PASSIVE DISPERSAL OF ALGAE AND PROTOZOA INTERNALLY AND EXTERNALLY BY SELECTED AQUATIC INSECTS

DISSERTATION

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

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This investigation was concerned with three aspects of the problem of passive dispersal of algae and protozoa by aquatic insects.

The first part was undertaken to further clarify the role of odonates in passive dispersal of viable small aquatic organisms. Special attention was given to (1) sampling odonate species previously unstudied in this role, (2) additional sampling of species for which only anecdotal information is available and (3) noting geographic variations in passive dispersal of algae and protozoa between odonates from the Southwest and the Pacific Northwest.

The studies showed that 24 odonate species were transporting 54 genera of small aquatic organisms. The most commonly transported algae were the Chlorophyta (green algae). Microorganism dissemination data on nine previously unstudied Southwestern dragonflies and seven Northwestern odonates showed 20 and 25 genera of microorganisms carried, respectively. The green alga Mesotaenium was found for the first

time on aquatic insects and the algae Gloeothece, Asterococcus, Hormidium, Rhizoclonium, Cymbella, Diatoma, and Ochromonas are new records for odonates.

The percentages of Chlorophyta to total genera carried were about the same for odonates from the Pacific Northwest and Southwest. A greater percentage of Cyanophyta were transported in the Southwest, whereas the Chrysophyta were carried in greater numbers in the Pacific Northwest. These regional differences in organisms transported are undoubtedly related to multiple factors of temperature, light and dissolved organic and inorganic materials. Algal morphology and specialized reproductive structures such as statospores and akinetes interact with insect behavioral patterns to contribute to disseminule pickup also.

Part II demonstrated the passage of viable algae and protozoa through digestive tracts of field-collected herbivorous and carnivorous aquatic insects. The results indicate that aquatic beetles are more important than dragonflies in algal and protozoan dispersal by internal transport. Thirty-two genera of viable algae and protozoa were identified from 36 cultures inoculated with beetle hind-guts. Eighty-six percent of the beetle cultures yielded organisms. Nineteen genera of algae and protozoa were identified from 107 cultures

inoculated with dragonfly feces. Sixty-five percent of the dragonfly cultures yielded organisms.

Results are ecologically significant since aquatic insects periodically disperse, carrying a variety of aquatic microorganisms adapted for alimentary survival during overland transport. Not only is internal transport of spores, cysts and resistant structures possible, but transport of vegetative algal cells was also demonstrated. Although the data indicate a more important role in passive internal dispersal of algae and protozoa by herbivorous beetles, they also show that accidental or incidental internal dispersal may be accomplished by carnivorous aquatic insects such as dragonflies.

Minimal viability duration of selected algae, during insect transport under monitored conditions, and longevity of beetles held in an environmental chamber, under simulated flight conditions, were determined during Part III of the study. The data indicate that sustained or short successive beetle flights could result in overland dispersal of algae and protozoa. It is estimated that Chlorella, Hormidium and Stichococcus used in this study could be dispersed up to 450 km by dragonflies flying at speeds of about 30 km per hour over a 24-hour period.

Survival of T. lateralis out of water under test conditions declined rapidly after 24 hours. T. ornaticollis survivorship started a steep decline after 48 hours. Under test conditions both algal viability duration and beetle survivorship times were sufficiently long to allow for dispersal of algae and protozoa.

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CHAPTER I

THE DISPERSAL OF ALGAE AND PROTOZOA BY SOUTHWESTERN AND PACIFIC NORTHWESTERN ODONATA

Introduction

An understanding of the population dynamics of small aquatic organisms, such as algae and protozoa, requires information regarding their means of dispersal across land barriers. Dispersal into and subsequent colonization of suitable new or isolated aquatic situations by emigrants from established populations enables their maintenance, especially during periods of stress, and also aids in range extension.

Since algae and protozoan populations do not exhibit effective adaptations for terrestrial motility, they are, to a large extent, dependent on physical vehicles (winds, flowing water) or biological agents for dissemination over land.

Winds as vehicles for dispersal of algae and protozoa have been discussed by Overeem (1936, 1937) and Schlichting (1961, 1964). Schlichting (1958, 1960), Proctor (1959, 1965, 1968) and Malone (1965) have demonstrated the importance of

waterfowl as disseminators of viable algal and protozoan disseminules.

Earlier anecdotal reports of the occurrence of small aquatic organisms on insects (Migula, 1888; Scott, 1910; Iréneé-Marie, 1938; Messikomer, 1943) pointed up the need for elucidating the role of aquatic insects as passive biological dispersal agents for viable free-living disseminules. Recent works have dealt with defining this role for Odonata, aquatic Diptera, aquatic Hemiptera, aquatic Coleoptera, Trichoptera and other aquatic insects (Stewart and Schlichting, 1966; Parsons, Schlichting and Stewart, 1966; Revill, Stewart and Schlichting, 1967a, b; Milliger and Schlichting, 1968; Stewart, Milliger and Solon, 1970; Milliger, Stewart and Silvey, 1971).

Few such studies have dealt with insect dissemination of microorganisms outside the Southwestern USA. Maguire (1959, 1963) presented a descriptive higher classification of algae, protozoa and moss spores washed from mosquitoes, dragonflies and other insects in New York State and Texas. Schlichting and Sides (1969) studied the transport of aquatic microorganisms by Hemiptera in the Seattle, Wash. area and Oklahoma.

The current study was undertaken to further clarify the role of odonates in passive dispersal of viable small aquatic organisms. Special attention was given to (1) sampling odonate species previously unstudied in this role, (2) additional sampling of species for which only anecdotal information is available, and (3) noting geographic variations in passive dispersal of algae and protozoa between odonates from the Southwest (including previous studies) and the Pacific Northwest.

Methods and Materials

Dragonflies were captured in the field, using sterile nylon nets. Nets in envelopes were autoclaved at 121 C under 15 lb. pressure for 15 min before each collection date. A different net was used for every five individuals collected or when contamination of a net occurred through accidental contact with water. A random section of each net was washed in a culture vial at the end of its use. Such washings will be hereafter termed "sham net washings." This practice gave some indication of contamination that might be attributed to the net. Sterile forceps were used to transfer insects from nets to culture vials. Damselflies were picked from vegetation, using sterile forceps and then transferred directly to culture vials.

The 8-dr screw-cap culture vials were charged with 16 ml of equal parts of sterile soil-water extract and Bristols medium (modified by Bold, 1949). The pH of the medium was 6.5 to 6.8. Culture vials were transported in ice-filled styrofoam bait boxes to avoid heating.

After inoculation in the field with captured odonates, vials were returned to the laboratory and agitated with a Vortex Jr. mixer to dislodge adhering disseminules. Insects were removed from the inoculae (to be subsequently called cultures), using sterile forceps and aseptic technique.

Cultures were maintained in plant growth chambers at 21 ± 2 C with a 16-hr photophase. They were homogenized with a Vortex Jr. mixer and sampled with sterile pipettes at 3, 6, 9 and 12 weeks. Two-drop samples from each culture were examined microscopically along three horizontal and three vertical transects. Algal identifications were made using Smith (1950), Starr (1955) and Prescott (1964). Jahn (1949), Kudo (1954) and Pennak (1953) were used in protozoan identification.

In addition to sham net washings, two other types of controls were taken as checks on possible atmospheric contamination and culture medium sterility. Sham controls consisted of simulation of all field and laboratory procedures,

including inoculation with "imaginary" insects. One such control was made for every five field inoculations. Ten percent of the medium vials prepared were used as medium controls. All controls were held in plant growth chambers with experimental cultures.

Species of Pacific Northwest odonates studied included the dragonflies Aeschna californica Calvert, Anax junius Drury, Cordulia shurtleffi Scudder, Leucorrhinia hudsonica Selys, Libellula forensis Hagen, Libellula incesta Hagen, Sympetrum occidentale Bartenev, and one damselfly, Ishnura cervula Selys. Texas and Oklahoma species previously not studied as microorganism dispersers included Anax junius Drury, Dromogomphus spoliatus Hagen, Dythemis fugax Hagen, Libellula flavida Rambur, Libellula pulchella Drury, Orthemis ferruginea Fabricius and Pantala hymenea Say. Additional records of microorganism dispersal were obtained for Celithemis eponina Drury, Dythemis velox Hagen, Erythemis simplicicollis Say, Gomphus militaris Hagen, Gomphus sp., Plathemis lydia Drury, and Tramea lacerata Hagen.

Results and Discussion

The eight Pacific Northwest odonates carried 26 genera of viable algae, 4 protozoa and 3 fungi (Table 1). Seventy-five percent of the experimental inoculations resulted in

culture populations of one or more species of small aquatic organisms.

A. californica carried 23 genera. Of these there were 3 blue-green algae (Chroococcus, Gloeothece and Phormidium), 14 green algae (Asterococcus, Bracteacoccus, Chlorella ellipsoidea, cf. Chlorella, Chlorococcum, Cladophora, Gloeocystis, Hormidium, Mesotaenium, Nannochloris, Oocystis, cf. Palmellococcus, Rhizoclonium and Stichoccus), and 4 diatoms (Cymbella, Diatoma, Navicula and Pinnularia). In addition, one golden-brown alga (Ochromonas) and one protozoan (Bodo) were found. Fungal spores were transported by four of the 28 individuals. This is the first report of passive transport of Mesotaenium by an aquatic insect. Carriage of the blue-green Gloeothece, green Asterococcus, Hormidium and Rhizoclonium, diatoms Cymbella and Diatoma and the golden-brown alga Ochromonas are new records for odonates.

A. californica is a wide-ranging, early-season western species according to Needham and Westfall (1955), ranging from British Columbia, Canada, southward to California and eastward to Idaho and Arizona. Usinger (1963) cited reports of A. californica nymphs in brackish water. Its wide range, relative abundance and number of organisms carried suggest

that A. californica is a major aquatic insect disperser of small aquatic organisms.

The pickup of the blue-green alga Gloeothece is facilitated by its adhesive envelope. It occurs free-floating or adhering to rocks. Vegetative cells probably are picked up by dragonflies making contact with water or while perching. Since Smith (1950) cited cell division and colony fragmentation as the only methods of reproduction in this organism, vegetative cell transport is suggested. Pickup of vegetative forms of Asterococcus, Cymbella and Diatoma probably are facilitated also by their sticky gelatinous sheaths, although Diatoma has been known to form auxospores. Vegetative cells of Mesotaenium generally are found in watery mucilagenous sheaths on rocks or dripping cliffs, or as solitary, free-floating cells (Smith, 1950). Ornamented zygotes are formed sometimes. Hormidium occurs as unattached floating filaments that probably become entangled on the legs and other appendages of insects. Vegetative cells remain viable for at least 24 hours (Solon and Stewart, unpublished). Rhizoclonium, a filamentous form found in quiet waters, probably is transported also as vegetative cells or as akinetes which differ little from the vegetative cells (Smith, 1950).

The variously ornamented silicified walls of the statospores of Ochromonas most likely contribute to its transport on the external surfaces of dragonflies. Many direct contacts with water by A. californica were observed at Sportsmans Lake on San Juan Island, Lone Lake on Whidby Island and streams and lakes in the Cle Elm area of Washington. At Sportsmans Lake, A. californica was observed to fly down and forcefully make contact with the water along its entire ventral surface, including its wings. This appeared to be in response to minute ripples caused by fish fry swimming close to the surface. This behavior results in the most direct environmental contact necessary for pickup of planktonic and other algal forms.

L. forensis carried seven genera of small organisms, including one blue-green alga (Gloeothece), three green algae (Chlorella, Chlorella ellipsoidea, Nannochloris), one golden-brown alga (Ochromonas) and the protozoan Bodo.

The range of L. forensis is from British Columbia, Canada, southward to California and westward to Montana and Colorado (Needham and Westfall, 1955). This is a common and relatively abundant species and, like A. californica, probably contributes substantially as a passive dispersal vehicle for small aquatic organisms.

Other dragonfly species carried lesser numbers of organisms (Table 1) with proportions of blue-green algae, green algae and protozoa similar to those in previous studies by Stewart, Schlichting and co-workers. Population densities of dragonflies were low in the Seattle study area during the course of the investigation (summer, 1967), requiring many man-hours for appropriate collection of relatively few samples for some species. A. californica, L. forensis and the damselfly, I. cervula, comprised 77% of the individuals encountered in the Pacific Northwest; they carried 88% of the total genera of microorganisms observed during the study.

I. cervula is widely distributed, from southwestern Canada southward through the western United States to Baja, California, and New Mexico (Usinger, 1963). It carried 10 genera of microorganisms (Table 1). Included were one blue-green (Chroococcus), six greens (Chlorella ellipsoidea, cf. Chlorella, Gloeocystis, Hormidium, Nannochloris and cf. Palmellococcus), one golden-brown (Ochromonas), two protozoans (Amoeba and Bodo), fungal spores and protozoan cysts. The data suggest a major role by this species in passive dispersal of small aquatic organisms in this geographical area.

Seventeen southwest odonate species carried 36 genera of algae, 15 protozoa and 2 fungi. One, O. ferruginea, carried a moss protonema and another, L. flavida, carried a nematode (Diplogaster). Information about encystment and desiccation among aquatic nematodes is scant according to Pennak (1953), although "some species show an ability to thrive in a wide variety of habitats and a wide variety of ecological conditions." Diplogaster is found in fresh water, and in all probability, was picked up in an encysted state.

Seventy-eight percent of the cultures resulting from "odonate inoculation" yielded one or more species of small aquatic organisms. D. fugax carried 12 genera of algae (Anacystis, Phormidium and cf. Polycystis), seven greens (cf. Arthrospira, Bracteacoccus, Chlorella, Chlorella ellipsoidea, Chlorococcum, cf. Chlorococcum, and Nannochloris), three protozoans (Bodo, cf. Bodo and Colpidium) and one fungus (Alternaria).

Three dragonflies, E. simplicicollis, L. pulchella and T. lacerata, each carried 11 different microorganisms. These dragonflies are common and abundant in southern Oklahoma and northern Texas and widely distributed over most of the USA. Needham and Westfall (1955) state that

E. simplicicollis makes many environmental contacts as it perches on floating or emergent vegetation waiting for suitable prey. L. pulchella and T. lacerata are both strong-flying species, widely distributed and capable of rapid flight.

P. flavescens and P. lydia carried nine and eight different microorganisms, respectively. A. junius, G. militaris and T. onusta each carried seven genera of algae and protozoa.

Southwestern dragonflies previously studies for which additional data were obtained include C. eponina, E. simplicicollis, G. militaris, L. luctuosa, P. lydia and T. lacerata (Table 2).

Frequencies of occurrence of viable algae, protozoa and fungi in 74 cultures from eight species of Pacific Northwest odonates are given in Table 3. Bodo occurred most frequently in culture. Chlorella ellipsoidea, Gloeothece and Nannochloris occurred in 18.9, 12.1 and 12.1% of the 74 cultures, respectively. Chlorella ellipsoidea, Chlorella and Bodo were all found on 50% of the insect species studied.

As in recent studies of various aquatic insects by Stewart, Schlichting and co-workers, green algae (Chlorophyta) were the most commonly transported groups of organisms by

both Southwest and Pacific Northwest insects. Eight genera of green algae carried were common to both Northwest and Southwest odonates. This probably is related to special morphological modifications of vegetative or reproductive structures and the particular behavioral habits of dragonflies which pick them up.

Frequencies of occurrence of algae, protozoa and fungi in 119 cultures from 17 species of Southwestern dragonflies are given in Table 4. The protozoan Bodo was most frequent in cultures (21.8%); Chlorella and Chlorococcum were second and third in frequency with 19.3 and 13.4%, respectively. Bodo was carried by 64.7% of the insects. Chlorella was second, occurring on 58.8%. The blue-green Phormidium, the the green Chlorococcum and the fungus Alternaria occurred on 47% of the species studied. All other microorganisms, with the exception of cf. Bodo, occurred on less than 25% of the insects studied. Fungal hyphae were found in 36.8% of the cultures and occurred on 94.1% of the insects washed.

The microorganisms and their relative frequencies on odonates in the Southwest study are somewhat similar to those reported by Stewart and Schlichting (1966). Among the blue greens, the percent frequencies of 11.7%, 23.5% and 47% for Nostoc, Oscillatoria and Phormidium, respectively,

are closely comparable, as are those of 5.8%, 5.8%, 17.6%, 47.0% and 5.8%, respectively, for the greens Ankistrodesmus, Bracteacoccus, Chlamydomonas, Chlorococcum and Scenedesmus. Other frequencies that are closely comparable are Navicula, 23.5%, Euglena, 11.7% and a protozoan ciliate Colpoda, 5.8%.

Comparisons by groups of organisms transported by aquatic insects between geographic areas during the present study and in previous studies are tabulated in Table 5.

There is consistency in percentages of groups of organisms transported within geographic areas (Table 5). Three odonate dispersal studies in the Southwest show percentages of Cyanophyta carried as 23.8, 26.3 and 29.0 in studies by Solon (current), Stewart and Schlichting (1966) and Parson et al. (1966), respectively. Percentages of Chlorophyta are similarly close as indicated by the percentages 47.6, 29.8 and 41.6 reported by Solon (current), Stewart and Schlichting (1966) and Parsons et al. (1966), respectively. Reporting Chrysophyta in the same order, the percentages are 9.5, 8.3 and 14.0.

A comparison of major groups obtained from Pacific Northwest odonates in the present study and those from aquatic Hemiptera from that area by Schlichting and Sides (1969) show similarities in percentages for the

Cyanophyta (11.5 and 10.7), Chlorophyta (50.0 and 42.8), Euglenophyta (7.6 and 3.5) and protozoa (11.5 and 10.7). The percentages of Chrysophyta carried by the Gerridae and Notonectidae in the previous study were considerably higher than in the odonata studied, 32.1 and 19.2, respectively.

Comparison between these two geographical regions indicate that a greater percentage of blue-green algae are transported in the Southwest. No one factor accounts for large populations of algae at any given time. A combination of factors including highly-dissolved organic content of the water and low concentrations of inorganic nutrients along with the ability to survive high temperatures favors abundance and blooms of blue-green algae (Fogg, 1965). Green algae were transported at about the same rate in both geographic areas. It appears that a greater percentage of Chrysophyta is transported by insects in the Northwest than in the Southwest; 10% more in the current study and 25% more than in the study by Schlichting and Sides (1969). Euglenophyta comprised less than 10% of the genera carried in all studies. The protozoa ranged from 7.6 to 24.5%, the pattern being less clear for these two groups.

Evidence thus far indicates that the aquatic coleoptera are among the most important aquatic insect disseminators of small aquatic organisms. The average number of genera carried per aquatic beetle species was 5.2, higher than for any other group (Milliger et al., 1970). The average number of genera carried per insect species in the Southwest odonate studies ranged between 2.0 and 2.4%. A slightly higher percentage (3.7) was reported in the Northwest, perhaps indicating a somewhat more important role for odonates as microorganism disseminators in this area.

A. junius was the only insect species studied which was common to the Northwest and Southwest. A. junius is a very large, strong flier and is difficult to catch even when there is no concern for net sterility. It is of particular interest since it is wide-ranging over much of the world. Felt (1928) described reports of mass flights in Connecticut and observations of establishment of this species in the Hawaiian Islands. Three specimens collected in the Pacific Northwest carried four genera of algae and one protozoan. In the Southwest during the following summer, two specimens yielded five genera of algae and one genus each of protozoa and fungi. The green alga Chlorococcum and the protozoan Bodo were carried in both regions. These

anectodotal observations suggest the possibility of regional dissemination of microorganisms. Corbet (1962) described migratory flights of A. junius adults of one generation flying north and the next generation flying south. "Summer populations in Canada are thought to be maintained solely by migration. Adults arrive from the south in May, and their progeny, which emerge in August and September, are said to fly south again" (Corbet, 1962:193). Migratory movement of this type could make A. junius a very important long-range disseminator of small aquatic organisms. Further investigation of this species is needed.

All media controls were negative. Only one sham washing yielded an unclassified ciliate contaminant.

Summary

1. The combined studies showed that 24 odonate species were transporting 54 genera of small aquatic organisms, representing 26 genera of Chlorophyta, 11 Cyanophyta, eight Chrysophyta, two Euglenophyta, five protozoans and two fungi. The most commonly transported algae were Chlorophyta (green algae).

2. Microorganism dissemination data on nine previously unstudied Southwestern dragonflies and seven

Northwestern odonates show 20 and 25 genera of microorganisms carried, respectively.

3. The green alga, Mesotaenium, was found for the first time on aquatic insects and the algae Gloeothece, Asterococcus, Hormidium, Rhizoclonium, Cymbella, Diatoma and Ochromonas are new records for odonates.

4. Green algae were transported in approximately the same percentages of total genera carried by aquatic insects in the Northwest and Southwest. Blue-green algae were carried in the Southwest to a greater extent than in the Northwest although these data were not correlated with population densities or availability for pickup of small aquatic organisms. Chrysophyta were carried in greater numbers in the Northwest. Regional differences in organisms transported are undoubtedly related to multiple factors of temperature, light, dissolved organic and inorganic materials which affect the abundance of various algal groups. Morphological characteristics of the algae and specialized reproductive structures such as statospores and akinetes may play a part in pickup of disseminules by aquatic insects. In addition, behavioral patterns in insects also contribute to probability and frequency of disseminule pickup.

5. A summary of recent dispersal studies of algae and protozoa by aquatic insects shows the Chlorophyta to be the major group dispersed in both geographic regions.

TABLE 1. Organisms cultured from eight Pacific Northwest odonate species

Insect Species	No. Washed	No. Different Cultures	No. Different Cultures Yielding Organisms	Organisms
Anisoptera (Dragonflies)				
A. californica	28	28	21	Asterococcus, Bodo, cf. Bracteacoccus, Chlorella ellipsoidea, cf. Chlorella, Chlorococcum, Chroococcus, Cladophora, Cymbella, Diatoma, Gloeothece, Gloeocystis, Hormidium, Mesotaenium, Nannochloris, Navicula, Ochromonas, Oocystis, cf. Palmellococcus, Pinnularia, Phormidium, Rhizoclonium, Stichococcus, fungal spores
A. junius	3	3	3	Bodo, Chlorococcum, Euglena, Gloeothece, Rhizoclonium, fungal spores
C. shurtleffi	2	2	1	Chlorella
L. hudsonica	7	7	7	Bodo, Chlorella, Chlorella ellipsoidea, Chroococcus

L. forensis	19	19	11	*Bodo*, *Chlorella*, *Chlorella ellipsoidea*, *Gloeothece*, *Nannochloris*, *Ochromonas*, fungal hyphae, fungal spores
L. incesta	1	1	1	*Ameba*, cf. *Valkamfia*
S. occidentale	4	4	2	*Chlorella*, cf. *Chlorococcum*, *Rhizoclonium*
Zygoptera (Damselflies)				
I. cervula	10	10	10	*Ameba*, *Bodo*, *Chlorella ellipsoidea*, cf. *Chlorella*, *Chroococcus*, *Gloeocystis*, *Hormidium*, *Nannochloris*, *Ochromonas*, cf. *Palmellococcus*, unclassified ciliate, unclassified fungal spores, unclassified protozoan cysts

TABLE 2. Organisms cultured from 17 Southwestern odonate species

Insect Species	Number Washed	Number Different Cultures	Number Different Cultures Yielding Organisms	Organisms
Anisoptera (Dragonflies)				
A. junius	2	2	2	Alternaria, Chlamydomonas, cf. Chlorococcum, Bodo, cf. Navicula, Navicula, cf. Polycystis, protozoan cyst, unclassified fungal spores
C. eponina	10	10	10	Chlorella, Euglena, Nannochloris, Navicula, Oocystis, Phormidium
D. spoliatus	1	1	1	Alternaria, Bodo, cf. Bodo, protozoan cysts, unclassified fungal spores
D. fugax	15	15	12	Alternaria, Anacystis, cf. Arthrospira, Bodo, cf. Bodo, Bracteacoccus, Chlorella ellipsoidea, Chlorella, cf. Chlorococcum, Chlorococcum, Colpidium, Nannochloris, cf. Phormidium, cf. Polycystis, protozoan cyst, unclassified fungal spores

D. velox	4	4	2	Chlorococcum, Peranema, cf. Phormidium, unclassified fungal spores
E. simplicicollis	10	10	9	Bodo, Chlorella ellipsoidea, Chlorella, Chlorococcum, cf. Chroococcus, Lyngbya, Nannochloris, Nostoc, Oocystis, Oscillatoria, Phormidium, unclassified fungal hyphae
G. militaris	3	3	3	Ameba, Anacystis, Chlorella, Chlorococcum, Chlorosarcinopsis, Chroococcus, Navicula, protozoan cyst, unclassified fungal spores
Gomphus sp.	2	2	2	Anacystis, Chlorella, Euglena, Lyngbya, Navicula, unclassified fungal spores
L. flavida	11	11	8	Chlorella, cf. Chlorella, Chlorococcum, Diplogaster, Oocystis, Scenedesmus
L. luctuosa	3	3	3	Alternaria, Bodo, protozoan cysts, unclassified fungal spores
L. pulchella	11	11	8	Alternaria, Ankistrodesmus, cf. Asterococcus, Bodo, Chlorella ellipsoidea, Chlorella, Chlorococcum, Chroococcus, Colpoda, Cosmarium, Nannochloris, protozoan cysts, unclassified fungal spores

TABLE 2--Continued

Insect Species	Number Washed	Number Different Cultures	Number Different Cultures Yielding Organisms	Organisms
O. ferruginea	1	1	1	Alternaria, Bodo, moss protonema, Phormidium, Stigeonema, unclassified chrysophyte, unclassified fungal spores
P. flavescens	5	5	5	Alternaria, cf. Bodo, Bodo, cf. Chlorella, cf. Chroococcus, cf. Nannochloris, Navicula, cf. Navicula, cf. Nitzschia, Phormidium, protozoan cysts, unclassified fungal spores
P. hymenaea	6	6	6	Alternaria, Chlorella, protozoan cyst, Stichococcus, unclassified fungal spores
P. lydia	11	11	6	cf. Bodo, Chlamydomonas, Chloragonium, Chlorella, Chlorococcum, Chlorosarcina, cf. Closterium, Phormidium protozoan cysts, unclassified fungal spores

T. lacerata	11	11	8	Alternaria, cf. Bodo, Bodo, Chlorella, Chlorococcum, Chlorosarcinopsis, Fusarium, Navicula, Nostoc, Oscillatoria, Phormidium, unclassified fungal spores
T. onusta	13	13	7	Bodo, Chlorella, cf. Chlorocloster, cf. Chlorococcum, Oscillatoria, Phormidium, protozoan cyst, Stichococcus, unclassified fungal spores

TABLE 3. Percent frequency of algae, protozoa and fungi in 74 cultures from 8 species of Pacific Northwest odonates

Organism	% Frequency In Cultures	% Frequency from Insect Species
Algae		
Division Cyanophyta		
Chroococcus	9.4	37.5
Gloeothece*	12.1	37.5
Phormidium	5.4	12.5
Division Chlorophyta		
Asterococcus*	2.7	12.5
cf. Bracteacoccus	2.7	12.5
Chlorella	6.7	50.0
Chlorella ellipsoidea	18.9	50.0
cf. Chlorella	8.1	25.0
Chlorococcum	2.7	25.0
Cladophora	2.7	12.5
Gloeocystis	2.7	25.0
Hormidium*	4.0	25.0
Mesotaenium**	1.3	12.5
Nannochloris	12.1	37.5
Oocystis	1.3	12.5
cf. Palmellococcus	8.1	25.0
Rhizoclonium*	8.1	37.5
Stichococcus	4.0	12.5
Division Chrysophyta		
Cymbella*	1.3	12.5
Diatoma*	1.3	12.5
Navicula	1.3	12.5
Ochromonas*	5.4	37.5
Pinnularia	1.3	12.5
Division Euglenophyta		
Euglena	1.3	12.5

TABLE 3--Continued

Organism	% Frequency in Cultures	% Frequency from Insect Species
Protozoa		
Class Sarcodina		
Amoeba	1.3	12.5
cf. Valkamfia	1.3	12.5
Class Mastigophora		
Bodo	20.2	50.0
Class Ciliata		
Unclassified ciliates	1.3	12.5
Fungi		
Unclassified Fungal hyphae	4.0	12.5

*New records for odonata.

**First report on aquatic insects.

TABLE 4. Percent frequency of algae, protozoa and fungi in 119 cultures from 17 species of Southwest dragonflies

Organism	% Frequency in Cultures	% Frequency from Insect Species
Algae		
Division Cyanophyta		
Anacystis	2.5	17.6
Chroococcus	1.6	11.7
cf. Chroococcus	2.5	11.7
Lyngbya	3.3	11.7
Nostoc	5.0	11.7
Oscillatoria	3.3	23.5
Phormidium	10.9	47.0
cf. Phormidium	1.6	11.7
cf. Polycystis	2.5	11.7
Stigeonema*	.8	5.8
Division Chlorophyta		
Ankistrodesmus	.8	5.8
cf. Asterococcus	.8	5.8
cf. Arthrospira	.8	5.8
Bracteacoccus	.8	5.8
Chlamydomonas	2.5	17.6
Chlorogonium*	.8	5.8
Chlorella	19.3	58.8
Chlorella ellipsoidea	5.0	17.6
cf. Chlorella	4.2	17.6
Chlorococcalean cells	2.5	17.6
Chlorococcum	13.4	47.0
Chlorosarcina*	.8	5.8
Chlorosarcinopsis*	2.5	11.7
cf. Closterium	.8	5.8
Cosmarium	1.6	5.8
Nannochloris	5.8	23.5
cf. Nannochloris	.8	5.8
Oocystis*	4.2	17.6
Scenedesmus	3.3	5.8
Stichococcus	1.6	11.7

TABLE 4--Continued

Organism	% Frequency in Cultures	% Frequency from Insect Species
Division Chrysophyta		
cf. Chlorocloster	.8	5.8
Navicula	3.3	23.5
cf. Navicula	4.2	17.6
cf. Nitzschia	.8	5.8
Division Euglenophyta		
Euglena	1.6	11.7
Peranema	.8	5.8
Protozoa		
Class Sarcodina		
Amoeba	.8	5.8
Class Mastigophora		
Bodo	21.8	64.7
cf. Bodo	5.0	29.4
Class Ciliata		
Colpidium*	.8	5.8
Colpoda	.8	5.8
Unclassified Protozoan cysts	27.7	64.7
Fungi		
Alternaria*	15.9	47.0
Fusarium*	.8	5.8
Unclassified hyphae	36.8	94.1
Unclassified spores	26.8	82.3

*New records for odonata.

TABLE 5. Percent comparisons by groups of organisms transported by aquatic insects between geographic areas during the present study and in previous studies

Study	Insects Studied	Number Species	Number Insects	Total Number Genera of Micro-org.	% Genera Carried per Study Organisms by Groups						Average Genera/ Insect Species
					Cyanophyta	Chlorophyta	Chrysophyta	Euglenophyta	Protozoa	Other	
Solon (current)											
Pacific N.W.	Odonates	7	74	26	11.5	50.0	19.2	7.6	11.5	0	3.7
Southwest	Odonates	17	119	42	23.8	47.6	9.5	2.3	11.9	4.7	2.4
Milliger, Stewart, Silvey (1970)											
Southwest	Coleoptera	23	204	120	18.3	41.6	20.8	1.6	15.8	1.6	5.2
Stewart, Milliger, Solon (1970)	Hemiptera, Trichoptera, Plecoptera	16	112	26	30.7	53.8	3.8	0	7.6	3.8	1.6
Revill, Stewart, Schlichting (1967)	Diptera	14	221	28	25.0	53.5	3.5	0	17.8	0	2.0
Stewart and Schlichting (1966)	Odonates	26	82	57	26.3	29.8	14.0	5.2	24.5	0	2.2

Parsons, Schlichting Stewart (1966)	Odonates	11	50	24	29.1	41.6	8.3	8.3	12.5	0	2.2
Schlichting and Sides (1969)	Hemiptera										
Seattle, Wash.		6	27	28	10.7	42.8	32.1	3.5	10.7		4.6
Southwest		4	82	19	31.5	42.1	5.2	5.2	15.7		4.7

CHAPTER II

DISPERSAL OF ALGAE AND PROTOZOA VIA THE ALIMENTARY TRACTS OF SELECTED AQUATIC INSECTS

Introduction

Prior to 1959, it was generally believed that freshwater algae and protozoa were dispersed across land barriers by winds or by external transport on migratory waterfowl. Proctor (1959) and Schlichting (1960) both showed that dispersal could be accomplished also by overland flights of birds while viable algae and protozoa were passing through their alimentary tracts. Colonization of new suitable aquatic situations was possible through fecal deposition of internally transported disseminules.

Several recent papers indicate that aquatic insects also play a significant role in overland transport of viable algae and protozoa (Maguire, 1959; Stewart and Schlichting, 1966; Parsons, Schlichting and Stewart, 1966; Revill, Stewart and Schlichting, 1967a, b; Schlichting and Sides, 1969; Stewart, Milliger and Solon, 1970; Milliger, Stewart and Silvey, 1971). These workers have demonstrated dispersal of small, viable, aquatic organisms by Odonata,

aquatic Diptera, aquatic Hemiptera, Trichoptera, aquatic and terrestrial Coleoptera and other aquatic insects, utilizing inoculation techniques that did not allow distinction as to whether disseminules were internally or externally transported.

Stewart and Schlichting (1966) pointed up the possibility of internal transport by insects after finding Nostoc and fungi in cultures inoculated with hind-gut samples aseptically collected from five species of odonates. Anacystis, Gloeocapsa, Chroococcus, protozoan cysts and fungal hyphae were found in mid-gut cultures, and several additional species were cultured from fore-gut contents. These observations were inconclusive since only one hind-gut culture was made for each of three odonate species, and two each were made for two others. They stated that "if algae or protozoa or their disseminules are later identified in number in the posterior gut of insects, it would be possible to imply ingestion at one aquatic site (directly or accidentally on aquatic prey), transport via the gut and during digestion, and deposition in a different environment by fecal elimination" (p. 560).

Milliger and Schlichting (1968) demonstrated in a laboratory experiment that fecal pellets of Tropisternus

lateralis contained viable algae and protozoa. They also suggested that the number of organisms transported internally might exceed the number transported externally.

The purpose of this study was to demonstrate the passage of viable algae and protozoa through the digestive tracts of field-collected herbivorous and carnivorous aquatic insects, and thereby to determine potential internal transport-dispersal of these microorganisms by certain aquatic insects.

Materials and Methods

Hydrophilid beetles were collected during the late summer and early fall of 1968 and 1969 near Denton, Texas. Species studied included Hydrochara obtusata (Say), Hydrophilis triangularis (Say), Tropisternus lateralis nimbatus (Say), and Tropisternus striolatus (LeC.). They were usually collected under lights at night and were transferred to the laboratory in vials kept in a styrofoam box containing crushed ice. Beetles were dipped and agitated in 99% isopropanol for about one minute. They were then air-dried and embedded in paraffin. The hindgut was exposed by dissection with sterile microsurgical instruments, pinched off and transferred to a vial of culture medium composed of equal parts of sterile soil-water extract

and Bristols medium. A sterile scalpel was used to open the hind-gut in the vial. Cultures thus inoculated were agitated for two minutes on a Vortex Jr. mixer and held in plant growth chambers at 22 to 24 C with a 16-hr photophase. Cultures were examined after 2, 4, 8 and 12 weeks after inoculation. A two-drop sample was examined microscopically along three vertical and three horizontal transects, followed by a scanning check.

Dragonflies were collected with nets during the summer of 1966 from ponds in the vicinity of Lake Texoma, Okla. They were transferred live to the laboratory in a shaded, wire screen box to minimize activity. They were wrapped in sterile cotton except for about one-fourth inch of the distal abdomen. This exposed portion was washed by dipping into 75% ethyl alcohol for about one minute to reduce the possibility of external contamination. The live specimens then were taped in position on a sterile 1.5-ml vial so that the exposed portion of the abdomen extended downward into it. The sterile cotton closed the top of the vial and the exposed abdomen was suspended so that it did not touch the sides. Fecal pellets deposited subsequent to this procedure were collected the following morning and used to inoculate 8-dr screw-cap vials containing 16 ml of

the same culture medium used for beetle gut inoculations. From 2 to 10 fecal pellets from one specimen constituted a sample; pellets were broken with a sterile spatula, and the inoculum was agitated with a vortex mixer for one minute. Controls consisted of inoculations made with distilled water-rinses of vials receiving no fecal deposits.

Cultures and controls thus obtained were held in plant growth chambers and examined at the same intervals with similar methods as utilized for hydrophilid beetles.

Odonates studied included Celithemis eponina Drury, Dromogomphus spoliatus Hagen, Dythemis fugax Hagen, Erythemis simplicicollis Say, Libellula luctuosa Burmeister, Libellula pulchella Drury, Pachydiplax longipennis Burmeister, Perithemis tenera Say, Plathemis lydia Drury, Tramea lacerata Hagen, and Tramea onusta Hagen.

Results

Table 6 shows a comparison of viable organisms carried internally by beetles and dragonflies. Thirty-two genera of viable algae and protozoa were identified from 36 cultures inoculated with beetle hind-guts. Eighty-six percent of the beetle cultures yielded organisms. Nineteen genera of algae and protozoa were identified from 107 cultures

inoculated with dragonfly feces. Sixty-five percent of the dragonfly cultures yielded organisms.

All medium controls were negative as were all beetle controls. Two of the dragonfly controls were contaminated with a Bodo-like organism (Table 7). Algae and protozoa carried by beetle and dragonfly species are shown in Table 7. All of the T. striolatus gut cultures yielded algae and/or protozoa. Of the 28 genera carried, there were 17 Chlorophyta, 3 Chrysophyta, 3 Euglenophyta, 2 Cyanophyta, 3 protozoans and unidentified fungal hyphae and spores.

H. triangularis transported 10 genera of algae and protozoa as well as fungal spores. Included were 5 Chlorophyta, 2 Cyanophyta, 1 Euglenophyta, 1 diatom and 1 protozoan.

H. obtusata carried 3 Chlorophyta, 2 Cyanophyta and 1 protozoan. T. lateralis inoculae yielded only fungal hyphae and spores.

Among the dragonflies studied, E. simplicicollis carried the greatest number of different algae and protozoa (Table 7). The 11 genera included 5 green algae, 3 blue-greens, 1 diatom and 2 protozoans. The fungus Helminthosporium and other unidentified fungal spores were also carried. T. lacerata carried 7 genera of small aquatic organisms. The other species C. eponina, L. luctuosa,

D. spoliatus, D. fugax, P. lydia, L. pulchella, P. tenera and T. onusta carried 5, 5, 4, 2, 2, 2 and 1 organisms, respectively. P. longipennis carried only protozoan cysts.

The top five algal and protozoan genera carried based on culture-frequency from the four beetles studied were the following: Bodo, Chlorococcum, Navicula, Scenedesmus (combined), and Ankistrodesmus (Table 8). Forty-seven percent of the cultures also contained unidentified fungal hyphae. Those organisms most frequently carried according to beetle species-frequency were as follows: Chlorococcum, Bodo, Oscillatoria, Chlorella, Chlorosarcinopsis, Closteridium, Fragilaria, Navicula, Euglena and Peranema. Fungal hyphae were found in some cultures of all four species.

The five most frequently carried algae and protozoa from dragonflies based on culture frequency (Table 9) were as follows: Bodo, cf. Bodo, Polycystis, Phormidium, and Chlorella. The most frequently carried algae and protozoa from dragonflies based on carrier species frequency (Table 9) were the following: Bodo, cf. Bodo, Polycystis, Chlorella, Gloeocystis, Oocystis, and Stigeonema. Bodo and Bodo-like flagellates were the most commonly occurring organisms in both percent frequency in culture and percent frequency from insect species in beetles and dragonflies.

Discussion

The results indicate that aquatic beetles are more important than dragonflies as dispersal agents for algae and protozoa (Table 6).

Stewart and Schlichting (1966) noted the problems in correlating insect activity with pickup of a given disseminule. However, alimentary transport directly implicates disseminule pickup with feeding behavior. Usinger (1963) describes hydrophilids as largely vegetarian, which accounts perhaps in part for greater numbers of algae transported internally. The period of time that food material is retained within the gut is unknown and presents another problem for investigation. Also, the period of time that algae and protozoa might remain in viable condition is important in determining dispersal distances.

Resistant cells such as zygotes, akinetes, auxospores and statospores, taken in with greater frequency, probably survive the digestive process and pass to the outside in feces. Specific studies of the enzymes secreted by beetle species studied, nor effects upon particular resistant cells by insect enzymes in general are available. Some vegetative cells apparently escape digestion due to sheer numbers ingested, possible delay of enzyme action due to gelatinous

sheaths and/or cell walls and possible compacting within the peritrophic membrane or encasement by matrices of other cells or impervious fragments.

Fecal pellets of many herbivorus beetles, including those from T. lateralis and H. triangularis, are enclosed by segments of peritrophic membrane. This membrane is produced continuously at the fore-mid-gut juncture and extends into the lumen of the mid and hind-guts, forming a protective sleeve for the mid-gut epithelium. Segments of it break off in the hind-gut and enclose the fecal pellets. Viability of any vegetative cells in the feces perhaps might be extended by the investment of fecal materials in cases where the feces are dropped on dry or even moist surfaces. A material "inoculation" of water might then occur after a washing rainfall or even by wind action.

Corbet (1962) described adult dragonflies as obligate carnivores, feeding in flight on almost any animals small enough to be captured, including chiromonids, tipulids, mosquitoes and other diptera. In doing so, algal and protozoan disseminules are passively ingested. Revill et al. (1967a, b), Schlichting and Sides (1969) and Stewart et al. (1970) showed passive transport of algae and protozoa by insects of a size suitable as food for dragonflies. Since

the primary digestive enzymes for carnivorous insects are proteases and lipases, digestion of plant material (cellulose) is limited.

There was little correlation between Cyanophyta carried in beetles and dragonflies (Table 6). The dragonflies carried almost twice as many including Lyngbya, Phormidium and Stigeonema which are not particularly well adapted to survive passage through the gut. Beetles carried Anacystis and Gloeothece, both well adapted to surviving passage through the gut of an herbivorous beetle since they are typically surrounded by double or multiple mucilagenous sheaths. The only other two blue-greens found in beetles were Phormidium and Oscillatoria, both very common and abundant algae.

Organisms well adapted to internal transport, common to both beetles and dragonflies, were Phormidium, Chlamydomonas, Chlorella, Oocystis, Scenedesmus, Navicula and Bodo. Oocystis cells are often surrounded by a gelatinized, greatly expanded cell wall and viable cells of Scenedesmus, Navicula and Chlorella might easily become embedded in the matrix of other algal cells such as Gloeocapsa or Gloeocystis, thus allowing for passage in viable form through the beetle's gut. Chlamydomonas is capable of forming

resistant spores, and Bodo, a bacterial feeder often found in fecal material, forms cysts capable of surviving over 40 years (Manwell, 1961). These adaptations suit these organisms to external as well as internal dispersal by insects. Phormidium, Chlamydomonas, Chlorella, Oocystis and Navicula also have been reported transported internally by waterfowl (Schlichting, 1960). The genus Cosmarium transported by beetles (Table 6) was also reported from the intestinal tracts of Killdeer (Proctor et al., 1967).

No new records of algae or protozoa are reported in this study, perhaps because in previous studies there was a mixture of externally transported and internally transported disseminules due to fecal contamination of cultures.

Anectodotal reports of organisms from gut cultures by Stewart and Schlichting (1966) compare favorably with current data showing all four organisms, Anacystis, Gloeocapsa, Chroococcus and Nostoc, reported from midgut and hindgut cultures.

Based on the present study, T. striolatus appears to be the best agent for dispersal of microorganisms of the four beetles studied. Further study is necessary to verify this because of relatively few specimens of other beetles collected. It is expected that further study of H. triangularis

will reveal a more important role for this species due to its size, range and reported migratory ability (Usinger, 1963). Further investigation of T. lateralis (Table 7) is needed also since only five specimens were obtained and may have been out of water for a number of days. Milliger and Schlichting (1968) in a well-planned laboratory experiment, suggested that T. lateralis is capable of dispersing many viable algae via the digestive tract.

The observation that E. simplicicollis carried the greatest number of genera internally (Table 7) may be due in part to its feeding habits. This species feeds close to the water roosting on floating or emergent vegetation, darting out to capture prey (Usinger, 1963). If it feeds on organisms in close proximity to the water it is more likely to be carrying a greater number of algae and protozoa. E. simplicicollis is widely distributed over most of the USA and southward to Mexico, Cuba, Haiti and Jamaica.

T. lacerata (Table 7), another wide-ranging dragonfly over much of the USA, Mexico and Hawaiian Islands, is potentially a significant internal disperser of microorganisms. It was included in a study on inter-pond dispersion by Stewart and Murphy (1968) in which marked dragonflies were released and subsequent sightings attempted. Marked

T. lacerata were not seen in the study area after release, indicating possible movement beyond the 5.8-km square study area. The same study showed L. luctuosa and D. fugax dispersing at least 1 km from the marking site and D. spoliatus and C. eponina dispersing at least .5 km.

The blue-green algae, Phormidium and Oscillatoria; the green algae, Chlorella, Chlorococcum, Gloeocystis, Oocystis; the diatoms, Fragilaria and Navicula; the euglenoids, Euglena and Peranema; and the protozoan, Bodo, all compare closely with previous studies as the most frequent genera of algae and protozoa transported by aquatic insects (Tables 8 and 9).

Confidence in the results obtained in this study is based on the use of aseptic techniques within the limits imposed by the living organisms used, careful handling of organisms and instruments and sufficient controls to rule out accidental contamination.

This study demonstrates that viable disseminules of algae and protozoa may be transported through the digestive tracts of both carnivorous and herbivorous aquatic insects. Ecologically, this is significant since aquatic insects periodically disperse, carrying with them a variety of aquatic microorganisms adapted for alimentary survival

during overland transport. Colonization of aquatic habitats by aquatic insects after periodic dispersal flights are well known. A recent study by Sublette and Sublette (1967) discussed seasonal recolonization of the shallow Playa Lakes of western Texas and eastern New Mexico. Aquatic insects moved to various types of above-ground tanks as the Playas diminished in size and dried up during late summer and winter. When rains refilled the Playas, aquatic insects returned to recolonize them, carrying aquatic microorganisms with them both externally and internally. Not only is internal transport of spores, cysts and resistant structures possible, but transport of vegetative cells has been demonstrated.

Although the data indicate a more important role in passive internal dispersal of algae and protozoa by herbivorous aquatic beetles, they also show that accidental or incidental internal dispersal may be accomplished by carnivorous aquatic insects such as dragonflies.

TABLE 6. Comparison of organisms transported internally by dragonflies and beetles

Organism	Internally Transported by	
	Dragonflies	Beetles
Algae		
Division Cyanophyta		
Anacystis		X
cf. Chroococcus	X	
Gloeocapsa		X
Lyngbya	X	
Nostoc	X	
Oscillatoria		X
Phormidium	X	X
cf. Polycystis	X	
Stigeonema	X	
Unclassified cocooid blue-green alga	X	
Division Chlorophyta		
Ankistrodesmus		X
cf. Asterococcus		X
Bracteacoccus	X	
Chlamydomonas	X	X
Chlorella	X	X
Chlorella ellipsoidea		X
Chlorococcum		X
cf. Chlorococcum	X	
Chlorosarcinopsis		X
Closteridium		X
Coelastrum		X
Cosmarium		X
Crucigenia		X
Cylindrocapsa		X
cf. Gleocystis	X	
cf. Golenkinia	X	
Nannochloris		X
cf. Nannochloris	X	
Oocystis	X	X
Protococcus	X	
Scenedesmus	X	X
Scenedesmus acuminatus		X
Scenedesmus quadricaudata		X

TABLE 6--Continued

Organism	Internally Transported by	
	Dragonflies	Beetles
Division Chlorophyta (Cont.)		
Schroederia		X
Selenastrum		X
Stichococcus		X
cf. *Tetrahedron*		X
Ulothrix		X
Division Chrysophyta		
Fragilaria		X
Navicula	X	X
Ochromonas		X
Division Euglenophyta		
Euglena		X
Peranema		X
Phacus		X
Protozoa		
Class Mastigophora		
Bodo	X	X
cf. *Bodo*	X	
Class Sarcodina		
Ameba		X
Class Ciliata		
Colpoda		X

TABLE 7. Organisms identified in fecal cultures from selected aquatic beetles and dragonflies

Insect	Number Cultures	Number Negative	No. of Genera Carried/Insect Species	Organisms (Alphabetical)
Hydrophilidae				
H. obtusata	10	3	7	Bodo, Chlorella ellipsoidea, Chlorococcum, Cylindrocapsa, Gloeocapsa, Lyngbya, Fungal hyphae and spores
H. triangularis	4	0	11	Bodo, Chlorella, Chlorella ellipsoidea, Chlorococcum, Chlorosarcinopsis, Closteridium, Euglena, Navicula, Oscillatoria, Phormidium, Ulothrix, fungal hyphae
T. lateralis	5	2	1	Fungal hyphae and spores
T. striolatus	17	0	29	Ameba, Anacystis, Ankistrodesmus, cf. Asterococcus, Bodo, Chlamydomonas, Chlorella, Chlorococcum, Chlorosarcinopsis, Closteridium, Coelastrum, Colpoda, Cosmarium, Crucigenia, Euglena, Fragilaria, Nannochloris, Navicula, Oocystis, Ochromonas, Oscillatoria, Peranema, Phacus, Scenedesmus sp., Scenedesmus acuminatus, Scenedesmus quadricaudata, Schroederia,

				Selenastrum, Stichococcus, cf. Tetrahedron, fungal hyphae and spores
Anisoptera				
C. eponina	19	3	6	cf. Bodo, Bodo, cf. Chlorococcum, cf. Polycystis, Protococcus, protozoan cysts, Stigeonema, fungal hyphae and spores, unclassified blue-green alga
D. spoliatus	7	3	5	Alternaria, Bodo, cf. Gloeocystis, Oocystis, fungal hyphae and spores, protozoan cysts
D. fugax	10	1	3	Bodo, cf. Bodo, cf. Polycystis, fungal hyphae and spores, protozoan cysts
E. simplicicollis	25	11	12	Bodo, cf. Bodo, Bracteacoccus, Chlamydomonas, Chlorella, cf. Chlorococcum, Chroococcus, cf. Golenkinia, Helminthosporium, Navicula, Phormidium, cf. Polycystis, fungal hyphae and spores, protozoan cysts
L. luctuosa	27	9	5	Bodo, cf. Bodo, Lyngbya, Phormidium, cf. Polycystis, fungal hyphae and spores, protozoan cysts
L. pulchella	2	1	2	cf. Bodo, Scenedesmus

TABLE 7--Continued

Insect	Number Cultures	Number Negative	No. of Genera Carried/Insect Species	Organisms (Alphabetical)
P. longipennis	2	1	0	Protozoan cysts
P. tenera	2	0	2	*Bodo*, cf. *Bodo*, fungal hyphae and spores, protozoan cysts
P. lydia	4	0	3	*Bodo*, cf. *Polycystis*, fungal hyphae and spores, protozoan cysts
T. lacerata	8	3	8	*Bodo*, cf. *Bodo*, *Chlorella*, *Nannochloris*, *Nostoc*, *Oocystis*, cf. *Polycystis*, *Stigeonema*, fungal hyphae and spores
T. onusta	1	0	1	Fungal hyphae and spores
Controls				
Hydrophilidae	5	5	0	
Anisoptera	12	10	1	cf. *Bodo*
Media	12	12	0	

TABLE 8. Percent frequency of algae and protozoa in 36 fecal cultures from 4 aquatic beetles species

Organism	% Frequency in Cultures	% Frequency from Insect Species
Algae		
Division Cyanophyta		
Anacystis	8.3	25
Gloeocapsa	2.7	25
Oscillatoria	8.3	50
Phormidium	2.7	25
Division Chlorophyta		
Ankistrodesmus	22.2	25
cf. Asterococcus	8.3	25
Chlamydomonas	8.3	25
Chlorella	30.5	50
Chlorella ellipsoidea	5.5	50
Chlorococcum	41.6	75
Chlorosarcinopsis	8.3	50
Closteridium	13.8	50
Coelastrum	19.4	25
Cosmarium	16.6	25
Crucigenia	8.3	25
Cylindrocapsa	2.7	25
Nannochloris	22.2	25
Oocystis	5.5	25
Scenedesmus	22.2	25
Scenedesmus acuminatus	8.3	25
Scenedesmus quadricaudata	2.7	25
Schroederia	8.3	25
Selenastrum	5.5	25
Stichococcus	5.5	25
cf. Tetrahedron	8.3	25
Ulothrix	11.1	25
Division Chrysophyta		
Fragilaria	13.8	50
Navicula	33.3	50
Ochromonas	2.7	25

TABLE 8--Continued

Organism	% Frequency in Cultures	% Frequency from Insect Species
Division Euglenophyta		
Euglena	16.6	50
Peranema	5.5	50
Phacus	5.5	25
Protozoa		
Class Sarcodina		
Ameba	2.7	25
Class Mastigophora		
Bodo	55.5	75
Class Ciliata		
Colpoda	2.7	25
Fungi		
Unclassified fungal hyphae	47.2	100

TABLE 9. Percent frequency of algae and protozoa in 107 fecal cultures from 11 odonata species

Organism	% Frequency in Cultures	% Frequency from Insect Species
Algae		
Division Cyanophyta		
cf. Chroococcus	0.9	9.1
Lyngbya	1.9	9.1
Nostoc	0.9	9.1
Phormidium	2.8	18.2
cf. Polycystis	8.4	54.5
Stigeonema	1.9	18.2
Unclassified coccoid blue-green algae	0.9	9.1
Division Chlorophyta		
Bracteacoccus	0.9	9.1
Chlamydomonas	0.9	9.1
Chlorella	2.8	18.2
cf. Chlorococcum	1.9	18.2
cf. Gloeocystis	0.9	9.1
cf. Golenkinia	0.9	9.1
cf. Nannochloris	0.9	9.1
Oocystis	1.9	18.2
Protococcus	0.9	9.1
Scenedesmus	0.9	9.1
Division Chrysophyta		
Navicula	0.9	9.1
Protozoa		
Class Mastigophora		
Bodo	37.4	72.7
cf. Bodo	21.5	54.5

CHAPTER III

VIABILITY DURATION OF EXTERNALLY CARRIED DISSEMINULES OF ALGAE AND PROTOZOA ON SELECTED AQUATIC INSECTS

Introduction

Sufficient evidence exists to show that aquatic insects passively disperse a variety of small aquatic organisms. Beginning with Darwin (1890) documentation of passive dispersal of small organisms by aquatic insects has continued to the present. Migula (1888) found algae on aquatic beetles and believed that aquatic insects were more important dispersers of these forms than birds were. Irénee-Marie (1938) found desmids on the legs of an aquatic beetle and also found them on dragonflies. Dragonflies as dispersers of algae and protozoa also were suggested by Messikomer (1943). Maguire (1957, 1959, 1963) studied passive dispersal of small aquatic organisms and found dragonflies carrying algae, protozoa and rotifers. Stewart and Schlichting (1966) cultured 40 algae, 3 euglenoids and 13 protozoa from 26 species of aquatic insects. Parsons, Schlichting and Stewart (1966) cultured 24 genera of algae and protozoa from external washings of dragonflies and damselflies. The legs of

Libellula auripennis Burmeister were primary sites of carriage. Revill et al., (1967b) found 28 different algae and protozoa carried by 221 specimens of 14 species of Tabanids and Culicids. Milliger and Schlichting (1968) investigated the role of fecal deposits of Tropisternus lateralis nimbatus (Say) in dispersal of algae and protozoa. Schlichting and Milliger (1969) cultured 91 genera of microorganisms from Lethocerus uhleri (Montandon), an aquatic hemipteran. Schlichting and Sides (1969) cultured 13 genera of algae and protozoa from corixids, 41 genera from gerrids and 39 from notonectids. Stewart et al., (1970) found 15 species of aquatic insects (Hemiptera, Trichoptera, Plecoptera, Megaloptera and Ephemeroptera) transporting 27 genera of viable small aquatic organisms. A reviewer of this paper posed the question of how long algae and protozoa might survive on insects and how long aquatic insects out of water might survive. Solon and Stewart (unpublished) found 54 genera of small aquatic organisms transported externally by 24 odonate species from the Pacific Northwest and Southwest regions of the USA. Solon and Stewart (unpublished) also demonstrated internal passive dispersal of algae and protozoa via the alimentary tracts of beetles and dragonflies.

Most of these previous studies of insect dissemination have dealt with documenting occurrence of viable disseminules externally and/or internally on dispersing insects. Of further interest in determining potential dispersion distance in this insect-microorganism relationship is knowledge of viability duration of the disseminules during dispersion. Studies of algal viability are scant. Bristol (1919, 1920) investigated algal viability in old stored soil samples. Lipman (1941) described the revival of Nostoc commune from an herbarium specimen reported to be 87 years old. Drouet (1968) states that certain Cyanophyceae may be dried on filter paper and stored indefinitely.

The purposes of this investigation were (1) to determine minimal viability duration for selected algae during insect transport under controlled environmental conditions and (2) to determine longevity of beetles held in a controlled environment under simulated flight conditions.

Materials and Methods

Species of aquatic insects studied were two odonates, Libellula luctuosa Burmeister and Erythemis simplicicollis Say, and two aquatic beetles, Tropisternus lateralis nimbatus (Say) and Thermonectus ornaticollis (Kby.).

The experimental design involved five basic techniques:

1. Three groups of 35 T. lateralis each, were autoclaved at 121 C, 15 lb. pressure for 15 min. One group each was then immersed in cultures of Chlorella pyrenoidosa Chick from the NTSU Collection, Hormidium barlowi Pringsheim from the Indiana Culture Collection #321, and Stichococcus bacillaris Naeg. from the Indiana Culture Collection #314. Beetles were then agitated in culture vials for two minutes on a Vortex Jr. mixer and hand-agitated for an additional five minutes. They were then removed with sterile forceps and suspended from a taut nylon line by means of sterile stainless steel hair clips in an environmental chamber at 21 ± 2 C, with a 16-Hr photophase and a relative humidity between 68 and 80%. The hair clips were fashioned so that the tip of an elytron was held. Beetles were spaced in the chamber in such a way that contact between individuals was avoided.

2. Three additional groups of live T. lateralis were decontaminated by washing through a series of three sterile distilled water baths then exposed to cultures of the same three algae. Beetles were agitated in culture vials for two minutes on a Vortex Jr. mixer then allowed to swim about for an additional five minutes. Removal from vials and

suspension in the chamber were the same as for dead, autoclaved T. lateralis.

3. A group of 40 field-exposed T. lateralis were transported to the laboratory in pond water containing emergent vegetation. These beetles were observed to climb the emergent vegetation and assume flight when placed in open pans. A net was placed over the pans and beetles were picked from the net with sterile forceps. One group of five was immediately used to inoculate culture medium; the remaining 35 were suspended in a manner already described in the environmental chamber. Twenty field-collected T. ornaticollis were handled in the same way, except they assumed flight directly off the surface of the water and were picked from nets and suspended in the environmental chamber. Beetles were spaced in the chamber so that flight-wing movements did not result in contact with adjacent specimens. Beetles exhibited wing movements over the first few hours of suspension.

4. Two groups of 40 live dragonflies each, one of L. luctuosa and another of E. simplicicollis, were collected during flight with sterile nets and transferred with sterile forceps to sterile 32-ml cotton-stoppered vials. They were transferred to the laboratory in an iced Styrofoam bait box

and then suspended in the environmental chamber. Sterile stainless steel clips, suspended from a taut nylon line, held the thorax in such a way that freedom of wing movement was possible.

5. Twenty roosting L. luctuosa were collected with sterile forceps after 2.5 and 8 hours and used immediately to inoculate culture medium.

Five individuals of each of nine groups (excepting the T. ornaticollis group) held in the chamber were used to inoculate a culture medium consisting of equal parts of sterile soil-water extract and Bristol's medium at 2, 4, 8, 12, 16, 20 and 24-hr intervals. An additional inoculation with five L. luctuosa and five E. simplicicollis was made after 36 hours in the chamber. Five individuals of the smaller group of T. ornaticollis were used in making inoculations at 4, 8, 12 and 16-hr. intervals. Insects were agitated for two minutes on a Vortex Jr. mixer after inoculation and were removed aseptically. After time-lapse inoculations were made, beetles were placed in a dry, paper-covered beaker and returned to the environmental chamber to determine minimal longevity under the test conditions. Beetles were considered dead when they could no longer orient themselves in an upright position. Cultures thus obtained were

held in a growth chamber under the same temperature and light conditions used for the insects. Cultures were sampled with sterile pipettes after 2, 4, 8 and 12 weeks. A two-drop sample was examined by taking three horizontal and three vertical transects followed by a scanning of the entire slide.

The possibility of airborne algae contaminating chamber-held insects was controlled by suspending two sterile insects for each time-lapse series of five in the environmental chamber adjacent to experimentals. In the case of the live, field-exposed T. lateralis and T. ornaticollis, two specimens for each time-lapse series of five were autoclaved prior to suspension in the chamber. Two L. luctuosa and two E. simplicicollis for each series were likewise sterilized prior to experiments with field-exposed dragonflies to serve as controls.

The possibility of contamination of roosting dragonflies with forceps or the cultures during inoculation was controlled by making four sham inoculations for this group. A total of 142 controls were made during the study. Twelve vials containing culture medium were kept in the chamber under the same conditions as all the other cultures as a check on medium sterility.

Dragonfly longevity in the chamber was determined daily. Dragonflies were considered alive if leg and mandible response resulted from needle stimulation.

Results

All of the C. pyrenoidosa and S. bacillaris cultures from sterile, dead T. lateralis were positive for all time periods through 24 hours (Figure 1). Eighty percent of the H. barlowi cultures were positive after 2, 4, 8 and 24 hours under simulated flight conditions. All of the 12, 16 and 20-hour H. barlowi washings were positive. All controls were negative, indicating no contamination of specimens due to air currents in the chamber.

Viability duration for C. pyrenoidosa, H. barlowi and S. bacillaris on live T. lateralis beetles are shown in Figure 2. C. pyrenoidosa and S. bacillaris, present in 100% of the cultures after two hours, continuously decreased through the four-hour interval cultures to 20% after 24 hours. H. barlowi found in 80% of the two-hour washings decreased to 40% after 24 hours. A definite decreasing trend is seen for all three algal species. Of the 42 controls only one, number five, of the two-hour time-lapse series yielded two contaminants, Nannochloris and Ankistrodesmus. All beetles

were alive at the end of their respective periods in the environmental chamber and at the time of inoculation.

Figure 3 represents 55 time-lapse inoculations and shows viability duration for naturally acquired algae and other microorganisms on T. lateralis and T. ornaticollis, held for periods up to 24 hours under simulated flight conditions. The decreasing trend in number of positive cultures in the time-lapse series for T. lateralis is more evident than for T. ornaticollis.

Twenty-four algae and five protozoa were found in cultures inoculated with live T. lateralis (Table 10). Sixteen of the algae and all five protozoa occurred in the cultures inoculated immediately from laboratory collection. Three algae, Phormidium, Chlorella and Nannochloris, and one protozoan, Bodo, survived 24 hours on this insect under chamber conditions. Viability duration varied with the algal genera. Phormidium, Chlorella, Nannochloris and Gloeochloris were found with greater frequency throughout the culture period. Eleven genera of algae and one protozoan were found in cultures inoculated with live T. ornaticollis (Table 10). Five algae, Oscillatoria, Polycystis, Chlorella, Chlorococcum and Peranema survived 16 hours on this insect. The ciliate Colpodium was the only protozoan found on this

insect, surviving for a period of 12 hours. All 22 controls for both beetle species were negative. Twelve algae, one protozoan and four fungi were found in the field-exposed cultures of L. luctuosa (Table 11). Four algae, Chroococcus, Nostoc, Chlorella and Cladophora, survived 36 hours. Four fungi, Alternaria, Arthoderma, Fusarium and Dactylaria, were found also on this insect. Eleven algae were found on E. simplicicollis (Table 11). Three algae, Oscillatoria, Chlorella and Cladophora, survived 36 hours on this insect. Oscillatoria, Chlorella and Anabaena were the three algae found most frequently on both dragonflies.

Seventy-five percent of the field-collected roosting L. luctuosa inoculations yielded organisms (Table 12). Five algae, Oscillatoria, Chlorococcum, Cladophora, Stichococcus and Navicula, and one protozoan, Bodo, survived 8 hours on this insect under field (natural) conditions. The most frequently carried alga was Chlorococcum found in 30% of the 2.5-hour cultures and 40% of the eight-hour cultures. Four sham inoculations (controls) were negative in this series.

All of the T. lateralis and T. ornaticollis were alive after 12 and 36 hours, respectively (Figure 5). After 48 hours 68% of T. lateralis were alive but a steep decline in numbers was noted after 48 hours.

Discussion

Knowledge of viability duration of small aquatic organisms carried externally has ecological significance in that this knowledge is essential in determining distances that these organisms might be transported during appentential and non-appentential flights. Flight speeds, distances, migration patterns and routes and weather conditions are other factors essential to the determination of distances that algae, protozoa and other small aquatic organisms may be transported by an aquatic insect. A great deal of work is needed in all these areas before accurate answers to the ecological problem of dispersal can be supplied. Insect dispersal has been observed and described many times for many semi-aquatic and aquatic species although specific details are not definitely known.

Moore (1954) states that some dragonflies are able to colonize new habitats promptly and efficiently up to 60 km away. Flight-speed records in still air by Demoll (1918), Magnan (1934) and Jacobs (1955) indicate Anisoptera capable of speeds between 25 and 35 km per hour. Hankin (1921) describes gliding capabilities for dragonflies that can out distance a swallow, and Hocking (1953) calculated that Aeschna costalis was capable of an air speed of 57.6 km

per hour. Using 30 km per hour as a reasonable estimate of flight speed of the Aeschnidae, algae such as Chlorella, Hormidium and Stichococcus could conceivably be dispersed up to 450 km in a 24-hour period, assuming that odonates would land and roost overnight. Wind and air currents, of course, could possibly increase or decrease the distance. Gibson-Hill (1950) found dragonflies from the Cocos-Keeling Islands which are situated 530 miles from Christmas Island and over 600 miles from the nearest land. Long-distance flight or transport of dragonflies along with viable disseminules of smaller aquatic forms is suggested. Pantala flavescens and Tramea rosenbergi have been reported from these islands even though the fresh-water bodies needed for breeding and naiad survival are lacking. As in dragonflies, some brief reports of beetle migrations are available, but the details regarding distances and patterns are lacking. Distances traveled by beetles are usually the sum of many successive flights of limited duration according to C. G. Johnson (Rockstein, 1965). Sublette and Sublette (1967) found species of aquatic coleoptera recolonizing the Playa Lakes of New Mexico and Texas annually. This recolonization also involves recolonization of micro-fauna and flora as

well, by means of internally and externally insect-transported disseminules.

The similarity of viability duration of C. pyrenoidosa, H. barlowi and S. bacillaris on dead, sterile beetles probably is due to the increased permeability and wettability of the cuticle brought about by autoclaving (Ebeling, 1964). Droplets of the algal culture medium clung to each beetle as it was lifted by forceps and suspended by an elytron in the chamber. In contrast, beetles that were alive and swimming actively in the algal culture appeared dry when removed with forceps from the cultures. Viability durations of algae on live T. lateralis beetles (Figure 2) shows quite a different pattern from Figure 1 in that there is a more pronounced decrease in the number of inoculations that yielded organisms over the 24-hour period. In addition to differences in the wettability of the cuticle, this is thought to be related to disseminule loss by beetles due to movement during suspension in the chamber which simulates, to some extent a natural overland flight. Such loss would potentially contribute to overland dispersal of small organisms during natural flights.

The absence of algae in 41 of the 42 control cultures for live beetle experiments attested to the adequacy of the

"decontamination" procedure. Only one culture contained Nannochloris and Ankistrodesmus as contaminants.

Algal growth was present in cultures for organisms shown in Figures 1 and 2 after 4 to 5 days, using the scanning techniques described under methods, and obvious growth (without the aid of a microscope) was seen after 6 to 10 days. The same was true for some of the naturally-acquired organisms represented by Figure 3. This was probably due to inoculation with viable vegetative cells since a short period of time yields large populations of algae. Field-exposed dragonflies, however, presented a different picture (Figure 4). Organisms from inoculae made over a 24-hour period showed no appreciable decreasing trend in viability duration. In fact, for L. luctuosa the 12-hour series of inoculations all produced growth, whereas only 60% of the earlier 2, 4, and 8-hour inoculations produced growth. This investigator has found that dragonfly inoculations usually do not yield sufficient numbers of organisms for identification (using methods described) before three weeks have elapsed. This suggests that viable disseminules carried by dragonflies are primarily in the form of cysts, spores or other refractive bodies that require a period of time to excyst. A comparison of Figures 2 and 3 shows a marked

similarity between viability duration of naturally acquired and artificially acquired microorganisms on live T. lateralis.

The decline in number of specific naturally-acquired algae and protozoa from T. lateralis and T. ornaticollis over the simulated flight period is shown in Table 10. Twenty-one genera of microorganisms were present in the 5 immediate inoculations and only 4 apparently survived 24 hours in the chamber. The organisms cultured most frequently were Chlorella, Phormidium, Bodo, Chlorococcum and Coelastrum. The first 4 are the same as those found most frequently in culture from aquatic beetles in studies by Milliger et al. (1971). T. lateralis may be more important than T. ornaticollis in number of species of microorganisms transported. This perhaps is due in part to feeding habits, since T. lateralis is primarily herbivorous and T. ornaticollis is predaceous.

The algae most frequently cultured from field-exposed dragonflies held in the chamber (Table 11) were Oscillatoria, Chlorella, Nostoc and Phormidium. Their frequencies in culture correlate closely with those reported for odonates by Stewart and Schlichting (1966) and Parsons et al. (1966). L. luctuosa and E. simplicicollis seem to be of equal

importance as far as number of algae and protozoa dispersed. L. lucutosa, however, was carrying more fungi.

One organism that is conspicuously absent from Table 11 is the protozoan flagellate, Bodo. This organism feeds on bacteria and is commonly associated with fecal material. It was found transported in fecal pellets of dragonflies by Solon and Stewart (unpublished). In the present study, dragonflies were held suspended in simulated flight in an environmental chamber prior to inoculation of the cultures. Many fecal dragonfly pellets were evident on the floor of the chamber during the experiment. The absence of Bodo gives some indication that fecal contamination of inoculae did not occur and that the organisms reported in Table 11 did come from externally carried disseminules.

T. lateralis held out of water under the test conditions survived in high numbers (95%) during the first 24 hours (Figure 5). After that there is a rapid decline in numbers so that, after 48 hours, 68% were alive and after 60 hours only 20% survived. This information, in addition to the viability data, seems to indicate that viable disseminule dispersion by T. lateralis probably is most effective during the first 24 hours after it embarks on a migratory flight. T. ornaticollis survived longer under

the test conditions, but its survivorship curve showed a great similarity to T. lateralis, with a steep decline after 48 hours and continuing until all specimens were dead at the end of 84 hours.

Fernando (1958a, 1960, 1961) reported that weather conditions are related to dispersal flights of beetles. Milliger et al. (1971) indicated two main dispersal periods in North Texas, one in the spring and another in the fall, both coinciding with periods of heavy rainfall. Periods of dispersion also coincide with warm, humid nights following a rainfall. Weather conditions of this sort would help maintain algal viability and insect longevity, both necessary for dispersal.

The data showing viability of naturally-acquired algae and protozoa at 2.5 and 8 hours from dragonflies collected on the roost resemble those from specimens collected in the field and held in the chamber (Table 11). There is little difference in number of organisms cultured in the shorter time-lapse series and little difference in frequency in culture. This bears out the previous postulate that viable disseminules carried by dragonflies are primarily resistant structures, and their frequency does not greatly diminish due to extended periods of air exposure. The presence of

Oscillatoria, Chlorococcum and Chlorella as major, common organisms agrees with data in Tables 10 and 11. Although dragonflies primarily disperse microorganisms in resistant stages, a non-spore-forming alga, Stichococcus was found, indicating dispersal of some vegetative cells as well. Bodo was present also, probably indicating fecal contamination.

Summary

Minimal viability duration was determined for three algae, C. pyrenoidosa, H. barlowi and S. bacillaris. All three were shown to remain viable on the external surfaces of dead, sterile and live, decontaminated T. lateralis for periods up to 24 hours under controlled conditions. The data indicate that sustained or short, successive beetle flights would result in overland dispersal of algae and protozoa.

Field-exposed T. lateralis beetles held in an environmental chamber over 24 hours carried viable Phormidium, Chlorella, Nannochloris and Bodo. Twenty-one additional algae and 5 additional protozoans also were found surviving for varying periods less than 24 hours.

A field-exposed predaceous beetle, T. ornaticollis, held under chamber conditions, yielded five viable algal

genera after 16 hours in an environmental chamber. Both herbivorous and predaceous aquatic beetles are capable of algal and protozoan dissemination.

Field-exposed L. luctuosa carried 12 algae, 1 protozoan and 4 fungi. Four of the algae, Chroococcus, Nostoc, Chlorella and Cladophora, survived on the external surface of the dragonfly for 36 hours. E. simplicicollis carried a total of 11 algal generas surviving varying periods up to 36 hours. Oscillatoria, Chlorella and Cladophora were viable after 36 hours on the external surface of E. simplicicollis.

Twenty roosting L. luctuosa collected aseptically in the field carried a total of 6 viable algae, 1 protozoan and fungal hyphae. The data, showing viability of naturally-acquired algae and protozoa at 2.5 and 8 hours (Table 12), resemble that from those collected in the field and held in the chamber.

It is estimated that Chlorella, Hormidium and Stichococcus used in this study could be dispersed up to 450 km by dragonflies flying at speeds of about 30 km per hour over a 24-hour period under suitable weather conditions.

Survival of T. lateralis out of water under test conditions declined rapidly after 24 hours. By 48 hours, 65% were left alive, and after 60 hours only 20% were alive.

T. ornaticollis survivorship started a steep decline after 48 hours, continuing until all specimens were dead after 84 hours. Under test conditions both algal viability duration and beetle survivorship times were sufficiently long to allow for dispersal of viable algae and protozoa.

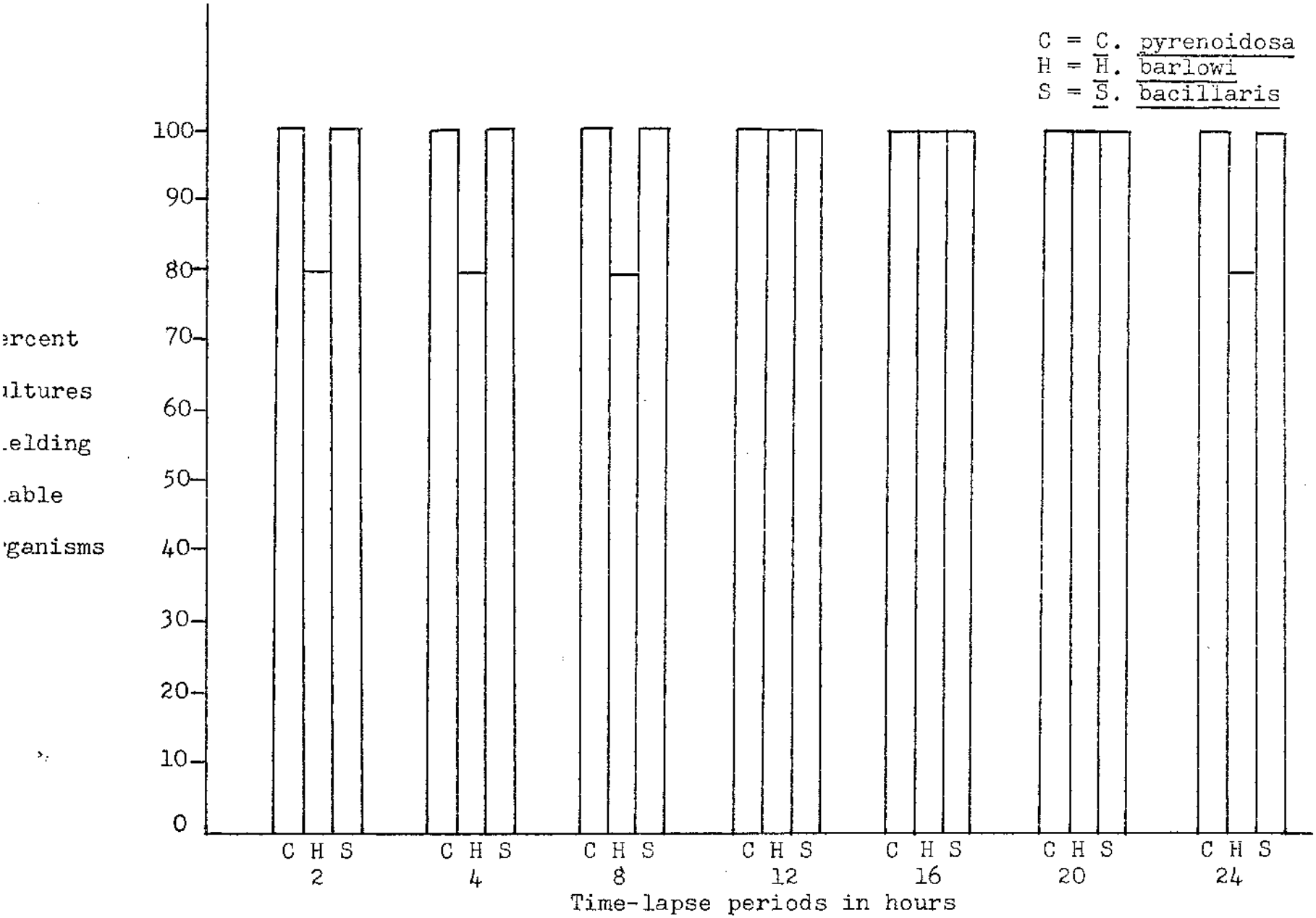

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Fig. 1. Viability duration of 3 artificially acquired algae at 21C±2C, 16-hr photophase and 68-80% R.H. on sterile dead T. lateralis.

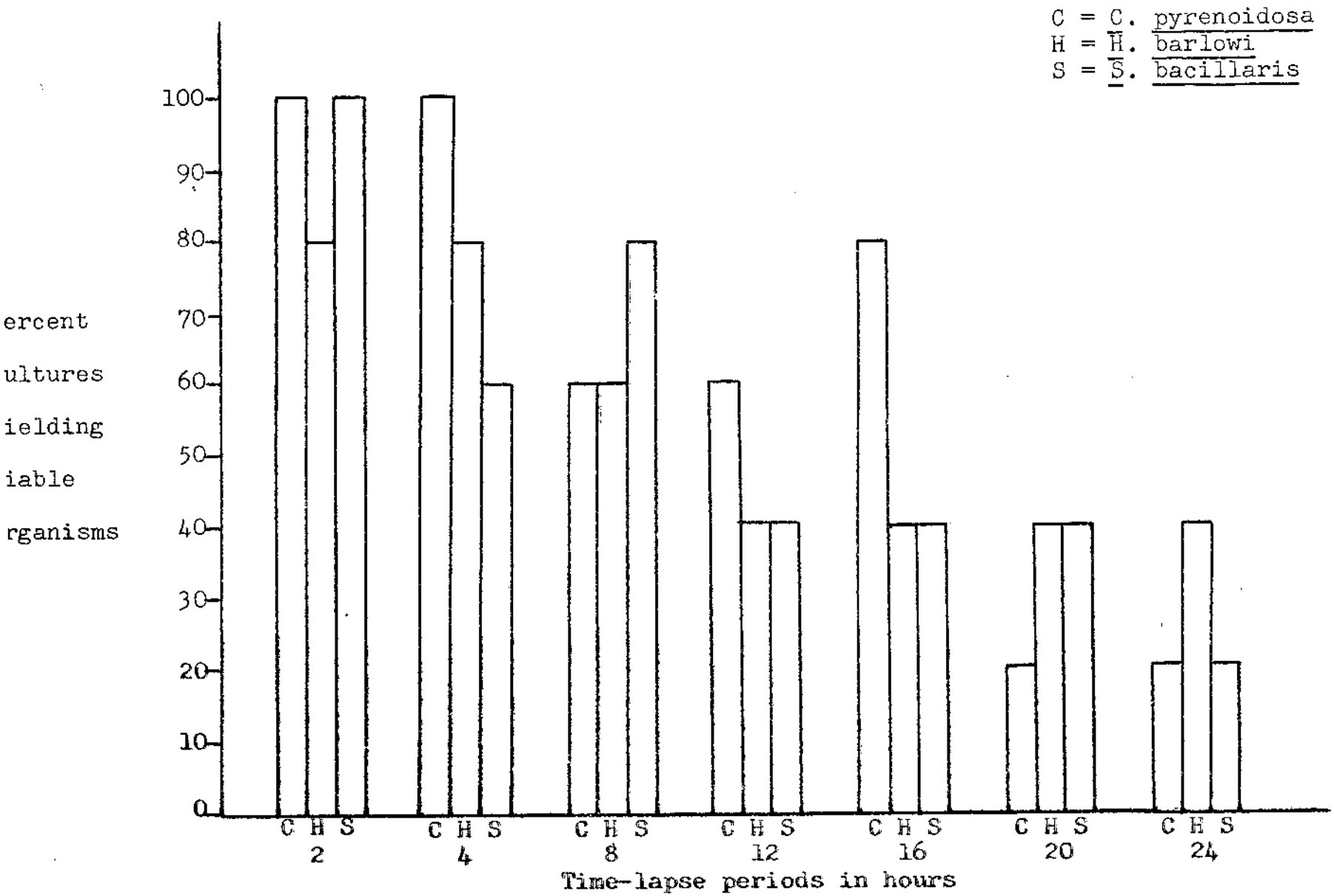

Fig. 2. Viability duration of 3 artificially acquired algae at 21C±2C, 16-hr photophase and 68-80% R.H. on live T. lateralis.

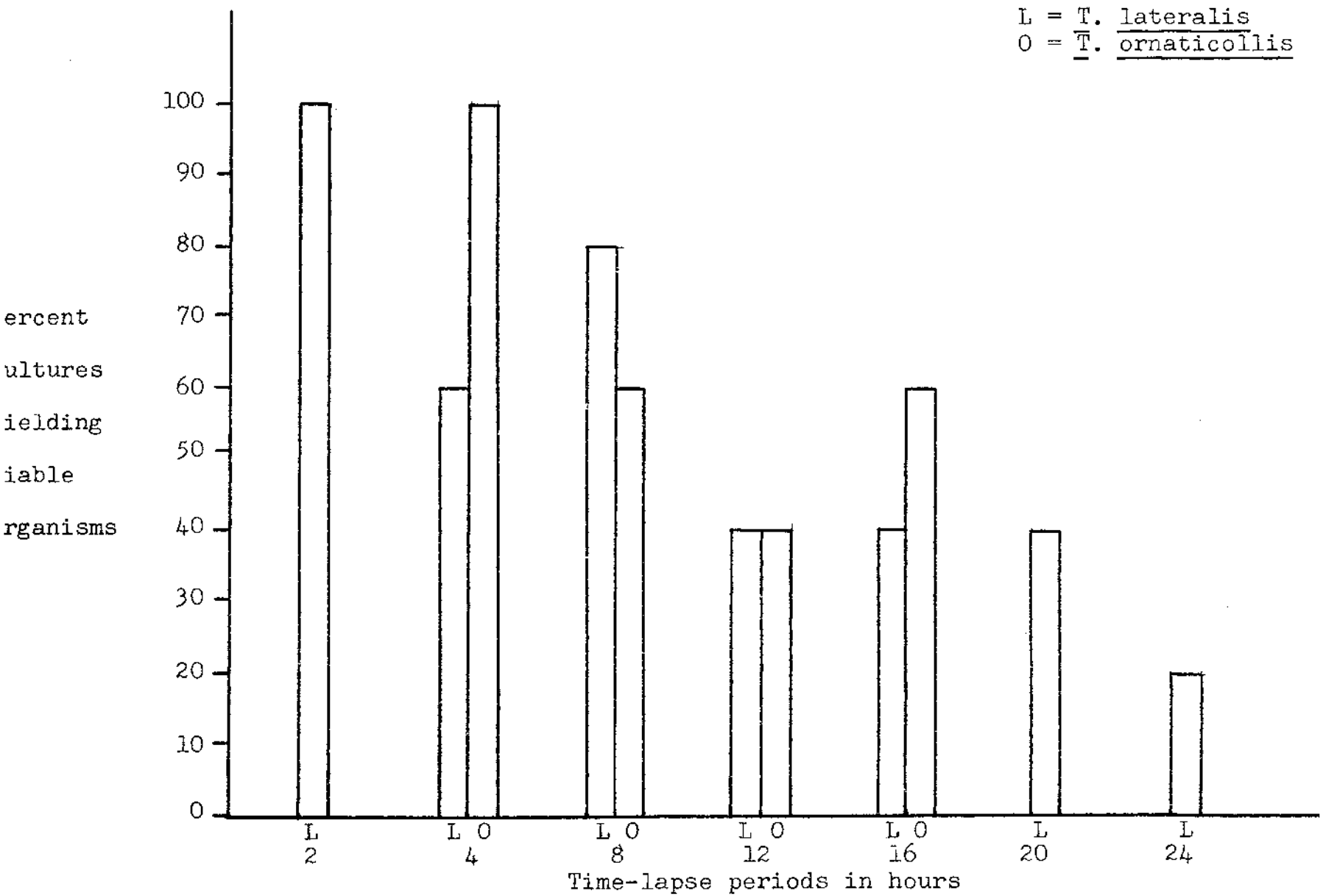

Fig. 3. Viability duration of naturally acquired algae and other micro-organisms at 21C±2C, 16-hr photophase and 68-80% R.H. on live T. lateralis and T. ornaticollis.

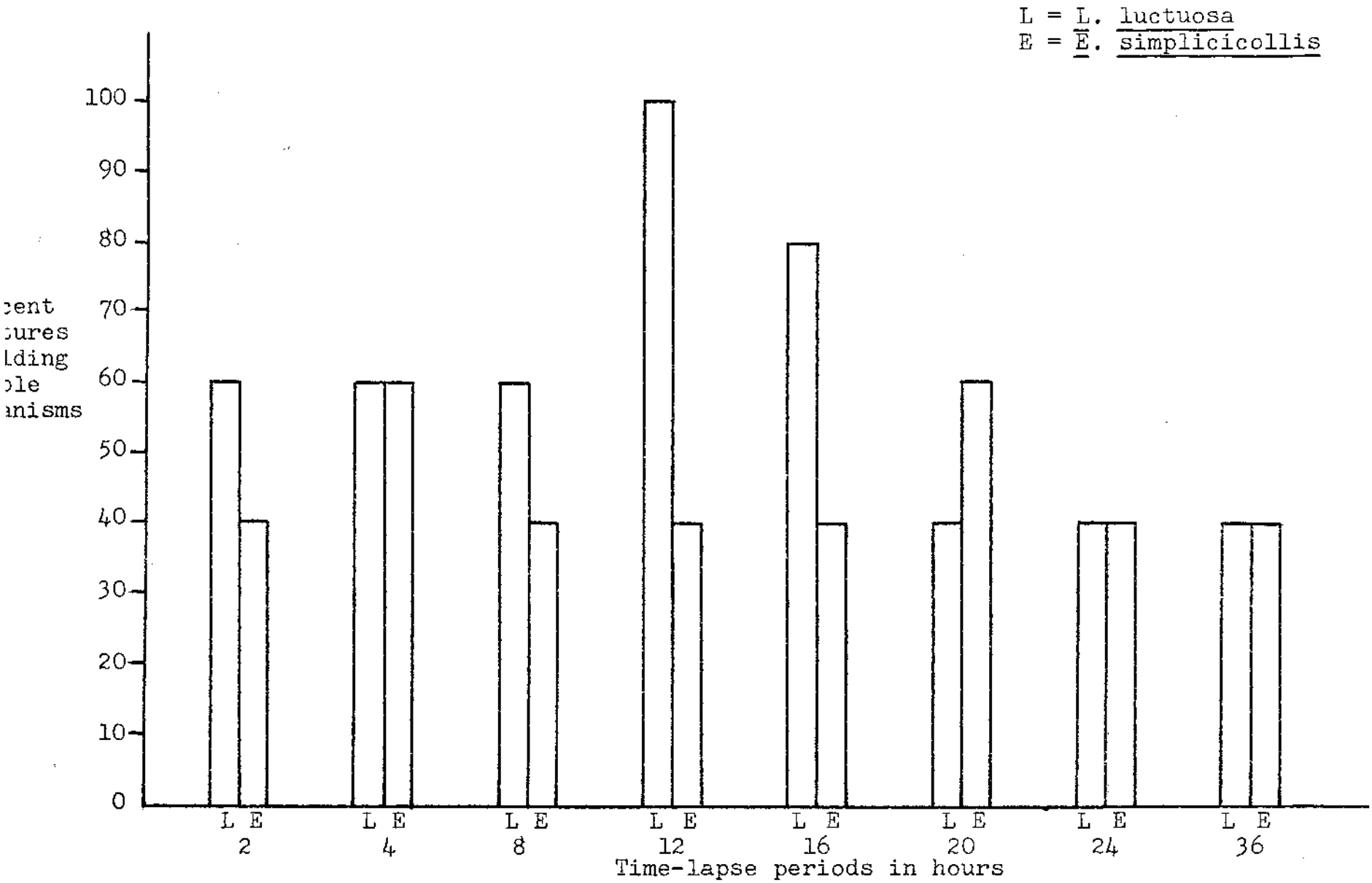

Fig. 4. Viability duration of naturally acquired algae and other microorganisms at 21C±2C, 16-hr photophase and 68-80% R.H. on live L. luctuosa and E. simplicicollis.

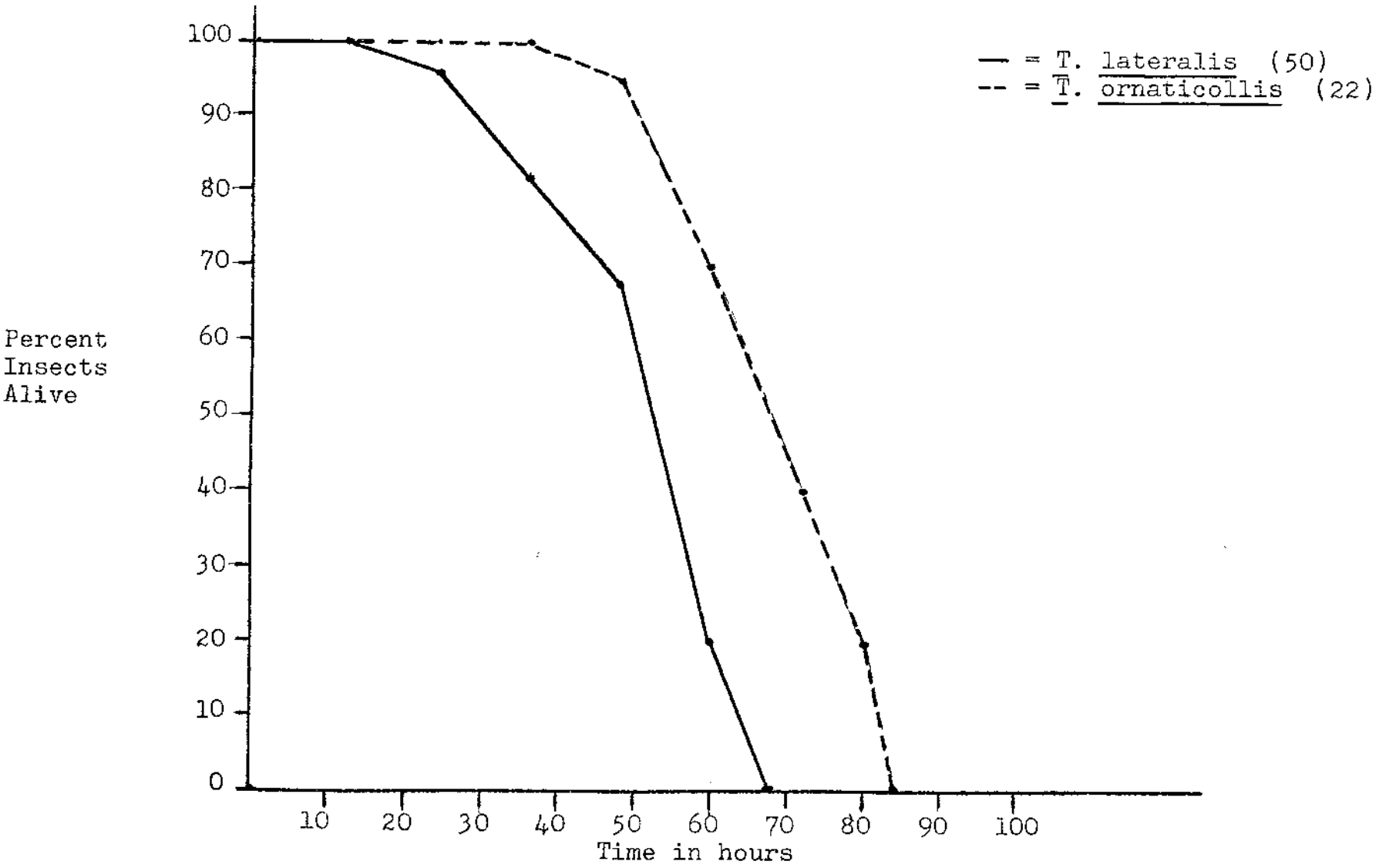

Fig. 5. Survivorship curves for T. lateralis and T. ornaticollis out of water at 21C±2C, 16-hr photophase and 68-80% R.H.

TABLE 10. Naturally acquired algae and protozoa cultured from time-lapse inoculations of T. lateralis and T. ornaticollis under simulated flight conditions

Organisms Cultured from Washings	T. lateralis Time-lapse Periods in Hours								T. ornaticollis Time-lapse Periods in Hours			
	Imed.	2	4	8	12	16	20	24	4	8	12	16
Algae												
Div. Cyanophyta												
Anacystis				1					1	1		
Chroococcus		1	1									
Oscillatoria	1	2				1			2	2		2
Phormidium	3	1	2	1	1	1	1	1	1	2		
Polycystis		1					1		1			1
Div. Chlorophyta												
Ankistrodesmus	1			1								
Asterococcus	1											
Characium	1											
Chlamydomonas			1									
Chlorella	2	3	1		2		1	1	2	2		1
Chlorococcum	2	2	1		1				3	1	1	1
Closterium	1											
Coelastrum	3	1	1	2	1				2	1	1	
Cosmarium			1								1	
Gloeocystis			1									
Nannochloris			1	1	1	1		1				
Scenedesmus									2	1		
Schroderia	1											
Selenastrum	2					1	1				1	
Stigeoclonium	1											
Div. Chrysophyta												
Diatoma	2			1								
Gloeochloris	2	2	3	1		1	1					
Navicula	3	3					1					
Ophiocytium	1											
Div. Euglenophyta												
Peranema									1			1
Phacus		1										

TABLE 10--Continued

Organisms Cultured from Washings	*T. lateralis* Time-lapse Periods in Hours								*T. ornaticollis* Time-lapse Periods in Hours			
	Imed.	2	4	8	12	16	20	24	4	8	12	16
Protozoa												
Class Sarcodina												
Ameba	2											
Class Mastigophora												
Bodo	4	4	1	1	1	1	1	1				
Oikomonas	1											
Class Ciliata												
Chilodonella	1											
Colpidium	1									1		

TABLE 11. Naturally acquired algae, protozoa and fungi cultured from time-lapse inoculations of L. luctuosa and E. simplicicollis under simulated flight conditions

Organisms Cultured from Time-Lapse Washings	Libellula luctuosa Time-Lapse Periods in Hours								Erythemis simplicicollis Time-Lapse Periods in Hours							
	2	4	8	12	16	20	24	36	2	4	8	12	16	20	24	36
Algae																
Div. Cyanophyta																
Anabaena			2	1						1				1	2	
Chroococcus			1					1				1	1			
Nostoc								1	1	2		1	1			
Oscillatoria	1			2		1			2	3				1	1	1
Phormidium	2				1	2							1			
Div. Chlorophyta																
Ankistrodesmus													1			
Asterococcus			2		1											
Chlamydomonas													1			
Chlorella			1					2			2	1	1	1		1
Chlorococcum					2					1			1			
Chlorosarcinopsis		1														
Cladophora			1				2	1	1							1
Gloeocystis			2	1	2											
Nannochloris											1			1		
Rhizoclonium			1													
Protozoa																
Class Sarcodina																
Ameba			1													
Fungi																
Alternaria			1	2	1											
Arthroderma				1		1										
Fusarium						1										
Dactylaria					1											

TABLE 12. Naturally acquired algae and protozoa cultured from ten 2.5-hr and ten 8-hr inoculations of L. luctuosa taken in the field

Organisms Cultured from Inoculations	L. luctuosa Time-Lapse Periods 2.5 hr	8 hr
Algae		
Div. Cyanophyta		
Oscillatoria		2
Div. Chlorophyta		
Chlorella	1	
Chlorococcum	3	4
Cladophora		1
Stichococcus	1	2
Div. Chrysophyta		
Navicula	1	1
Protozoa		
Class Mastigophora		
Bodo		2
Uncl. Protozoan Cysts	2	1
Fungi		
Uncl. Fungal Hyphae	5	3
Uncl. Fungal Spores	4	3

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