 27, 1956  Aphanocapsa sp. Oscillatoria spp. (2) Euglena sp. Protozoa: Monas sp.; Amoeba radiosa-like cell; Oikomonas-like flagellate; un- classified flagel- late (2)	April 2, 1957  Anabaena affinis Chlamydomonas (encysted) Euglena sp.	
September 8, 1956 Bacteria Protozoa: unclas- sified flagellate	October 27, 1956  Euglena sp. Glenodinium sp. Protozoa: unclassified amoeba (lobopodia)	April 3, 1957  Euglena sp. (encysted)  Gloeocystis gigas  Fungal hyphae  Bacteria	

TABLE VIII (Continued)

			<u> </u>		
Flask No.	Date Planted	Soil- Water Medium	Time in Mud Pen	Time in Air	Boot Wash in Min.
78	Aug. 19	CaCO <sub>3</sub>	l hr.	24 hrs.	15
79	Aug. 19	plain	l hr.	24 hrs.	15
80	Aug. 19	plain	l hr.	24 hrs. Bill W	15 Vashing

TABLE VIII (Continued)

Time By	Pl	Planted Culture Examinations				
Air / x li	I	II	III			
ars.   ii	September 8, 1956	October 27, 1956	April 3, 1957			
	Bacteria: Spirillum sp.	Bacteria	Anabaena sp. Euglena sp. Oscillatoria sp.			
15	September 8, 1956	October 27, 1956	April 3, 1957			
	Navicula sp. Protozoa: unclassified flagellate	Euglena sp. Glenodinium sp.	Chlamydomonas sp. Euglena sp. Ulothrix sp. Bacteria			
	September 8, 1956	October 27, 1956	April 3, 1957			
Navicula sp. (en- cysted) Protozoa: unclas- sified flagellate		Euglena spp. (2)  Navicula sp.  Oscillatoria spp. (2)  Protozoa: unclassified flagellate	Anabaena sp. Oscillatoria spp. (2) Euglena sp. Phacus sp. Navicula sp.			

TABLE IX

DATA FROM CONTROLLED EXPERIMENTS (CONTROL FLASKS)
WITH CLASSIFICATION OF ORGANISMS, 1956

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>1</sub>	July 18	CaCO <sub>3</sub>	Culture flasks control
C <sub>1</sub>	July 18	plain	Culture flasks control
C <sub>2</sub>	July 19	plain	Exposed to air 45 minutes
С <sub>3</sub>	July 20	plain	Culture flasks control
C <sub>4</sub>	July 20	CaCO <sub>3</sub>	Culture flasks control

TABLE IX (Continued)

	Culture Examinations	
I	II	III
August 25, 1956	November 9, 1956	No check made
Debris	Bacteria <sup>a</sup>	
August 25, 1956	November 9, 1956	No check made
Fungal hyphae	Bacteria Fungal hyphae	
August 25, 1956	November 9, 1956 April 19, 1957	
Debris	Bacteria	Bacteria
August 25, 1956	November 9, 1956	April 19, 1957
Debris	Debris	Bacteria Fungal hyphae
August 24, 1956	November 9, 1956	No check made
Debris	Debris	

<sup>&</sup>lt;sup>a</sup>Bacteria were recorded only when present in large numbers.

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>5</sub>	July 21	CaCO <sub>3</sub>	15 ml. of Lake Water
c <sub>6</sub>	July 21	CaCO <sub>3</sub>	30 minute Air Sample
C <sub>7</sub>	July 21	plain	30 minute Air Sample

TABLE IX (Continued)

Culture Examinations				
I	II	III		
August 24, 1956	November 9, 1956	April 19, 1957		
Chlorella sp.  Navicula sp.  Spirulina princeps  Protozoa: Oikomonas sp.; Codonaeca-like cell; unclassified ciliates	Ankistrodesmus sp.  Scenedesmus sp. Arthrospira sp. Oscillatoria spp. (2) Euglena sp. (encysted) Diatoms (2 sp.) Protozoa: unclassified flagellates (2 sp.)	Oedogonium sp. Scenedesmus bijuga Aphanocapsa sp. Arthrospira sp. Gloeocapsa calcarea Oscillatoria sp. Phormidium sp. Pelogloea bacillifera Euglena sp. (encysted) Navicula sp. Protozoa: Euplotes sp.; unclassified ciliate Bacteria		
August 24, 1956	November 9, 1956	April 19, 1957		
Bacteria Fungal hyphae	Bacteria	Bacteria		
August 24, 1956	November 10, 1956	April 20, 1957		
Bacteria Debris	Debris	Bacteria		

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
С <sub>8</sub>	July 23	CaCO <sub>3</sub>	4 hour Air Sample
C <sub>9</sub>	July 23	plain	4 hour Air Sample
C <sub>10</sub>	July 23	plain	10 ml. Lake Water

TABLE IX (Continued)

Culture Examinations				
I	II	III		
August 22, 1956 Bacteria	November 10, 1956 Bacteria Fungal hyphae	April 20, 1957  Bacteria: Spirillum sp.		
August 22, 1956  Fungal spore: Alternaria sp. Bacteria	No check made	No check made		
August 22, 1956  Chlorella vulgaris Protozoa: Parame- cium bursaria Fungal hyphae	Chromulina sp. Oscillatoria sp. Scenedesmus sp. Protozoa: Ento- siphon sp.; un- classified flag- ellate (3)	April 20, 1957  Scenedesmus bijuga Nannochloris bacil- laris Oscillatoria acutis- sima Phormidium sp. Protozoa: unclas- sified flagellates (2 spp.)		

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>11</sub>	July 24	CaCO <sub>3</sub>	10 ml. Lake Water
C <sub>12</sub>	July 24	CaCO <sub>3</sub>	6 hour Air Sample
C <sub>13</sub>	July 24	plain	6 hour Air Sample

TABLE IX (Continued)

Culture Examinations				
I	II	III		
August 22, 1956	November 10, 1956	April 20, 1957		
Chlamydomonas sp.  Microcystis aerugi- nosa Protozoa: unclas- sified flagellate Bacteria	Cosmarium sp. Palmella sp. Scenedesmus bijuga Scenedesmus sp. Arthrospira sp. Oscillatoria spp. (2) Euglena sp. (encysted) Navicula sp.	Scenedesmus bijuga Chlamydomonas (encysted) Arthrospira sp. Oscillatoria spp. (2) Navicula sp. Protozoa: unclassified flagellate; unclassified ciliate		
August 22, 1956  Bacteria  Fungal hyphae	November 16, 1956 Bacteria	April 20, 1957  Chlorella vulgaris  Nannochloris bacil-  laris Oedogonium sp.		
		Sphaerocystis sp. Protozoa: Colpoda sp.		
August 22, 1956	November 16, 1956	April 20, 1957		
Bacteria Fungal hyphae	Bacteria	Chlamydomonas (encysted) Nostoc sp. Oedogonium sp. Ulothrix sp.		

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>14</sub>	July 25	CaCO <sub>3</sub>	10 ml. Lake Water
C <sub>15</sub>	July 25	CaCO <sub>3</sub>	5 hour Air Sample
C <sub>16</sub>	July 25	plain	5 hour Air Sample

TABLE IX (Continued)

Culture Examinations				
I	II	III		
August 22, 1956  Protozoa: Cyclidium sp.; Peranema sp.; unclassified ciliate; unclassified flagellate  Bacteria: Spirillum sp.	(contaminated)			
August 22, 1956 Fungal hyphae	November 16, 1956 Bacteria	April 20, 1957  Chlorella vulgaris  Nannochloris bacil-  laris  Ulothrix sp.  Fungal spore:  Alternaria		
August 22, 1956 Bacteria Fungal hyphae	November 16, 1956 Bacteria	April 20, 1957  Higher plant:  Utricularia sp.		

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TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>17</sub>	July 26	CaCO <sub>3</sub>	Filamentous Mat; 5 minute Roccal 1:625 wash
C <sub>18</sub>	Aug. 1	plain	5 minute Tide wash, rinse with sterile water (wash bottle); boot wash 15 minutes
C <sub>19</sub>	Aug. 1	plain	1:625 Roccal wash, rinse with sterile water (wash bottle); boot wash 15 minutes

TABLE IX (Continued)

Culture Examinations				
I	II	April 20, 1957  Gloeocystis sp. Scenedesmus bijuga Nostoc sp. Navicula sp. Nematode (round worm)		
August 22, 1956  Euglena sp. Protozoa: Chilomonas paramecium; Cyclidium sp. Bacteria	Chlamydomonas sp. Protococcus-like cell Scenedesmus bijuga Scenedesmus sp. Oscillatoria sp. Spirulina sp. Euglena sp. Navicula sp. Protozoa: Peranema sp.			
August 22, 1956  Protozoa: Peranema granulifera Bacteria: Spirillum sp.	November 16, 1956  Chlorella sp.  Euglena sp.  Navicula sp.  Oscillatoria sp.  Fungal hyphae	April 20, 1957  Chlamydomonas globosa Navicula sp. Nostoc sp.		
August 22, 1956 Debris	November 21, 1956  Nannochloris bacil- laris Protococcus sp.	April 20, 1957  Chlorella ellipsoidea  Cyanarcus sp.  Nannochloris sp.		

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TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>20</sub>	Aug. 2	CaCO <sub>3</sub>	5-1/2 hour Air Sample
C <sub>21</sub>	Aug. 2	plain	5-1/2 hour Air Sample
C <sub>22</sub>	Aug. 2	plain	10 ml. Lake Water

TABLE IX (Continued)

	Culture Examinations				
I	II	III			
August 21, 1956	November 21, 1956	April 20, 1957			
Bacteria	Bacteria Fungal hyphae	Euglena sp. (encysted) Gloeocystis sp. Bacteria			
August 21, 1956	November 21, 1956	April 26, 1957			
Bacteria	Debris	Pelogloea bacillifora			
August 21, 1956	November 27, 1956	April 26, 1957			
Oscillatoria sp. Protozoa: Chilomonas sp.; Entosiphon sp.; unclassified flagellate Bacteria	Chlamydomonas sphagnicola Chlamydomonas sp. Chlorella sp. Scenedesmus bijuga Scenedesmus sp. Oscillatoria tenuis Oscillatoria sp. Euglena sp. (2) Diatoms (2 sp.) Protozoa: Monas- like flagellate Fungal hyphae	Ankistrodesmus convolutus Chlamydomonas sp. C. sp. (encysted) Gloeocystis ampla Nannochloris bacillaris Scenedesmus arcuatus S. armatus S. bijuga S. dimorphus Stigeoclonium sp. Tetraedron minimum Unclassified phytoflagellate Chroococcus limneticus Oscillatoria sp. Phormidium sp. Euglena sp. (encysted) Fragilaria sp. Navicula sp.			

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TABLE IX (Continued)

Flask No.	Date. Planted	Soil- Water Medium	Flask Description
C <sub>23</sub>	Aug. 2	CaCO <sub>3</sub>	Culture flask control
C <sub>24</sub>	Aug. 2	plain	Culture flask control
C <sub>25</sub>	Aug. 3	CaCO <sub>3</sub>	10 ml. Lake Water
C <sub>26</sub>	Aug. 3	CaCO <sub>3</sub>	25-1/2 hour Air Sample
C <sub>27</sub>	Aug. 3	plain	25-1/2 hour Air Sample

TABLE IX (Continued)

	Culture Examinations	
I	II	III
August 21, 1956	November 27, 1956	April 27, 1957
Bacteria	Nothing observed	<b>Ba</b> cteria
August 21, 1956	November 27, 1956	April 26, 1957
Bacteria	Nothing observed	Bacteria
August 21, 1956	November 27, 1956	April 26, 1957
Protozoa: Chilo- monas sp.; un- classified flagel- late Bacteria	Euglena sp. (en- cysted) Oscillatoria tenuis Diatoms (2 sp.) Protozoa: unclas- sified flagellate	Chlorella vulgaris  Euglena sp. (en- cysted)  Navicula sp. Oscillatoria tenuis
August 21, 1956	November 27, 1956	April 27, 1957
Fungal hyphae Bacteria	Chroococcus sp. Bacteria	Bacteria
August 21, 1956	November 27, 1956	April 27, 1957
Bacteria	Chlorella vulgaris Gloeocapsa sp.	Chlorella vulgaris Euglena sp. (en- cysted) Bacteria

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>28</sub>	Aug. 3	plain	Faecal sample cultured
C <sub>30</sub>	Aug. 3	plain	Faecal sample cultured
C <sub>31</sub>	Aug. 5	plain	10 ml. Lake Water

TABLE IX (Continued)

	Culture Examinations	
I	П	III
August 21, 1956	December 1, 1956	April 27, 1957
Bacteria	Chlamydomonas sp. Euglena sp. Oocystis pusilla Fungal spore	Nostoc sp. Oocystis eremos- phaeria
August 21, 1956	December 1, 1956	April 27, 1957
Bacteria	Bacteria	Bacteria
August 20, 1956	December 8, 1956	April 27, 1957
Chlorella vulgaris  Microcystis aeru- ginosa Oscillatoria spp. (2)  Navicula sp. Protozoa: Coleps sp.; Peranema sp.; protozoan cysts; unclassi- fied ciliate; un- classified flagel- late	Ankistrodesmus sp.  Protococcus sp.  Scenedesmus ar-  cuatus Oscillatoria sp.  Euglena sp.  Phacus sp.  Diatoms (3 sp.)  Protozoa: Bodo sp.;  Bodo-like flagel- late; Entosiphon sp.; Heliochona sessilis; Monas sp.  Rotifer: Philodina- like Fungal hyphae	Navicula sp. Oscillatoria lim- netica Selenastrum minu- tum Synedra sp.

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>29</sub>	Aug. 3	CaCO <sub>3</sub>	Faecal sample cultured
C <sub>32</sub>	Aug. 6	CaCO <sub>3</sub>	29 hour Air Sample
C <sub>33</sub>	Aug. 6	plain	29 hour Air Sample
C <sub>34</sub>	Aug. 8	CaCO <sub>3</sub>	10 ml. Lake Water

TABLE IX (Continued)

Culture Examinations			
I	II	III	
August 21, 1956 Bacteria	December 1, 1956  Oscillatoria sp.	April 27, 1957  Phormidium sp.  Bacteria	
August 20, 1956  Bacteria	December 11, 1956 Bacteria	April 27, 1957 Bacteria	
August 20, 1956 Debris	December 11, 1956  Rhizoclonium sp. Higher plant: Elodea (young plant)	April 27, 1957  Higher plant: <u>Utricularia</u> sp.	
August 20, 1956  Chlorella sp. Oscillatoria sp. Protozoa: Monas sp.; Chilomonas paramecium Bacteria: Spirillum sp.	Chlamydomonas sp.  Chlamydomonas sp.  Oscillatoria spp. (2)  Euglena spp. (2)  Phacus sp.  Phacus sp.  Diatom (2 sp.)  Protozoa: Monas-		

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TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>35</sub>	Aug. 8	plain	10 ml. Lake Water
C <sub>35</sub>	Aug. 8	CaCO <sub>3</sub>	10 ml. Lake Water

TABLE IX (Continued)

	Culture Examinations	
I	II	III
August 20, 1956	December 13, 1956	April 27, 1957
Chlorella sp. Gloeocystis gigas Pediastrum Borya- num Fragilaria sp. Navicula sp. Protozoa: Entosiphon sp; unclassified ciliate; unclas- sified flagellate	Ankistrodesmus sp. A. convolutus Chlorella sp. Gloeocystis sp. Nannochloris sp. Palmodictyon varium Pediastrum tetras Protococcus sp. Scenedesmus ar- matus S. bijuga S. quadricauda Tetraedron sp. Desmid Aphanocapsa sp. Euglena sp. (en- cysted) Phacus anacoelus Navicula sp. Diatoms (2 sp.) Protozoa: Monas sp.; Halteria sp.; unclassified cili- ate; unclassified flagellate	Ankistrodesmus fal-  catus variety acicularis Scenedesmus bijuga Scenedesmus sp. Tetraedron mini- mum Oscillatoria sp. Navicula sp. Protozoa: Monas sp.; unclassified amoeba
August 20, 1956	December 13, 1956	April 27, 1957
Bacteria	Bacteria	Bacteria

TABLE IX (Continued)

		<del></del>	
Flask No.	<b>Da</b> te Planted	Soil- Water Medium	Flask Description
C <sub>36</sub>	Aug. 14	CaCO <sub>3</sub>	10 ml. Lake Water
C <sub>37</sub>	Aug. 15	CaCO <sub>3</sub>	32 hour Air Sample
C <sub>38</sub>	Aug. 15	plain	32 hour Air Sample

TABLE IX (Continued)

	Culture Examinations	
I	II	III
September 10, 1956  Lyngbya sp. Oscillatoria sp. Protozoa: Chilo- monas parame- cium; Cyclidium sp.; Peranema sp.; unclassified flagellate	Protococcus sp.  Scenedesmus spp. (2) Oscillatoria spp. (2) Euglena sp. Navicula sp. Synedra sp. Protozoa: Cyclidium sp.; Peranema sp.	April 27, 1957  Chroococcus dispersus Navicula sp. Oscillatoria spp. (2) Protozoa: Peranema sp.; unclassified flagellate
September 10, 1956  Bacteria Debris	December 13, 1956 Bacteria	April 27, 1957 Bacteria
September 10, 1956  Bacteria Debris	Chroococcus sp.  Euglena sp. (encysted)  Protococcus sp.  Sphaerocystis  Schroeteri	April 29, 1957  Gloeocystis gigas  Gloeocystis vesicu-  losa  Rhizoclonium sp.

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>39</sub>	Aug. 15	CaCO <sub>3</sub>	5 ml. Mud and Water
C <sub>40</sub>	Aug. 15	plain	5 ml. Mud and Water

TABLE IX (Continued)

	Culture Examinations	
I	II	Ш
September 10, 1956	December 13, 1956	April 29, 1957
Phacus acuminata Phacus sp. Oscillatoria granu- lata Euglena elongata Euglena sp. Protozoa: Holo- phyra-like ciliate; unclassified flag- ellate	Oscillatoria spp. (4)  Euglena sp. Phacus sp. Diatom (2 sp.) Protozoa: Monas- like cell; Vorti- cella sp.; unclas- sified ciliates (2) Nematode (round worm)	Scenedesmus bijuga Anabaena sp. Oscillatoria amphibia Navicula sp. Diatom Nematode (round worm)
Microcystis aeru- ginosa Oscillatoria sp. Navicula sp. Synedra sp. Protozoa: Coleps hirtus; Cyclidium sp.; Euplotes sp.; Peranema sp.; Pyxidium vernale; Trachelophyllum sp. Rotifers (2 sp.) Bacteria	Closterium spp. (2) Rhizoclonium sp. Unclassified phytoflagellate Anabaena sp. Oscillatoria sp. Euglena sp. Diatoms (2 sp.) Unclassified zygospores Bristleworm (annelid) Copepod: nauplius Nematode (round worm)	April 29, 1957  Chlamydomonas   (encysted)  Nostoc sp. Oscillatoria sp. Phormidium sp. Navicula sp. Diatom Nematode (round worm)

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>41</sub>	Aug. 16	CaCO <sub>3</sub>	4 hour Air Sample
C <sub>42</sub>	Aug. 16	plain	4 hour Air Sample
C <sub>43</sub>	Aug. 16	CaCO <sub>3</sub>	Culture flask control
C <sub>44</sub>	Aug. 16	plain	Culture flask control

TABLE IX (Continued)

	Culture Examinations	
I	II	III
September 10, 1956 Bacteria	December 14, 1956 Bacteria	April 29, 1957 Bacteria
September 10, 1956  Debris	December 14, 1956  Oscillatoria lacus- tris Protococcus sp. Bacteria	April 30, 1957  Chlorella vulgaria Oscillatoria lacus- tris
September 10, 1956 Debris	December 14, 1956 Debris	May 1, 1957 Nothing observed
September 10, 1956  Debris	December 14, 1956 Debris	May 1, 1957 Fungal hyphae Bacteria

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sup>45</sup>	Aug. 17	plain	5 ml. of Mud cultured
C <sub>46</sub>	Aug. 17	CaCO <sub>3</sub>	7 hour Air Sample
C <sub>47</sub>	Aug. 17	plain	7 hour Air Sample

TABLE IX (Continued)

	Culture Examinations	
I	II	III
September 10, 1956  Lyngbya sp. Oscillatoria Agardhii O. articulata O. sp. Navicula sp. Protozoa: Chilomonas paramecium; Cyclidium sp.; Euplotes sp.; Monas sp.; unclassified Heliozoan	Protococcus sp. Gloeocapsa sp. Oscillatoria spp. (3) Euglena sp. (encysted) Navicula sp. Diatom Nematode (round worm)	May 1, 1957  Oscillatoria sp. O. acutissima Navicula sp. Synedra sp. Diatom Nematode (round worm)
September 11, 1956  Bacteria Debris	December 14, 1956 Fungal hyphae Bacteria	May 1, 1957 Fungal spore Bacteria
September 11, 1956 Debris	December 15, 1956  Debris	May 1, 1957  Oscillatoria lacus- tris Protozoa: unclas- sified amoeba

TABLE IX (Continued)

	_	Soil-	
Flask No.	Date Planted	Water Medium	Flask Description
C <sub>48</sub>	Aug. 19	CaCO <sub>3</sub>	16 hour Air Sample
C <sub>49</sub>	Aug. 19	plain	16 hour Air Sample
C <sub>50</sub>	Aug. 20	CaCO <sub>3</sub>	24 hour Air Sample
C <sub>51</sub>	Aug. 20	plain	24 hour Air Sample

TABLE IX (Continued)

Culture Examinations				
Ι	II	III		
September 11, 1956 Bacteria	December 15, 1956 Bacteria	May 1, 1957 Bacteria		
September 11, 1956	December 15, 1956	May 1, 1957		
Fungal spores Fungal hyphae	Fern prothallis Fungal hyphae Bacteria	Fern prothallis Bacteria		
September 11, 1956	December 15, 1956	May 1, 1957		
Bacteria	Bacteria Debris	Phormidium sp. Bacteria		
September 11, 1956	December 15, 1956	May 1, 1957		
Protozoa: unclas- sified flagellate	Lyngbya-like filament Bacteria	Gloeocystis sp. Oscillatoria sp. Phormidium tenuis		

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>52</sub>	Aug. 20	CaCO <sub>3</sub>	5 ml. of Mud cultured

TABLE IX (Continued)

Culture Examinations					
I	II	III			
September 11, 1956  Anabaena sp.  Microcystis aeruginosa Oscillatoria spp. (2)  Euglena acus variety-rigida  Euglena sp.  Lepocinclis acuta  Phacus sp.  Diatoms (2 spp.)  Protozoa: Monas	December 15, 1956  Scenedesmus spp. Anabaena sp. Arthrospira sp. Lyngbya sp. Oscillatoria spp. (2) Euglena sp. (encysted) Phacus sp. Diatoms (2 spp.) Protozoa: Frontonia-like ciliate;	May 1, 1957  Ankistrodesmus falcatus A. convolutus Cladophora sp. Oscillatoria sp. Euglena sp. Navicula sp. Diatom Nematode (round worm) Copepod			
sp.; Chilomonas paramecium; Coleps sp.; un- classified Heli- zoan; unclassified ciliate; unclassi- fied flagellate	unclassified amoeba; unclas- classified flag- ellate Gastrotrich (annelid) Rotifer: Philodina sp.				

TABLE IX (Continued)

Flask No.	Date Planted	Soil- Water Medium	Flask Description
C <sub>53</sub>	Aug. 20	plain	5 ml. of Mud cultured
С <sub>54</sub>	Aug. 20	CaCO <sub>3</sub>	Culture flask control
C <sub>55</sub>	Aug. 20	plain	Culture flask control

TABLE IX (Continued)

Culture Examinations				
I	II	III		
Rhizoclonium sp. Protozoa: Coleps sp.; Chilomonas paramecium; Euplotes sp.; Halteria sp. Rotifer	Chlamydomonas Chlorella sp. Closterium sp. Pediastrum sp. Pediastrum duplex Scenedesmus spp. (2) Navicula sp. Synedra sp. Diatoms (3 spp.) Ostracod Copepod: naupulus Rotifer	May 1, 1957  Closterium sp. Oscillatoria sp. Euglena sp. (encysted) Nematode Rotifer		
September 11, 1956  Bacteria Debris	December 17, 1956 Bacteria	May 1, 1957 Bacteria		
September 11, 1956  Debris	December 17, 1956  Debris	May 1, 1957 Bacteria		

TABLE X

FEATHER WASHINGS PLANTED NOVEMBER 2, 1956,
WITH CLASSIFICATION OF ORGANISMS

		Cultura Fr	caminations
Flask		Culture Ex	rammations
No.	Description	I	II
1	12 feathers from lower breast of a Mallard duck (1/2" × 2")	November 15, 1956  Navicula sp. Bacteria	April 6, 1957  Chlamydomonas sp. Scenedesmus abundans Oscillatoria sp. O. limnetica Euglena sp. Navicula sp. Bacteria
2	7 undertail covert feathers from a Mallard duck (3/4" × 3-3/4")	November 15, 1956  Navicula sp. Protozoa: Frontonia-like ciliate	April 12, 1957  Chlamydomonas sp. Scenedesmus bijuga Oscillatoria sp. O. limnetica Navicula sp. Unclassified diatom Protozoa: Fronto- nia-like ciliate; unclassified ciliate Bacteria

TABLE X (Continued)

Flask	Flask	Culture Examinations		
No.	Description	I	П	
3	15 feathers from lower breast of a Mallard duck	November 16, 1956  Navicula sp. Protozoa: unclassified flagellate	April 12, 1957  Chlamydomonas sp. Oscillatoria sp. Navicula sp. Unclassified diatom Fungal hyphae Bacteria	
4	10 undertail covert feath- ers from a Mallard duck	November 16, 1956  Lyngbya sp. Synura sp. Protozoa: unclassified flagellate Unclassified spore	April 12, 1957  Chlamydomonas sp. Scenedesmus abundans Oscillatoria granudata Navicula sp. Protozoa: Amoeba radiosa	

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TABLE XI

FEATHER AND OTHER WASHINGS PLANTED NOVEMBER 23,
1956, WITH CLASSIFICATION OF ORGANISMS

Flask No.	Flask Description	Time in Water Pen	Time in Air
1	Feet washing	1 hr.	l hr.
2	Bill washing	l hr.	l hr.
3	Feet washing	1/2 hr.	l hr.

TABLE XI (Continued)

	Culture Examinations	
I	II	III
December 17, 1956	January 21, 1957	April 14, 1957
Bacteria	Bacteria Fungal hyphae Unclassified cyst or spore	Chroococcus mini-  mus Oscillatoria lacus-  tris Oscillatoria sp.  Trichodesmium  lacustre  Euglena sp.  Bacteria
December 17, 1956	January 21, 1957	April 14, 1957
Bacteria	Euglena sp. (en- cysted) Bacteria	Chlamydomonas sp. Chlorella vulgaris Nannochloris bacil- laris Scenedesmus sp. Oscillatoria sp. Phormidium sp. Euglena sp.
December 17, 1956	January 23 1957	April 4, 1957
Fungal spore	Gloeocystis vesicu- losa Oscillatoria lim- netica Bacteria	Chlamydomonas sp. Chlorella ellip- soidea Euglena sp. Oscillatoria sp.

TABLE XI (Continued)

Flask No.	Flask Description	Time in Water Pen	Time in Air
4	Bill washing	1/2 hr.	l hr.
5	l hour Air Sample		
6	18 Lower breast feathers from a Mallard duck		

TABLE XI (Continued)

Culture Examinations			
I	II	III	
December 17, 1956	January 23, 1957	April 14, 1957	
Debris	Chlorella ellip- soidea C. vulgaris Scenedesmus abun- dans S. bijuga	Ankistrodesmus falcatus Chlamydomonas sp. Scenedesmus abundans S. bijuga S. quadricauda Euglena sp. Bacteria	
December 17, 1956	January 26, 1957	April 17, 1957	
Bacteria	Chlorella ellip- soidea C. vulgaris Scenedesmus bijuga Bacteria	Chlamydomonas sp. Scenedesmus abun- dans S. quadricauda Euglena sp. Bacteria	
December 17, 1956	January 31, 1957	April 17, 1957	
Bacteria Fungal hyphae	Chlamydomonas  (encysted)  Chlorella ellip-  soidea  C. vulgaris  Scenedesmus quad- ricauda  Bacteria	Chlamydomonas sp. Nannochloris bacil- laris Scenedesmus quad- ricauda Phormidium tenue Euglena sp.	

TABLE XI (Continued)

Flask No.	Flask Description	Time in Water Pen	Time in Air
7	7 Undertail covert feathers from a Mallard duck		
8	21 Lower breast feathers from a Mallard duck		
9	8 Undertail covert feathers from a Mallard duck		
10	Culture flask control		

TABLE XI (Continued)

	Culture Examinations		
I	II	III	
December 17, 1956	January 31, 1957	April 17, 1957	
Protozoa: unclas- sified flagellate Bacteria Unclassified spore	Ankistrodesmus Braunii Chlorella vulgaris Scenedesmus abun- dans S. quadricauda Euglena sp. (en- cysted)	Characium sp. Nannochloris bacil- laris Scenedesmus abun- dans Euglena sp.	
December 17, 1956	February 1, 1957	April 17, 1957	
Bacteria Fungal hyphae	Chlorella vulgaris Scenedesmus bijuga Merismopedia tenuissima Navicula sp. Bacteria	Scenedesmus bijuga  Merismopedia  tenuissima Phormidium sp.  Euglena sp.  Navicula sp.	
December 17, 1956  Bacteria  Fungal hyphae	February 1, 1957 <u>Microcystis incerta</u> Bacteria	April 17, 1957  Anabaena sp. Chroococcus dispersus	
December 17, 1956	February 1, 1957	April 17, 1957	
Debris	Nothing observed	Debris	

## CHAPTER VI

## **DISCUSSION**

A thorough review of the literature concerning the role of waterfowl in the dispersal of algae revealed that there is a lack of direct experimental evidence for the transmission of microorganisms by waterfowl. It is clearly illustrated with one or two exceptions that this means of dispersal has been largely an assumption of most ecologists. Ingold (1953, pp. 137, 148) stated:

Waterfowl almost certainly play an essential part in the long-distance dispersal of freshwater aquatic fungi.

In spite of the fact that these fungi have no air borne spores, the distribution of individual species is just as wide, if not wider, than that of terrestrial species. This raises the problem of the dispersal of these fungi from one isolated freshwater system to another, and there can be little doubt, in spite of the absence of direct evidence, that, as with aquatic plants, water birds play an essential part in their long-distance dispersal.

The dispersal of micro-organisms is also attributed to chance or accident. Gulick (1932, p. 423), referring to Pacific oceanic islands. stated

Through their lists of species we have been able to verify the ability of certain rather restricted types of organisms to suffer transportation into such distant spots by rare and rather accidental means. Fritsch (1931, p. 253) also mentioned that an element of chance becomes a factor in the populating of ponds with algae.

Palmgren (1926, pp. 593, 594, 595) has pointed out:

In this multitude of conditions, and of various possibilities for their combination into complexes, probably lies the chief cause of the accidental characteristic of the conditions of occurrence-the stamp of mere chance.

In phytogeographical discussion the notion of 'chance'' consequently means an effective complex of causes, so constituted that scientific research, for the present at least, is unable to propound the problem of the ultimate essential dependence on natural laws.

When it appears to be absolutely impossible to anticipate these phenomena of occurrence, then we may characterize the circumstance referred to as chance. When on the other hand we have been able to predict the occurrence, it must be ascribed to law.

Therefore, in the author's opinion, as the unknown becomes known, the various "accidental" or "chance" occurrences used to explain dispersal, will be found to be predictable. These occurrences can then be ascribed to some of the various factors governing dispersal of organisms in a more orderly fashion. This research has demonstrated some of these factors and conditions heretofore attributed to chance in the dispersal of micro-organisms.

The hunters' data sheets and the field collections were used only as a preliminary attack toward an explanation of the dispersal of algae by waterfowl. This preliminary investigation showed that

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birds washed in sterile water after being shot in the field, give us an incomplete representation of the forms which might be externally transported.

In gathering field data the investigator was faced with the following problems:

- 1. The exact location of the bird prior to being flushed and shot is difficult to determine.
- 2. Even if the exact location from which the bird was flushed were known, extensive sampling would be necessary to determine the qualitative population of the micro-organisms in this habitat.
- 3. The period of time the bird had been in the air prior to being shot is either unknown or is usually less than a minute in duration. The organisms removed and cultured from a bird flushed from the water which had been in the air less than a minute only demonstrates that these forms are taken from an environment. This does not determine the time that they remain viable.
- 4. Frequently the exact place where the bird falls after being shot and the micro-organisms which might become adherent to it from that area are unknown.

Acquiring birds in the field for sterile washings would best be accomplished by flushing a bird from a body of water and shooting it down over dry land. For example, a high grassy bank where there

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would be little likelihood of finding plankton-type organisms. An attempt was made by the author to secure birds under these specific conditions (pp. 31-36). In these situations the plankton organisms washed from the birds were actually carried out of the natural environment by the bird and not by the hunter as he scooped up the bird.

The organisms washed from birds shot in the field vary considerably in species and in number depending upon many factors.

The micro-organisms in the environment from which the bird was taken, the period of time the bird was in the air, the sky conditions, air temperature, relative humidity, and wind velocity could all play an important part in determining which organisms are to be dispersed in a given situation. For these reasons the birds used in the controlled experiments were used as a check on the field collected specimens.

Results from the controlled experiments did not lessen the value of the field research done previously by Darwin, De Guerne, Klingle, Irènèe-Marie, and others. They were intended to supplement and clarify their findings and give us a more lucid understanding of some of the previously unconsidered factors concerned with the dispersal of micro-organism.

I had originally hoped that I would be able to determine the micro-organisms a duck could pick up in a certain period of time from a particular body of water. Conclusions based on my experimental data indicated that this was not possible. For example, just as many varieties of micro-organisms were attached to the ducks that were in the water pen one hour as to ducks in the water pen for 24 or more hours (Table III, pp. 63, 64). I had expected that more organisms both quantitatively and qualitatively would be gathered with each increase in time interval; i.e., 1/2, 1, 2, 4, and 8 hours. The relationship of numbers of organisms to time was not demonstrated by the results of the research.

Frequently statements in the literature indicated that ducks arise from a body of water with filamentous algae and higher aquatic plants adhering to their feet, feathers, and bills. The author noted in the controlled experiment that although ducks were removed from the water with Lemna minor, Spirogyra, and other filamentous green algae adhering to their feet, these forms were not present after being hung in the air for ten minutes.

When placed in the pen situated in the mud and organic debris, the feet of the ducks collected a large amount of material.

After exposure to the air for one-half hour, the feet appeared perfectly clean to the naked eye. Even beneath the toe nails no dirt

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particles were visible. Perhaps the oily secretion from the feet prevents the adherence of materials. Yet many micro-organisms were present; the algal forms being much the same as those found in the water pen series. The protozoa were much more numerous in the mud series (Table VIII, p. 136). This occurred with essentially no wind velocity so birds flying at an air speed of 20 or more miles per hour would be apt to carry the material for even shorter periods of time.

The effect of the detergent Tide upon micro-organisms was briefly studied. The cleansing effect of Tide is attested to by the following statement from a letter written by Mr. Owen Carter (1956):

... The Tide solution used to wash the waterfowl would act in two ways to eliminate microorganisms from the skin and feathers of the birds (1) by physical removal of the organisms by means of detergent action, and (2) by cidal action. We would expect the washing procedure you described to be very effective in removing all types of surface micro flora and fauna. Specifically regarding antimicrobial activity, our invitro test shows that a five-minute exposure to Tide under ordinary washing conditions will kill 90-95% of the common Gram (+) and Gram (-) bacteria. We do not have similar information relating to the cidal effect of Tide solutions on algae and protozoa.

Ten ml. of lakewater with a piece of the filamentous algal mat which was floating abundantly in the lake, was placed in an equal amount of concentrated detergent solution (proportion: 1/4 cup of Tide in nine cups of tap-water). This was shaken for five minutes and then poured into a sterile flask of soil-water medium for

culturing. Later upon microscopic examination of the culture the following organisms were found to have survived the detergent wash: green algae--Ankistrodesmus convolutus, Chlamydomonas sp.,

Gloeocystis gigas, Lepocinclis acuta, Oedogonium sp., Scenedesmus armatus, S. spp. (2); blue-green algae--Anabaena sp., Spirulina sp.; other algae--Arachnochloris-like cell, Navicula sp., Phacus acuminata, P. orbicularis, P. pyrum, protozoa: Amoeba verrucosa, Frontonia-like ciliate, Monas-like flagellate, and rotifer: Euchlanis (Table IV).

Ten ml. of the detergent solution were added to a culture flask which had been inoculated with 10 ml. of lakewater. Later examinations showed the following organisms: Chlamydomonas globosa, Nannochloris sp., Navicula sp., Oscillatoria limnetica, protozoa: an amoeba and a Holotricha.

In both of the culture flasks containing the detergent solution, growth was more rapid and richer than in the other experimental flasks. Growth was especially abundant in the former culture when the organisms were exposed directly to the concentrated Tide solution (Table IV, p. 73).

Although some of the feet of the experimental ducks were washed for five minutes with the detergent solution, some organisms appeared in the culture flasks. They were Arachnochloris-like cell, Gloeocy stis gigas, Protococcus viridis, Rhizoclonium fontanum,

Scenedesmus abundans, and an unclassified blue-green unicell. The presence of the organisms could possibly have been contributed to the 30-minute exposure to the air before the boot wash. These experiments would indicate that Tide is not an algacide, but on the contrary it seems to accelerate growth in culture flasks.

The author noted that the ducks washed in Tide prior to being placed in the water pen tended to pick up a greater variety of forms than did ducks which were trapped from the wild and placed directly in the water pen. One reasonable explanation would be that the detergent removes the oily secretion from the ducks' feet facilitating the adherence of micro-organisms.

In another instance when a duck had died in the water pen, more algal forms were found to be adherent than occurred on the live birds. Charles Darwin experimented with severed ducks' feet and found that the larval stage of fresh-water mollusks became firmly attached (p. 14). Perhaps the decrease in the amount of oily secretion as well as the lower temperature and lack of movement of the feet of a nonliving duck may contribute to an increase in a dherence of organisms.

Roccal was also found to be an ineffective algacide. Organisms found in the cultures which had survived a five-minute Roccal wash were: green algae--Chlamydomonas sp., Chlorella vulgaris,

Nannochloris bacillaris. Rhabdoderma irregulare, Scenedesmus abundans, and S. quadricauda; blue-green algae--Lyngbya sp., Microcystis aeruginosa, Oscillatoria sp., Plectonema-like filament; other algae--Euglena sp.

Tide was more effective than Roccal in the physical removal of micro-organisms.

Much work has been done in respect to the dispersal of airborne fungal spores, bacteria, and pollen grains. Studies have also been completed concerning the various environmental factors which affect their numbers in the air: Pady (1957), Feinberg (1949), Ingold (1953), Meier and Lindberg (1935), Zobell (1942), and others. The study of algae and protozoa carried by air currents, however, has been largely neglected. When boiled pondwater having been exposed to the air for various periods of time was cultured (p. 86), my results were very similar to those found by Pushkarew (1913).

To expose sterile culture media to the air in the laboratory does not demonstrate the exact nature of micro-organism dispersal by air currents. The cultured forms may have been carried into the medium from a dried-up culture in the same room or from the very table where the cultures were placed.

There was little correlation between the time of the exposure of the boiled pondwater and the humidity, wind velocity, air

obtained from the cultures. The boiled pondwater exposed to the air for the longer period of time generally, but not always, produced the most algal forms. As shown in Table IX, a six-hour exposure yielded more algal forms than did a 24-hour exposure.

Wind velocity, humidity, and air temperature may be more important than the time of exposure. A steady breeze would perhaps keep more organisms aloft than would short gusts of wind at a greater air speed (Table XV, p. 228). Insufficient data were gathered to reach any conclusions on the effect of these environmental factors on the algal and protozoan content of the air.

Faecal material was collected from several birds and cultured under sterile conditions (Tables III, VII, and IX). All of the forms recorded were apparently in a healthy vegetative condition and had multiplied, giving rise to a very rich growth in some of the culture flasks.

Organisms found in the field collections of faecal material aring the summers of 1955 (p. 57) and 1957 (p. 35) were: green gae--Chlamydomonas sp., Gloeocystis gigas, Spirogyra sp.; blue-reen algae--Arthrospira sp., Phormidium sp., Spirulina sp.; other gae--Navicula sp.; protozoa--Paramecium bursaria, unclassified otozoan cysts and small flagellates.

The following forms were taken from three faecal samples during the summer of 1956: green algae--Chlamydomonas sp.,

Oocystis Eremosphaeria, Oocystis pusilla; blue-green algae--Nostoc

sp., Oscillatoria sp., Phormidium sp., and unclassified zooflagellates

(pp. 132-135; 164-165).

The organisms found compared favorably with the work done by Messikommer (1943). His method differed from mine in that his microscopic examinations were made directly from the fresh material without use of cultures. Usually, he did not indicate whether his orms were in a viable condition.

Messikommer's findings are listed below: green algae-licrospora quadrata, Oedogonium spp. (2), Staurastrum cingulum,
enedesmus ecornis, Spirogyra sp., Tribonema vulgare; other algae-mbella cymbiformis, Epithemia zibra, Fragilaria capercina, Gomonema angustatum, Navicula gracilis, N. radiosa, and a living
ate. He also found much debris and pieces of both plant and

Various culture media for use in this research were considd and soil-water medium was selected. This medium provided near as possible the natural conditions for the growth of algae d protozoa. Certain artificial media have been recommended for are cultures of particular organisms, but have been found to be unsatisfactory for growing mixed cultures. According to Dr. E. G. Pringsheim of Pflanzenphysiologisches Institut, Gottingen, Germany (1946, also personal communication in 1956), "when only one medium is to be used for the culturing of algae, the soil-water medium would be the best selection. Since nutrient requirements for many species of algae are as yet unknown, many forms which can not be grown in artificial medium will thrive in soil-water medium."

In 1955 three or four grams of <u>Sphagnum</u> peat were added to several of the flasks (Table III, pp. 67, 68) to increase the acidity in an attempt to encourage new forms to develop. There was very little change in the rate of growth or the forms found.

In 1956 a pinch (approximately 1/16 of a teaspoon) of both CaCO<sub>3</sub> and starch was added to half of the soil-water medium flasks to produce a more basic medium (Table VI). The pH readings of the various culture flasks are given in Table XVI (Appendix, p. 232). Algal and protozoan growth in quantity and variation of forms was far superior in the plain medium than in the more basic ones. The bacterial and fungal growth occurred at a greater rate in the basic medium while the algal and protozoan growth was extremely poor (Table VI).

Those organisms which can form spores or cysts and those with a matrix were expected to be favored in the dispersal by

waterfowl. Some cells such as <u>Chlorella</u> and <u>Scenedesmus</u> might become embedded in the matrix of other cells (<u>Gloeocystis</u>). In this way they could be carried externally by waterfowl for greater periods of time.

An illustrative point may be made of the phytoplankton of the lakes in the Faeröes (Borgesen, 1903). The more common plankton forms found frequently had a matrix such as Cosmarium, Crucigenia, Cyclotella, Gloeocystis, Raphidium, and Sphaerocystis, Staurastrum, and Xanthidium. These islands are located in the Atlantic Ocean about 400 miles from the coast of Norway.

The cultured washings which were made showed, however, that forms of algae both with and without a matrix and those capable of cyst- or spore-formation were taken from the ducks exposed to the air for short periods of time. The spore- or cyst-forming algae such as Chlamydomonas and Euglena were more prominent in the longer air exposure.

To observe the growth rates of the various algae and protozoa in the culture flasks under these experimental conditions was very interesting. The succession of protozoan forms was more rapid than that of the algal forms. The greatest variety of protozoa occurred usually within the first two to four weeks after inoculation. The algal succession was very slow and some cultures, once the

growth peak was reached, were relatively unchanged quantitatively or qualitatively during the remainder of the nine months of culturing.

Rao (1953, pp. 173-175) stated the peak of algal growth in numbers was determined by placing 50 gm. of dried soil in various types of media. These cultures were kept in bright light at all times.

Medium	Days of Growth	pH Readings
Molischs' solution	130	5.1
Knop's medium	191	7.4
Distilled water	70	6.7

The increase in numbers in a mixed culture was much slower than that of a pure culture. In general, it was not until after a two-month period that the increase of algal numbers reached a peak and the forms that appeared first were not always present later. Sometimes different species would appear from latent forms after four or five months of culturing. In pure cultures the rate of growth was usually far more rapid with the maximum peak in numbers occurring within less time than two months.

Spores, cysts, and single cells were found which could not be classified without being cultured. Therefore, microscopic examinations of the uncultured washings did not give a complete

analysis of the organisms present, and only a few were made in 1956.

De Guerne (Appendix B, p. 237) also observed that some forms appearing in his cultures were either not detected or not identifiable in the uncultured state.

Bristol (1920, p. 39) mentioned some of these same difficulties in his research of culturing organisms from soil.

A great deal of difficulty was experienced in identifying the algae found in the cultures for various reasons. In the first place, the preliminary treatment of the soils was such as to preclude the possibility of the presence of all algae except in a resting condition [in] the initial stages of the cultures. The length of time taken for the germination of these resting forms varied in individual species, and for some months the cultures contained largely developmental stages which it was impossible to identify with any degree of certainty. Again, the somewhat abnormal conditions of excessive moisture under which the algae were growing tended to produce forms which in some cases were rather different from those of typical species already described, and it was necessary to decide whether such variations were the result of these conditions or whether they might perhaps characterize new species or varieties.

Therefore, the use of cultures seems extremely important to the author for two reasons: (1) to determine the viability of the organisms found, and (2) to aid in classification of spores and cysts of algae and protozoa which may produce vegetative cells.

Other taxonomic difficulties were experienced as follows:

(1) only one or a few cells were observed; (2) no reproductive structures were available for study; and (3) some cultures contained

species which varied only slightly from species already described, i.e., size, thickness of matrix, pyrenoid or flagellum lacking or not visible, and cells such as Scenedesmus not being in their usual coenobium form (cells existed singly, in pairs, triples, and in the regular coenobium of four or eight cells). Blue-green cells of approximately  $l_{\mu}$  in diameter which might be classified either as bacteria or blue-green algae were also difficult to identify to species using the present taxonomic keys (see Bibliography).

Separate listings of the organisms cultured from the washings of the feet, bills, feathers, gullets, and faecal material of the waterfowl are given in Table XIII (Appendix, p. 214) as well as a listing of the specific waterfowl studied (Table XII, p. 213). The organisms found in the research area of Wintergreen Lake are listed in Table XIV.

Some forms of blue-green algae (Anabaena, Aphanizomenon, Microcystis, etc.) which do "bloom" in a fairly short period of time with proper environmental conditions, are known to give off toxic substances in sufficient quantity to cause poisoning of livestock (Ingram and Prescott, 1954, p. 86).

The author does not know if any relationship exists between the large numbers of migrant waterfowl flocking to the small ponds, watering holes, et cetera, in Texas and the toxic poisoning of livestock.

The death of waterfowl (Gray, 1943, p. 39) and fish (Ingram and Prescott, 1954, p. 83) also have been attributed to phytoplankton organisms, some of which are capable of being carried by waterfowl from one aquatic environment to another.

#### CHAPTER VII

#### CONCLUSIONS

- 1. Various parts of 106 waterfowl from seventeen species (Table XII) were washed with boiled pondwater. Data from the examination of 41 birds were obtained from the hunters' data sheets and field washings. Twenty-three ducks were used in the controlled experiments of 1955, whereas 42 were used in 1956. Organisms collected by washing the gullet, bill, feathers, and feet, as well as organisms from the faecal material of the ducks, were examined directly and after culturing.
- 2. The research shows that various micro-organisms can be carried both internally and externally by waterfowl. The contents from the gullets sampled produced good algal growth in culture, whereas only a few of the faecal samples contained viable forms.
- 3. The length of time the birds were in the water pen prior to boot washing did not seem to be correlated with the various forms of organisms which would adhere to the feet of the ducks.

  Thus, in general, the ducks in the water pen for one hour yielded

as many organisms as those ducks which had been in the water pen twelve or more hours (Table VI).

- 4. Generally the ducks exposed to the air for one-half, one, two, and four hours carried a great variety of organisms in a viable condition. Those in the air eight hours transported some organisms on their feet, but a greater variety were found to be carried in their bills. The birds exposed to the air longer than eight hours yielded very few organisms. In one instance three genera were recorded after the duck was in the air for 24 hours (pp. 69-70).
- 5. The controlled experiments gave a more lucid understanding of dispersal of micro-organisms by waterfowl than did the field data.
- 6. The morphology of algal cells with respect to their ability to be dispersed is not clear. Cells without spines and matrix were carried, but encysting, spore-producing, and matrix-producing forms were most commonly found on the waterfowl.
- 7. Planktonic organisms from the water pen were not picked up as readily by the ducks or at least did not survive after exposure to the air as long as the organisms taken from the mud pen.
- 8. Plain soil-water medium with a pH between 6.5 and 7.0 gave the best results for algal growth under these experimental

conditions. A more basic or acidic medium retarded growth in this research.

- 9. Findings indicate that waterfowl play a major role in the dispersal of algae and protozoa for short distances but become less important with an increase in distance between bodies of water.

  Dispersal over a distance of five hundred or more miles, for example, may take place under certain conditions but would be rather rare, according to information derived from birds sampled in this research. When considering the vast numbers of migratory birds in existence, however, it seems probable that micro-organisms have been dispersed to distant oceanic islands and that certain algal forms would be favored as implied by Warming (1901-1908).
- were not found in the cultured washings taken from ducks exposed to the air for over two hours. However, these organisms were present in the lakewater taken from the water pen while the ducks were there and were cultured in the soil-water medium.
- 11. Chance happenings in dispersal are explainable, in part, in terms of cell morphology, reproductive structures, the nature of the dispersing agent, and environmental conditions.
- 12. Not only the biological and physical nature of the aquatic environment determines the organisms which are to be found in a

given environment but also their modes of dispersal. Although often not considered, these are also important in explaining the distribution of aquatic micro-organisms throughout the world. Future investigations will probably reveal "laws of dispersal" which will show that one form is less favored in dispersal than another. Apparently natural selection is operating not only in respect to the physical and biological nature of a given aquatic environment, but also as to which organisms will reach these new environments by various modes of dispersal. Specific organisms are not found in all suitable habitats and restriction may be attributed to competition in reaching the suitable environment.

## APPENDIXES

- A. Additional Tables
- B. The Author's Translation:

"Sur la dissemination dissemination des organismes d'eau douce par les Palmipedes" by J. M. de Guerne

# APPENDIX A

ADDITIONAL TABLES

.

#### TABLE XII

## WATERFOWL USED FOR WASHINGS

Black duck (Anas rubripes)

Blue goose (Chen caerulescens)

Buffle-head duck (Bucephala albeola)

Canada goose (Branta canadensis)

Coot (Fulica americana)

Eastern Belted Kingfisher (Megoceryle alcyon)

Gadwall (Anas strepera)

Goldeneye (Glaucinetta clangula americana)

Green-winged teal (Anas carolinensis)

Mallard (Anas platyrhynchos)

Purple Martin (Progue subis)

Redhead duck (Aythya americana)

Ring Billed Gull (Larus delawarensis)

Ruddy duck (Oxyura jamaicensis)

Spotted Sandpiper (Actitis macularia)

Common Snipe (Capella galinago)

Wood duck (Aix sponsa)

A total of 106 waterfowl were washed in the field and in controlled experimentation during 1955-56. Thirty-two were collected by hunters and nine by the author to provide field data. Twenty-three birds in 1955 and forty-two birds in 1956 were used in the controlled experiments.

#### TABLE XIII

# MICRO-ORGANISMS FOUND ON WATERFOWL USED IN THE CONTROLLED EXPERIMENTS<sup>a</sup>

#### Feet

Green Algae: Ankistrodesmus Braunii, A. convolutus, A. falcatus,

Arachnochloris-like cells, Arthrospira Gomotiana, A. Jenneri,

Chlamydomonas globosa, C. mucicola, C. pseudopertyi, C.

sp., b Chlorococcum sp., Chlorella ellipsoidea, C. vulgaris,

C. sp., b Closteriopsis-like cell, Dactylococcopsis acicularis,

Franceia sp., Glenodinium sp., Gloeocystis gigas, b Mougeotia

sp., Nannochloris bacillaris, b Oedogonium sp., Oocystis

Borgei, Palmodictyon sp., Protococcus sp., Rhabdoderma

irregulare, Rhizoclonium fontanum, Scenedesmus abundans, b

S. dimorphus, S. quadricauda, S. sp., Sphaerocystis Schroeteri, Tetraedron minimum, T. wisconsinense, T. sp., and

Ulothrix sp.

Blue-green Algae: Anabaena affinis, Aphanocapsa sp., Aphanothece

castagnei, A. nidulans, Chroococcus dispersus, C. minutus,

Gloeocapsa sp., Gloeothece linearis, Lyngbya attenuata,

a Bacteria have been excluded in the table.

b
The most common forms found on the feet.

L. limnetica, L. sp., Microcystis aeruginosa, Nostoc sp.(?),
Oscillatoria angustissima, O. limnetica, O. subbrevis, O.
tenuis, O. terebriformis, O. sp., Pelogloea bacillifera,
Phormidium mucicola, P. tenue, P. sp., Plectonema nostocorum, Synechococcus aeruginosus

Euglenophyta: Euglena gracilis, E. minuta, E. sp., b Phacus sp.

Chrysophyta: Gomphonema sp., Navicula sp., b Synedra sp.

Protozoa: Anisonema-like cell, Bodo-like cell, Carteria multifilis,

C. sp., Chromulina sp., Chrysidella sp., Cosmarium sp.,

Cryptoglena pigra, Holotricha ciliate, Monas sp., Monas-like cell, Oikomonas sp., Peranema sp., Phacotus-like cell,

Scytomonas-like flagellate, Stylonchia-like cell, unclassified Heliozoan.

Fungi: Alternaria sp.

Rotifer: Philodina sp.

# Feathers

Green Algae: Ankistrodesmus sp., Characium sp., Chlamydomonas sp., Chlorella ellipsoidea, C. vulgaris,

The most common forms found on the feet.

Nannochloris bacillaris, Scenedesmus abundans, S. bijuga,
S. quadricauda

Blue-green Algae: Anabaena sp., Chroococcus dispersus, Lyngbya sp., Merismopedia tenuissima, Microcystis incerta, Oscillatoria granulata, O. limnetica, O. sp.

Chrysophyta: Navicula sp., unclassified diatom, Synura sp.

Euglenophyta: Euglena sp.

Protozoa: Amoeba radiosa, Frontonia-like ciliate, unclassified ciliate and flagellate

Fungi: hyphae

#### Bills

- Green Algae: Chlamydomonas sp., Chlorella vulgaris, Nannochloris

  bacillaris, Occystis pusilla, O. eremosphaeria, Protococcus sp.,

  Scenedesmus abundans, S. bijuga, S. quadricauda, S. sp.
- Blue-green Algae: Anabaena sp., Aphanothece sp., Nostoc sp. (few cells), Oscillatoria subbrevis, O. sp., Phormidium mucicola, P. sp.
- Chrysophyta: Arachochloris-like cell, Diplonesis sp., Fragilaria sp.,

  Navicula sp., Pleurosigma sp.

Euglenophyta: Euglena sp., Phacus sp.

Protozoa: unclassified ciliate and flagellate

#### Gullets

Green Algae: Ankistrodesmus convolutues, Gloeocystis sp., Mougeotia sp., Nannochloris sp., Spirogyra sp.

Chrysophyta: Cyclotella sp., Pleurosigma sp., Navicula sp.

Euglenophyta: Euglena sp., Lepocincles sp.

Protozoa: Chromulina sp., Monas-like flagellate

Rotifer: Bdelloidea. Higher plant: Potomogeton sp.

## Faecal Material

Green Algae: Chlamydomonas sp., Gloeocystis gigas

Blue-green Algae: Arthrospira sp., Oscillatoria sp., Phormidium

sp., Spirulina sp.

Protozoa: Paramecium bursaria, unclassified flagellates

#### TABLE XIV

# PLANKTONIC MICRO-ORGANISMS TAKEN FROM WINTERGREEN LAKE DURING THE SUMMER OF 1956 --QUALITATIVE STUDY

## Water Pen

Plankton Bloom Sample, July 20

Blue-green Algae: Anabaena affinis, A. Bornetiana(?), Chamaesiphon incrustans, Chrococcus sp., Lyngbya Hieronymusii, L.
sp., Microcystis aeruginosa, Oscillatoria lacustris, O. limnetica, O. sp.

Euglenophyta: Euglena sp.

Chrysophyta: Navicula sp.

Fungi: Alternaria sp. spores

Rotifer: unclassified cells

Other organisms: ostracod

Slides planted in the Water Pen 3-6 inches below the surface of the water on July 2; removed and examined July 15

Green Algae: Chlorococcum sp., Closterium sp., Cosmarium sp.,

Oocystis sp., Pediastrum Boryanum, Scenedesmus quadricauda, Sirogonium sticticum, Spirogyra spp. (2)

<sup>&</sup>lt;sup>a</sup>Organisms causing bloom.

Blue-green Algae: Anabaena sp., Microcystis aeruginosa, Oscillatoria sp., Rivularia sp.

Euglenophyta: Euglena sp.

Chrysophyta: Fragilaria sp., Navicula sp., Synedra sp.

Protozoa: Arcella vulgaris, Bodo sp., Frontonia sp., Loxodes sp.,

Peranema sp., Stylonychia sp., Valkomphia-like amoeba

Other organisms: gastrotrich, ostracod

Slides planted July 15; removed and examined for 23

- Green Algae: Chlamydomonas sp., Oedogonium spp. (2), Oocystis sp., Pediastrum tetras var. tetraodon, P. sp., Rhizoclonium sp., Scenedesmus sp., Sphaerocystis Schroeteri, Staurastrum sp.
- Blue-green Algae: Anabaena sp., Chroococcus sp., Microcystis

  aeruginosa, Oscillatoria sp., Synechococcus aeruginosus
- Chrysophyta: Arachnochloris sp., Cyclotella sp., Cymbella sp.,
  Fragilaria sp., Gomphonema sp., Navicula sp., Surirella sp.
- Protozoa: Amphileptus sp., Arcella sp., Stentor sp., Vorticella sp., unclassified Heliozoan

# Slides planted August 3; removed and examined August 20

Green Algae: Apiocystis sp., Coleochaeta orbicularis, C. scutata,

Cosmarium sp., Pediastrum Boryanum, P. tetras, P. sp.,

Rhizoclonium sp., Scenedesmus sp.

Chrysophyta: Gomphonema sp., Navicula sp., Stauroneis sp., Synedra sp.

Blue-green Algae: Anabaena spp. (2), Calothrix sp., Gloeotrichia sp.,

Merismopedium sp., Microcystis aeruginosa, Oscillatoria

spp. (2).

Euglenophyta: Euglena sp.

Protozoa: Actinophrys sp., Arcella sp., Epistylis sp., Lionotus sp.,

Stentor sp., Valkamphia-like amoeba

Other organisms: amphipod, cladoceran, flatworm (Planaria), gastrotrich, rotifer (Bdelloidea), roundworm (nematod).

Plankton net tow preserved in 6-3-1 (Transeau's) solution on July 8 (1 gallon of lakewater)

Green Algae: Eudorina alegens, Pediastrum integrum, Pleodorina californica, Spirogyra sp., Volvox tertius

Blue-green Algae: Anabaena affinis, A. sp., Microcystis aeruginosa

Other organisms: Ceratium hirudinella, copepod nauplius, rotifer

# Plankton net tow July 8 (planted in CaCO<sub>3</sub> culture)

Green Algae: Pleodorina californica, Spirogyra sp.

Euglenophyta: Euglena sp.

Bacteria: Spirillum sp.

Other organisms: Chromulina sp., two unclassified phytoflagellates

Plankton net tow (planted in culture media) July 8

Green Algae: Chlamydomonas globosa, C. sp., Pandorina sp.,

Scenedesmus sp., Spirogyra sp.

Blue-green Algae: Anabaena sp., Aphanizomenon flos-aquae, Micro-cystis aeruginosa, Oscillatoria sp.

Euglenophyta: Euglena sp., Lepocinclis acuta

Chrysophyta: Navicula sp.

Protozoa: Peranema sp.

Other organisms: unclassified phytoflagellates

Plankton net tow--July 21

Green Algae: Oedogonium sp., Pandorina morum, Spirogyra Weberi,

Staurastrum pentacerum var. tetracerum, S. sp., Tetraedron

sp.

Blue-green Algae: Anabaena affinis, Microcystis aeruginosa a

Chrysophyta: Cyclotella sp., Navicula sp.

Other organisms: cladoceran, copepod, rotifer (3 spp.)

Plankton net tow--July 23

Green Algae: Eudorina elegans, Oedogonium sp., Oocystis sp.,

Pleodorina californica, Spirogyra Weberi, Staurastrum pentacerum var. tetracerum

Blue-green Algae: Anabaena affins, Chroococcus dispersus, Microcystis aeruginosa

Other organisms: Ceratium hirudinella, rotifer (2 spp.)

Plankton net tow--July 24

Green Algae: Pandorina morum, Pleodorina californica, Spirogyra

Weberi, Staurastrum pentacerum var. tetracerum, S. sp.

Blue-green Algae: Anabaena affinis, A. sp., Merismopedia Trolleri,

Microcystis aeruginosa

Other organisms: copepod nauplius, rotifer: Keratella sp., Brycella sp.

<sup>&</sup>lt;sup>a</sup>Organisms causing bloom.

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Plankton net tow--July 25

Green Algae: Oedogonium sp., Oocystis sp., Pandorina morum,
Pleodorina californica

Blue-green Algae: Anabaena affinis, A. sp., Chroococcus dispersus,

Microcystis aeruginosa

Chrysophyta: Navicula sp.

Other organisms: Ceratium hirudinella, rotifer: Keratella sp.,

Brycella sp.

Plankton net tow--August 2

Green Algae: Oocystis sp., Pleodorina californica, Staurastrum sp.

Blue-green Algae: Anabaena affinis, Microcystis aeruginosa, Oscillatoria tenuis

Other organisms: Ceratium hirundinella, a copepod nauplius

Plankton net tow--August 3

Green Algae: Spirogyra Weberi

Blue-green Algae: Microcystis aeruginosa

Other organisms: Ceratium hirundinella, cladoceran

Plankton net tow--August 6

Green Algae: Oedogonium sp., Spirogyra Weberi

<sup>&</sup>lt;sup>a</sup>Organisms causing bloom.

Blue-green Algae: Gloeotrichia sp., Microcystis abundans, Nostoc punctiforme

Chrysophyta: Navicula sp.

Plankton net tow--August 9

Green Algae: Occystis pyriformis, Pandorina morum, Pediastrum

duplex, Pleodorina californica, Volvox tertius

Blue-green Algae: Anabaena affinis, A. sp., Chroococcus dispersus,

Microcystis aeruginosa

Chrysophyta: Navicula sp.

Protozoa: Arcella vulgaris

Other organisms: Ceratium hirundinella, rotifer

Plankton net tow--August 8

Green Algae: Pandorina morum, Pleodorina californica, Rhizoclonium sp., Sphaerocystis Schroeteri

Blue-green Algae: Anabaena affinis, A. subcylindraca, A. sp.,
Microcystis aeruginosa

Chrysophyta: Navicula sp.

Protozoa: Diffulgia sp.

Other organisms: copepod nauplius, rotifer (3 spp.)

<sup>&</sup>lt;sup>a</sup>Organisms causing bloom.

## Plankton net tow--August 14

Green Algae: Pandorina morum, Pleodorina californica, Sphaerocystis Schroeteri

Blue-green Algae: Anabaena affinis, A. subcylindrica, A. sp.,

Chroococcus dispersus, Microcystis aeruginosa, Oscillatoria
sp.

Chrysophyta: Navicula sp., Synedra sp.

Fungi: Alternaria sp. spore

Other organisms: Ceratium hirudinella, rotifer: Keratella Brycella sp., Brachionus sp.

Filamentous Mat of Algae Floating near the Water Pen--Living and Preserved Material, July 9

Green Algae: Oedogonium sp., Oocystis sp., Pediastrum Boryanium,
Rhizoclonium sp., Spirogyra spp. (2)

Blue-green Algae: Anabaena affinis, A. sp., Aphanizomenon flosaquae, Aphanocapsa sp., Aphanothece gelatinosa, A. microspora, Microcystis aeruginosa, Oscillatoria spp. (2)

Euglenophyta: Euglena sp.

Chrysophyta: Gomphonema sp., Navicula sp.

<sup>&</sup>lt;sup>a</sup>Organisms causing bloom.

Rotifers: unclassified cells

Protozoa: not recorded

## Mud Pen

Five-milliliter sample--August 16

Blue-green Algae: Anabaena affinis, Chroococcus minimum, Lyngbya sp., Microcystis aeruginosa, Oscillatoria sp.

Euglenophyta: Phacus orbicularis

Chrysophyta: Cocconeis sp., Navicula sp., Synedra sp.

Fungi: Alternaria sp. spore

Five-milliliter sample--August 17

Green Algae: Cerasterias sp.

Blue-green Algae: Anabaena affinis, Aphanocapsa pulchra, Chroococcus sp., Microcystis aeruginosa, Oscillatoria sp.

Euglenophyta: Phacus sp.

Chrysophyta: Cocconeis sp., Navicula sp.

Fungi: Alternaria sp. spores

Other organisms: nematode

Five-milliliter sample--August 19

Green Algae: Ankistrodesmus sp., Pandorina morum, Scenedesmus quadricauda

Blue-green Algae: Anabaena affinis, Lyngbya limnetica, Microcystis

aeruginosa, Oscillatoria lacustris, O. sp., Synechococcus

aeruginosus

Chrysophyta: Cocconeis sp., Navicula sp., Stauroneis sp.

Protozoa: Holotrich ciliate

Fungi: Alternaria sp. spore

TABLE XV

ENVIRONMENTAL FACTORS IN THE CONTROLLED EXPERIMENTS, 1956<sup>a</sup>

	Organisms (Genera)	Time of	Rela- tive	Sky Con-	Air Temp.	Wind Velocity <sup>C</sup>			Total Miles
Date	from Air Sample	Ex- po- sure	Hu- midity (pct.)	di- tion <sup>b</sup>	Max Min.	8:00 a.m.	1:00 p.m.	5:00 p.m.	of Wind
7/12	Vaucheria	45 min.	75-88	С	62-80	1	4	4	36
7/13	Chlorella Nanno- chloris Pleodorina Oscillatoria Euglena	l hr.		PC	62-79	2	4	2	36
7/17	Euglena	45 min.	66	PC	59-77	2	4	1	38
7/19	nothing	45 min.	77	C	57-75	1	0	4	24
7/21	nothing	30 min.	72	PC	63-83	2	3	2	20

a Bacteria and fungi are not listed.

bC = cloudy; PC = partly cloudy; Cl = clear.

Wind velocity data were obtained from W. K. Kellogg Forest Station.

TABLE XV (Continued)

Date	Organisms (Genera) from Air Sample	Time of Ex- po- sure	Rela- tive Hu- midity (pct.)	Sky Con- di- tion	Air Temp. Max Min.		1:00 p.m.	5:00 p.m.	Total Miles of Wind
7/23	nothing	4 hr.	59-63	PC	59-79	1	3	2	31
7/24 (d)	Chlamydo- monas Chlorella Nanno- chloris Oedogonium Sphaero- cystis Ulothrix Nostoc Colpoda	6 hr.	51-63	Cl	59-89	2	2	2	24
7/25	Chlorella Nanno- chloris Ulothrix Utricularia	5 hr.	72-82	С	66-81	1	1	0	17
8/2	Euglena Gloeocystis Pelogloea	5- 1/2 hr.	48-56	Cl	54-77	3	2	5	38

Cheese cloth netting was placed over the remainder of the fingerbowls.

TABLE XV (Continued)

	Organisms	Time of	Rela- tive	Sky	Air	Wind	l Velo	city	Total Miles
Date	(Genera) from Air Sample	Ex- po- sure	Hu- midity (pct.)	y di- y tion	Temp. Max Min.	8:00 a.m.	1:00 p.m.	5:00 p.m.	of Wind
8/3	Chlorella Chroococ- cus Gloeocapsa Euglena	25- 1/2 hr.	51-87	С	55-77	1	1	1	31
8/6	Rhizoclo- nium Utricularia	29 hr.	86-90	С	64-81	2	3	1	31
8/15	Gloeocystis Protococ- cus Rhizoclo- nium Sphaero- cystis Chroococ- cus Euglena	32 hr.	44-90	PC	61-85	1	1	2	19
8/16	Chlorella Oscillatoria Protococ- cus	4 hr	54-6	PC	65-8	7 1	4	0	22

TABLE XV (Continued)

	Organisms (Genera)	Time of	e Rela- tive	Sky Con-	Air Temp.	Wind Velocity			Total Miles
Date	from Air Sample	Ex- po- sure	Hu- midity (pct.)	di- tion	Max Min.	8:00 a.m.	1:00 p.m.	5:00 p.m.	of
8/17	Oscillatoria Unclas- sified amoeba	7 hr.	59-70	PC	63-88	2	2	1	15
8/19	Fern pro- thallus	16	53-78	PC	55-76	-	4	1	45
8/20	Gloeocystis Lyngbya- like cell Oscillatoria Phormid- ium Unclas- sified flagellate	24 hr.	53-78	PC	48-70	2	0	1	33

TABLE XVI

pH OF CULTURE FLASKS, 1956

Flask No. <sup>a</sup>	pH before Swirl- ing	pH after Swirl- ing	Flask No. <sup>a</sup>	pH before Swirl- ing	pH after Swirl- ing		
		July 3	0, 1956				
$CaCO_3^b$	7.3	8.3	Plain	6.2	6,8		
$CaCO_3^b$ $CaCO_3$	7.4	-					
	January 5, 1957						
7(c) <sup>c</sup>	7.7	8.1	19(p)	7.4	7.4		
8(p)	6.9	6.9	20(c)	6.3	6.5		
9(c)	7.9	8.0	21(p)	7.2	7.4		
10(p)	7.0	7.1	22(c)	7.3	7.4		
11(c)	7.8	8.0	23(p)	7.4	7.4		
12(p)	7.4	7.4	24(c)	8.3	8.4		
17(p)	6.5	6.6	25(p)	7.6	7.7		
18(c)	8.0	7.9	26(p)	7.4	7.5		

 $a(c) = CaCO_3$ ; (p) = plain.

bColman pH electrometer (Model 18) was used for these readings.

Beckman pH meter (Serial No. 126747) was used for the remainder.

TABLE XVI (Continued)

Flask No.	pH before Swirl- ing	pH after Swirl- ing	Flask No.	pH before Swirl- ing	pH after Swirl- ing	
		January	5, 1957			
27(c)	-	8.1	39(c)	-	7.5	
28(p)	7.1	7.0	40(p)	7.2	7.0	
29(c)	8.3	8.3	41(c)	8.0	7.9	
30(p)	7.6	7.5	42(p)	7.4	7.4	
31	7.4	7.4	43(c)	8.1	8.3	
32(c)	8.4	8.4	44(p)	7.3	7.2	
33(p)	7.3	7.3	45(c)	8.3	8.4	
34(c)	7.8	8.0	46(p)	7.5	7.3	
35(p)	7.4	7.4	47(c)	8.0	8.0	
36(c)	7.5	7.5	48(c)	-	8.3	
37(p)	7.2	7.1	49(p)	-	7.2	
38(p)	7.2	7.2	50(c)	7.3	7.4	
	March 29, 1957					
51(p)	8.1	8.2	53(p)	7.6	7.5	
52(c)	8.1	8,1	54	7.3	7.6	

TABLE XVI (Continued)

Flask No.	pH before Swirl- ing	pH after Swirl- ing	Flask No.	pH before Swirl- ing	pH after Swirl- ing		
		March	29, 1957				
55(c)	7.8	7.8	59(c)	8.3	-		
56(p)	7.3	7.1	60(p)	6.9	6.8		
57(c)	8,2	8.2	61(c)	8.2	-		
58(p)	7.2	7.0	62(p)	7.6	7.6		
	April 6, 1957						
66(c)	8.1	-	80(p)	7.4	7.5		
70(p)	7.5	7.5	C(c)	8.3	8.3		
72(p)	7.1	-	D(p)	7.4	7.3		
74	6.8	6.7	E(p)	7.4	7.4		
75	7.2	7.2	G(p)	7.7	7.7		
79(p)	7.4	7.3	I(p)	7.2	-		
		May !	5, 1957				
C <sub>47</sub> (p)	7.5	6.8	C <sub>55</sub> (p)	7.5	7.4		
C <sub>49</sub> (p)	7.0	-					

Note: The pH of the water pen during 1956 experimental period varied from 10.0 in June to 7.5 the latter part of August.

#### TABLE XVII

## FIELD DATA SHEET FOR DUCK HUNTERS

Research is being carried out to determine what microscopic organisms are carried externally on waterfowl from one body of water to another. PLEASE CUT OFF THE HEAD AND FEET OF THE DUCK SHOT AND PLACE THEM WITH THIS DATA SHEET IN THE PAPER BAG PROVIDED. LEAVE IT AT THE ROSE LAKE EXPERIMENTAL STATION OR RETURN TO H. E. SCHLICHTING, ROOM 131, NATURAL SCIENCE BUILDING, MICHIGAN STATE COLLEGE. Your assistance in the collection of material will be greatly appreciated.

Please check the appropriate items:

Waterfowl shot (check only one):

Coot	Blue-winged teal	Wood duck			
Mallard	Ruddy duck	Ring-neck duck			
Canada Goose	Canvasback	White-winged Scooter			
Blue Goose	Greater Scaup duck	American Scooter			
Black Duck	Lesser Scaup duck	Hooded Merganser			
Baldpate	American Goldeneye	American Merganser			
Pintail	Bufflehead	Red-breasted Merganser			
Green-winged teal	Old Squaw	Other (or if in doubt)			
Sex of bird: male	female				
_	the body of water. body of water.				
Name and location	of the body of water				
Date	Time of day that the	bird was shot			
Name and address	of hunter				
Marine with	<del></del>				
Remarks:					
Kemarks.					

### APPENDIX B

# SUR LA DISSEMINATION DES ORGANISMES D'EAU DOUCE PAR LES PALMIPEDES

#### APPENDIX B

## ON THE DISSEMINATION OF ORGANISMS FROM FRESHWATER BY THE WEB-FOOTED

by Mr. Jules de Guerne

The possibility of the transport of organisms by the birds is admitted by the majority of naturalists. However, if one begins to look on what basis this opinion almost always rests, we recognize that but for the plants the number of observed facts is extremely limited.

Lyell and Darwin, who have specially studied the mode of dispersion applied to plants are far from having unrecognized its importance concerning the aquatic animals. But they did not use the microscope, and it is probably the main reason which has prevented them from going deeply into the question. Even in the last years of his life, Darwin was to be preoccupied by it. The more startling observations that we possess on the transport of the Lamelibranchs by winged beings, birds and insects, were published by him in 1878 and 1882.

<sup>&</sup>lt;sup>1</sup>J. M. de Guerne. Sur la dissemination des organismes d'eau douce par les Palmipedes. Societe de Biologie. 8 (March, 1888), pp. 294-298.

Aside from these documents, rather few in number, collected by Darwin, I only know but one precise fact on this subject which was reported by Professor F. A. Forel in 1876 according to Alois Humbert. This naturalist has found sticking to the feathers of ducks and of Grebes some winter eggs of Cladocerans.

This question was therefore hardly scratched when upon the return from the third voyage of the Hirondelle, completed under the direction of S. A. the Prince Albert of Monaco, after having discovered at the Azores a lacustral fauna composed almost entirely of European types, spread over a considerable geographical area, I attempted to bring about new arguments in favor of this doctrine of transport.

My researches started in the fall of 1887, at the time of the arrival of birds from the North, and have been continuing during each winter under circumstances more or less favorable. I limited myself, until now, in examining the web-footed, and especially of the common wild ducks, Anas boschas, ordinarily very abundant and easy to obtain. Two species of Teal (Querquedula circia and Q. crecca) and also various birds not specified, likewise, have furnished me some objects for study.

I have had at my disposal the game coming from the hunting of S. A. the Prince Albert of Monaco, at Marchais, Aisne, and sent

forth directly to Paris. Besides, a well known zoologist, M. Chevreaux, has been kind enough to send me the product of the washing from the feet of several Teal (Querauedula crecca L.) killed at Croisic, (Loire-Inferieure). I have examined as well a certain number of web-footed killed in January, 1888, in the marshes of Arleuz, near Douai (Nord).

Finally at different times I myself have procured wild ducks in different parts of Paris, in the markets, or from peddlers. person in charge of buying being ignorant of the purpose pursued could not be tempted, consequently, to choose among those birds whose feet appear particularly dirty. By a trick of fate which is certainly permissible to call upon as a favorable argument in this case, that it is on the feet of the duck handled many times from the marsh, to the central market, and at the retail store where I have found some of the most interesting objects mentioned below (Cytheridea torosa, for example). Except for the Teal of the Croisic which had been examined immediately by M. Chevreaux, the inspection of the birds had not occurred until nearly twenty-four hours after death. It is on the average the time necessary for the arrival of the ducks from the region of the Nord (Bay of Somme, etc.) from place of the hunting to the hands of the consumers in Paris. state of freshness of the viscera (the digestive apparatus several

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times supplied valuable indications about the last stop of the Bird) showed me that in several circumstances this delay had not even been attained.

Two procedures have been followed for the researches:

- 1. The direct examination, practiced either immediately on the material collected and diluted with water or at the end of a certain period on the product of the washing of the feet and of the bill in the water with the addition of alcohol immediately after the operation.
- 2. The culture of the material collected.

The direct observation furnished me the following results.

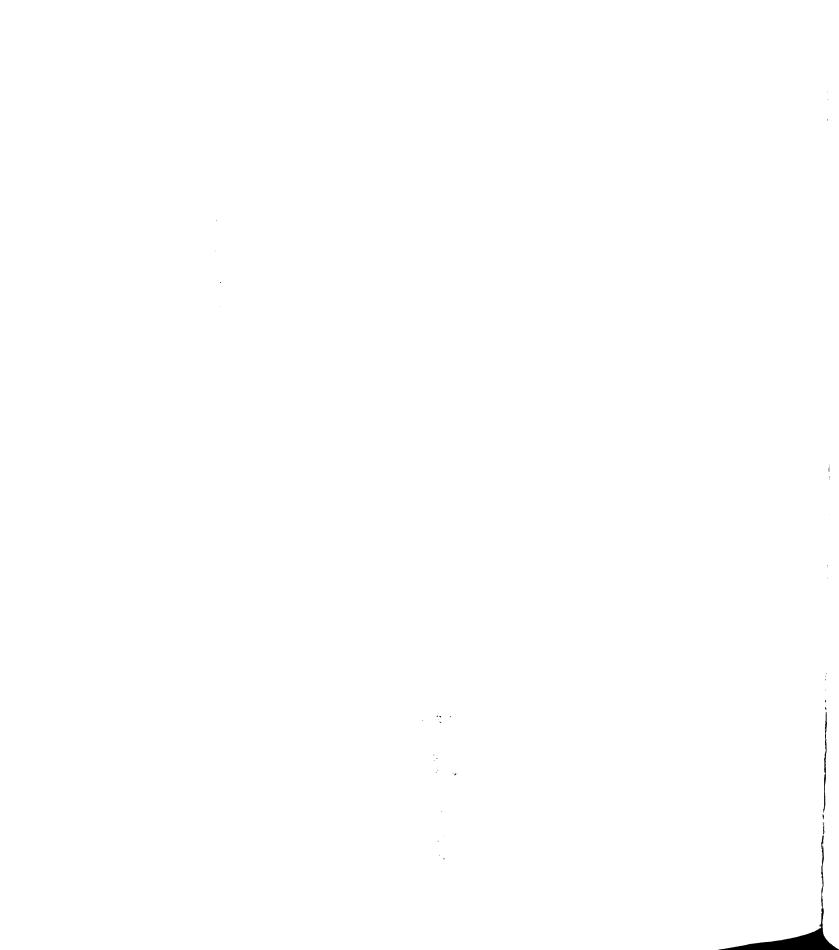
All the webbed-feet examined, with a few exceptions, carried foreign material on various parts of the body. From the point of view of the quantity transported, the feet should be cited as the most important, next are the edge of the tongue and the bill, finally the feathers. The latter, oily and compact, appear to be generally very clean.

However, it has happened to me to find a few spatters on the neck, on the face, and inside of the secondary wing feathers. Those on the wing feathers are produced in all probability when the bird has shaken itself on the bank or else even in open water, as is so often the case. The specks of dirt whose composition I have studied were entirely made up of microscopic plant debris. I do not doubt

that in the future researches, one will meet such spatters or of organisms in the state of latent life (spores, winter eggs, etc.) capable of being transported from one lake or from one marsh to another. These specks have a good hold on the feather while dry, but are dissolved quickly in the water: this circumstance appears to be most favorable to the dissemination.

I insist on the transport by the feathers, it is on them, in fact, that can be removed the bodies that float far from the banks on clear and deep water; the question offers a great interest from the point of view of the dispersion of some lacustral pelagic types; but it will be necessary, in order to definitely solve it, to undertake series of observations on places of hunting near large surfaces of water. If the preceding observations allow some doubt as to the subject of transport of pelagic organisms by the birds, those that follow show the important role which these last play in the dissemination of the littoral forms.

As I have said, it is upon the feet that one finds most of the material carried. On November 18, 1887, it happened to me to gather on the upper part of the membranes between the toes of a wild duck a quantity sufficient to entirely cover the bottom of a plate 15 centimeters in diameter. It was the murk from one peaty marsh, a bit brown, formed almost exclusively of plant debris (many



attaining 1 centimeter in length) mixed with a very small number of round quartz grains. In this deposit, I have found the presence of a large number of microscopic cysts, animal and plant, of many diatoms, of one desmid, of one cladoceran egg (Lyndeide?), of the half of a Pluetella repens statoblast, and one Ostracod valve. The latter, thanks to a particular definite character, has been able to be determined. It belongs to a species unknown in France, Cytheridea torosa Jones, but whose geographic distribution is very extensive. They have been reported in England, in the Azo Sea, in the east, etc. It lives in fresh and brackish waters and occurs especially in estuaries. Among these easily recognizable bodies are found many others which the specialists may succeed in naming. The fragments of insects are numerous. The majority hairy or even thorny in appearance should easily hook themselves and retain in addition some diverse matter. One of the fragments, a Dipteran femur three milimeters long, formed a true protective tube where some delicate beings incapable of surviving the dessication could find protection. The compression had forced out some pieces of trachea and a very large number of infusoria cysts. It is worth while to remember this fact: some similar debris is to be found frequently; in conserving small quantities of water by protection from evaporation, they

render perhaps possible the dissemination of certain aquatic organisms in their normal surroundings.

The case which I have explained in detail is absolutely typical; it is the most interesting that I have seen yet, however the examination of matters adhering to the feet of other ducks have furnished me with some different objects: some rotifers of the family Philodinadae (Arleuz, Marchais), a large number of setae from Oligocheates, one antenna of Cyclops?, some debris of Acariens (possible parasites on the bird?), one capsule of Turbellaria? having oviform fruit, and many carapaces of a cladoceran of the genus Alona (Marchais).

In the same way that the fragments of insects, these contained some diatoms and various corpuscles of which they facilitate for sure the dissemination. The edge of the carapace is bordered with numerous thorns. The objects encountered on the tongue and on the bill have gained my attention many times by their volume. Thus I have collected in the interior of the bill of a duck some plant fragments attaining up to three centimeters in length and by no means desiccated. In one other case, on the edge of the tongue were found some ovoid particles of quartz being three millimeters in diameter longitudinally and about two millimeters in transverse diameter.

This shows that some Molluscs, for example, of a certain size can be transported in the same manner.

I will be forced to be brief on the subject of the cultures I have still to speak about. One of them continued for two monthsfrom November 18, 1887, to January 17, 1888, with some material taken from the duck mentioned above, has furnished aside from other living animals, some Nematodes, and some very lively Rotifers (Philodinadae). The direct examination had not shown these types. Some Rhizopods (Trinema enchelys Ehv. for example) seem to have made their appearance there as well. But one should never rely on this experiment, the winter being an unfavorable season during which many aquatic organisms remain inactive in our climate. I dare to hope that the cultures where the presence of some living beings is certain will not delay in changing its appearance; the study of which will immediately be resumed.

Nevertheless, the range of general observations which precedes is by now obvious. They show the important role played by the birds, and the web-footed in particular in the dissemination of organisms from fresh water. They explain the cosmopolitan character of certain types, at the same time that their presence in these isolated points and notably on some oceanic island; they explain also the introduction of these types in the basins Lacustres of recent

origin, or in the artificial ponds. They help form the understanding of the singular uniformity of certain animal associations in the fauna of lakes and account also for the irregularities apparent in the distribution of various species.

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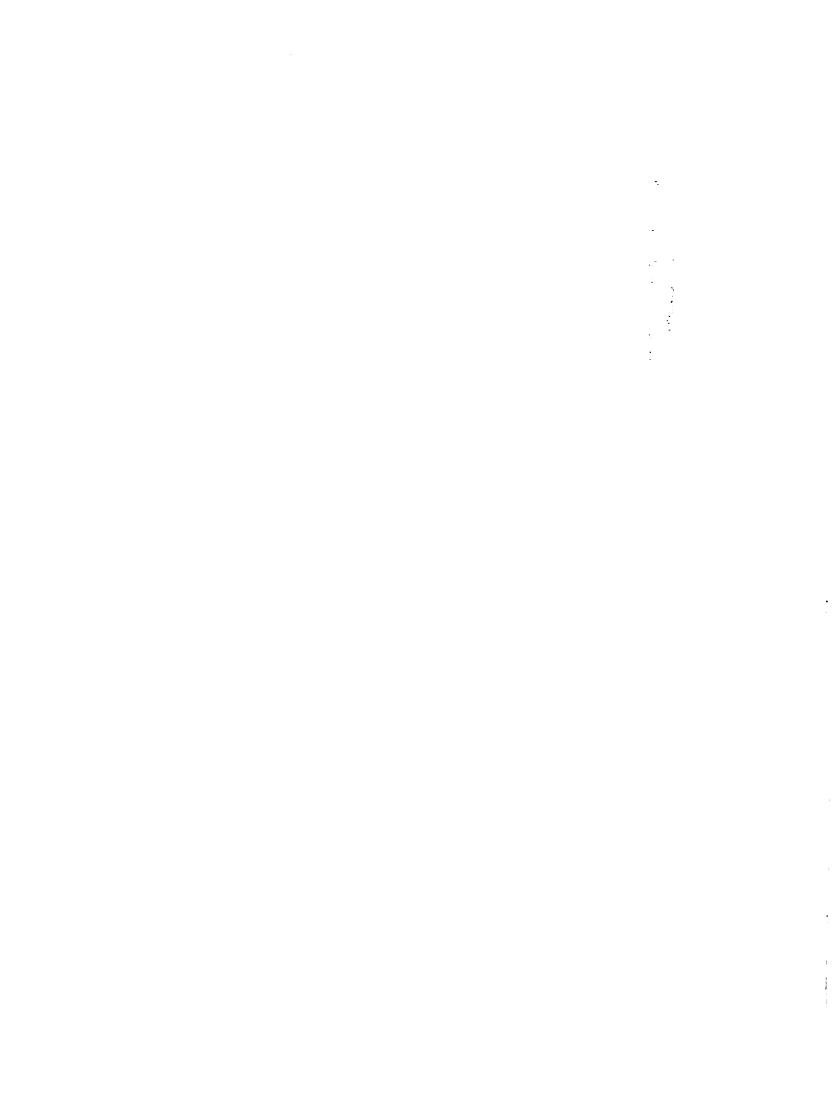
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