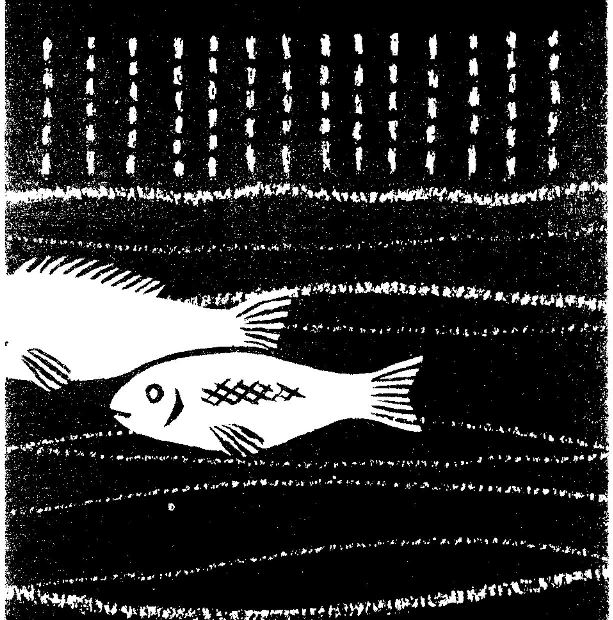

Effects of monolayers on insects, fish and wildlife

—A RESERVOIR EVAPORATION REDUCTION STUDY

A Water Resources Technical Publication

RESEARCH REPORT NO. 7

United States Department of the
INTERIOR

Bureau of Reclamation

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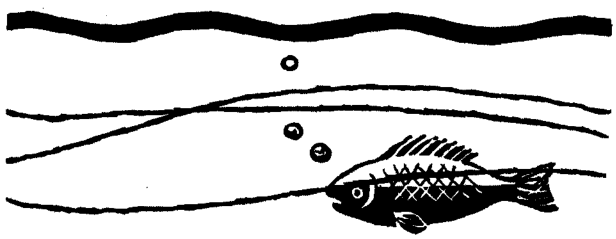

Effects of monolayers on insects, fish and wildlife

prepared by William J. Wiltzius,

Colorado State University,

for the Bureau of Reclamation

UNITED STATES DEPARTMENT OF THE INTERIOR

STEWART L. UDALL, Secretary

Bureau of Reclamation

Floyd E. Dominy, Commissioner

In its assigned function as the Nation's principal natural resource agency, the Department of the Interior bears a special obligation to assure that our expendable resources are conserved, that renewable resources are managed to produce optimum yields, and that all resources contribute their full measure to the progress, prosperity, and security of America, now and in the future.

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PREFACE

This report, the seventh in the Bureau of Reclamation's Water Resources Technical Group, evaluates the influence on insects, fish and wildlife of long-chain alcohol monomolecular films, commonly called monolayers, being used in the Bureau's long-range research program to reduce evaporation losses in reservoirs. The report concludes that the monolayer-forming materials investigated are not a limiting factor to the biota in or associated with most of the reservoirs now proposed for treatment, considering the present monolayer materials in use, application rates, and the duration of the film coverage maintained under present field conditions.

The importance of reducing evaporation losses in reservoirs is emphasized by the 1958 report of the Senate Committee on Interior and Insular Affairs on "Control of Evaporation Losses," which states that the annual loss by evaporation in the United States exceeds the amount taken for use in cities and towns. In the 17 Western States, the losses from large lakes and reservoirs amount to over 14 million acre-feet annually.

Evaporation reduction through the use of monolayers was first considered by the Bureau of Reclamation in 1952, and a long-range program of laboratory and field investigations was initiated in 1954.

The field and laboratory research performed by the Bureau has centered on the selection of the most effective monolayer-forming materials, techniques of applying and maintaining the monolayers, development of methods of measuring the reduction in evaporation loss to determine the economics of the system, and the investigation of the biological effects of the films. Much of the Bureau's research has been performed in collaboration with other Federal, State, and local agencies, and with educational institutions under research contracts.

This report was prepared under research contract No. 14–06–D–3777 with Colorado State University by William J. Wiltzius, now with the Colorado Department of Game, Fish, and Parks. The work was directed by Dr. Howard A. Tanner, who was Fish Research Chief, Colorado Game, Fish, and Parks Department at the time.

Limited dissemination was made of the original report, revised March 29, 1965, but since the research work is of broad interest to ecologists, biochemists, engineers, and others directly concerned with the use of monolayers in reducing evaporation, it has been reprinted as a Water Resources Technical Publication.

Other Bureau or Bureau-sponsored reports containing information on various phases of evaporation reduction research are listed immediately following this preface.

Future research is needed to develop or discover films which will resist losses by the various forms of attrition, to develop practical and less costly methods of evaluating the effectiveness of the reservoir surface treatment in reducing evaporation losses, to improve the techniques of film application and replenishment, and to develop methods of reducing evaporation losses by means other than monolayers.

Included in this publication is an informative abstract and list of descriptors, or keywords, and "identifiers." The abstract was prepared as part of the Bureau of Reclamation's program of indexing and retrieving the literature of water resources development. The descriptors were selected from the *Thesaurus of Descriptors,* which is the Bureau's standard for listings of keywords.

List of Reports on Reservoir Evaporation Reduction by Bureau of Reclamation and Cooperating Groups and Individuals

Evaporation Reduction Investigations Relating to Small Reservoirs in Arid Regions, by C. Brent Cluff and Sol D. Resnick, Agricultural Experiment Station, University of Arizona, Tucson, Ariz., October 1964.

Wind Effects on Chemicals for Reducing Evaporation from Small Reservoirs, by F. R. Crow, Oklahoma State University, Stillwater, Okla., May 1964.

Aerial Application of Evaporation-Reducing Chemicals, Development and Evaluation of Equipment and Techniques, by C. Earl Israelsen and Vaughn E. Hansen, Utah State University, Logan, Utah, July 1963.

Evaporation Reduction Investigation, Pactola Reservoir, South Dakota, by Agronomy Department, Agricultural Experiment Station, South Dakota State University, Brookings, S. Dak., August 1964.

Selection of Material for Use in Water Evaporation Reduction by Monolayers, by Ralph J. Bunker, Bureau of Reclamation, Denver, Colo., May 13–17, 1963.

Evaporation and Its Reduction, by W. U. Garstka, Bureau of Reclamation, Denver, Colo., July 24, 1962.

Water-Loss Investigations; Lake Hefner 1958 Evaporation Reduction Investigations by City of Oklahoma City, Oklahoma State Department of Health, U.S. Department of Health, Education, and Welfare, U.S. Department of Commerce, and U.S. Department of the Interior, June 1959.

Water Conservation Laboratory Report No. WC–1, Aerial Application Technique Development and Monolayer Behavior Study, Elephant Butte Reservoir—1962, Bureau of Reclamation, Mar. 27, 1963.

Chemical Engineering Laboratory Report No. SI–32, 1960 Evaporation Reduction Studies at Sahuaro Lake, Arizona, and 1959 Monolayer Behavior Studies at Lake Mead, Arizona-Nevada and Sahuaro Lake, Arizona, Bureau of Reclamation, Aug. 15, 1961.

Chemical Engineering Laboratory Report No. SI–33, Water Loss Investigations Lake Cachuma—1961, Evaporation Reduction Investigations, Bureau of Reclamation, Jan. 1, 1962.

Equipment and Techniques for Aerial Application of Evaporation-Reducing Monolayer-Forming Materials to Lakes and Reservoirs, Utah State University, Logan, Utah, December 1964.

Effect of Bacterial Decomposition of Long-Chained Alkanols and Related Compounds in Monolayer Films on the Evaporation Losses of Water and Means To Combat this Effect, by Drs. Shih Chang and Mark A. McClanahan, Robert A. Taft Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, Ohio, July 1964.

Evaporation Reduction Investigation, Elephant Butte Reservoir, New Mexico, 1963–1965, Technical Report No. 25, by Narendra N. Gunaji, Bruce A. Tschantz, William W. G. Yeh, Cesar Morales, Engineering Experiment Station, New Mexico State University, University Park, N. Mex., November 1965.

Evaporation Investigation at Elephant Butte Reservoir Using Energy-Budget and Mass-Transfer Techniques, Thesis for D. Sc., C. E. by Bruce Allen Tschantz, New Mexico State University, University Park, N. Mex., January 1965.

The Relative Reflectance of a Monolayer-Covered Water Surface, Thesis for Ph. D. by James Taylor Beard, Oklahoma State University, Stillwater, Okla., August 1965.

Colorado State University Report, "Evaluation of the Influences of Long-Chain Alcohol Monomolecular Films on Insects, Fish, and Wildlife"

AUTHOR'S ACKNOWLEDGMENTS

The author wishes to convey appreciation and recognition to the following organizations and persons for their part in the completion of this study:

The Bureau of Reclamation, which supplied the monolayer-forming materials, literature, and financial support for conduct of this study as part of the research program of the Colorado Cooperative Fishery Research Unit under auspices of the Colorado State University Research Foundation. Again, to the Bureau of Reclamation, which exhibited considerable patience in awaiting the final report of this study after the termination of the contract completion date.

Dr. Howard A. Tanner, who supervised this study and rendered guidance and encouragement.

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Special thanks are due James Kollath and Kenneth Thompson for their part in the construction of the field pens in College Lake and in the collection of field data pertinent to this study.

Paul Byron, Farm Manager at Colorado State University, and the Northern Colorado Water Conservancy District for cooperation in minimizing water level fluctuations of College Lake during the study.

The Colorado Game, Fish, and Parks Department for the use of facilities and equipment at their Research Center in Fort Collins, Colo.

Special appreciation to secretaries Chonita Lowry and Carol Seligmann of the Colorado Game, Fish, and Parks Department Research Center, and to my wife Nancy, who all rendered assistance by either drafting plates or typing sections of the final draft of this manuscript.

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and forms a slight diffusion barrier to the absorption of gases by the water. Furthermore, the film causes increases of a few degrees Fahrenheit to the surface waters and usually reduces the surface tension of the water by 50 percent or more. Although these changes are confined to the surface waters, they could either directly or indirectly upset the aquatic ecosystem by adversely affecting members of the biota which associate with the surface. This is especially true for the change involving the considerable reduction in the surface tension of the water because some important members of the biota, especially many of the insects, depend upon the normal surface tension of the water for support while at the water surface during some stages in their life histories.

Theoretically, when the surface tension of water is reduced, the wetting property of the water increases and the lifting force, which some members of the biota use for support, decreases (Fair, Chang, and Richart, 1951). The long-chain alcohol materials usually reduce the surface tension of water from its normal 60 to 72 dynes per centimeter to less than 36 dynes per centimeter. Russell and Rao (1941) and Fair, Chang, and Richart (1951) have shown that at such reduced surface tensions *Anopheles* mosquito larvae could not support themselves at the water surface and many of them drowned. Adverse effects have also been described by Manzelli (1941) to mosquito pupae, to the mosquito adults while egg laying, and to the eggs themselves when the surface tension of the water was reduced to levels near those of monolayer-treated water surfaces. In addition, Hayes (1959) reported that water striders drowned, and that the emergence of insects such as mosquitoes, mayflies, and midges was hindered by the presence of a hexadecanol monomolecular film.

The importance of the insects in the aquatic ecosystem cannot be overemphasized. In the productive littoral zone of lakes and reservoirs, herbivorous insects such as mayfly nymphs and midge larvae serve as primary converters of plant materials into animal protoplasm. This same function is almost exclusively performed by the midge larvae which dominate in the comparatively large profundal ooze areas of most reservoirs. Furthermore, some aquatic insects, especially the midges of the family Tendipedidae (Chironomidae), are very important in the diet of certain fish and wildlife. Wirth and Stone (1956) mention that many shore and water birds eat the aquatic immature stages of these insects. After extensively studying the food of fishes, Forbes (1888) found that dipterous insect larvae, composed primarily of Tendipedid midges made up nearly one-tenth of the food of all fishes. According to Townes (1938), both the immature stages and the adults of Tendipedid insects are eaten by the young of many species of fish and may be almost the exclusive food of adult fish such as bass, trout, and white-fish. Many other examples of the importance of the midge insects in the diets of fish are presented in the voluminous work of Thienemann (1954).

Since long-chain alcohol monomolecular films cause some fundamental changes in the aquatic environment, especially the considerable reduction in the surface tension of the water that adversely affects some insects which are important both in the biology of reservoirs and in the diets of certain fish and wildlife, it is not surprising that concern has developed regarding the adversity monomolecular films may inflict on the fish and wildlife of our reservoirs. The reservoirs in the arid areas of the West are the prime target for the use of monomolecular films for evaporation control; however, these reservoirs may also be the only locations at which the sportsmen of these areas can obtain recreational fishing and waterfowl hunting. Much money is spent on trips and equipment by sportsmen and large sums of money are also spent by State and Federal agencies to stock and restock fish and wildlife to these reservoirs. Because of the concern for the well-being of the valuable fish and wildlife of monolayer-treated reservoirs, the present study was initiated to evaluate the influences several of the long-chain alcohol monomolecular films have on fish and wildlife.

Problem

Do long-chain alcohol monomolecular films used to suppress evaporation from reservoirs affect the aquatic ecosystem to a magnitude which would jeopardize fish and wildlife?

Problem analysis.—The problem may be further analyzed into the following questions:

1. In what manner and degree do long-chain alcohol monomolecular films affect the life processes of aquatic insects, especially the midges of the family Tendipedidae?

2. Do the commercial film-forming materials which contain small amounts of long-chain alcohols other

Chapter I

INTRODUCTION

Water, besides being essential to life, is fundamental to the economic strength, prosperity, and well-being of man. Without an abundant supply of good quality water, agriculture and industry would be considerably less efficient, and contemporary metropolitan life would become uncomfortable or even unbearable. The United States, as well as other nations, is entering a period of enormous population and industrial growth which will require additional water. Instead of the present population of about 180 million in the United States, by 1980 the population probably will be at least 250 million people. The gross national product by then should be in excess of 1 trillion dollars per year, or twice its present size, and industrial production should be about three times its present level. According to Senator Murray (1962), the need for water will grow from the present level of 250 billion gallons per day in the United States to about 600 billion gallons per day by 1980. Because we are presently using most of the water that is readily available and fit to use, together with the fact that our basic supply of water is usually fixed by inelastic factors of precipitation and runoff, conserving present supplies and a more efficient use of our water resources will be necessary to meet the water needs of the future.

A common practice today is to conserve water by storing it in reservoirs for later use; however, evaporation from the water surfaces of these reservoirs is a major cause of water loss. Eaton (1958) reported that at least 11.5 million acre-feet of water is lost each year by evaporation from water surfaces in the 11 Western States. This is enough water to supply the average annual domestic needs of 46 million people. Over a 6-month period, July–December 1956, Crow and Daniel (1957) found that the evaporation loss from farm-water-supply ponds in Oklahoma was over 10 times as great as the volumes for home-water use. To exemplify the economic loss due to evaporation, Moran and Garstka (1957) calculated that the average loss from Lake Hefner, the water supply reservoir for Oklahoma City, is in the order of $660,000 per year.

In attempts to reduce reservoir evaporation losses, engineers have considered and sometimes applied various techniques. Since the water of many reservoirs today may be used simultaneously for domestic consumption, irrigation, industry, swimming, etc., and also support an important fish and wildlife resource, the evaporation suppression technique which is finally selected must take such multipurpose uses into consideration. According to Garstka (1962), the most promising technique is to use surface coverings of compressed monomolecular films derived from heavy alcohols of 16-carbon chains and longer. The commercial materials which are most frequently used in forming the monomolecular film are powdered alcohols primarily composed of hexadecanol ($CH_3\ (CH_2)_{14}\ CH_2OH$) and octadecanol ($CH_3\ (CH_2)_{16}\ CH_2OH$) with smaller amounts of other long-chain alcohols. Monomolecular films formed from such materials generally reduce evaporation losses by 10 to 25 percent. Because current and future research are almost certain to improve these savings, the film method appears to be susceptible to practical applications at costs within economic limits in the very near future. Besides this, the method probably is the least harmful to most other reservoir uses.

Biochemical studies have shown that hexadecanol and octadecanol, which together usually comprises well over 80 percent of the commercial alcohol materials most frequently used in forming the monomolecular film, are normal intermediates in fat metabolism. According to the Association of Food and Drug Officials of the United States (Anonymous, 1958), the use of these two alcohols as monomolecular films for evaporation control of potable water supplies presents no health hazards.

Despite the apparent nontoxicity of these materials, early research on their application as monomolecular films has indicated changes in some physical and chemical characteristics in the aquatic environment. The monomolecular film causes an initial calming of the water surface similar to that produced by an oil slick, than nontoxic hexadecanol and nontoxic octadecanol exhibit toxic qualities to fish and wildlife?

3. Would the temperature and chemical changes of monolayer-treated water of reservoirs adversely affect fish and wildlife?

4. What influence would the increased wetting property of monolayer-treated water have on the waterproofing mechanisms of waterfowl plumage.

Delimitation.—This study was delimited to some physical and biological effects of several long-chain alcohol materials used as monomolecular films to reduce water evaporation in laboratory aquaria and on a group of small pens in College Lake near Fort Collins, Colo. The overall changes in the aquatic insect communities with particular reference to effect on fish and wildlife were of primary concern. Other effects, such as those pertinent to the physical and chemical characteristics of the water and to particular species or groups, were investigated insofar as they concerned the primary problem.

Definitions of terms.—As used herein, a *monomolecular film* is a stratum of a long-chain alcohol material one molecule deep on a water surface. The molecules are so oriented that all of the long-chain molecules are more or less parallel to one another with their head groups downward toward the water. The head groups interact mainly with the aqueous subphase and with the head groups of adjacent molecules, while the hydrocarbon chains interact primarily with adjacent hydrocarbon chains. *Monolayer, monomolecular layer, monomolecular film,* and *monofilm* are synonymous terms.

Surface tension is that property of a liquid surface that tends to change the shape of the surface by the inward pull (contractile force) on the molecules at the surface to produce a minimum potential energy value. The mathematical equivalent of this energy value is usually expressed as the force in dynes acting perpendicularly to a section of the liquid surface 1 centimeter in length.

A *dyne* is such a force that under its influence a particle whose mass is 1 gram would experience during each second an acceleration of 1 centimeter per second.

Film pressure is defined as the difference in the surface tension between a clean water surface and one treated with a film-forming material. *Surface tension depression* and *surface pressure* are synonymous terms.

Chapter II

METHODS AND MATERIALS

Some effects of seven long-chain monomolecular film materials used to suppress evaporation were either studied in the fishery laboratory at Colorado State University or at field pens in College Lake during the period July 10, 1961, until October 20, 1963. Although some data were collected on how monomolecular films affect the physical and chemical characteristics of water, data on the emergence of the aquatic insects were emphasized. Generally, methods were those common in limnology and fishery biology as described by Welch (1948) and Lagler (1956).

Field Problems

Certain problems usually are associated with applications of monolayer-forming materials under natural field conditions. The total area of the film coverage together with its duration are usually reduced by winds and wave action. In addition, the abundance and occurrence of organisms in the aquatic environment are quite variable even in small ponds. Hayes (1959) sampled an untreated 1-acre pond and found that the total insect emergence in each half of the pond varied by 36 percent. Furthermore, certain aquatic organisms are very mobile and could migrate away from film-covered areas. Such interrelating factors could easily influence the effects due to monomolecular films.

Selection, Description, and Construction of Experimental Apparatus

To alleviate the field problems mentioned above, an artificial experimental field approach was taken. Small pens, which minimized wind and wave action, eliminated migration of organisms, and permitted complete film coverage for long durations with little disturbance to the film, were constructed in the northwest end of College Lake. This northeastern Colorado Lake, which capacitates 550 acre-feet of water, is located approximately 4 miles due west of Fort Collins, Colo., township 7 north, range 69 west, in the southeast corner of section 11 and northwest corner of section 14. Thomas (1952) described College Lake as being similar to the littoral zone of larger lakes. Organic material has accumulated; the bottom is chiefly a pulpy, peat-muck bottom type; and littoral vegetation has gained a foothold on the periphery. The lake is in late eutorphic successional stage, and its productivity probably is near maximum. Characteristics considered in selecting the experimental site in the northwest end of the lake were: (1) Access; (2) privacy; (3) wind protection; (4) abundant and varied populations of bottom dwelling invertebrates, especially insects; (5) even gradation of bottom; and (6) consistent density of aquatic weed stands.

College Lake water is mostly water released from Horsetooth Reservoir, but includes snowmelt and runoff from the surrounding land, two known surface springs, and seepage water that moves through the ground water table from higher elevations. During this study, water from the lake was used primarily to irrigate some experimental farms of Colorado State University. An agreement was obtained with Paul Byron, farm manager at Colorado State University, and the Northern Colorado Water Conservancy District to minimize water level fluctuations. Water used for irrigation was replaced with water from Horsetooth Reservoir during the irrigating or soon afterwards.

Preliminary investigation of the site in 1961 revealed environmental stratification as to bottom type and weed composition. Two distinct strata were observed parallel to the shoreline; the first at 3 to 5 feet in depth (hereafter referred to as tier 1) and the second at 6 to 8 feet in depth (hereafter referred to as tier 2). Tier 1 had a sandy clay bottom type with parrot feather, *Myriophyllum exalbescens*, the dominant vegetation type while tier 2 had a pulpy peat-muck bottom with coontail, *Ceratophyllum demersum*, the dominant vegetation type. The original plan called for constructing 12 equal sized (16 by 16 feet) polyethylene pens which could be sampled without disturbing a monomolecular film. Because of the environmental differences between the two tiers, six pens were constructed in each tier.

Poles of 4-inch diameter were driven into the lake bottom to form the corners of the pens. Next, 3- by 8-inch bridge planks were nailed to the posts approximately 8 inches above the water line, forming three sides to each pen. One side was left open to permit the floating of a polyethylene structure into each pen area.

A 16- by 16-foot jig was built on the shore to facilitate constructing the polyethylene structures. A square was constructed around the jig by corner nailing 1- by 4-inch boards. Waterproof calking was applied on all sides of the square and 4-mil reinforced polyethylene was then stapled to the square in the calked area. Calking was applied over the staples and a 12-inch strip of 20-gage sheet metal was nailed around the square. The vertical seam of the polyethylene structure was sealed with a plastic heat welder.

Each of the polyethylene structures was floated into an open pen area, sunk, and the sheet metal was driven into the mud to anchor the structures and prevent migration of bottom organisms in or out of the pen areas. A bridge plank was then laid across the open side of each pen area to form the "above water square". The polyethylene was pulled up and over and stapled to this square (fig. 1). Two flat planks were laid across the top of each pen to permit sampling in any area. A boardwalk was then constructed from the shore to the deep tier, facilitating access to any pen without disturbing the water surface.

Each pen was hypothetically divided into nine numbered sampling areas, beginning in the upper left corner. As shown in figures 2 and 3, three areas were randomly selected from each pen and emergent insect traps, identical to those used and described by Hayes (1959), were secured in the center of those areas. These same sample areas were used for the traps in 1962.

It was believed that the planking structures at the top of each pen and the windbreak around the pen areas would sufficiently break waves to permit a complete film coverage. On two or three occasions during 1961 when winds were very strong, waves splashed over the boards, broke up the film coverage, upset the emergent traps, and ripped the polyethylene. Damage was greatest when the water level was below the plankings. Consequently, for added protection in 1962, the sides of each pen were reinforced with ⅜-inch-thick

FIGURE 1.—Early construction view of the 12 experimental pens at College Lake, 1961.

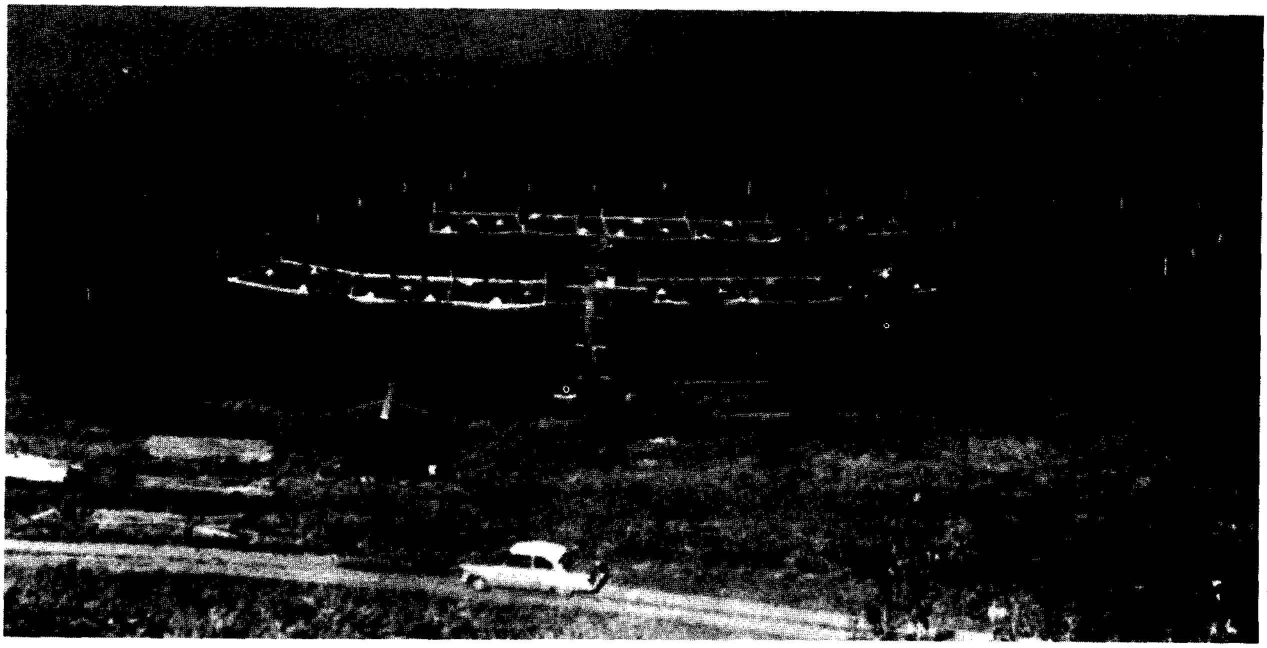

FIGURE 2.—View of completed experimental pen apparatus at College Lake, 1961.

tempered masonite sheets. All seams and corners were sealed to prevent migration of organisms.

Monolayer-Forming Materials and Methods

Materials.—In table 1 are shown the percent carbon constituents of the seven alcohol materials procured from the Bureau of Reclamation for use in this study. Because more repetitions of a treatment usually result in greater accuracy, the original field plan called for testing only two materials. In this case the alcohol materials with the lowest and highest average carbon values were selected; namely Adol 52 with a 16.06 average carbon value and Stenol 1820 with a 19.0 average value. However, at the time field studies were started in 1961, adequate amounts of Stenol 1820 were not available and T.A. 1618 was used instead. To obtain comparable data, T.A. 1618 was used again as the second material in the 1962 field studies.

Selection of pens for treatment.—Since variation in depth, vegetation type, etc., existed between the two tiers of pens and since nothing was known of the insect distribution, a strict random selection of the treatment a pen received in a given tier was employed during 1961. Consequently, since two materials were to be tested, two pens were randomly selected for each treatment in each tier. Pens 1 and 4 (tier 1) and pens 9 and 12 (tier 2) received the T.A. 1618 treatment, while pens 2 and 5 (tier 1) and pens 7 and 11 (tier 2) received the Adol 52 treatment. The remaining pens (3 and 6 in tier 1 and 8 and 10 in tier 2) received no treatment and were used as controls (fig. 3).

A different technique of selecting the pens for the treatments was employed in the 1962 studies because it was found that the distribution of the insect larvae in the pens of the same tier varied considerably. Originally, it was believed that the distribution of the insects in the pens of a given tier would be comparable because of the similiarity of ecological conditions (small similar pen area, depth, vegetation, etc.). Distribution here refers both to the abundance and the occurrence of the species of insect larvae in the pens. Two pens in the same tier, for example, could have both had a particular insect species present but one pen could have had considerably more numbers (abundance) of this species. In addition, a particular insect species could have been present in one of the pens and not the other (occurrence).

Because of the considerable variation in the abundance and occurrence of the insect larvae in the pens, it was believed that better homogeneity of pens for each treatment-pair in a given tier could be attained by purposely selecting similar pen-pairs. Such

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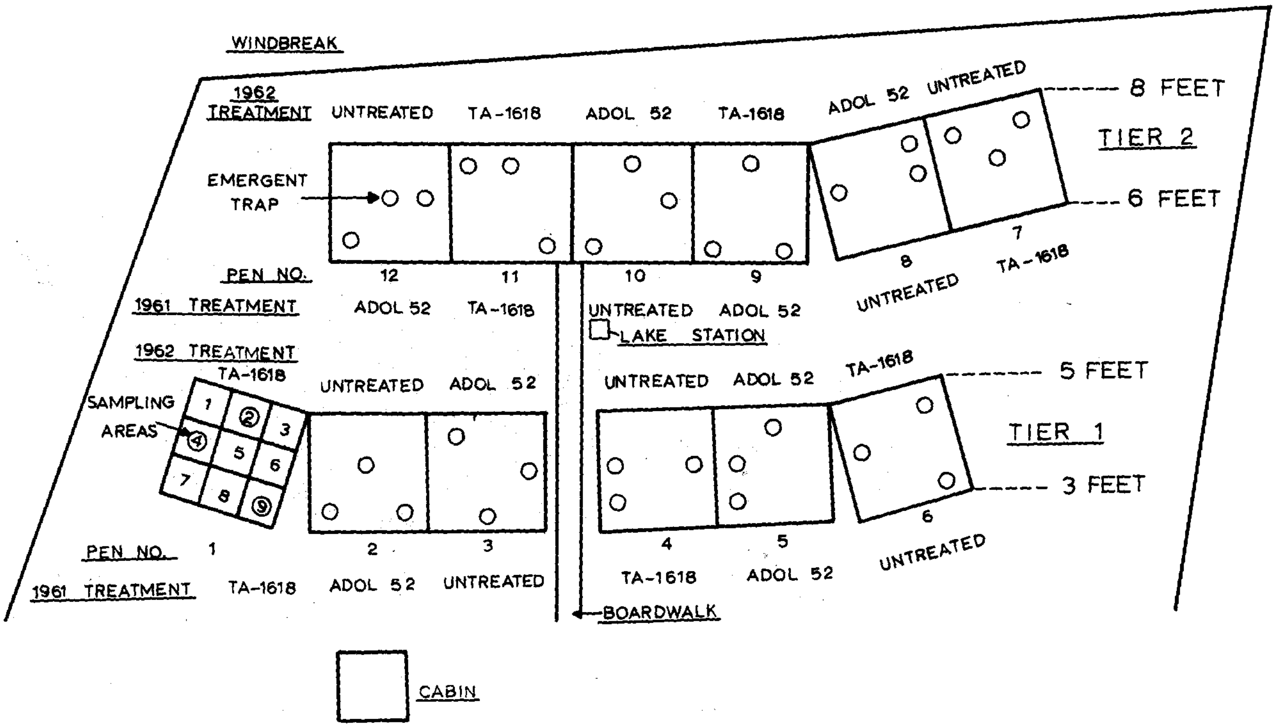

FIGURE 3.—Important characteristics of experimental design at College Lake during 1961 and 1962.

TABLE 1.—*The Percentage Carbon Constituents and Average Carbon Values of the Seven Experimental Monolayer-Forming Materials*

Carbon values	Adol 52	Micronized cachalot	Percentage film materials			Adol 62	Stenol 1820
			Stenol PC	Adol 54	T.A. 1618		
C 12	0. 1			0. 1			
C 13							
C 14	2. 4	4–6		3. 7	2. 0	0. 1	
C 15	. 8			. 6			
C 16	89. 3	44–49	50. 0	42. 6	32. 5	8. 8	
C 17	3. 2			3. 2		2. 0	
C 18	4. 2	43–46	50. 0	41. 7	65. 5	72. 3	64. 0
C 19				2. 9		1. 6	
C 20		4. 0		5. 1		10. 0	22. 0
C 21						1. 9	
C 22						3. 3	14. 0
Average C	[1] 16. 06	16. 95	17. 0	17. 06	17. 27	18. 20	19. 0

[1] The addition of the values obtained from multiplying each C value by its percentage for each material.

a procedure was employed during 1962 using the coefficient of similarity technique described by Oosting (1956). Data from 1961 were used to compute an average coefficient of similarity between the abundance and between the occurrence of insect larvae for each pen-pair combination. In each tier, 15 possible pen-pair combinations existed. From these combinations, the three pen-pairs which did not contain pen repetition and had the least amount of deviation between their average coefficients were selected and then randomly assigned a treatment. Pens 1, 6, 9, and 11 received the T.A. 1618 treatment, while pens 3, 5, 8, and 10 received the Adol 52 treatment. The remaining pens (2, 4, 7, and 12) received no treatment and were used as controls during 1962. It can be seen in figure 3 that pens 1, 5, and 11 were the only pens which received the same treatment in both years.

Monomolecular film formation.—Twenty grams of compound applied by hand in the center of each treated pen every other day were sufficient to maintain a film coverage. To assure a complete surface coverage, a small portion of the compound was applied inside each emergent trap even though the base of the traps was constructed of 12-gage screen which permitted the film to pass under the trap area.

Preliminary performances of the films indicated an almost repelling effect to any objects breaking the water surface. The films approached objects such as emergent weeds or any floating debris and appeared to work around them without making contact. As a result, all emergent vegetation was clipped approximately 12 to 18 inches below the water surface and each pen was cleaned daily of its surface debris during 1961. If debris was allowed to accumulate longer than 1 day, the compounds had a tendency to adhere to the debris (fig. 4). In addition, film coverage was much slower if the treatment compounds were allowed to accumulate in the pens for more than 3 days. Consequently, it was necessary to clean the water surface. This was accomplished by moving a fine-mesh, cheese-cloth seine through the surface waters. To determine if cutting the weeds and cleaning the water surface had significant effects on the emergence of insects, only half of the pens during 1962 (pens 1, 3, 4, 9, 10, and 12) were handled in the above described manner.

Chemical Methods

After the completion of the experimental design in late July 1961, the basic characteristics of dissolved oxygen, carbon dioxide, carbonate and bicarbonate alkalinities, along with pH data were collected from each pen and the lake station every week until mid-September 1961. These data were collected using standard equipment and techniques as described in Welch (1948) and Lagler (1956). Samples were taken at the surface and at a 4-foot depth in the center of each pen. Because of low water, 4-foot depth samples sometimes could not be obtained from pens in tier 1. Effects of the films to these chemical characteristics were slight during 1961, so consequently, weekly

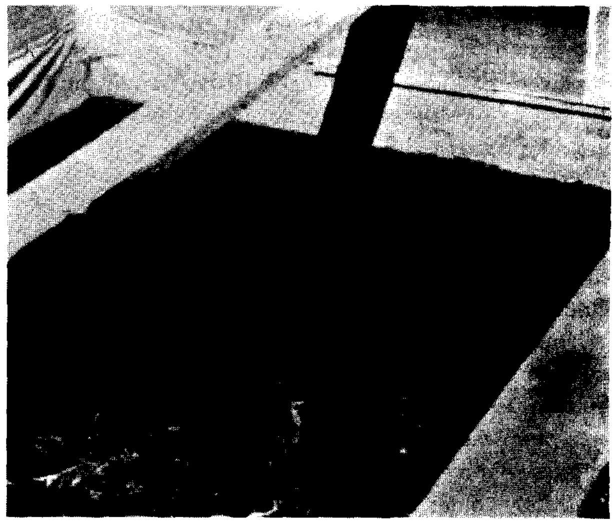

FIGURE 4.—Photograph of film material adhering to surface debris in an experimental pen at College Lake.

sampling from every pen was dispensed with in 1962. Some samples were taken during 1962, however, to check on the consistency of their performance.

Physical Factors

Temperatures.—Because little information was available on how monomolecular films affect the water temperature below the treated water surface, water temperatures were taken daily at the surface, 4-foot depth, and bottom of each pen and the lake station by means of a Foxboro Resistance Thermometer.

Wind.—Although it is realized that a film coverage is directly dependent upon wind force and direction, no actual measurements of wind were taken during this study. The masonite-modified pen structures of 1962 eliminated wind as an affecting variable.

Biological Factors

Fish methods.—Because Hayes (1959) showed little effect of hexadecanol to fish but a greater effect to emergent insects, fish were eliminated from the pen areas immediately after construction was completed. Since fish could eat insect larvae, it would be impossible to attribute changes in the larvae population due to the effects of the film or due to the fish. However, the contents of several stomachs of fish taken from the pens in early 1962 contained very few, if any, insect larvae. Consequently, the fish were not completely eliminated from the pens during 1962 but their numbers were considerably reduced by hook and line fishing. These fish had gained access into the pens when the pens were modified.

Insect larvae sampling.—Insect larvae were found both on the aquatic weeds and on the bottom of College Lake. A standard 6- by 6-inch Eckman dredge was found inadequate because it failed to collect representative samples of both weeds and bottom soil. When the weeds were dense, the gravity force of the sampler failed to penetrate the soil. To obtain proper bottom penetration, an 8-foot length of ½-inch-diameter pipe was attached to the dredge, allowing it to be manually forced through the weeds into the bottom soils. By soldering a fine screen over the top of the Eckman dredge, it was possible to collect representative samples of the weeds. To eliminate the muck and still retain the larvae, samples were washed through a screen bucket (60 meshes to the inch) similar to that described by Anderson (1959). Further separation of remaining debris was difficult. At first, standard Tyler screens were used but this technique proved very time consuming. The sugar-flotation technique of Anderson (1959) was found to be a more rapid process of separation and was employed during 1961.

The procedure used each week during 1961 was as follows: One of the nine possible sample areas from each pen was chosen randomly and a sample was taken in the center of this area with the modified Eckman dredge. The sample was washed through the screen bucket and the wet weight of the weeds was recorded. All debris including the weeds was then placed in a white enamel pan and a sugar solution (specific gravity of 1.12) was poured over it allowing the organisms to float out of the debris. All visible organisms from two separate flotations of each sample were handpicked and preserved in 10 percent formalin for later analyses.

An important drawback to the sugar-flotation is that strong-swimming organisms like mayflies and damselflies grasp debris and do not float to the surface as well as other insect larvae such as the midges. Tanner and Leik (1954) used interrupted direct current in flowing tap water for sorting bottom fauna and reported at least 90 percent recovery of strong-swimming mayflies and damselflies but only 57 percent recovery of the midges. Research was conducted as to the feasibility of combining these two techniques because it was believed that a very high percentage recovery and separation of insect larvae at College Lake could be attained.

Despite larvae recovery in excess of 95 percent, this combined technique was considered inefficient for samples at College Lake and, consequently, details of the apparatus and the procedures used in the technique will not be described. Much of the dead organic debris and live vegetation present in the College Lake samples floated in the sugar solution and the time required to separate the larvae from this debris was not sufficiently improved over the hand-picking technique employed during 1961. In addition, by subjecting the 1961 larvae data to the species-area-curve technique described either by Rice and Kelting (1955) or by Oosting (1956), the number of modified Eckman-dredge samples necessary to attain a representative sample of the larvae occurring in any pen was found to range between two and four samples per pen. Greater numbers of samples would be necessary to estimate the abundance of larvae in the pens. Because of the increased number of samples needed from each pen, together with the fact that a more efficient technique of separating the larvae from the debris had not been developed, quantitative sampling of the insect larvae was not attempted during 1962.

Insect emergence sampling.—As previously pointed out, emergent insects were sampled by using traps identical to those described by Hayes (1959) (fig. 5). Total counts of these insects were taken each morning from every trap during 1961. The quart collecting jars were washed and replaced immediately after counting the insects. Since these counts did not contain information on the midge species variation, a different technique and procedure was used to tabulate the emergent counts during 1962. Each sample jar, which was numbered to identify the pen and trap it came from, was removed from its trap each morning, capped, and a second jar was immediately substituted. This second jar was also numbered for pen and trap. Next, a tea bag containing a powdered insecticide known commercially as cyanogas, was placed in each capped jar to kill the insects. These insects were then identified to "types" and their total number was recorded. Later in the laboratory, samples of these "types" were identified to species when possible.

During 1961, it was suspected that certain insects were avoiding the emergent traps. In addition, it was

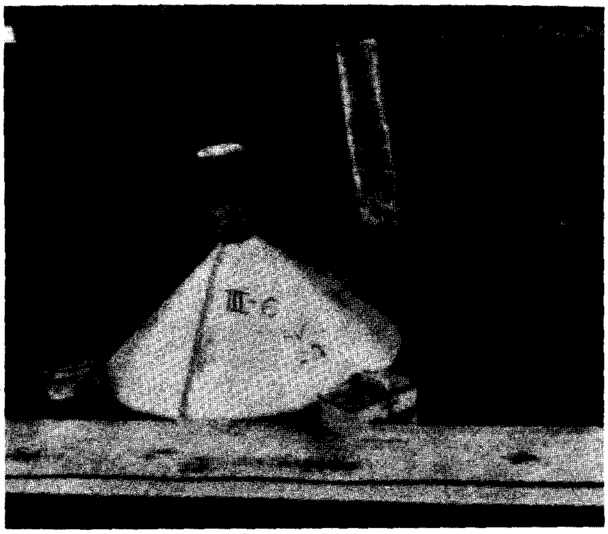

FIGURE 5.—Photograph of an emergent insect trap floating on an experimental pen surface at College Lake.

believed that the trap counts in some pens did not yield representative counts of the emergence which actually occurred. Consequently, to test the efficiency of the traps, four tents, which completely covered an entire pen, were employed in the 1962 field studies and are shown in figure 6.

Each tent was made from dacron curtain material of approximately 26 meshes to the inch, and unshrinkable, mildew-resistant canvas. The tent culminated at a sewn-in canvas cone approximately 6 feet above the center of the pen. Two-inch-wide strips of canvas were sewn from the base of the tent to the 18-inch-base-diameter canvas cone along the tent's four main seams. Over the entire base of the tent was sewn a 6-inch strip of canvas, which was calked and secured to a wooden frame. This frame was constructed with 1- by 12-inch boards laid flat to result in inside dimensions of 15.5 feet square and outside dimensions of 16.5 feet square. Such dimensions permitted placing a tent structure over any pen.

As can be seen in figure 7, the canvas cone which tapered to a 4-inch-diameter opening fitted over a galvanized cone structure. This 16-inch-base-diameter cone terminated at a wide-mouth mason jar screw-ring to which a one-quart collecting jar could be attached. Within the screw-ring was soldered a 4-inch-diameter, hardware-cloth entrance cone terminating in a ½-inch-diameter opening in the collecting

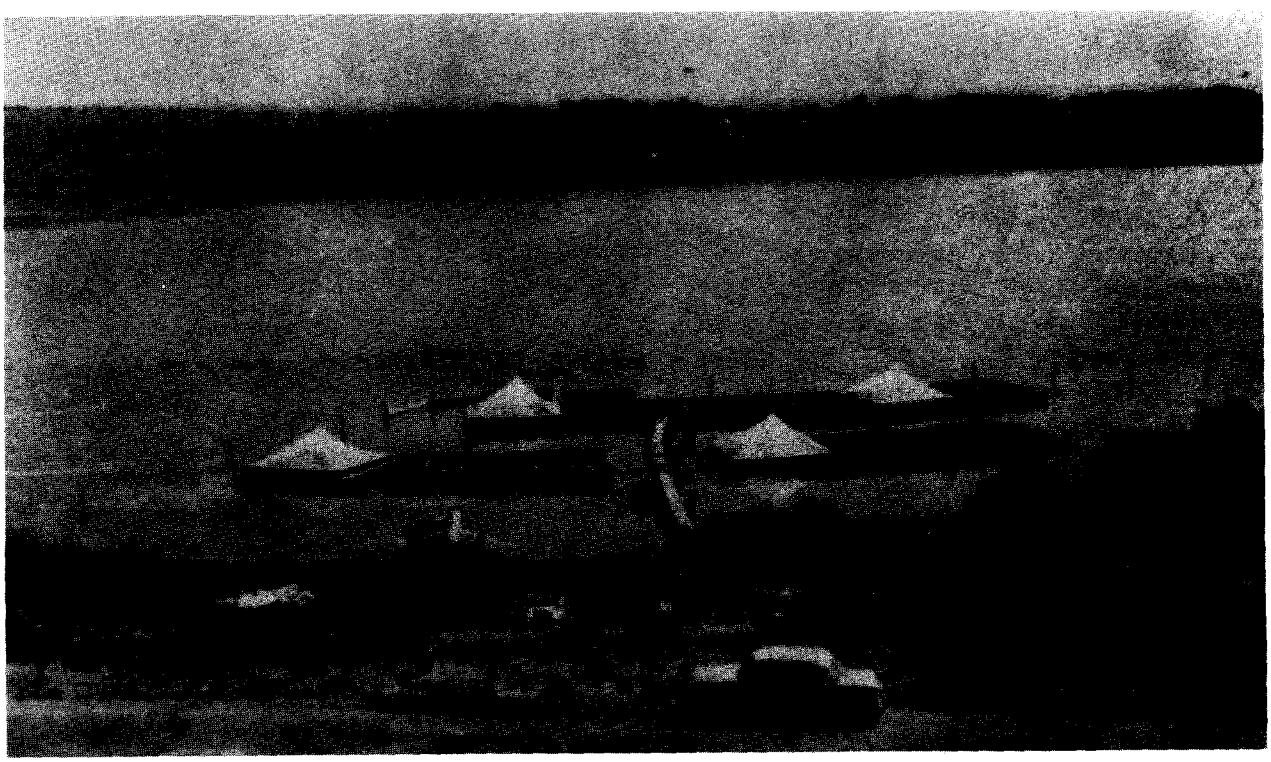

FIGURE 6.—Photograph of the four tents used to determine the efficiency of the emergent trap samples in pens at College Lake, 1962.

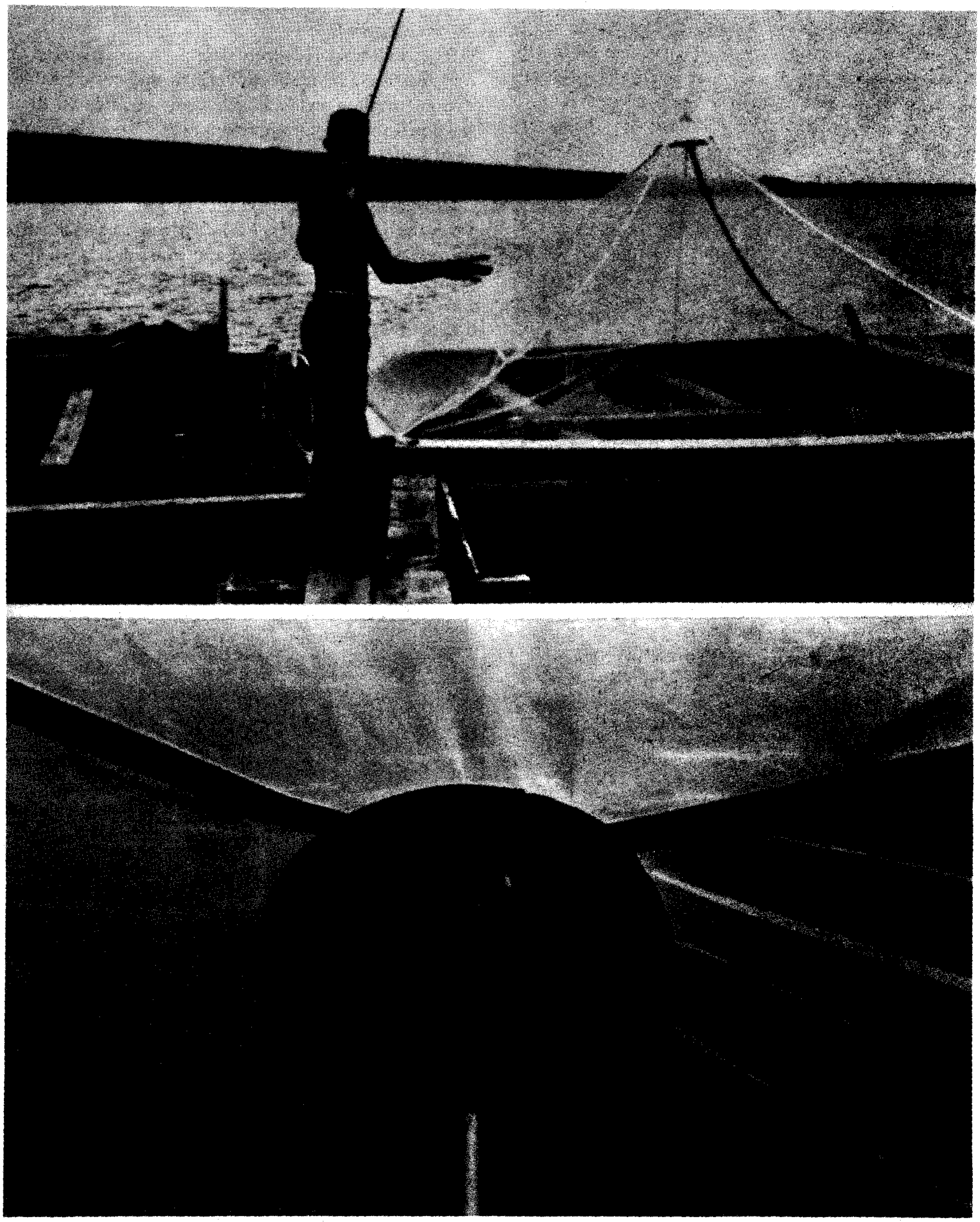

FIGURE 7.—Photographs of tent showing details of its construction. The upper picture shows the access zipper into the tent, the suspension technique employed, and the collecting jar at the peak of the tent. The lower picture is an inside-the-tent view of the galvanized insect trapping mechanism located in the peak of the tent.

jar. A 16-inch metal cross was riveted in the base of the galvanized cone to add support for the structure and to form a platform from which a standard flashlight battery could be mounted. Light supplied by the battery was used at night to attract insects into the sampling jar. The top of each jar was equipped with a metal reflector to concentrate the light. In the center of the metal cross, a 2-inch length of ⅜-inch-diameter rod was welded to form a pin, over which a ½-inch-diameter pipe could be slipped. This pipe, which held the tent upright, was anchored in a plank suspended across the two walkways of the pen and is shown in figure 7. Access into the tent was through a 32-inch zipper sewn into the front side. By opening a 12-inch zipper near the peak of the tent, the sample jar could be removed from inside the tent.

After the tents were completed in late June 1962, the tents were placed over an untreated and a treated pen in each tier and rotated so that each pen was covered at least 8 to 10 days. The control pens were covered longer because there were half as many of them compared to the treated pens. An estimate of the efficiency of the sampling by the traps in any pen was obtained by comparing the total tent-trap count with the total trap count during the tent-covered period. The total trap count during this period was added to the total tent count because the tents should have caught those insects that the traps caught. Since the traps sample 5 percent of the area in a pen, 5 percent of the tent-trap total was taken to estimate the total insects the traps should have caught. This total was compared to the total insects the traps actually caught and resulted in the sampling efficiency for a pen. The same procedure was used to determine the trap efficiency for each species.

Laboratory Procedures

In the laboratory, insects were identified, counted, reared, and in some instances sexed and photographed. Coefficients of similarity and trap efficiencies for the pens were calculated and some effects of the seven film materials to some fish and insects were investigated. In addition, experiments relating to the surface tension reduction of monolayer-treated water were conducted and pertinent data were statistically analyzed and compared.

Identification techniques.—Aquatic plants were identified by the use of Matsumura and Harrington (1955). When possible, insects were identified to species. The larvae were identified with the aid of keys in Johannsen (1934, 1935, 1937), Johannsen and Thomsen (1937), Paine (1956), Pennak (1953), Ross (1944), and Usinger (1956). Identifications of the adult insects were facilitated through keys presented in Cook (1956), Johannsen (1905, 1946), Townes (1945), and Usinger (1956).

Some of the identification techniques and nomenclature procedures used in this study warrant further explanation, especially for the midges of the family Tendipedidae. This group of insects is very difficult to work with because of its many species, small sizes, and various immature stages, all of which is compounded by inadequate and out-of-date keys especially for the species in many areas of the United States. The classic works of Malloch (1915), Johannsen (1934, 1935, 1937), and Townes (1945) for the North American species are sadly out of date particularly in regards to nomenclature. Work on the Tendipedid insects has been much more extensive in Europe and the nomenclature employed there has consequently been more frequently accepted. The system of nomenclature used in this study is the Dendy and Sublette (1959) modification of that suggested in the monographic series on the African Chironomids by Freeman (1955, 1956, 1957, 1958).

The procedure used to identify the midges was as follows: Both the larvae and the adults were assigned "types" on the basis of anatomical features, coloration, size, etc. The larval "types" were reared to adults to facilitate the use of the species keys of the Nearctic Tendipedini presented in Townes (1945). This work, like many others, does not include species keys for the immature forms. Adult midge "types" not in the tribe Tendipedini were taken through the keys of Wirth and Stone (1956) to generic standing and then identified to species with the aid of Johannsen (1905, 1946). After a "type" was identified as a particular species, if possible, the nomenclature system of Dendy and Sublette (1959) was applied to it. For example, a particular "type" was taken to the genus *Harnischia Kieffer* in the keys of Wirth and Stone (1956) and later identified to *Harnischia* (*Harnischia*) *edwardsi* (Kruseman) with the aid of keys in Townes (1945). The generic and subgeneric nomenclature used by Townes for this species has been grouped by Dendy and Sublette (1959) to *Tendipes* (*Cryptochironomus*); thus, the nomenclature used in this study for this species would be *Tendipes* (*Cryptochironomus*) *edwardsi* (*Kruseman*). Non-midge species were identified primarily with the aid of Usinger (1956), along with specific works such as Ross (1944) for the Trichoptera and Cook (1956) for the Chaoborinae. Table 2 summarizes the insects encountered in this study and the sources used in their identification. For brevity, the subgeneric classification and the authors of the species are not included in the body of this report.

Emergent aquatic insect experiments.—Immature stages of mosquitoes and midges were collected by dredge or dip net from waters near Fort Collins, Colo., and brought into the laboratory to complete their development. Identical numbers of these immature forms were introduced and reared in several 2-gallon, wide-mouth glass fishbowls using pond water and bottom debris as the rearing media. Some of the fishbowls were untreated, while others were treated with the seven film materials used in this study. To retain any insects that emerged between observations, the bowls were covered with small traps similar to those used in the field studies (fig. 8).

Water strider experiments.—Monomolecular films are known to reduce the surface tension of water, and insects such as water striders use the surface tension for their support while on water surfaces. Consequently, experiments were conducted with water striders in treated and untreated fishbowls to determine any detrimental effects of the film materials to these insects.

Surface tension reduction experiments.—Because a reduction in the surface tension is probably the greatest single effect of monomolecular films on water, it was necessary to determine the magnitude of the reduction for each film material in order to analyze properly the data in this study. This was accomplished by first measuring the surface tension of distilled water and then measuring the surface tension of distilled water treated with the various monolayer-forming materials. A Cenco du Nouy ring tensiometer was used to measure the surface tensions of these liquids at 25.5° C. This temperature was selected because that was the approximate temperature at which the emergent insect experiments were conducted.

Duck feather experiments.—Theoretically, when the surface tension of water is reduced the wetting property of the water increases. Consequently, it was of interest in this study to determine if monomolecular-treated water would significantly influence the waterproofing mechanisms of duck plumage. Some experiments were conducted with individual mallard duck feathers in monomolecular-treated and untreated water, while a few experiments were conducted using dead ducks in treated and untreated water.

TABLE 2.—*List of the Insect Species Encountered From College Lake and the Sources by Which They Were Identified*

Nomenclature	Source of identification
Diptera:	
Tendipedidae:	
Tendipes (Tendipes) plumosus (Linnaeus).[1]	Townes (1945).
Tendipes (Endochironomus) subtendens (Townes).[1]	Do.
Tendipes (Cryptochironomus) monochromus (Wulp).[1]	Do.
Tendipes (Cryptochironomus) edwardsi (Kruseman).[1]	Do.
Tendipes (Cryptochironomus) fulvus (Johannsen).[1]	Do.
Tendipes (Dicrotendipes) nervosus (Staeger).[1]	Do.
Glyptotendipes (Phytotendipes) lobiferus (Say).[1]	Do.
Pentaneura (Ablabesmyia) monilis (Linnaeus).[1]	Johannsen (1946).
Prodiamesa spp.	Wirth and Stone (1956).[2]
Procladius (Procladius) culiciformis (Linnaeus).[1]	Johannsen (1905).
Pelopia stellatus (Coquillett)[1]	Do.
Corynoneura celeripes (Winnertz).	Do.
Cricotopus trifasciatus (Panzer)	Do.
Calopsectra spp.	Wirth and Stone (1956).[2]
Hydrobaenus spp.	Do.[2]
Heleidae:	
Bezzia glabra (Coquillett)	Do.[2]
Bezzia opaca (Loew)	Do.[2]
Other Diptera:	
Chaoborus punctipennis (Say)	Cook (1956).
Parascatella melanderi (Cresson).	Wirth and Stone (1956).[2]
Odonata:	
Libellula spp.	Smith and Pritchard (1956).[2]
Enallagma clausum (Morse)	Do.
Ephemeroptera:	
Caenis simulans (McDunnough)	Needham, Traver, and Hsu (1938).
Trichoptera:	
Agraylea multipunctata (Curtis)	Ross (1944).

[1] Nomenclature after Dendy and Sublette (1959).
[2] These keys are presented in Usinger (1956).

Fish toxicity experiments.—Green sunfish were maintained for approximately 3 months in holding

FIGURE 8.—Photograph of typical laboratory rearing experiments showing the rearing bowls with their emergent trap attached.

tanks and fed a control diet or a diet containing 50 percent film material. Each of the seven film materials was prepared into a diet by melting the material in a double-boiler apparatus and mixing it for 15 minutes with finely ground trout pellets. The resulting paste was spread on glass plates, allowed to harden, and repelleted into ⅛-inch cubes. The control diet was prepared in exactly the same manner except water was used instead of the monolayer-forming material.

Statistical techniques.—Generally, statistical analyses in this study were computed using procedures described by Snedecor (1957); however, some means were analyzed by using the multiple range tests of Duncan (1955). As used herein, a significant difference refers to the 95-percent level of significance (0.05 level of probability) and a highly significant difference refers to the 99-percent level of significance (0.01 level of probability).

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Chapter III

ANALYSIS OF DATA

In the first portion of the analyses, temperature and chemistry characteristics of the water in the pens were analyzed and discussed. Next, the distribution of the insects from the pens, both larvae and emergents, were analyzed in regard to the accuracy of the sampling techniques employed. The effects of the monolayers on the emergence of various species, their sexes, and differences between these effects due to the various monolayer-forming materials were analyzed from data collected both in the pens at College Lake and in laboratory experiments. In the second phase of the analyses, many speculations were postulated as to the cause-effect relationship monolayers have on the emergence of some insects. In addition, monolayer effects on various developmental stages of some insects, effects on waterfowl plumage, and effects on green sunfish fed each of the seven film-forming materials were analyzed and discussed. Throughout these analyses, data from other studies were in some cases presented and compared with the findings of this study.

Temperature and Chemistry

Temperature and chemistry data presented in table 3 suggest effects due to the lake, effects due to the pens, and effects due to monomolecular film coverage. The "lake effect" was considered the overall influence the lake had on the experimental apparatus, while the "pen effect" was considered the difference between observations of the lake station and those of the untreated pens. The difference between data of the treated and untreated pens was considered the "film effect". By comparing data from the lake with those from the untreated pens it can be seen in table 3 that the pens had a tendency to decrease the temperature of surface water and increase the temperatures of water below the surface. Dissolved oxygen and alkalinity tended to increase slightly in the surface waters of the pens. Such pen effects, however, probably were consistent in all pens regardless of the treatment and should not have influenced the effects attributed to the films. The lake effect will be discussed later in conjunction with its probable influence on the film effects.

In pens treated with monomolecular films, dissolved oxygen concentrations and temperatures generally increased. Surface water temperatures of T.A. 1618 treated pens had an average increase of 0.4° F., compared to 0.5° F. increase in Adol 52 pens. These temperature increases were greatest in the surface waters and tended to diminish with depth. Dissolved oxygen concentrations in the surface water increased by an average of 0.6 p.p.m. in T.A. 1618 pens and by 0.7 p.p.m. in Adol 52 pens. Although analysis of variance tests between untreated and treated pens clearly demonstrated that the increases in temperature and oxygen in the surface water were effects of the films, no statistical difference was found between the two film materials for those variables.

Increases in the surface temperature of film-treated waters are common in the literature (La Mer, 1962); however, the temperature increases noted in table 3 for the College Lake treated pens are considerably less than those reported in other studies. Hayes (1959) treated ponds with hexadecanol, a material identical to the Adol 52 used in this study, and found that the surface waters of two treated ponds averaged 2.6° and 3.8° F. warmer than that of control ponds. Such increases are approximately five to eight times greater than the 0.5° F. average temperature increase in the Adol 52 treated pens in College Lake. It is believed that the lake water, which was generally cooler and which surrounded the pens, probably buffered the waters in the treated pens thus preventing these waters from increasing their temperatures to the magnitude found in other studies. In addition, the fact that the temperatures were taken early in the morning before the surface waters could regain the heat they had lost during the previous evening and early that morning, may have contributed to the minimal increases in temperature noted in the treated pens.

TABLE 3.—*Average Temperature and Chemistry Characteristics of Treated and Untreated Pens at College Lake*

Treatment	Water temperature (° F.)				Dissolved oxygen (p.p.m.)		Carbon dioxide (p.p.m.)		Phenolphthalein alkalinity (p.p.m.)		Methyl orange alkalinity (p.p.m.)		pH	
	Surface	3 feet	4 feet	Bottom	Surface	4 feet	Surface	4 feet	Surface	4 feet	Surface	4 feet	Surface	4 feet
Lake	[1] 72.9	------	70.2	68.9	8.9	------	0.0	0.0	18.3	------	67.3	------	9.2	------
Untreated	72.2	70.2	70.3	69.2	9.0	7.7	0.0	0.0	19.0	18.0	71.2	72.6	9.2	9.1
T.A. 1618 treatment	72.6	70.5	70.3	69.2	9.6	9.1	0.0	0.0	19.7	20.2	68.7	69.0	9.3	9.3
	[2] (.4)	(.3)	(.0)	(.0)	(.6)	(1.4)	------	------	------	------	------	------	------	------
Adol 52 treatment	72.7	70.6	70.5	69.4	9.7	8.5	0.0	0.0	19.8	20.8	69.8	70.8	9.3	9.2
	(.5)	(.4)	(.2)	(.2)	(.7)	(.8)	------	------	------	------	------	------	------	------

[1] All values except those from the lake are averages of 4 pens for both years.

[2] Figures in parentheses are the temperature and oxygen increases resulting from the films and were obtained by subtracting the untreated pen values from the treated pen values at the indicated depths.

Increases in the oxygen concentration of film-treated waters such as occurred in the College Lake treated pens are seldom corroborated in the literature but Hayes (1959) also found increases of dissolved oxygen in a treated half of a pond. Most studies have described the oxygen concentration to decrease in film-treated waters because the films reduced the diffusion of oxygen from the atmosphere into the water. If the films can reduce the diffusion of oxygen into the water, they should be able to reduce the diffusion of oxygen out of the water. Since the pens at College Lake contained many aquatic weeds which are known to release oxygen into the water, it appears that the films reduced the diffusion or "loss" of oxygen to the atmosphere and permitted the oxygen being released by the weeds to accumulate in the pens.

Although there was an indication for phenolphthalein alkalinity and pH to increase in the treated pens, their increases were not great enough when the accuracy of the methods used in their determination was considered. The lack of carbon dioxide was influenced probably by the shallowness of the study areas and the abundance of aquatic plants.

Insect Larvae Distribution and Importance

Midge larvae, Diptera, dragonflies and damselflies, Odonata, mayflies, Ephemeroptera, and caddisflies, Trichoptera, were observed in the study areas during 1961 and their distribution and abundance characteristics are presented in table 4. Diptera larvae comprised 75 percent of the species observed and made up 37 percent of the total number sampled. The damselfly, *Enallagma clausum,* which made up 33.8 percent and the mayfly, *Caenis simulans,* which comprised 28.1 percent of the total number of larvae were found in all pens. The caddisfly, *Agraylea multipunctata,* and the dragonflies, *Libellula* spp., made up only 1.1 percent of the insect larvae by number.

In most biological populations, homogeneous distribution of species rarely occurs and is dependent on many interrelating factors such as, in this instance, depth, bottom type, vegetation type, etc. It was for this reason that the small pen sites were selected with many of the variables as consistent as possible. From data presented in table 4 the effect of changing many of the variables ("tier effect") is demonstrated for the occurrence and/or abundance of a species. The midge, *Procladius culiciformis,* the dragonfly, *Libellula* spp., and the caddisfly, *Agraylea multipunctata,* were all found in tier 1 but were absent in tier 2. Both the midge, *Tendipes plumosus* which was the second most important insect in tier 2 and the phanthom midge, *Chaoborus punctipennis* were not found in tier 1.

Quantitative comparisons of the frequency of occurrence of insect larvae were made by computing coefficients of similarity between the pens. Such coefficients have been used by plant ecologists in evaluating comparisons of vegetation types or communities and as described in the methods and materials used to select pen pairs for the treatments during 1962. A coefficient value of 1.0 is perfect similarity. Coefficients were

TABLE 4.—*Abundance and Distribution of Insect Larvae at College Lake, 1961*

Larvae	Tier 1		Tier 2		All samples	
	A	B	A	B	A	B
Diptera (Midges):						
Hydrobaneus spp	14	1. 1	5	0. 4	19	0. 7
Tendipes (Cryptochironomus) spp	33	2. 5	16	1. 2	49	1. 9
Crictopus trifasciatus	4	. 3	11	. 8	15	. 6
Tendipes nervosus	17	1. 3	8	. 6	25	1. 0
Calopsectra spp	162	12. 4	67	5. 0	229	8. 7
Pentaneura monilis	10	. 8	5	. 4	15	. 6
Procladius culisciformis	5	. 4	0	0	5	. 2
Pelopia stellatus	5	. 4	25	1. 9	30	1. 1
Tendipes subtendens	33	2. 5	16	1. 2	49	1. 9
Tendipes plumosus	0	0	409	30. 8	409	15. 5
Heleidae (Biting midges)	117	9. 0	1	. 1	118	4. 5
Chaoborous punctipennis	0	0	11	. 8	11	. 4
Odonata (Damsel and Dragonfly):						
Enallagma clausum	387	29. 7	503	37. 9	890	33. 8
Libellula spp	3	. 2	0	0	3	. 1
Ephemeroptera (Mayflies):						
Caenis simulans	488	37. 5	251	18. 9	739	28. 1
Trichoptera (Caddisfly):						
Agraylea multipunctata	25	1. 9	0	0	25	1. 0
Pooled total	1, 303	----------	1, 328	----------	2, 631	----------

A=The total number of larvae from 18 Eckman dredge samples in each tier (3 random samples from each pen).

B=Percent relative abundance determined by $\frac{A}{\text{Pooled total A}}$ (100) for each species.

computed for all of the possible comparisons of the 12 pens and were considerably variable even between pens in the same tier. Within tier 1 the coefficients ranged between 0.73 and 0.90, and within tier 2 between 0.67 and 0.94. In each tier these coefficients for species similarity averaged 0.81. The "tier effect" demonstrated previously is exemplified when the coefficients for comparisons of pens located in different tiers are analyzed. The coefficients for these comparisons ranged between 0.52 and 0.83, with an average of only 0.69. This latter average is less than the 0.81 average noted for comparisons of pens in the same tier and clearly indicates the dissimilarity for the occurrence of species between the two tiers. Comparisons of similarity coefficients were also made on the abundance of the species. Such coefficients ranged between 0.35 and 0.76 with an average of 0.59 for pens within tier 1, while tier 2 coefficients ranged between 0.47 and 0.86 with an average of 0.74. Since both tiers had identical average coefficients for occurrence of species, it appears that, overall, tier 2 is more homogeneous than tier 1.

Emergence of Insects

From tables 5 and 6, it can be seen that much variation existed between the total number of insects caught by the traps in pens with the same treatment and tier location in both 1961 and 1962. The numbers are not directly comparable between the years because the collection times were different. In 1961, emergent insects were collected only during August, but in 1962 they were collected between July 13 and September 10. Within tier 1 in 1961, the totals of untreated pens 3 and 6 varied 55.6 percent, those of Adol 52 treated pens varied 27.1 percent, and those of T.A. 1618 treated pens 56.4 percent. Variation was apparently less in tier 2 except for the Adol 52 pens, which varied 46.9 percent in 1961. As was found for larvae occurrence and abundance in 1961, most of the variation for insect emergence appeared between the totals of pens located in different tiers. Similar variations existed in the total trap emergences from the pens during 1962; however, they usually were consistently less than those

TABLE 5.—*Comparison of the Total Numbers of Emergent Insects Caught by Traps from Untreated and Monolayer-Treated Pens at College Lake During 1961 and 1962*

Pen	Untreated		T.A. 1618				Adol 52			
	1961	1962	1961	PD [1]	1962	PD [1]	1961	PD [1]	1962	PD [1]
Tier 1:										
1			234		668					
2		1,628					94			
3	[2] 675								405	
4		1,367	102							
5							129		724	
6	300				675					
Total	975	2,995	336	(65.6)	1,343	(55.1)	223	(77.1)	1,129	(62.3)
Tier 2:										
7		832	136							
8	157								489	
9					547		60			
10	102								396	
11			97		258					
12		1,007					113			
Total	259	1,839	233	(10.0)	805	(56.2)	173	(33.2)	885	(51.9)
Grand total	1,234	4,834	569	(53.8)	2,141	(55.6)	396	(67.9)	2,014	(58.3)

[1] PD = Percentage difference in the total number of emergent insects between untreated and treated pens for each year.
[2] Totals of the 3 traps in each pen.

TABLE 6.—*Percentage Variation of the Total Number of Emergent Insects Trapped in Variously Treated Pens at College Lake During 1961 and 1962*

Pens	Within tier 1		Within tier 2		Between tiers		Between totals of each tier	
	1961	1962	1961	1962	1961	1962	1961	1962
Untreated	55.6	15.4	35.7	17.3	84.4	48.9	73.4	38.6
T.A. 1618	56.4	1.0	28.7	52.8	58.9	61.8	30.7	40.1
Adol 52	27.1	44.1	46.9	19.0	53.5	45.3	22.4	21.6
Total	139.1	60.5	111.3	89.1	196.8	156.0	126.5	100.3

in 1961. This is exemplified by comparing the totals in table 6. The most likely explanation for less variation in 1962 is that the pen pairs used for the treatments in each tier were more homogeneous. Better homogeneity probably occurred because the selection of these pen pairs was based on insect presence and abundance rather than the strict random selection of pens for treatments in a tier in 1961.

Larva-emergence diversity.—Regardless of the year or treatment shown in table 5, greater numbers of insects generally emerged from pens in tier 1 even though samples taken in 1961 indicated that the numbers of insect larvae were slightly greater in tier 2. Comparisons of the abundance of the larval and adult stages of the species are limited in this study because quantitative samples of the larvae were not collected in

1962, and many of the midge species of the family Tendipedidae were not differentiated in the emergent counts of 1961. Diversity between the larvae abundance and the emergence abundance can be caused by many interrelating factors, some of which will be discussed in this section. The most likely explanation for fewer insects emerging in tier 2 is that most larval members of the midge *Tendipes plumosus*, which occurred only in tier 2 and which were relatively abundant, were immature and did not enter into the emergence. According to Townes (1938), *Tendipes plumosus* usually has only one complete generation a year with the peak of adult emergence in May. This, together with the fact that the larvae were comparatively small, suggests that most of the emergence of *Tendipes plumosus* at College Lake probably occurred prior to emergent sampling which began in August during 1961 and in July during 1962.

Larva-emergence diversity was also observed for mayflies and damselflies. These insects comprised 61.9 percent of the insect larvae present in the entire study area, but constituted only 2 percent of the total number of insects caught by the traps in 1961. Some probable explanations for this phenomenon were believed to be found in the interreaction of: (1) the ability of mayfly and damselfly larvae to avoid the traps; (2) the greater majority of mature larvae may have emerged prior to the start of emergent sampling; and (3) the cutting of weeds below the water surface to ensure complete film coverage may have interfered with the damselfly emergence. Damselflies usually crawl out of the water on the weeds before transforming into adults. These factors were investigated during 1962 using emergent tents and weed-cutting techniques as described in the methods and materials.

The avoidance theory was supported in 1961 by the occurrence of many mayfly castskins on objects including the outer surface of the traps while very few were caught inside the traps. Furthermore, both mayfly and damselfly larvae are strong swimmers, which makes possible avoidance of the traps. If the monomolecular films were preventing their emergence, they should have been caught at least by traps in the untreated pens but were not. Data collected during 1962, however, indicate that avoidance of mayflies and damselflies to the traps probably was negligible even though the traps did not catch any of these insects in tent-covered pens. It can be seen in table 7 that a total of only 25 mayflies and damselflies was taken from tent-covered pens between July 30 and September 10, 1962, a period comparable to and even overlapping the 1961 sampling period. Since the traps sample 5 percent of the pen area, 5 percent of the 25 mayflies and damselflies which emerged in the pens indicates that only 1.25 of these insects should have been caught by the traps and it is not surprising that none were taken. Furthermore, the mayfly castskins which were observed on the outside of the traps during 1961 were not found on the traps of the tent-covered pens during 1962, but were only found on the traps in pens not covered by tents. Such evidence indicates that these castskins were blown in from other areas of the lake and were not indicative of the emergence of mayflies in the pens.

Although damselflies usually crawl out of the water on the weeds before transforming into adults, the cutting of the weeds in half of the pens probably had little effect on their emergence because 10 of the 18 damselflies which emerged in tent-covered pens were from pens with the weeds cut. Those damselflies from the cut pens probably crawled up the sides of the pens and traps and emerged successfully without the aid of the weeds. It appears then that most of the larva-emergence diversity for the mayflies and damselflies resulted because their larval forms were immature and consequently did not enter into the emergent counts. This is supported by the fact that comparatively large numbers of small, immature larvae of these insects were taken during the period when emergence data were collected in 1961, and by the fact that considerable numbers of their adults were observed in the study area prior to emergent sampling in 1962.

Besides diversity of the larval-emergence abundances occurring for a species, diversity can occur between species. For example, two species which have identical relative abundances at a given time in the larval stage may have emergent abundances which vary considerably. Two factors which could influence such diversity are variable natural mortality of the larvae and differences in the life-histories of the species. Borutsky (1939) presented evidence that "dying out" of *Tendipes plumosus, Chaoborus* spp., and *Pelopia* spp. occurred in considerable numbers in Lake Beloie in Russia and in some instances reached proportions of 99 percent. He attributed this mortality both to cannibalism and predation by the larvae and to predation of the larvae by fish. If variation in the natural mortality of the larvae occurs for two species requiring the same amount of time to complete a generation in their life-history, divergence in their emergent abundance probably will occur. This is especially true if the natural mortality varies after the larval abundance has been estimated. If the larvae of two species have identical relative abundances and identical natural mortalities but one species has more generations than the other during the same time period, larval-emergence diversity between the species would result because the species with the greater number of generations would contribute more individuals to the total emergence.

Direct comparisons of the relative abundance data presented in table 4 for the larvae, and in table 7 for the emergence of the various species, are not applicable because the data were collected in different years. However, if it is assumed that the larvae abundance did not change significantly during 1962, comparisons of *Tendipes* (*Cryptochironomus*) spp. and *Tendipes subtendens* may illustrate the larval-emergency diversity resulting from life-history differences. In table 4 it is seen that the larval members of *Tendipes* (*Cryptochironomus*) spp. and *Tendipes subtendens* had identical 1.9 relative abundances; however, the emergent data in table 7 shows that members of *Tendipes* (*Cryptochironomus*), i.e., *T. edwardsi, T. monochromus* and *T. fulvus,* were much more abundant than *Tendipes subtendens*. The three species of *Tendipes* (*Cryptochironomus*), which were much smaller than *Tendipes subtendens* at College Lake, probably had more generations during the period when emergence data were collected and this could account for their greater numbers in the emergence despite the identical larval abundances.

Several discrepancies are apparent when the list of species in tables 4 and 7 are compared. Species such as *Prodiamesa* spp., *Corynoneura celeripes, Parascatella melanderi,* and *Glyptotendipes lobiferus* were all taken as emergents during 1962 but were not differentiated in the larval counts of 1961. The most likely explanation for this is that some species were missed in the larval samples while others were included with similar species. The larvae of *Prodiamesa* spp., *Corynoneura celeripes,* and *Parascatella melanderi* probably were missed because they were too small for the collector to see. It has been pointed out previously in the methods and materials that only the visible larvae were picked from the samples. Because of similarities, the larvae of *Glyptotendipes lobiferus* probably were tabulated as *Tendipes plumosus,* while those of *Tendipes edwardsi, Tendipes monochromus* and *Tendipes fulvus* were not separated from *Tendipes* (*Cryptochironomus*). Adult dragonflies, *Libellula* spp., probably were not taken because of their sparsity in the study area.

Film effects and sampling efficiency.—Despite all of the variations encountered in species occurrence, abundance, and emergence, data presented in table 5 show generally that fewer insects emerged in the traps of pens treated with monomolecular films. In both years, statistical analyses demonstrated highly significant reductions in the grand total number of insects emerging in monolayer-treated pens. These reductions are expressed as the percentage difference (PD) between the total emergence of untreated and treated pens and were similar in both years. In 1961 a 53.8-percent grand total reduction was observed in T.A. 1618 pens, compared to a 55.6-percent reduction in 1962. A 67.9-percent reduction in the insects was observed in Adol 52 pens during 1961, compared to a 58.3-percent reduction in 1962. The similarity of the reductions is most striking when considering that only 3 of the 12 pens (1, 5, and 11) received the same treatment in both years. All of the untreated pens in 1962 were treated in 1961.

The accuracy of the reductions attributed to monomolecular film coverage shown in table 5, however, is dependent upon how efficiently the traps sampled the insects emerging in the pens. The three traps used in each pen constituted only a 5-percent sample of the pen. Before proceeding further, a review of the procedure used to determine the trap efficiencies is suggested (p. 13).

Chi-square analyses of the data presented in table 7 clearly show that the trap sampling was inefficient both for the pooled data and for most of the species. Chi-square values larger than 3.841 demonstrate significant differences or inefficiencies, and values larger than 6.635 demonstrate highly significant inefficiencies with 1 degree of freedom. Efficient sampling usually was limited to species which were comparatively few in number. The pooled data imply the traps sampled 8.6 percent fewer insects than was expected because a total of 2,174 was caught compared to a total of 2,379 expected. Consistently fewer insects than expected in the traps was not indicative for all of the species. In fact, members of *Corynoneura celeripes, Crictopus trifasciatus, Bezzia* spp., etc., were all taken in greater numbers than expected in the traps. Such heterogeneity (inconsistency) between the species data and the pooled data is exemplified by the large 2,078.13 highly significant chi-square interaction.

TABLE 7.—*Relative Abundance and Trap Efficiency of the Total Numbers of Insects Emerging in Tent-Covered Pens at College Lake, July 30 to September 10, 1962*

Emergent insect classification (species)	A Tent total	B Trap total	C Pen total A+B	D Percent RA	E Expected trap total (5 percent of C)	F Expected tent total (95 percent of C)	Chi-square $\frac{(B-E)^2}{E}+\frac{(A-F)^2}{F}$
Corynoneura celeripes	1, 557	'439	1, 996	4. 2	100	1, 896	[1] 1, 209. 82
Hydrobaenus spp.	804	41	845	1. 8	42	803	. 03
Prodiamesa spp.	13, 553	615	14, 168	29. 8	708	13, 460	[1] 12. 86
Tendipes edwardsi	3, 009	31	3, 040	6. 4	152	2, 888	[1] 101. 39
Crictopus trifasciatus	770	93	863	1. 8	43	820	[1] 61. 19
Tendipes nervosus	1, 207	60	1, 267	2. 7	63	1, 204	. 15
Calopsectra spp.	5, 519	46	5, 565	11. 7	278	5, 287	[1] 203. 79
Tendipes monochromus	9, 298	277	9, 575	20. 1	479	9, 096	[1] 89. 68
Tendipes fulvus	988	61	1, 049	2. 2	53	996	1. 27
Pentaneura monilis	4, 867	178	5, 045	10. 6	252	4, 793	[1] 22. 87
Procladius culiciformis	9	0	9	. 02	0	9	. 00
Pelopia stellatus	457	18	475	1. 0	24	451	1. 58
Tendipes subtendens	1, 684	90	1, 774	3. 7	89	1, 685	. 01
Glyptotendipes lobiferus	962	15	977	2. 0	49	928	[1] 24. 84
Tendipes plumosus	128	12	140	. 3	7	133	3. 76
Bezzia glabra	182	27	209	. 44	11	198	[1] 24. 56
Bezzia opaca	141	41	182	. 38	9	173	[1] 119. 70
Chaoborus punctipennis	26	0	26	. 05	1	25	. 04
Parascatella melanderi	116	15	131	. 3	7	124	[1] 9. 66
Enallagma clausum	18	0	18	. 04	1	17	. 06
Caenis simulans	7	0	7	. 01	0	7	. 00
Agraylea multipunctata	86	106	192	. 4	10	182	[1] 142. 80
Others	16	9	25	. 05	1	24	[1] 66. 67
Sum							[1] 2, 096. 73
Pooled totals	45, 404	2, 174	47, 578	100. 00	2, 379	45, 199	[1] 18. 60
Interaction							[1] 2, 078. 13

A=Total numbers for each species taken in the tents between July 30 and Sept. 10, 1962.

B=Total numbers for each species taken in the traps of the tent-covered pens between July 30 and Sept. 10, 1962.

D=Percent relative abundance: $\frac{C(100)}{\text{Pooled total of C}}$ for each species.

[1] Highly significant difference.

The inconsistency of the trap data seems to suggest both avoidance and affinity of the insects to the traps. Where the number of insects caught by the traps (col. B) is larger than the expected number (col. E), affinity of that species to the traps is probable, whereas the reverse condition implies that avoidance of that species to the traps is probable. It appears, however, that the inconsistencies of the trap catches could have been influenced by the traps being located in the pens over areas where a species was either abundant or sparse. Because insect species rarely are distributed evenly throughout a given area, it is not known whether the suggested avoidance or affinity was purposeful or due to the distribution of the insects. The caddisflies, *Agraylea multipunctata,* however, appeared to exhibit purposeful affinity to the traps because more of them were caught in the 5-percent trap samples than in the 95-percent tent samples. Furthermore, such affinity occurred in the traps of 9 of the 12 pens and, since the traps were all randomly located, it does not appear likely that these traps could have been located consistently over areas where caddisflies were the most abundant. It may be possible that the affinity is phototropic in response.

Despite the inefficiencies of the traps, it was believed that by calculating whether the traps sampled fewer or greater total numbers of insects from each pen, the 1962 trap totals shown in table 5 could be adjusted

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accordingly and increased accuracy of the overall reducing effects of the monomolecular films could be procured. For example, in table 8 it is seen that a total of 58 insects was caught in the traps of pen 1 when a total of 94 insects should have been caught during the indicated tent-covered period. This means the traps in pen 1 actually caught 38.3 percent fewer insects than expected and, if the trap total of this pen shown in table 5 is adjusted accordingly, it becomes 924 instead of 668. Such adjustments appear to be futile, however, because an assumption of this technique is that the trap efficiency in a given pen during its tent-covered period is representative of the entire trap-sampling period when data in table 8 show otherwise. Between July 30 and August 8, 1962, the traps in pen 4 caught 35 percent more insects than expected but between August 30 and September 10, 1962, these identically located traps caught 48.1 percent fewer insects than expected. Because similar inconsistencies occurred in all other pens which were covered in different time periods (2, 7, and 12), it is not likely that the indicated estimate of the trap efficiency for the pens covered in one time period is representative of the entire sampling period and adjustments would therefore be useless.

When the data of table 8 are rearranged in table 9 to show the reduction effect of the monomolecular films, a striking phenomenon is seen. Regardless of the tent-covered period, monomolecular treatment, or tier location, the reductions derived from the trap samples are consistently greater than the actual reductions. The actual reductions are considered those determined from the pen totals, which are the summations of the tent and trap catches in the pens. The greater reduction effects noted by the traps are exemplified by the grand totals. In T.A. 1618 treated pens the overall actual reduction was 30.92 percent compared to 61.80-percent reduction demonstrated by the traps, whereas, in Adol 52 pens the actual reduction was 46.54 percent in contrast to a 66.35-percent reduction by the traps. Such evidence, together with the consistency of the traps to yield greater reductions in all tent-covered periods, suggests that the grand total reductions indicated by the traps in table 5 for the entire 1962 sampling period are definitely high.

TABLE 8.—*Trap Efficiency of the Total Number of Insects Emerging From the Pens During Various Tent-Covered Periods, College Lake, 1962*

Tent-covered period	Pen number	A Tent total	B Trap total	C Pen total	D Expected trap total (5 percent of C)	E Expected tent total (95 percent of C)	Percent trap efficiency (B compared to D)	Chi-square $\frac{(B-D)^2}{D}+\frac{(A-E)^2}{E}$
1962								
July 30–Aug. 8	1	1,823	58	1,881	94	1,787	−38.3	[1] 14.51
Aug. 9–Aug. 18	2	4,818	320	5,138	257	4,881	+19.7	[1] 16.26
Aug. 19–Aug. 29	2	7,548	384	7,932	397	7,535	−3.3	.45
Aug. 30–Sept. 10	3	2,939	58	2,997	150	2,847	−61.3	[1] 59.35
July 30–Aug. 8	4	2,126	177	2,303	115	2,188	+35.0	[1] 35.18
Aug. 30–Sept. 10	4	4,578	122	4,700	235	4,465	−48.1	[1] 57.20
Aug. 9–Aug. 18	5	2,457	89	2,546	127	2,419	−29.9	[1] 11.97
Aug. 19–Aug. 29	6	5,140	160	5,300	265	5,035	−39.6	[1] 43.79
Aug. 9–Aug. 18	7	3,661	199	3,860	193	3,667	+3.0	.20
Aug. 19–Aug. 29	7	2,504	199	2,703	135	2,568	+32.2	[1] 31.94
July 30–Aug. 8	8	776	29	805	40	765	−27.5	3.18
Aug. 30–Sept. 10	9	1,281	77	1,358	68	1,290	+11.7	1.25
Aug. 9–Aug. 18	10	1,420	69	1,489	75	1,414	−8.0	.51
Aug. 19–Aug. 29	11	1,671	37	1,708	85	1,623	−56.5	[1] 28.53
July 30–Aug. 8	12	876	87	963	48	915	+44.8	[1] 33.35
Aug. 30–Sept. 10	12	1,786	109	1,895	95	1,800	+12.8	2.17
Pooled total	------	45,404	2,174	47,578	2,379	45,199	−8.6	[1] 18.60

[1] Highly significant difference demonstrating inefficient sampling.

TABLE 9.—*Comparison of the Total Numbers of Insects Caught by Tents and Traps From Untreated and Monolayer-Treated Pens During Various Tent-Covered Periods at College Lake, 1962*

Tent-covered period		Tier 1				Tier 2			
		Untreated	Treated		PR	Untreated	Treated		PR
			T.A. 1618	Adol 52			T.A. 1618	Adol 52	
1962									
July 30–Aug. 8	Pen number	*4*	*1*			*12*		*8*	
	Tent total	2,126	1,823		[1] 14.3	876		776	[2] 11.4
	Trap total	177	58		[1] 67.2	87		29	[1] 66.7
	Pen total	2,303	1,881		[1] 18.3	963		805	[1] 16.4
Aug. 9–Aug. 18	Pen number	*2*		*5*		*7*		*10*	
	Tent total	4,818		2,457	[1] 49.0	3,661		1,420	[1] 61.2
	Trap total	320		89	[1] 72.2	199		69	[1] 65.3
	Pen total	5,138		2,546	[1] 50.5	3,860		1,489	[1] 61.4
Aug. 19–Aug. 29	Pen number	*2*	*6*			*7*	*11*		
	Tent total	7,548	5,140		[1] 31.9	2,504	1,671		[1] 33.3
	Trap total	384	160		[1] 58.3	199	37		[1] 81.4
	Pen total	7,932	5,300		[1] 33.2	2,703	1,708		[1] 36.8
Aug. 30–Sept. 10	Pen number	*4*		*3*		*12*	*9*		
	Tent total	4,578		2,939	[1] 35.8	1,786	1,281		[1] 28.3
	Trap total	122		58	[1] 52.5	109	77		[2] 29.4
	Pen total	4,700		2,997	[1] 36.2	1,895	1,358		[1] 28.3
Grand total (all pens): T. A. 1618 treatment.	Tent total	13,964	9,915		[1] 29.00				
	Trap total	869	332		[1] 61.80				
	Pen total	14,833	10,247		[1] 30.92				
Adol 52 treatment	Tent total	13,933		7,592	[1] 45.51				
	Trap total	728		245	[1] 66.35				
	Pen total	14,661		7,837	[1] 46.54				

[1] Highly significant reduction; see app. A for type of statistical analyses used.
[2] Significant reduction.
PR=The percentage reduction (overall reducing effect) or percentage difference between the untreated and treated totals. That determined from the pen totals is considered the actual percent reduction effect of the treatment.

Despite only four tent-covered pens being used at any given time, the pen total data appear to be the most accurate from which reduction effects of the monomolecular films can be derived because they represent actual total counts from the pens. This is especially true when reductions are derived for the species because considerably greater numbers of the species were taken from the four tent-covered pens than from the traps of all pens during the entire sampling period. The statistical benefit of working with larger numbers can be seen when the data from pens 12 and 9 in table 9 are examined. Between August

30 and September 10, 1962, the traps in untreated pen 12 caught 109 insects, whereas the traps in the T.A. 1618 treated pen 9 caught 77 insects. This represents a 29.4-percent difference or reduction due to the film which is statistically significant. However, identical statistical comparison of the pen totals yields a percentage reduction which is less (28.3 percent) but which is highly significant.

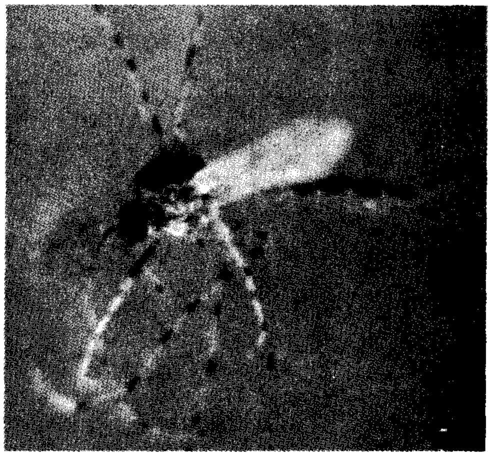
Although the actual reductions in all tent-covered periods were less than the trap reductions, they were all highly significant reductions. The analyses used to determine the statistical difference between untreated and treated pen totals are presented in appendix A.

Besides the two monomolecular films causing definite reductions of the insect emergence, a highly significant difference was found between the magnitude of the reductions, with Adol 52 more adverse to the emergence of insects. The overall reductions with Adol 52 was 46.54 percent compared to only 30.92 percent for the T.A. 1618 treatment. The chi-square analysis used to determine this treatment difference warrants some explanation. According to Snedecor (1957), percentages cannot be used directly in the calculation of chi-square except in the case where the sample size is an even hundred, which is seldom the case for field data. In all other samples, before chi-square can be computed the percentage must be converted to the actual number of individuals found with the attribute, or else some appropriate device must be employed for accomplishing the same end. The procedure employed to the percentage reduction data is illustrated in appendix B.

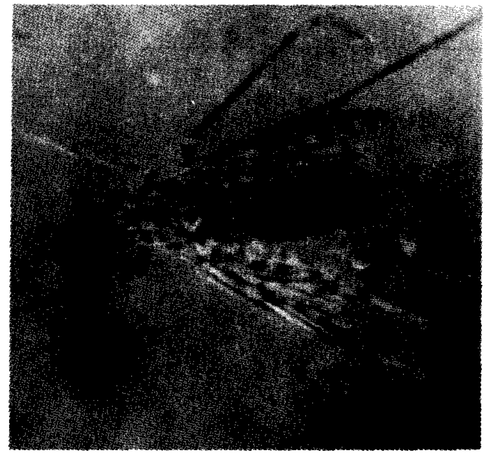
Species-sex effects.—The 15 species of the family Tendipedidae taken at College Lake between July 30 and September 10, 1962, comprised 65.0 percent of the species observed and 98.3 percent of the total number of insects which emerged in the tent-covered pens. As pointed out in the methods and materials, the insects were tabulated as "types" in the field and later identified to species in the laboratory. Color and body appearances were the characteristics used for assigning a "type" and in many instances the "type" was indicative of the sex of the insect species. This was especially true for insect species of the family Tendipedidae which exhibit a marked sexual dimorphism (fig. 9). The male has long antennae which appear plumelike, comparatively narrow wings, and an abdomen which is very long, straight, somewhat depressed, with conspicuous forceps at its posterior. In contrast, the female has short unplumelike antennae, broader wings, and a shorter abdomen which is decurved, somewhat compressed, without conspicuous forceps at its posterior. Many of the above described characteristics can be seen in the photographs of figure 9; however, it is warned that the actual sizes of the insects are not comparable. For example, the actual wing length of the *Tendipes plumosus* specimen, the largest of all tendipedids, is 5.93 mm., whereas, the actual wing length of the *Corynoneura celeripes* specimen is .8 mm. These two species represent the smallest and largest insects from the family Tendipedidae taken at College Lake.

Because of the striking sexual dimorphism characterized by the species of Tendipedidae, it was felt that there may be differences in the reducing effects of the monomolecular films for the sexes of these species. Such data are presented in table 10. Although differences in the reducing effects for the sexes occurred in some species, the differences were not great enough to be considered statistically significant. A calculated chi-square value of at least 3.841 is needed to demonstrate a significant difference between the sexes of a species and none of the values shown in table 10 are that great. Consequently, the data of the sexes were combined for the species and are presented in table 11. In this table the differences in the reducing effect the two monomolecular films have on the various species can be seen. The more adverse reducing effect of the Adol 52 treatment to the emergence of insects is evident in eight of the nine species for which treatment differences were demonstrated. *Pentaneura monilis* was the only species more adversely reduced by the T.A. 1618 treatment. It should be pointed out, however, that *Parascatella melanderi* and *Agraylea multipunctata* were also more adversely reduced by the T.A. 1618 treatment, but the numbers of these species were not large enough to demonstrate statistical differences between the treatment effects.

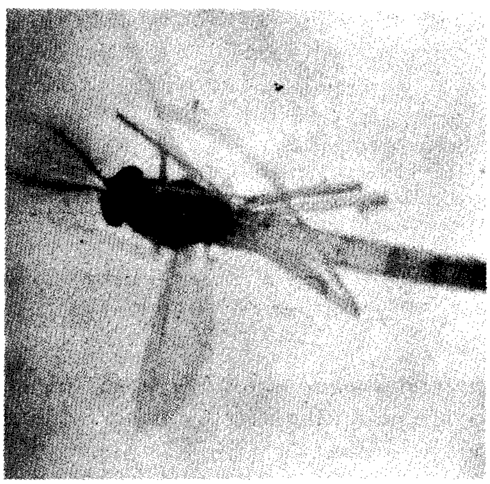
It is apparent from the data in table 11 that the monomolecular films did not affect the species consistently. In Adol 52 pens the reductions to the species ranged between 0 and 73.16 percent, while in T.A. 1618 treated pens the reductions ranged between 0 and 53.13 percent. Although exceptions can be seen in these data, the reductions usually were greatest for the smaller sized species. Size is a very difficult characteristic to measure on the species of Tendipedidae because of their marked sexual dimorphism. The average wing length was the only size measurement



Pentaneura monilis—MALE



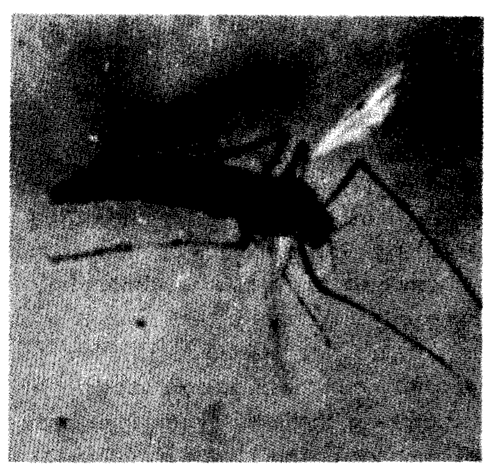
Pelopia stellatus—FEMALE



Tendipes subtendens—MALE

Tendipes subtendens—FEMALE

Corynoneura celeripes—MALE



Tendipes plumosus—FEMALE

FIGURE 9.—**Photographs of the sexual dimorphism exhibited by insects of the family Tendipedidae, College Lake, 1962. The sizes of the insects are not proportionate or comparable.**

TABLE 10.—*Comparison of the Reducing Effects of Monomolecular Films for the Emergence of Male and Female Insects of the Family Tendipedidae, College Lake, 1962*

Species	Adol 52						Chi-square	T.A. 1618						Chi-square
	Male			Female				Male			Female			
	U	T	PR	U	T	PR		U	T	PR	U	T	PR	
Corynoneura celeripes	390	258	33. 85	0	0	0	------	810	538	33. 58	0	0	0	------
Hydrobaenus spp	191	59	69. 10	148	32	78: 37	2. 1740	132	125	0	75	83	0	------
Prodiamesa spp	2, 984	937	68. 59	2, 037	630	69. 07	. 2349	2, 691	1, 755	34. 78	1, 874	1, 260	32. 76	0. 4274
Tendipes edwardsi	737	410	44. 37	701	363	48. 22	. 6974	199	194	0	225	211	0	------
Crictopus trifasciatus	176	76	56. 81	69	32	53. 62	. 0967	252	176	30. 16	50	32	36. 00	. 2294
Tendipes nervosus	87	57	34. 48	272	146	46. 32	1. 3468	125	54	56. 81	355	171	51. 83	. 4432
Calopsectra spp	875	554	36. 68	429	307	28. 43	1. 9919	1, 304	1, 071	17. 86	557	468	15. 97	. 1094
Tendipes monochromus	1, 109	526	52. 57	1, 589	837	47. 33	2. 4686	1, 594	784	50. 82	2, 157	981	54. 52	1. 8525
Tendipes fulvus	209	137	34. 44	137	94	31. 38	. 0693	119	153	+22. 22	86	114	+24. 56	. 0281
Pentaneura monilis	649	730	+11. 10	620	762	+18. 64	1. 3480	500	463	0	690	631	0	------
Pelopia stellatus	77	87	0	45	56	0	------	57	55	0	52	46	0	------
Tendipes subtendens	368	206	44. 02	161	102	36. 64	. 7602	301	292	0	166	178	0	------
Glyptotendipes lobiferus	166	90	45. 78	159	93	41. 50	. 1729	108	115	0	118	128	0	------
Tendipes plumosus	44	30	0	12	6	0	------	26	21	0	1	0	0	------

U=Total number of emergents from the untreated tent-covered pens.

T=Total number of emergents from the monomolecular-treated tent-covered pens.

PR=The percentage reduction or difference between U and T. If chi-square analysis of the type shown in app. A between the U and T totals for the sex of each species demonstrated a significant difference, the PR was computed; otherwise a 0 was indicated. A + indicates a significant difference between the U and T totals but, rather than a reduction of the insects in the treated pens, there was an increase.

Chi-square=Computed in the manner of app. B for the sex of each species when definite reductions were found for both sexes. A value of 3.841 is needed to show a significant difference in the reduction effects of the sexes.

found consistent enough between the sexes of a species to demonstrate the relative size of that species. The trend for greater reductions to smaller species can best be seen in the reductions of the species emerging from the pens treated with Adol 52. In addition, if one considers only those species which emerged in considerable numbers (over 1,000) the trend is much more pronounced. To exemplify this trend for the Tendipedidae insects, the data from table 11 were rearranged into three general size groups and are presented in table 12. It can be seen that in pens treated with Adol 52, Tendipedidae insects with average wing lengths less than 2.0 mm. were reduced 62.4 percent compared to a 43.5-percent reduction of the insects with wing lengths between 2.0 and 3.0 mm. and only a 6.0-percent reduction of the insects with wing lengths greater than 3.0 mm. Although this trend was not as pronounced for insects emerging from T.A. 1618 treated pens, the insects with wing lengths smaller than 3.0 mm. were more adversely reduced than those with larger wings.

Emergent aquatic insect laboratory studies.—The emergence of aquatic insects was studied in the laboratory by rearing the midge, *Tendipes* (*Tendipes*) *riparius* (Meigen) and mosquitoes (*Aedes* spp.) in untreated and monomolecular-treated 2-gallon fishbowls. These insects were used because of their abundance and availability in the Fort Collins, Colo. area. Thirty-two experiments, four control and four of each of the seven film materials, were run on both of these insects. In each midge experiment, a total of 30 larvae was used, whereas a total of 20 immature forms (10 larvae and 10 pupae) was used in each of the 32 mosquito experiments. The results of these experiments are summarized in table 13.

Analysis of variance tests demonstrated a highly significant difference between the treatments for the success of adult emergence of both midges and mosquitoes. A successful emergent is considered an adult which becomes airborne. Much of the treatment difference is attributed to the considerable differential in the success of the emergents between the untreated and

TABLE 11.—*Comparison of the Reducing Effects of Monomolecular Films for the Species of Insects, College Lake, 1962*

Species	Average[1] wing length (mm)	Adol 52		PR[2]	T.A. 1618		PR[2]	TRD[3]
		Untreated pens	Treated pens		Untreated pens	Treated pens		
Tendipedidae:								
Corynoneura celeripes	0.80	390	258	33.85	810	538	33.58	--------
Hydrobaenus spp	1.45	339	91	73.16	207	208	0	(4)
Prodiamesa spp	1.63	5,021	1,567	68.79	4,565	3,015	33.95	(4)
Tendipes edwardsi	1.86	1,438	773	46.25	424	405	--------	(4)
Crictopus trifasciatus	1.93	245	108	55.92	302	208	31.12	(4)
Tendipes monochromus	2.19	2,696	1,363	49.44	3,751	1,765	52.95	--------
Tendipes nervosus	2.50	359	203	43.45	480	225	53.13	--------
Calopsectra spp	2.80	1,304	861	33.97	1,861	1,539	17.30	(4)
Tendipes fulvus	3.00	346	231	33.24	205	267	+23.22	(4)
Pentaneura monilis	3.30	1,269	1,492	+14.94	1,190	1,094	8.07	(4)
Pelopia stellatus	3.35	122	143	0	109	101	0	--------
Procladius culiciformis	--------	3	1	0	2	3	0	--------
Tendipes subtendens	3.80	529	308	41.78	467	470	0	(4)
Glyptotendipes lobiferus	4.60	325	183	43.69	226	243	0	(4)
Tendipes plumosus	5.93	56	36	35.71	27	21	0	--------
Heleidae:								
Bezzia glabra	--------	56	74	0	48	31	0	--------
Bezzia opaca	--------	50	59	0	44	29	0	--------
Other Diptera:								
Chaoborous punctipennis	--------	10	8	0	3	5	0	--------
Parascatella melanderi	--------	35	31	0	42	23	45.24	--------
Odonata:								
Enallagma clausum	--------	10	6	0	1	1	0	--------
Ephemeroptera:								
Caenis simulans	--------	1	0	0	1	5	0	--------
Trichoptera:								
Agraylea multipunctata	--------	49	35	0	64	44	31.25	--------
Others	--------	8	6	0	4	7	0	--------
Totals	--------	14,661	7,837	46.54	14,833	10,247	30.92	(4)

[1] The average wing length of 10 specimens (5 of each sex) for the species indicated.

[2] PR=The percentage reduction or difference between untreated and treated pen totals (reducing effect). If chi-square analysis of the type shown in app. A between the untreated and treated totals for each species demonstrated a significant difference, the PR was computed; otherwise a 0 was indicated. A + indicates a significant difference between the untreated and treated totals but, rather than a reduction of the insects in the treated pens, there was an increase.

[3] TRD=Treatment differences to the species. Determined in the manner of app. B.

[4] Indicates a highly significant difference between the treatments. A dash indicates no significant difference was found between the treatment effects for that species.

monolayer-treated fishbowls. No significant difference was detected for the fishbowl interaction which indicates repeatable results for the various treatments. To determine specifically where the differences in the emergence success were between the eight treatments, a series of statistical tests using a procedure described by Duncan (1955) was employed and the results of these tests are also included in table 13. This table presents all of the possible comparisons of emergence success between the various treatments for both midges and mosquitoes. Data were arranged to facilitate comparisons of the differences in emergence success between the average of a particular treatment with that of other treatments. The figures in the right portion of the table are the numerical differences in the average number of successful emergents for each comparable treatment with respect to the average number of successful emergents for a particular treatment. For example, the 10.00 average number of midges which emerged successfully in the Micronized

TABLE 12.—*Comparison of the Reducing Effect (PR) of Monomolecular Films for Various Size Groups of Insects in the Family Tendipedidae, College Lake, 1962*

Wing length size group (mm)	Average wing length (mm)	Number [1] of species	Untreated pen emergence	Adol 52 pen emergence	PR [2]	Untreated pen emergence	T.A. 1618 pen emergence	PR [2]
Less than 2.0	1.58	5	7,433	2,797	62.4	6,308	4,274	30.7
2.0 to 3.0	2.46	4	4,705	2,658	43.5	6,297	3,796	39.7
Greater than 3.0	3.60	5	2,301	2,162	6.0	2,019	1,929	4.5

[1] *Procladius culiciformis* was not included because of lack of specimens.

[2] Percentage reduction (reducing effect)—obtained from $\frac{(\text{untreated}-\text{treated})}{\text{untreated}}$ 100.

TABLE 13.—*Emergence Difference Between Various Treatments for Midge and Mosquito Adults Reared in 2-Gallon Fishbowls*

Species	Particular treatment	A Average number successful emergents (4 bowls)	B Average (PR)	Numerical difference between average number emerging in any treatment with respect to a particular treatment.						
				MC	A 54	A 62	TA 1618	A 52	SPC	S 1820
Midges:										
Tendipes (*T.*) *riparius.*	Untreated	17.00	-------	[1] 7.00	[1] 7.75	[1] 9.00	[1] 9.00	[1] 9.25	[1] 11.50	[1] 15.00
	MC	10.00	41.2	-------	.75	2.00	2.00	2.25	[2] 4.50	[1] 8.00
	A 54	9.25	45.6	-------	-------	.75	.75	1.50	3.75	[1] 7.25
	A 62	8.00	52.9	-------	-------	-------	.00	.25	2.50	[2] 6.00
	TA 1618	8.00	52.9	-------	-------	-------	-------	.25	2.50	[2] 6.00
	A 52	7.75	54.4	-------	-------	-------	-------	-------	2.25	[2] 5.75
	SPC	5.50	67.6	-------	-------	-------	-------	-------	-------	3.50
	S 1820	2.00	88.2	-------	-------	-------	-------	-------	-------	-------
				SPC	MC	A 52	TA 1618	A 54	A 62	ST 1820
Mosquitoes:										
Aedes spp.	Untreated	18.75	-------	[1] 15.00	[1] 15.25	[1] 15.50	[1] 15.75	[1] 18.50	[1] 18.75	[1] 18.75
	SPC	3.75	80.0	-------	.25	.50	.75	[2] 3.50	[2] 3.75	[2] 3.75
	MC	3.50	81.3	-------	-------	.25	.50	[2] 3.25	[2] 3.50	[2] 3.50
	A 52	3.25	82.7	-------	-------	-------	.25	[2] 3.00	[2] 3.25	[2] 3.25
	TA 1618	3.00	84.0	-------	-------	-------	-------	2.75	[2] 3.00	[2] 3.00
	A 54	0.25	98.7	-------	-------	-------	-------	-------	.25	.25
	A 62	0	100.0	-------	-------	-------	-------	-------	-------	.00
	ST 1820	0	100.0	-------	-------	-------	-------	-------	-------	-------

[1] Highly significant difference.
[2] Significant difference.

A = 30 midge larvae in each bowl; 20 (10 larvae, 10 pupae) in each mosquito bowl; successful emergent means airborne adult. Error mean square = 6.00 for midges; 2.80 for mosquitoes.

$$B = (PR)\text{—percentage reduction: } \frac{\text{Untreated total} - \text{Treated total}}{\text{Untreated total}} \quad (100).$$

Cachalot (MC) treated fishbowls differed or was 7.00 midges less than the 17.00 average number of midges which emerged in the untreated fishbowls, the 9.25 emergents from the Adol 54 treated bowls was 7.75 emergents less than the untreated bowls, the 8.00 emergents from the Adol 62 bowls was 9.00 emergents less than the untreated bowls, etc.

Without exception, it can be seen in table 13 that the average number of successful emergents in monomolecular-treated fishbowls was highly significantly less than the emergents in untreated bowls regardless of whether the emergents were midges or mosquitoes. The magnitude of these differences or reductions in emergence is expressed as the percentage reduction (PR). Such reductions ranged between 41.2 and 88.2 percent depending upon the monomolecular treatment for the midges and between 80 and 100 percent for the mosquitoes. The reductions probably were greater to the mosquitoes because both the larva and the pupa of mosquitoes were in contact with the monolayer at the water surface much of the time while breathing, whereas the midge larvae were not in contact with the monolayer. This midge lives on the bottom in tubes that it constructs from debris and breathes through its integument by diffusion.

Some of the monolayer-forming materials were consistent in their effects to both the midges and the mosquitoes but most of the materials were inconsistent in their effects. For example, Stenol-PC (SPC) was significantly more adverse in reducing the midge emergence than was Micronized Cachalot (MC); however, inconsistency occurred when no difference could be detected in the reducing effects of these two materials for the mosquitoes. Other inconsistencies can be seen in table 13. A more consistent effect was observed for Stenol 1820 in that it produced the greatest reductions to both midges and mosquitoes. It should be pointed out, however, that statistically this material was not any more adverse in reducing the mosquitoes than was Adol 54 or Adol 62.

Cause for insect emergence reduction.—To analyze properly the cause-effect relationship for the reduction in the emergence of insects due to monomolecular films, especially in respect to the explanation for species and treatment differences, involves a detailed understanding of the insects' physiology and life history together with a thorough knowledge of the physical and chemical properties of the specific monomolecular film materials. At present, much of these data are only speculative because of their unavailability in the literature or because of lack of time and funds to determine many of these details in this study. This is particularly true for physiological details in the life history of the Tendipedid midges although some details are available in Miall (1895) and in the monograph by Thienemann (1954).

Adequate information appears to be available on the mosquitoes, possibly because of their greater economic importance. From information available in Bates (1949), Muirhead-Thomson (1951) and in particular, the long monograph by Christophers (1960), together with the specific works of Fair, Chang and Richart (1951) on mosquito larvae and the work of Manzelli (1941) on mosquito pupae, it was possible to construct a highly probable explanation of why monomolecular films reduce the success of the mosquito emergence. Since some of the midges are very similar to the mosquitoes both in structure and in physiology, it may be possible to interject the explanation for the mosquito reduction which will follow to some of the midge reductions. Probably the most logical place to begin is to analyze the effects monomolecular films have on the water surface because the film materials, being virtually insoluble in water, are found for all practical purposes only at the water surface and, consequently, the effects of the films to the insects should occur primarily when the insects are in contact with the treated water surface.

Natural and monolayer-treated water surfaces.—Water surfaces, like all liquid surfaces, have the hydrological property of surface tension. The surface tension of pure water varies inversely with temperature in that at 0° C. it is 75.64 dynes per centimeter, but at 100° C. it is only 58.85 dynes per centimeter. Almost any contaminating substance such as dust or powder, etc., coming in contact with the pure water surface depresses or reduces the surface tension of the water. Consequently, in nature, normal water surfaces have surface tensions less than would be expected at a given temperature because the surfaces are usually covered with natural films of one kind or another which result from the contaminating substances. The reduction in the surface tension due to these substances, natural or otherwise, is termed either film pressure, surface pressure, or surface tension depression by surface investigators. Hayes (1959) found that the surface tension depressions of 31 natural water surfaces in northern Colorado were less than 5 dynes per centimeter, except in protected locations in lakes and in paludal situations where the depressions reached 35 dynes per centimeter. These data agree generally with those of Adam (1937), Hardman (1941), and Goldacre (1949), who have measured natural surface tension depressions in other locations. The seven long-chain alcohol monolayer-forming materials tested in this study produced surface tension depressions which ranged between 33.5 and 41.1 dynes per centimeter (table 14). These depressions are considerably greater than the usual depression of less than 5 dynes per centimeter for natural water surfaces.

TABLE 14.—*Surface Tensions and Surface Tension Depressions of Untreated and Monolayer-Treated Distilled Water at 25.5° C.*

Treatment	Surface tension (dynes per centimeter)	Surface tension depression [1] (dynes per centimeter)	PD [2]
Untreated	71.9	0	0
Micronized Cachalot	30.8	41.1	57.1
Adol 52	31.9	40.0	55.6
Stenol 1820	34.6	37.3	51.9
Stenol-PC	37.0	34.9	48.5
Adol 62	37.6	34.3	47.7
T.A. 1618	38.4	33.5	46.6
Adol 54	38.4	33.5	46.6

[1] (Untreated surface tension − treated surface tension).
[2] Percent depression=

$$\frac{\text{Untreated} - \text{Treated surface tension}}{\text{Untreated surface tension}}(100)$$

or

$$\frac{\text{Surface tension depression}}{\text{Untreated surface tension}}(100)$$

Significance of surface tension in mosquito and midge life histories.—The normal high surface tension of water is used in a number of vital ways in the life histories of both mosquitoes and midges; however, since there are some important differences in the life histories of these insects, each life history will be discussed separately, the mosquitoes first.

A mosquito, being homometabolous in its development, completely changes its body form and function in its progressive transformation from the egg, to an aquatic larva, to an aquatic pupa, to an air-inhabiting, flying adult. All forms of the insect, including the adult, utilize the surface tension of the water at some time.

According to Milne and Milne (1948), females of ordinary biting mosquitoes literally lay a raft of eggs, which floats upon the water surface with only its lower surface wet. Although they do not mention the species which do this, egg rafts are most common among *Culex* spp. and *Aedes* spp. Mosquitoes of *Anopheles* spp. lay their eggs singly on the surface of the water. These eggs are provided with floats which prevent them from submersion; however, many mosquito eggs float upon the surface by utilizing the hydrofuge covering of the egg and the surface tension of the water. Christophers (1960) notes for *Aedes aegypti* eggs that, although they require submersion for the act of hatching, submersion before the eggs are ready for hatching may be fatal to the eggs. Eggs sunk immediately after oviposition may not even undergo the normal processes of swelling and darkening. This phenomenon has also been observed by Howard, Dyar, and Knab (1912).

The primary function of the aquatic larva in the overall development of the mosquito is growth. To accomplish growth, the larva must both feed and respire and, much of the time, it accomplishes these two functions simultaneously by "suspending" or "hanging" itself at the water surface. While in this position the larva obtains atmospheric oxygen through the spiracles of the siphon located at the posterior end of its body and feeds on small organisms associated with the surface film. However, since the larva is known to have a specific gravity greater than water, the only way it can suspend itself at the surface is to counteract the downward gravitational force of its body by utilizing the upward pulling force of surface tension (Fair, Chang and Richart, 1951). When disturbed, the larva closes the perispiracular valves, which are hydrofuge folds over the spiracles, and breaks away from the surface by a kicking movement. Much more detailed information regarding the function and structure of the perispiracular valves can be obtained from Keilin, Tate and Vincent (1935) and Watson (1941). While the larva is submerged it utilizes first the atmospheric oxygen which it had stored in its trachea and then it utilizes oxygen obtained through cutaneous respiration. Richards (1941) concluded that the survival of submerged mosquito larvae depends on cutaneous respiration, which in turn depends on at least seven factors: (1) Oxygen concentration; (2) carbon dioxide concentration; (3) temperature; (4) activity; (5) inherent differences in permeability between the cuticles of different species; (6) environmentally produced differences in cuticle permeability within a single species; and (7) size of the insect larvae.

The transformation which the mosquito must undergo in changing from an aquatic larva to an air-inhabiting, flying insect involves drastic changes in the entire body as well as the external form and is known as the pupal period. The kind of food which the adult will take is entirely different from that of the larva, so the alimentary canal undergoes a great change. Muscles for moving the legs, the wings, and the mouth parts must be formed and useless muscles of the larval stage eliminated. Many other changes must occur, one of the most important being a number of modifications of the breathing system.

Externally, the pupa seems to consist of a large, bulky saclike anterior portion and a flattened posterior abdomen. This anterior sac is usually termed the pupal shell, within which the adult mosquito is formed. The location of and the structures through which the mosquito now breathes has changed from the posterior siphon previously found in the larva to two anterior breathing trumpets. These trumpets, in their relationship with the surface tension, are important in maintaining the hydrostatic balance of the pupa. To understand fully this relationship; however, details of the pupal structure along with details of the passage of air within the pupa are necessary. The diagrams and descriptions which follow are those of Manzelli (1941).

The passage of air from the atmosphere to the living mosquito inside the pupal shell is rather complicated. Inside each trumpet shown in figure 10a, there is a breathing tube or trachea which runs along the inside wall of the pupal shell between it and the body of the adult, finally entering the adult through a breathing pore. This pore, or spiracle, is located on the wing-bearing segment of the thorax. On entering the spiracle, the tube joins the main respiratory system of the adult. Air is then distributed throughout the body of the adult by its normal breathing system.

Besides providing oxygen for the developing mosquito adult, the air taken in by the pupa enables the pupa to float at the surface of the water. A cavity exists between the bottom of the pupal shell and the underside of the adult's thorax (fig. 10b). Air enters this cavity from the breathing system of the adult by means of two spiracular openings, one at each side of the first abdominal segment.

The buoyant pupa normally rests at the surface, with the two trumpets protruding through the surface film of the water, and with the abdomen curved under the anterior portion of the body. When the pupa submerges, the trumpet is filled with an air bubble which is held there by the action of very small hairs within the trumpet. When disturbed, the pupa leaves the surface of the water and descends to the bottom. It overcomes its own buoyancy by whipping the water vigorously with its abdomen. This movement of the abdomen, however, produces pressure on the air content of the pupa. Unless a means were provided for closing the air tubes at such times, air would be forced out of the pupa by way of the trumpet and be replaced with water. This means for closing the air tube exists in the structure of the trumpet.

Simultaneous with the straightening of the abdomen, as it whips the water, the trumpets are bent back in a posterior direction, folding at the narrowed portion close to the pupal shell. As the trumpet bends back, the tracheal tube within it is pinched together, much as a rubber tube may be closed by sharply bending it. When the abdomen regains its normal position, the pressure on the trumpet is released, and the air tube is again opened.

The buoyancy of the pupa depends mainly upon the air cavity, and the pupa's center of buoyancy may be thought of as occurring within that cavity (fig. 10c). Since this cavity lies below the bulk of the body, there is a natural tendency for the body to be turned over in the water so as to bring the cavity uppermost. This action may be likened to a sealed can which contains a weight attached to one end and which floats in water. The can will always float with the weight down. If the can is inverted, so that the weight is uppermost, the can will at once return to its original position when released.

Since the pupa has, in effect, its weight uppermost, it faces a serious problem in maintaining itself in that position, for if it rolls over, it will be unable to keep the trumpets in communication with atmospheric air, and of course, it would then suffocate. The problem of keeping the weight uppermost is met by an interesting use of the trumpets. Figure 10c shows the position of the trumpets with respect to the air cavity. The center of buoyancy may be seen to lie between the two trumpets. The tips of the trumpets are maintained at the surface of the water by the action of the surface tension, or high surface-film strength of the water. Thus, when the air cavity tends to cause the pupa to turn over, the surface film would have to be broken in order to allow the trumpets to turn with it. This cannot occur as long as a strong surface film is maintained. Thus with a normal surface film, the pupa is sure of maintaining its equilibrium.

The above descriptions and diagrams by Manzelli (1941) are very general and probably are more characteristic for the pupae of *Culex* spp. and *Aedes* spp. than for other groups of mosquitoes. This is especially true in regards to the shape and structure of the breathing trumpets because many modifications of them are known to exist. Although it is known that the breathing trumpets of mosquito pupae are double-walled structures, this is seldom mentioned because in most species the two walls adhere closely to one another, and the trumpets commonly appear as simple thin-walled funnels leading direct to the spiracles. In some species, however, the walls are separated over a part of their area, leaving a space between. This may be a simple annular space as in *Harpagomyia,* or much more complex as in some groups of *Anopheles.* The outer wall of the trumpet is hydrophil (wettable) and the inner wall is hydrofuge (nonwettable). In species of the *Anopheles umbrosus* group, the trumpet has a tragus which may be thought of as an outgrowth of the posterior rim of the trumpet. The back or outer surface of the tragus in living pupae floating normally in water has been observed by Reid (1963) to be completely wetted while the inner surface of the tragus has remained dry. This outer surface is an extension of the outer wall of the trumpet which is hydrophil, while the inner surface of the tragus is part of the hydrofuge inner wall of the trumpet. In *Anopheles umbrosus,* although the tragus stands erect above the level of the water surface, the water film apparently extends to its tip. In fact, it seems possible that it is the surface tension of this water film which automatically pulls the tragus erect when the pupa comes to the surface and the

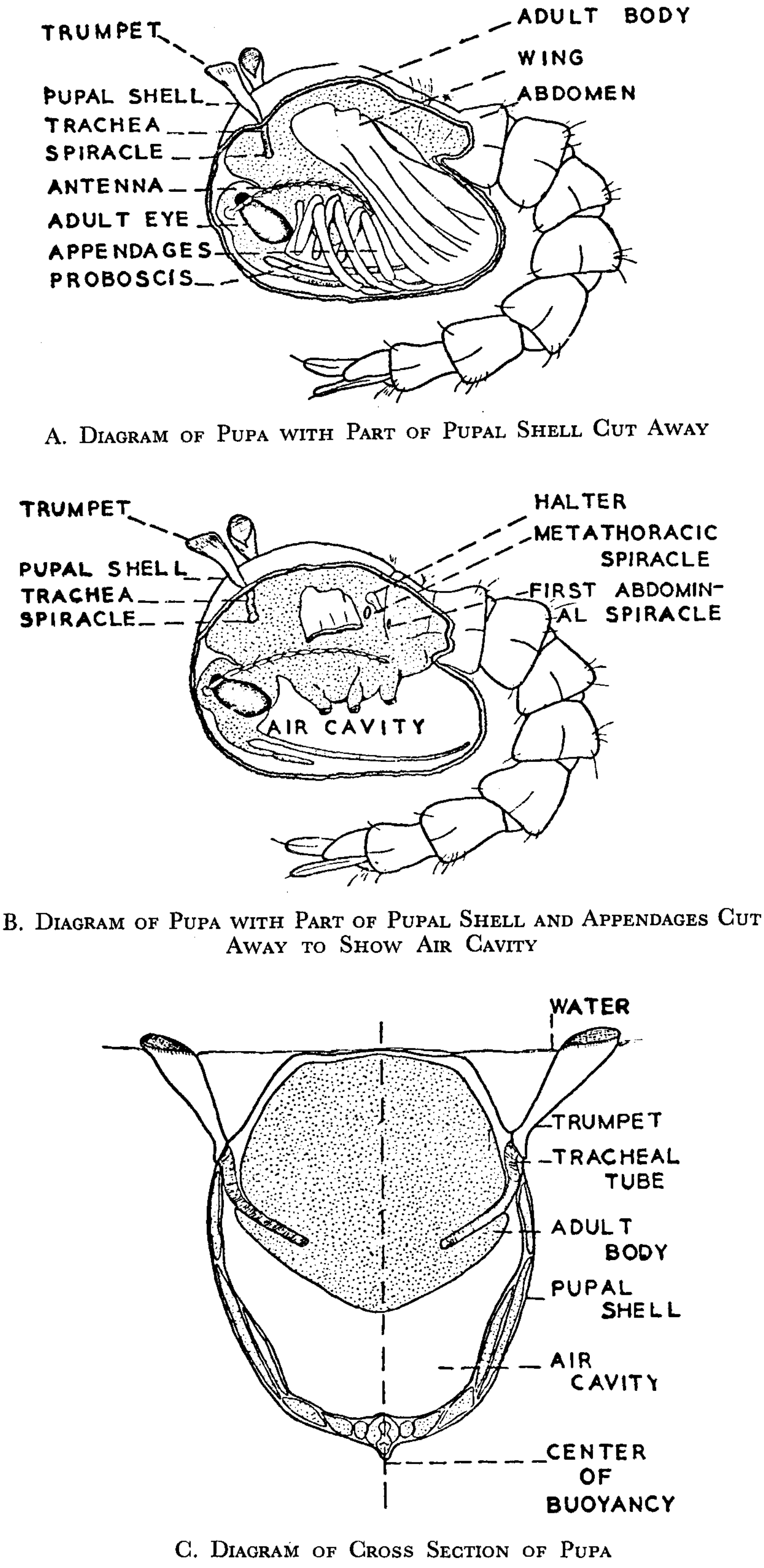


FIGURE 10.—Important structural and physical characteristics of mosquito pupae (after Manzelli, 1941).

trumpets expand. However, pupae seem able to make flicking movements of the trumpets, and this suggests the existence of some mechanism that might also play a part in erecting the tragus. In the commoner simple type of anopheline trumpet with a single split, expansion of the trumpet at the surface of the water is undoubtedly caused by surface tension on the hydrophil outer wall (Reid, 1963).

The process that the adult mosquito within the pupa uses when it emerges has been adequately described by Howard, Dyar and Knab (1912), Bates (1949), and Christophers (1960) and need not be repeated here. It will suffice to say that the adult uses the pupa as a dry, floating platform or "boat", from which it can become airborne. The pupa actually thrusts its prothorax through the surface film so as not to wet the adult while it emerges. No doubt, the pupa utilizes surface tension to maintain its stability while in this position; otherwise, it would tip over and the adult would be wetted. The new adult utilizes both the surface tension of the water and the floating pupal shell for leverage and support while emerging from the pupa. The adult again utilizes the surface tension for support during resting periods or when, in the case of the female adult, it lays the eggs on the surface of the water.

The significance of surface tension is not understood as fully in the life history of the midges as it is for the mosquitoes. The midge, like the mosquito, is homometabolous in that it transforms from an egg to an aquatic larva, to an aquatic pupa, to an air-inhabiting, flying adult. Despite this, however, there are some differences in structure, physiology, and habits of the developmental stages which come in contact with the water surface and further explanation is therefore necessary.

Many details in the egg-laying process of midges vary. Eggs may be deposited by the females singly, in clumps, or in gelatinous masses, most of which usually sink to the bottom or attach to submerged objects where the embryos develop. There are, however, a few midges whose egg masses float freely near the surface but it is not necessary for these eggs to float upon the surface as is the case for some mosquitoes. The brief description by Needham and Lloyd (1937) for *Chironomus meridionalis* (*Tendipes anthracinus*) should exemplify the utilization of the surface tension of the water in the egg-laying process for most midges. The following description has been slightly modified by the present author.

The adult female rests on some vertical stem by the water side and extrudes the eggs in a rounded clump of gelatin at the tip of her abdomen. She then flies over the water, coursing back and forth in nearly horizontal lines at shoulder height above the water surface often as long as 20 minutes before she finally settles down on the water surface with outspread feet to rest again. The elongated tarsi of her legs rest like outriggers radiately arranged upon the water surface, easily supporting her weight because of the upward pulling force of the surface tension. While in this position, she liberates the egg mass and lets it down into the water. At the top of the egg mass is a circular transparent disc which catches upon the surface film. This disc is pulled down into the surface film in a little rounded pitlike depression by the weight of the eggs. The eggs slowly descend, pulling out the gelatin attaching them to the disc into a slender thread that becomes stretched to a length of several inches. While the floating egg mass is being transported by waves and currents, the thread may break and the eggs would then settle to the bottom where the embryos will develop. The egg mass could also adhere to floating stems against which it is tossed, but in any event the egg embryos develop while completely submerged. Consequently, the surface tension of the water is not vital to the development of the embryos as it is in some mosquitoes, but it does aid in the dispersion of a species within a particular body of water. This is at least true for those species which have the disc attached to their egg mass.

Midge larvae vary in their habits. The larvae of some species may be free-swimming but most species construct tubes within which the larva normally is found living. These tubes may be found either in the bottom muds or on submerged vegetation. Berg (1950) has described several species associated with *Potamogeton* weeds. Regardless of where Tendipedid larvae live, Keilin (1944) points out that they all are apneustic. Apneustic is a term applied to a closed respiratory system and denotes the lack of functional spiracles. Atmospheric oxygen is not utilized. The oxygen requirements of the larvae are obtained exclusively by diffusion across the cuticle (cutaneous respiration) and the larvae may be considered water breathers. Midge larvae, therefore, are seldom associated with the water surface and probably do not utilize the surface tension of the water. The time a particular species spends as a larva may vary between a few weeks and 2 years, but according to Ford (1959), all fresh-water Tendipedid larvae undergo four metamorphic molts termed instars. Metamorphosis of fourth-instar larvae results in the pupae.

Tendipedid pupation normally occurs inside the larval tube or case and seldom lasts longer than 3 days. Those species with free-swimming larvae also have free-swimming pupae. The respiration of midge pupae is normally apneustic; however, in those species which have free-swimming pupae, the respiration usually is propneustic. Propneustic refers to an open respiration system with a functional spiracle located in each of the two anterior trumpets or horns. This is the type of respiration found in mosquito pupae. Many modifications in the respiration structures are found in the midge pupae regardless of whether they are apneustic or propneustic. Some apneustic pupae have no respiratory structures (abranchiate), while others have either horns or filaments. The horns are similar in shape to those of propneustic pupae, but they do not have open stigmata with functional spiracles. Other apneustic pupae have respiratory filaments or tufts. According to Thienemann (1954), these filaments are genuine tracheal gills. The general habits and respiration adaptations of larvae and pupae of College Lake midges are summarized in table 15. The larvae and pupae of some midges were not observed in this study and their habits are indicated with a question mark.

For adult midges to become airborne, the pupae must come to the water surface. The period of time the pupae are at the surface is extremely variable. Propneustic pupae may be at the surface only periodically, as are those of most midges in the subfamily Tanypodinae (*Pentaneura, Pelopia, Procladius,* etc.), or they may be at the surface almost constantly, as are biting midge pupae of the family Ceratopogonidae (*Palpomyia, Bezzia,* etc.) and phanthom midge pupae (*Chaoborus*). Those pupae in the Tanypogonid group are very active, much like the mosquito pupae which submerge at the slightest disturbance. Morgan (1949) found that many species of this group were bottom dwelling during most of the pupal period, apparently only coming to the surface to replenish oxygen requirements. She noted further, however, that before emergence occurred, the pupae often became more active and moved about in the water, eventually becoming stationary under the surface film where they remained motionless for some time, "hanging" by their respiratory trumpets.

"Hanging" by the trumpets has also been reported by Thienemann (1954) for the inactive ceratopogonid

TABLE 15.—*General Habits and Respiration Adaptations in the Larva and Pupa of Midge Insects, College Lake, 1962*

Species	Larva			Respiration	Pupa		Respiration			
	Free-swimming	Live in tube or case—			Free-swimming	In tubes or cases	Apneustic [1]		Propneustic [2]	
		On bottom	On vegetation				Filaments	Horns	No horns or filaments	Trumpets or horns
Corynoneura celeripes			X?	A [1]		X?			X?	
Hydrobaenus spp			X?	A		X?		X?		
Prodiamesa spp			X?	A		X?		X?		
Tendipes edwardsi		X		A		X	X			
Crictopus trifasciatus			X	A		X		X		
Tendipes monochromus		X		A		X	X			
Tendipes nervosus		X		A		X	X			
Calopsectra spp			X	A		X		X		
Tendipes fulvus			X	A		X	X			
Pentaneura monilis	X			A	X					X
Pelopia stellatus	X			A	X					X
Procladius culiciformis	X			A	X					X
Tendipes subtendens			X	A		X	X			
Glyptotendipes lobiferus			X	A		X	X			
Tendipes plumosus		X		A		X	X			
Bezzia glabra	X			A?	X					X
Bezzia opaca	X			A?	X					X
Chaoborous punctipennis	X			A	X					X
Tendipes riparius		X		A		X	X			

[1] A = Apneustic—no functional spiracles—cutaneous respiration.
[2] Propneustic—function spiracles located in trumpets or horns (atmospheric air breathers).
?—Not observed in this study.

pupae which are at the water surface almost constantly. When the water surface is disturbed slightly, these pupae sink temporarily, but rise again passively without swimming motions since they are a little lighter than water. No doubt, the "hanging" phenomenon sometimes involves the pupae utilizing the upward pulling force of surface tension. This is especially true if pupation occurs in open water before the pupae have an opportunity to build up their buoyancy. Weerekoon (1953), experimenting with *Palpomyia quadrispinosa* and *Bezzia* spp., has shown that contact of the respiratory trumpets with the air has to be maintained for almost a quarter of an hour before these pupae become buoyant enough to float in water. It should be pointed out, however, that the pupation of these ceratopogonid midges normally occurs outside the lake where contact with the air is assured.

Effects of surface tension reduction.—Before presenting the effects due to surface tension reduction, some of the physical factors affecting the relationship of an insect (solid) in contact with water (liquid) at the water surface must be discussed. When a substance in contact with water attracts water molecules more strongly than water molecules attract each other, the water wets the surface of the substance. The liquid creeps along, invading every crevice, clinging tightly to each irregularity. However, on various parts of an insect's body there usually are materials such as waxes, oils, or lipids, etc., sometimes in the form of thin monolayers which attract water molecules so little that the water draws away, pulling back into itself and leaving some portion of the surfaces of these insect parts comparatively dry.

Regardless of whether the water rests upon these insect parts, or the parts dip into the water, the water-air interface usually meets the insect part-air interface at a definite angle which usually is a physical constant for the part of the particular insect concerned. This angle, measured in the water, is the contact angle. Most authors consider that the contact angle depends on the adhesion forces between the water and the insect part. These forces are influenced by the surface tensions of the three interfaces involved (insect part-air, insect part-water, and water-air). Details of the mathematical considerations of these forces in their relationship with the contact angle have been adequately described by O'Kane, Westgate, Glover and Lowry (1930), Ebeling (1939), Adam (1941), Fair, Chang and Richart (1951), Holdgate (1955), etc., and will not be discussed here.

Water strider experiments.—Best known of all the insects which utilize the surface tension of water are the water striders. These insects, which walk dry-shod on pond and stream surfaces, have four long legs stretching out to their sides and two legs held under their head. Their slender feet are covered with greasy hairs which the water fails to wet. Each foot presses the water surface and makes a dimple in it, but the water does not run around and let the foot fall through the surface film as it would if the waxy hairs were absent. Instead, the insect's weight is supported partly by the bouyant force of the water displaced from the dimples and partly by the surface tension, which tends to erase the depressions and bring all the water film to the same level.

According to Milne and Milne (1948), a considerable length of surface must be called upon to support an insect as heavy as a full-grown water strider. If its hair-booted feet pressed on the film at only six small points, the insect would penetrate into the water and sink at once. However, the striders' legs are spread so widely that they make elongated dimples or furrows in the water film which aid in preventing the insect from sinking.

Theoretically, when a solid such as an insect like a water strider with a higher specific gravity than water is placed on a water surface, the insect will float on this surface if the contact angle is greater than 0°, that is, if the insect is not wetted and if that portion of the weight of the insect that is unbalanced by the weight of the displaced water can be supported by the surface tension of the water. For instance, if a paraffin-coated needle is placed on a water surface, the needle will float and the water surface under the needle will be depressed to a point where the vertical component of the surface tension becomes great enough to support the unbalanced weight of the needle. If the weight of the needle is increased, or the contact angle is decreased, the needle will sink in the water until a new position of equilibrium is established. However, when the surface tension of the water is lowered, the wetting property of the water is increased and both the lifting force of the surface tension and the contact angle are decreased. Furthermore, if the contact angle is decreased to 0°, it is impossible to generate any lifting force by surface tension and the needle will sink.

Hardman (1941) reported preliminary unpublished notes of G. Evelyn Hutchinson dealing with the effect of reduced surface tension on surface skating insects. Hutchinson showed that *Hydrometra* and a gerrid behaved differently toward reduced surface tensions resulting from the addition of ethyl alcohol to water. At a surface tension of about 50 dynes per centimeter (normal water is about 72 dynes per centimeter) the gerrid fell through the surface film with legs held rigid in the normal position; whereas, *Hydrometra* first collapsed, lying flat on the water surface.

Differences in behavior of insects to reduced surface tensions probably result from differences in the contact angles between species and between individuals of a species. Ebeling (1939) reported that not only does the contact angle of a given liquid vary greatly on leaves from different plant species, but also on leaves of different ages of a single species, or on different surfaces of a single leaf.

Since water striders were found by Hutchinson to sink when the surface tension was reduced to about 50 dynes per centimeter, it certainly can be expected that water striders would also sink when the surface tension is reduced even further. It is not surprising, therefore, that Hayes (1959) found that water striders drowned in water treated with hexadecanol, a long-chain alcohol monolayer-forming material which reduces the surface tension of the water to about 32 dynes per centimeter.

In the present study, experiments were conducted on how long it would take gerrid water striders to drown in monolayer-treated fishbowls. The results of these experiments are presented in table 16 and show that all water striders drowned in monolayer-treated water in less than 7 minutes. It is interesting to note that there is a correlation between the time required for drowning and the surface tension

TABLE 16.—*Relation of Drowning Time of Water Striders to the Surface Tension of Untreated and Monolayer-Treated Water*

Treatment	Surface tension (dynes per centimeter)	Drowning time [1] (minutes)
Untreated	71.9	(2)
Adol 54	38.4	6.9
T.A. 1618	38.4	6.5
Adol 62	37.6	5.5
Stenol-PC	37.0	5.0
Stenol 1820	34.6	2.5
Adol 52	31.9	1.8
Micronized cachalot	30.8	1.5

[1] Average time in minutes for 3 water striders to drown (completely submerge) in treated fishbowls.

[2] None drowned.

of the treated water. For example, there is a successive decrease in the time needed to drown a water strider when the surface tension is lowered. In other words, the lower the surface tension the faster the water strider drowns.

Mosquito experiments.—It has previously been pointed out that all developmental stages of mosquitoes utilize the surface tension. Fair, Chang, and Richart (1951) working with *Anopheles quadrimaculatus* mosquito larvae, which have a specific gravity greater than water, postulated and proved that there was a critical value of the surface tension of water at which the contact angle was 0°, and below which the larvae sank. By using various amounts of Aerosol-OT (dioctyl sodium sulfosuccinate) to reduce the surface tension they found this critical value of the surface tension of the water to be about 30 dynes per centimeter. Below this value the larvae were unable to "hang" themselves at the water surface where they accomplish normal respiratory and feeding functions.

At surface tensions between 30 and 35 dynes per centimeter the larvae hung themselves at the water surface for about 15 minutes before their respiratory apparatus became flooded and the larvae sank. Surface tensions above 39 dynes per centimeter were sufficient for the larvae to float at the water surface and accomplish both the normal respiration and feeding functions.

The behavior of several species of anopheline mosquito larvae under reduced surface tensions was also studied by Russell and Rao (1941) and they concluded that reduction in surface tension is directly responsible for drowning of larvae. Because small differences in the behavior between individuals of the same species were observed, they expressed the minimum surface tension at which the larvae drowned in a short critical range between 36 and 27 dynes per centimeter rather than as a precise critical point. This critical range was based upon the highest surface tension when the drowning effect was just visible, that is, when at least one larva of a particular species was drowned, and the lowest surface tension in which the effect was just complete, that is, when all larvae for that species were drowned. No significant difference was found between the critical ranges for the species studied, namely: *Anopheles annularis, barbirostris, culicifacies, hyrcanus, subpictus,* and *varuna.* Furthermore, they added that preliminary studies indicated the minimum surface tension required for culicine larvae was not much different from that found for the anopheline larvae.

Although Russell and Rao (1941) do not mention it, there was somewhat of a general trend in their data for successive increased drowning of larvae as the surface tension was lowered below 37 dynes per centimeter. For example, at a surface tension of 37 dynes per centimeter none of the *Anopheles culicifacies* larvae drowned, but at 36 dynes per centimeter 14 percent of these larvae drowned. The percentage of drowned larvae was 18 percent at 34 dynes, 47 percent at 33 dynes, 55 percent at 32 dynes, 33 percent at 31 dynes, 81 percent at 30 dynes, 94 percent at 29 dynes, 99 percent at 28 dynes, and 100 percent at 27 dynes per centimeter. This same general trend, much like that observed in the present study for water striders, was also apparent with *Anopheles subpictus* and *Anopheles annularis.*

The tests conducted on the rearing of *Aedes* mosquitoes in monolayer-treated fishbowls did not show the expected trend of increasing larval mortality with decreasing surface tension (table 17). In this table, the monomolecular-film treatments are arranged in order of decreasing surface tension. It is apparent that neither the larval mortality (col. C) nor the pupal mortality (col. F) increased as the surface tension of the film-treated waters decreased. In fact, no direct correlation existed between the surface tension of the monolayer-treated water in the fishbowls and the mortality of *Aedes mosquitoes.* Consequently, the reduction in surface tension resulting from monomolecular film application to water, although a definite factor, cannot be the exclusive cause for the mortality of these mosquitoes. Other factors which probably influenced their mortality will be discussed later in this chapter.

The fact that the pupal mortalities (col. F, table 17) were consistently greater than the larval mortalities (col. C) for any given film material was not unexpected. Mosquito larvae are capable of submersion for longer periods than the pupae because they can utilize oxygen from the water when pupae cannot. As a result, pupae usually are in contact with the film material at the water surface for longer periods than the larvae and the effects of the films to them should normally be greater. Besides this, the upsetting of the pupae's hydrostatic balance due to the reduction in the surface tension of the water caused, for example, by the application of the film materials results in the pupae drowning sooner than the larvae.

Manzelli (1941) found that all of the *Culex* mosquito pupae tested had drowned when the surface tension was reduced to about 36 dynes per centimeter, the surface tension at which Russell and Rao (1941) found that only 14 percent of anopheline larvae had drowned. Furthermore, Singh and Micks (1957) reported that none of the pupae of five mosquito species, *Aedes aegypti, Culex fatigans, Culex molestus, Culex pipiens,* and *Anopheles squadrimaculatus,* emerged successfully when the surface tension was reduced to 41 dynes per centimeter or less. At 41 dynes per centimeter they found that the emergence of some

TABLE 17.—*Comparison of the Development of Aedes Mosquito Larvae and Pupae Reared to Adults in Untreated and Monolayer-Treated Fishbowls*

Treatment	Surface tension of water (dynes per centimeter)	A[1] Number of larvae tested	B Number of dead larvae	C Percent larval mortality B/A(100)	D[1] Number of pupae tested 40+(A−B)	E Number of dead pupae	F Percent pupal mortality E/D(100)	Number of trapped adults	G Number of airborne adults	H Percent airborne adults G/80(100)
Untreated	71.9	40	0	0	80	2	2.5	3	75	93.8
A 54	38.4	40	12	30.0	68	60	88.2	7	1	1.3
T.A. 1618	38.4	40	8	20.0	72	50	69.4	10	12	15.0
A 62	37.6	40	24	60.0	56	54	96.4	2	0	0
SPC	37.0	40	11	27.5	69	50	72.5	4	15	18.8
ST 1820	34.6	40	20	50.0	60	58	96.7	2	0	0
A 52	31.9	40	5	12.5	75	54	72.0	8	13	16.3
MC	30.8	40	16	40.0	68	50	73.5	0	14	17.5

[1] 40 larvae and 40 pupae were initially introduced into the fishbowls for each treatment. The larvae which did not die (A−B) metamorphosed into pupae and were consequently added to the original 40 pupae to result in the number of pupae subjected to each treatment in D.

species occurred although it was incomplete, but at 31 dynes per centimeter total pupal mortality occurred to all species. They did not test the emergence at surface tensions between these two points.

It is apparent in table 17 that all of the monolayer-forming materials reduced the surface tension of the fishbowl water below the 41 dynes per centimeter critical point found by Singh and Micks (1957) for mosquito pupae. Yet, it can also be seen in column G that some *Aedes* mosquito pupae emerged sucecssfully at these lower surface tensions. The explanation for the above phenomenon may be found in the interrelationship of the following two factors. First, the *Aedes* mosquitoes used in these experiments could have been more tolerant to reduced surface tensions than those used by Singh and Micks; and second, the surface tensions indicated in table 17 which were taken only at the start of the experiments may have increased due to bacterial attrition of the films during the rearing of the mosquitoes. Chang, McClanahan, and Kabler (1962) have shown at least for a hexadecanol-treated water surface that the surface tension increases (film pressure decreases) when *Pseudomonas* and/or *Flavobacterium* which are common bacteria to surface waters attack this monomolecular film material.

Toxic influences.—Although not tested in this study, the monolayer-forming materials probably enter into the larva and pupa respiratory system when they are at the treated water surface for breathing. Flooding of this water into the system probably occurs because of increased wetting due to surface tension reduction by the film materials. Fair, Chang and Richart (1951) mention that flooding of water into the respiratory apparatus of anopheline larvae occured when the surface tension of the water was reduced to between 30 and 35 dynes per centimeter. Three of the seven materials tested in this study reduced the surface tension within this range and the other materials were not more than 3.4 dynes above this range.

No doubt, water enters into mosquito pupae when their hydrostatic balance is upset by surface tension reducing agents; otherwise, they would not drown. Manzelli (1941) noted that it may be thought that the inability of the pupae to maintain their equilibrium when the surface tension is reduced to about half is due to some toxic effect of the chemical employed in reducing the surface tension. This, however, Manzelli points out is improbable in view of the fact that pupae removed after a short time from such treated water behaved normally after being placed in fresh water, and later emerged as adults. Manzelli added, however, that although the initial effect of the wetting agent on the pupae is due to the reduction of the surface tension, chemical toxicity may occur if the concentration of the agent is sufficiently high. He found that all pupae placed in soap-water dilutions ranging between 1–100 and 1–1,000 lost their equilibrium at once and died if left in these solutions. When some of these pupae were transferred to fresh water after being in the various dilutions for only 3 minutes, they appeared to act normal but after 12 hours those from the 1–100 and 1–200 dilutions had died, while the pupae from 1–400 and 1–1,000 dilutions were normal. Such experiments have also been conducted by Senior-White (1943), but he found toxic effects to pupae in soap-water dilutions weaker than 1–200.

In the present study, toxicity experiments of the type described above were not conducted with the monolayer-forming materials. However, the film-treated, fishbowl, mosquito-rearing experiments seem to indicate that film materials which contain alcohols higher than C 18 are more lethal to *Aedes* mosquitoes than those which do not contain such alcohols. This is true regardless of how much the specific material reduces the surface tension of the water (table 18).

In table 18, the seven film materials are divided into two groups: Group I being composed of four different materials all of which contain alcohols higher than C 18; and group II being composed of three different materials which do not contain alcohols higher than C 18. It can be seen that 36 percent (22 percent C 20, 14 percent C 22) of the alcohols which make up the ST 1820 film material, 15.8 percent of A 62, 8 percent of A 54, and 4 percent of MC are of alcohols higher than C 18. Group II materials do not contain such alcohols.

The pupal and larval mortalities incurred with group I materials were consistently higher than those of group II. For example, the pupal mortalities ranged between 73.5 and 96.7 percent with group I materials, but were consistently less with group II materials in that these ranged between 269.4 and 72.5 percent. The same can be said for the larval mortalities although the percentages are not as high. The pupal mortalities seem to be closely correlated with the percentage of C 20 in the film materials. The materials with larger amounts of C 20 yield greater pupal mortalities. It becomes apparent, therefore, that the mortality of *Aedes* pupae and larvae in the monolayer-treated fishbowls is probably due in part

TABLE 18.—*Relation of Mosquito Larval and Pupal Mortality to the Surface Tension and Carbon Values of Long-Chain Alcohol Monolayer-Forming Materials*

Film material	Percent pupal mortality	Percent larval mortality	Surface tension[1]	Percent carbon value of alcohol constituents—									
				C 12	C 14	C 15	C 16	C 17	C 18	C 19	C 20	C 21	C 22
Group I:													
ST 1820	96.7	50.0	34.6	------	------	------	------	------	64.0	------	22.0	------	14.0
A 62	96.4	60.0	37.6	------	0.1	------	8.8	2.0	72.3	1.6	10.0	1.9	3.3
A 54	88.2	30.0	38.4	0.1	3.7	0.6	42.6	3.2	41.7	2.9	5.1	------	------
MC	73.5	40.0	30.8	------	4–6	------	44–49	------	43–46	------	4.0	------	------
Group II:													
STPC	72.5	27.5	37.0	------	------	------	50.0	------	50.0	------	------	------	------
A 52	72.0	12.5	31.9	.1	2.4	.8	89.3	3.2	4.2	------	------	------	------
T.A. 1618	69.4	20.0	38.4	------	2.0	------	32.5	------	65.5	------	------	------	------

[1] Surface tension of film-treated water in dynes per centimeter.

to alcohol constituents higher than C 18 being in the materials.

The mortalities cannot be the exclusive result of drowning due to surface tension reduction because, if this was true, one would expect to find the greatest mortality with materials such as MC and A 52 which reduce the surface tension the most. Such a relationship was not found. In fact, the least mortality to the larvae, for example, was observed in the A 52 treated fishbowls.

Midge experiments.—The analyzed laboratory data presented in table 13 leaves little doubt that monomolecular films adversely reduced the emergence of the midge, *Tendipes riparius.* The same can be said for many of the midge species tested in the field pens in College Lake (table 11). However, the explanation for these effects is still rather speculative. Little appears to be known on how surface tension reducing agents affect these insects; in fact, very few details are known on how and when these insects utilize the surface tension of the water. Those that are known to the present author have already been discussed.

The explanation of the effects which have just been described for the mosquitoes do not apply to the midges in every respect. Midge larvae do not utilize the surface tension and, probably because of this, no difference in the development of *Tendipes riparius* larvae in untreated and monolayer-treated fishbowls was found. Consequently, the effects of the films to these midges must occur either and/or during the pupal stage, during the process of emergence from the pupa when the female midge returns to the surface to lay its eggs, or during resting periods of the adult.

Mortalities to *Tendipes riparius* occurred during each of these periods but they were consistently less than those found with the mosquitoes. Like the mosquito mortalities, these midge mortalities were greatest during the pupal stage, but the trend found in the mosquito experiments for greater pupal mortality with increased amounts of alcohols higher than C 18 in the film materials was not as pronounced in these midge experiments. Again, the pupal mortality with the ST 1820 film material was the greatest; however, the other materials did not produce midge mortalities correlated with the amounts of alcohols higher than C 18.

The exact explanation for the mortality of *Tendipes riparius* midge pupae is not fully understood. Paralleling the explanation for the mosquitoes to that for these midges appears futile because *Tendipes riparius* pupae are both morphologically and physiologically different from mosquito pupae. They have respiratory filaments by which they utilize oxygen from the water (apneustic breathers) in contrast to the propneustic mosquito pupae which utilize atmospheric oxygen by means of two respiratory trumpets.

It has previously been shown that these two trumpets in their relationship with the surface tension of the water are paramount in maintaining the hydrostatic balance of the mosquito pupa. Such a relationship, however, does not appear to exist for *Tendipes riparius* midge pupa. Their respiratory filaments probably are the only structures this midge pupa could possibly utilize in the manner the mosquito pupa utilizes its two trumpets for balance, and Branch (1923) has shown that if these respiratory filaments break through the surface of the water before the close of the pupal stage the pupa cannot extricate itself and dies.

Normally, the respiratory filaments are held below the surface but if they happen to break through, they remain above the water surface due to the action of the surface tension around them. This action tends to prevent the pupa from freeing its filaments, thereby preventing it from obtaining oxygen from the water and death results. Factors closely associated with this phenomenon may be the most likely explanation for the *Tendipes riparius* pupal mortalities in monomolecular-treated fishbowls; however, more details regarding the habits of these pupae during their development are necessary to understand fully this phenomenon.

Tendipes riparius pupae normally lie upon their sides on the bottom, moving only slightly during the first 2 days of the pupal period and consequently do not come in contact with the water surface at this time. Usually, it is not until the third day that they become extremely active in swimming and/or floating and come in contact with the water surface. At this time the pupae usually hold their thorax upright and usually keep their respiratory filaments below the surface; otherwise, as just explained, they would die.

During the third day the cuticles of the pupae become transparent and the bodies of the adults within may be seen. Branch (1923) reports that the pupae now start to swim with their body in a horizontal position just under the water surface. Apparently, the pupae at this time either are already buoyant or are in the process of building up their buoyancy. Lewis (1957) points out that in similar *Tanytarsus* pupae which are about to emerge, a film of gas forms beneath the cuticle. This gas is believed to be the result of the absorption of the moulting fluid (Wigglesworth, 1953).

Although Branch (1923) did not say definitely that *Tendipes riparius* pupae were all buoyant when in the horizontal position at the surface during the third day, she did note that after swimming or floating in this position for about 1 day, the pupae suddenly pushed their respiratory filaments through the surface, stretched out upon the surface, and became quiet. Emergence of the adults would soon follow.

Observations in the laboratory indicated that most of the pupal mortalities of *Tendipes riparius* in monolayer-treated fishbowls occurred during the third day prior to the emergence attempt. If the pupae were nonbuoyant at this time, no doubt they could have accidentally pushed their respiratory filaments through the treated surface more easily due to the reduced surface tension, but death should not have resulted because it also should have been easier for these nonbuoyant pupae to free themselves due to downward gravitational forces. Conversely, buoyant pupae, which have a tendency to push against the water surface from below, may have found it more difficult to keep their respiratory filaments below the water surface, thus causing their death.

No doubt, other factors could have influenced the pupal mortalities. The probability that these mortalities simply could have been the result of the film materials clogging the respiratory filaments and/or a toxic influence from film-treated water entering into the respiratory system should not be overlooked. Entrance of film-treated water could have been difficult in the closed apneustic respiratory system of these midge pupae, but despite this, such water may have entered this system by osmotic invasion. Beament (1961) has shown that, when certain aquatic insects were placed in water of higher temperature than normal, osmotic invasion of water into their system occurred and resulted in their death. It is well established that monomolecular film materials increase the temperatures of the surface waters but it is not known if these increases are great enough to cause osmotic invasion of water into the midge pupae.

Mortalities to *Tendipes riparius* adults in monolayer-treated water regardless of whether the mortalities occurred while the midges were emerging, egg laying or resting, seemed to follow the explanation given for the sinking (drowning) of water striders. Only a few of these midges sank through monolayer-treated water while emerging. The reason that most of these midges did not sink is that the adults of this species usually flew straight up from the pupal skin and consequently did not come into contact with the treated water surface. However, those few that did sink, actually stepped off the pupal skin onto the treated water surface before they attempted to fly away. With their widespread legs slowly sinking, some were unable to free themselves by flying up from the treated water surface and they drowned. Lewis (1957) observed somewhat the same phenomenon with *Tanytarsus* midges emerging from water treated with a thin film of waste garage oil. He found that the mesonotum of the pupa rose above the water level and the adult emerged successfully, only to be trapped by the oil when it stepped off the old pupal skin. He did not mention if the oil had any effect on pupae before they emerged.

It does not appear valid to project the somewhat limited observations of the effects monomolecular films had on *Tendipes riparius* midges in the laboratory to all of the various midge species encountered in the field pens in College Lake. Many differences between the species are known to exist. Certain morphological, physiological, and ecological differences have already been listed in table 15 and, although not observed in this study, many others occur. For example, variation in the habits of the adult midges during emergence and while egg laying is frequently mentioned in the literature. Because of inadequate time and funds, determining the effects of monomolecular films to the various developmental stages of the field species was not accomplished in the laboratory. Furthermore, direct observations of such effects in the field pens were quite difficult and consequently few were obtained.

Several factors influenced the difficulty of direct observation in the field pens. First, the very small size of many of these midges limited visual observation. Second, the rapidity of the emergence process made detailed observations of this process difficult. As an example of such rapidity, Malloch (1915) remarked that many midge adults emerged in a few seconds, so soon after their pupae reached the water surface that few fresh adults could be caught on it. Finally, in most species at College Lake, the emergence of the adults usually occurred after dark when observations could not be made readily. Emergence after sunset appears to be characteristic of many midge species but not of all (Morgan and Waddell, 1961). The use of lights on the water surface was not helpful because it produced negative phototropic responses to most of the pupae that distorted their normal behavior.

Despite the fact that detailed observations of the effects of the film materials to the various developmental stages of the field midge species were not obtained either in the laboratory or in the field, some valuable trend data were obtained in the field-pen experiments. Data have already been presented in table 12 to demonstrate that a trend existed for the emergence of smaller sized Tendipedid species at College Lake to be more adversely reduced by monomolecular films than the emergence of the larger sized species. This trend, however, only occurred in certain midge species having apneustic pupae with horns or trumpets (table 19).

In species having apneustic pupae with filaments, the reductions were fairly consistent, ranging between 33.2 and 54.4 percent with the A 52 treatment. Although the reductions to these species were not as consistent with the T.A. 1618 treatment, *Tendipes monochromus* was the only species in this category taken in substantial numbers in the field pens to determine a reliable estimate of the emergence reduction. The underlined reductions in table 19 are considered the most reliable since they were derived from species in which over 1,000 adults emerged in the pens. Furthermore, the underlined reductions for the field species in this group compared well with those found in the laboratory for *Tendipes riparius.*

It also can be seen in table 19 that the emergence of species with propneustic pupae was not reduced by the A 52 treatment. In fact, in *Pentaneura monilis,* the species in this category with the most reliable estimate of the film effect, 14.9 percent more adults emerged in the treated pens than in the untreated pens. The reduction was only 8.1 percent with this species in T.A. 1618 treated pens.

The emergence reduction figures presented in table 19 should not be mistaken as reductions only to the pupae. Actually, these reductions are composites of the reductions to all developmental stages with the possible exception of the larval stage. Midge larvae do not come in contact with the monolayer as the other stages do and adverse effects to them should not occur. Although not observed, it is quite possible that the emergence reduction to midge species having apneustic pupae with respiratory filaments may be due primarily to pupal mortality before emergence occurs, as was the situation with *Tendipes riparius* midges in the laboratory.

Just why the emergences of midge species with propneustic pupae at College Lake were reduced less than those for most of the species with apneustic pupae is not known. It may be that the propneustic midge pupae by sometimes being in direct contact with atmospheric oxygen became more buoyant than apneustic pupae, and thus formed more stable platforms from which the adults within emerged. Apparently, the reduction in surface tension by the two film materials did not upset the hydrostatic balance of the propneustic midge pupae like it did the propneustic mosquito pupae; otherwise, comparable reductions in the midge emergence would have been observed. A maximum reduction of only 8.1 percent occurred to the emergence of these midges, whereas such reductions ranged

TABLE 19.—*Comparison of the Emergence Reduction Effect of Two Monomolecular Films for Various-Sized Midge Insects With Different Types of Pupae, College Lake, 1962*

Species	Average wing length of adult (mm)	Apneustic pupae						Propneustic pupae	
		Prothoracic structures—						Prothoracic structures—respiratory horns or trumpets	
		Respiratory filaments—		Horns or trumpets—		None—			
		A 52	T.A. 1618	A 52	T.A. 1618	A 52	T.A. 1618	A 52	T.A. 1618
Corynoneura celeripes	0.80					33.9	*33.6*		
Hydrobaenus spp	1.45			73.2	0				
Prodiamesa spp	1.63			*68.8*	*34.0*				
Tendipes edwardsi	1.86	[1] *46.3*	0						
Crictopus trifasciatus	1.93			55.9	31.1				
Tendipes monochromus	2.19	*49.4*	*53.0*						
Tendipes nervosus	2.50	43.5	53.1						
Bezzia glabra	2.53							0	0
Bezzia opaca	2.60							0	0
Calopsectra spp	2.80			*34.0*	*17.3*				
Tendipes fulvus	3.00	33.2	+23.2						
Pentaneura monilis	3.30							*+14.9*	*8.1*
Pelopia stellatus	3.35							0	0
Procladius culiciformis								0	0
Chaoborous punctipennis	3.50							0	0
Tendipes riparius	3.60	[2] 54.4	[2] 52.9						
Tendipes subtendens	3.80	41.8	0						
Glyptotendipes lobiferus	4.60	43.7	0						
Tendipes plumosus	5.93	35.7	0						

[1] Underlined percentage reductions are those derived from species in which substantial numbers (over 1,000 adults) emerged in the pens. (See bottom of table 11 for details as to the manner in which the reductions were computed.)

[2] Percentage reduction determined from fishbowl rearing experiments (table 13).

between 80 and 100 percent, depending upon the film material, for the mosquitoes.

Conceivably, the effects of the monolayers to midge species having apneustic pupae with horns or trumpets may have occurred primarily when these midges were in the adult stage. This is supported by indirect evidence since no observations were made on these insects in the pens. Adult midges are known to utilize the surface tension of the water for their support in the same manner as water striders. Observations in the laboratory indicated that smaller sized water striders became wetted and sank sooner than larger sized striders in all monolayer-treated fishbowls. If the smaller midges sank sooner than the larger ones, the smaller ones would have had greater difficulty and less time to free themselves by flying away from the treated surface to become successful airborne emergents. Consequently, fewer smaller midges should enter into the emergence totals and subsequent derivation of the reduction effects of the monolayers from these totals should yield a trend for greater reductions to the smaller midges.

Such a trend can be seen in the emergence reductions presented in table 19 for midge species having apneustic pupae with horns or trumpets. As the sizes of these species decreased, their emergence reductions successively increased. The only exception to this trend is that the emergence of *Hydrobaenus* midges was not reduced by the T.A. 1618 treatment more than the preceding larger *Prodiamesa* midges. However, *Hydrobaenus* midges were not abundant in the pens at College Lake and the indicated reduction estimate of zero may not be very accurate.

Another trend which appears in these species is that greater reductions in their emergences consistently occurred with the A 52 treatment. For example, the 68.8- and 34.0-percent reductions respectively for *Prodiamesa* and *Calopsectra* midges with the A 52 treatment were about twice as great as the 34.0- and 17.3-percent reductions to these midges with the T.A.

1618 treatment. Such greater reductions, which occurred to all other species in this category, probably are due to the A 52 material reducing the surface tension more than T.A. 1618. A 52 reduced the surface tension from its normal 72 dynes per centimeter to about 32 dynes per centimeter, whereas T.A. 1618 reduced it to only 38.4 dynes.

Theoretically, as surface tension decreases, the wetness of a liquid to a solid floating on a liquid increases and the solid sinks more rapidly. This has been shown in table 16 for water striders which support themselves similar to midge adults. As previously pointed out, increased wetting (sooner sinking) should subsequently result in greater emergence reductions. Therefore, since A 52 decreases the suface tension more than T.A. 1618 and surface tension decreases are correlated with greater emergence reductions, one would expect greater emergence reductions such as were observed with A 52 for these species.

Although the above suggests that the reduction effects of the monolayers to these midges were primarily the result of the adults being wetted, the probability that similar or identical reductions could have resulted from their pupae being wetted should not be overlooked. However, to attain similar results, either one of the following two sets of conditions involving the buoyancy and the utilization of surface tension by these pupae would have to be true and such conditions were not definitely substantiated in the literature nor investigated in the present study.

In the first set of conditions it is assumed that these pupae were nonbuoyant while at the water surface and that they utilized the surface tension to "hang" themselves by means of their prothoracic horns or trumpets similar to the way mosquito larvae "hang" themselves at the surface. Such conditions would permit the line of reasoning as used for the adults to obtain the observed trend. The only difference here is that the pupae would be supported by the surface tension from below rather than from above as was the case with the adults.

The nonbuoyancy of these pupae is suggested because Berg (1950) described pupae of one of these species, *Cricotopus trifasciatus,* as "swimming" into open water, where the adults emerged. "Swimming" does not appear to confirm that these pupae were buoyant since buoyant pupae should normally rise to the surface passively. Furthermore, the pupae of these five species were all from shallow water and for them to swim a few feet to the water surface should not have been an unaccomplishable task.

No mention of these pupae utilizing the surface tension with their horns or trumpets has been found in the literature and Thienemann (1954) has even noted that there does not exist any connection between the form (shape) of these structures, their relative size, and the way of life of the apneustic pupae which have them. Since these horns or trumpets do not contain functional spiracles, the pupae which have these structures do not use them in respiration either like the propneustic pupae use theirs or like other apneustic pupae use their respiratory filaments. In fact, no use other than taxonomic has been ascribed to the prothoracic horns or trumpets of these apneustic pupae and, consequently, it still is possible that such pupae could be using these structures to break through the water surface so as to utilize the surface tension for support.

In the second set of conditions necessary to result in the trend for greater emergence reductions of smaller sized species having apneustic pupae with horns or trumpets, it is assumed, contrary to the first set of conditions, that the pupae were buoyant while at the surface and that they utilized the surface tension with their horns or trumpets to maintain hydrostatic balance similar to the way mosquito pupae maintain their balance. Buoyancy of these apneustic midge pupae could be attained by gas formation from the absorption of the moulting fluid prior to the time they reach the surface. Consequently, sinking of these pupae should not occur as it would if they were nonbuoyant. However, sooner wetting of the horns or trumpets on the pupae of the smaller sized species could occur because smaller pupae probably have horns or trumpets with less surface area to wet. Therefore, the smaller pupae should lose their hydrostatic balance sooner and fewer successful emergents should subsequently result.

Quantitative estimation of the effects monolayers have on the deposition of midge eggs was not obtained in this study. No doubt, though, with many members of some species being lost before they become airborne adults, fewer adults would remain to mate and replenish these species. In addition, some of the females that do emerge successfully and mate probably will sink in the treated water when they return to the surface to lay their eggs. This, however, is not meant to imply that they would all drown before they could deposit their eggs.

Some of the *Tendipes riparius* females which emerged in monolayer-treated fishbowls apparently mated and deposited their eggs successfully through the monolayers because minute young larvae were found in some of the treated bowls a few days after the adults had emerged. This observation certainly vindicates some replenishment of the species but it does not show conclusively that this replenishment was accomplished by the normal processes of female egg deposition from the water surface. The females were not actually observed depositing their eggs through the film-treated water surfaces and they could have used the sides of the fishbowls for their support rather than the treated water surfaces, thereby eliminating the possibility of drowning before completing the egg-laying process.

Although a female midge adult may drown before she completes the egg-laying process, many of her eggs may still hatch provided her egg mass is extruded from her body before she dies and provided her eggs become submerged. The latter condition is assured because the eggs would sink with the adult if they had not already been deposited and submerged before she died. In regard to the former condition, females of some species, for example, *Tendipes anthracinus,* actually extrude an egg mass which remains attached to their bodies before they alight on the water surface and the subsequent hatching of these eggs in monolayer-treated water would therefore be assured because the attached eggs mass would sink with the adults. Consequently, the replenishment of species which exhibit this characteristic should be assured in monolayer-treated water.

Furthermore, Wigglesworth (1953) points out that the larvae and pupae of some midge species lay eggs parthenogenetically and display paedogenesis. Such characteristics would permit these species to replenish themselves without their adults emerging, mating, and egg laying. Consequently, the fact that monolayers may significantly hinder the emergence process and may subsequently hinder the egg-laying process would have little bearing on the replenishment of these species.

The fact that replenishment of aquatic insects can occur in a monolayer-treated situation was suggested by Hayes (1959), who reported that the numbers of immature aquatic insects living in the bottoms of hexadecanol-treated ponds remained at levels near those prior to treatment. This certainly indicates some replenishment of the insects; otherwise, larvae probably would have been fewer in the treated ponds. Successful replenishment of a midge species was also suggested in the present study by the emergence abundance of subsequent generations of *Prodiamesa* midges in the treated pens at College Lake. These midges were the most abundant emergents in the pens and it is believed that they underwent about four complete generations during the time emergence samples were collected. About every 8 or 9 days, definite "peaks" or increases in the number of emergents of this species were observed in both the untreated and treated pens. Despite the fact that the "peaks" usually were of lesser magnitude in the treated pens, each of the "peaks" was interpreted as indicating the completion of the life history (one generation) of this species. If replenishment of this species was being totally prevented by the monolayer, one would not expect any emergents in subsequent generations or consequently later in the summer. Even if replenishment was not totally prevented, one would probably expect a trend for the numbers of emergents of this species to decrease in the treated pens in subsequent generations or later in the summer because fewer adults would be present from previous generations. However, the numbers of emergents from the treated pens later in the summer were, in fact, comparable or of a greater magnitude than those for this species earlier in the summer. Consequently, this species must have replenished itself but the manner in which it accomplished this is not known.

Duck-feather experiments.—Under normal conditions, waterfowl plumage is considered water-repellant (waterproof) and it has been a common belief that waterfowl maintain this condition by anointing their feathers with secretions from their oil gland. Madsen (1941, 1943) and Elder (1954), however, have found that the water-repellant quality of moulted plumage on young birds was effective in birds with the oil gland removed. This excluded the possibility that secretion from the oil gland which could be present on the feathers of previous moults prior to the removal of the oil gland is the reason for the water-repellant quality of the feathers.

Madsen (1941) contended that the large amount of finely distributed air among the ramifications of the feathers was the principal factor in the water-repellency of waterfowl plumage. This has been confirmed by Fabricius (1959), who added that the ability of the plumage of birds to repel water was apparently not primarily dependent on the secretion of the oil gland, but primarily dependent on the delicate structure of the feathers. He found that the water-repellent quality of the plumage was lost if the fine barbules of the feathers became disarranged by contact with smearing substances or by mechanical action. Fabricius (1959) further pointed out, however, that the waterproof condition of the plumage could be restored by either the normal preening behavior or the nibbling movements of the bird's bill, which are both effective in bringing the barbules back into their normal positions.

The importance of waterfowl maintaining water-repellent plumage cannot be overemphasized because wet plumage sometimes leads to the death of waterfowl with symptoms resembling pneumonia (Fabricius, 1959). Consequently, since monolayers are known to increase the wetness of the water, an attempt was made to determine the extent of this wetting to plumage of mallard ducks in monolayer-treated water.

Originally, experiments were started by placing dead ducks of similar known weight in treated and untreated water for about 4 or 5 hours. The objective here was to compare the weights of the ducks after contact with the treatment to obtain a quantitative estimate of the uptake of water by their plumage due to the monolayer. In some such experiments, however, the plumage of the ducks in untreated water took on more water than that of the ducks in treated water. This was believed to be due to a greater disarrangement of the feather structure (barbule attachment) of the ducks in the untreated water. Consequently, since the structure characteristics of all of the feathers of particular ducks could not be assured comparable, experiments of this type were discontinued.

Instead of using dead ducks, experiments were conducted with breast feathers of similar size and structure in untreated and monolayer-treated water. Microscopic examination of the barbule attachment permitted a selection of feathers of similar structure to be used in the experiments. Such feathers in monolayer-treated water became wetted and actually sank in an average time ranging between 60 and 74 hours, depending on the monolayer used and the variation in the three replications of each monolayer. Although the feathers in the untreated water did not completely sink during these experiments, they were wetted and were about halfway submerged at the time the feathers in the treated water sank. No significant difference between the average times required to sink the feathers in the various monolayers was detected despite the fact that sinking (wetting) should theoretically be faster with monolayer-forming materials which reduce the surface tension the most.

Apparently, surface tension reduction is not the only factor that influences the wetting of feathers. To exemplify this, experiments were conducted with feathers placed on the surfaces of soap solutions and monolayer-treated waters having identical surface tensions (31 dynes per centimeter). The feathers placed on the surfaces of the soap solutions became wetted and sank in an average time of 22 seconds, which is approximately 12,000 times faster than the 72-hour average time it took the feathers to become wetted and sink in the monolayer-treated waters. Although not investigated, this considerable difference in sinking time is believed to be due to the soap decreasing the contact angle at this surface tension more than the monolayer-forming material reduced it. Decreases in the contact angle usually result in increased wetting.

Despite the fact that waterfowl plumage probably would take on water more rapidly (about twice as fast) in monolayer-treated situations, it appears doubtful that their plumage would become wet enough to result in adversity to the waterfowl. Waterfowl seldom would be on monolayer-treated water surfaces for the extended time periods necessary to completely wet their feathers because waterfowl frequently fly daily from one location to another and, while in flight, the additional water in their plumage may even evaporate. Besides this, their normal preening behavior would tend to restore water-repellency to their plumage.

Furthermore, no obvious adversities to waterfowl in the field have been reported by investigators of monolayer-treated water (Hayes, 1959). In the present study ducks, ducklings and geese were frequently observed in the treated pens in College Lake with no apparent ill effect. Laycock (1956) has even reported birds eating large amounts of hexadecanol pellets in Africa, again, with no apparent ill effects.

Fish-toxicity experiments.—The monomolecular film-forming materials used in this study are primarily composed of hexadecanol (C 16) and octadecanol (C 18) (table 1). According to the Association of Food and Drug Officials of the United States (Anonymous, 1958), hexadecanol and octadecanol are normal intermediates in fat metabolism and their use as monomolecular films for evaporation control of potable water supplies presents no health hazards. However, the film-forming materials shown in table 1 contain alcohols other than hexadecanol and octadecanol and, consequently, fish-toxicity experiments similar to those described by Hayes (1959) were conducted with each of the seven materials to determine if any of these film-forming materials exhibitited toxic qualities to green sunfish, *Lepomis cyanellus.*

Hayes (1959) fed a control diet and a diet consisting of 50 percent A 52 material and 50 percent trout pellets to carp, *Cyprinus carpio,* suckers, *Catostomus commersoni suckleyi,* and green sunfish, *Lepomis cyanellus* for 91, 58, and 49 days, respectively, with no mortality except for one green sunfish which died near the end of the 49 days in which the green sunfish were fed A 52. This death was believed to have resulted from aggressive behavior of the fish rather than from any effect of the A 52. All fish had been fed as much of the particular diet (control or treated) that they would consume in about 20 minutes three or four times a week.

In the present study, 40 green sunfish ranging between 4.5 and 5.0 inches in length were divided into 8 lots of 5 fish each. Similar sized fish were selected for the lots and probably because of this the average weights of the lots did not differ by more than 1.5 grams at the start of the experiments. Each lot of green sunfish was randomly assigned the diet it was to be fed. One lot was fed a control diet, while the other seven lots were each fed a diet containing 50 percent of a particular film-forming material. The diets were fed to the fish similarly to the manner described above by Hayes (1959), but the duration of the feeding was continued for 105 days rather than 49 days.

The results of these experiments are presented in table 20 and show that over the 105 days no mortalities resulted from feeding any of the film-treated diets to the green sunfish. It also can be seen that the average weight increases of the green sunfish after 105 days of feeding film-treated diets did not differ much from the average-weight increase of sunfish which were fed the control diet. Hayes (1959) similarly found that the average weight of carp which were fed an A 52 treated diet for 91 days did not differ from that of carp fed a control diet.

Such findings appear to indicate that carp and green sunfish apparently are able to metabolize much of the film-forming materials; otherwise, the average weight increases of these fish fed the treated diets should have been much less because the treated diets contained only half the amount of normal pellet material that the control diet contained. The possibility of these fish metabolizing much of the materials is not very surprising because these materials are primarily composed of hexadecanol and octadecanol, which are known to be normal intermediates in fat metabolism.

Considering the large concentration (50 percent by weight) of each of the seven film-forming materials fed to green sunfish for 105 days with no mortality or adversity, there does not appear to be any reason to suspect that these materials in the form of a thin monomolecular film on the water surface of a reservoir would be consumed by any fish in concentrations great enough to result in death by toxicity. This probably would be true regardless of whether the fish obtained the materials by direct feeding or through food networks.

Information from the literature contains no indication of toxic effect to aquatic animals resulting from long-chain alcohol monolayers and, though not quantitative, no obvious adverse effects to largemouth black bass, green sunfish, redear sunfish, frogs, turtles, water

TABLE 20.—*Effect of Feeding Diets Containing 50-Percent Monolayer-Forming Material to Green Sunfish, Lepomis Cyanellus, for 105 Days*

Lot number	Treatment diet	Number of fish	Average weight before feeding (grams)	Average weight after 105 days (grams)	Average weight increase (grams)	Mortality
1	Control	5	35.7	56.3	20.6	None.
2	ST 1820	5	34.9	54.7	19.8	Do.
3	A 52	5	34.6	54.8	20.2	Do.
4	ST 1618	5	36.1	56.6	20.5	Do.
5	MC	5	35.2	56.1	20.9	Do.
6	A 54	5	34.8	54.5	19.7	Do.
7	ST PC	5	35.5	55.4	19.9	Do.
8	A 62	5	35.4	54.2	18.8	Do.

snakes, muskrats, ducks, ducklings, or geese were noticed in the treated pens in College Lake.

The fact that toxic influences to mosquitoes were suggested for some of the materials should be of little consequence to these insects or to the biology of monolayer-treated reservoirs because mosquitoes usually prefer paludal environments and the reservoirs proposed for treatment usually are nonpaludal with few mosquitoes present. Furthermore, the effects of the monolayers to the important midge insects are believed to be primarily the result of surface tension reduction rather than any toxic influence of the monolayers.

Chapter IV

DISCUSSION

Evaluation of Monolayer Influences

In this chapter an attempt will be made to evaluate the influences long-chain alcohol monomolecular films have on fish and wildlife from the knowledge that is available at the present time. The evidence to date strongly indicates that long-chain alcohol materials which contain primarily hexadecanol and octadecanol are not directly toxic to fish and wildlife when in the quantities and manner proposed for use in evaporation reduction. This already has been discussed in the analysis chapter, as has the wetting influence of monolayers to waterfowl plumage which is not considered to be a problem.

Since nontoxicity of the film-forming materials has been suggested, any adverse influence of the monolayers to fish and wildlife would probably have to result indirectly from the monolayers influencing adversely environmental conditions on life processes of aquatic animals which fish and wildlife are dependent upon. The influences under scrutiny at the present time are temperature increases, retardation of gas diffusion, and reductions to insect populations, particularly midges which are important in the diets of fish and wildlife and in the biology of reservoirs.

Temperature increases.—Logically, for a number of reasons, concern has developed regarding any adversity the increases in water temperature associated with monolayers may have directly or indirectly on fish, and possibly wildlife. First, a warming of the water in a particular environment may exceed the maximal critical temperature for particular species. For example, trout usually cannot tolerate extended periods of water temperature over 68° F. in the deeper waters during the summer period and some trout environments normally approach this criticial temperature. Consequently, warming of these marginal waters by monolayers could change these normal trout environments to environments more suited for warm-water species.

Secondly, temperature increases may adversely affect fish indirectly by enhancing the development of algal "blooms". Dead and decaying blue-green algae may cause oxygen depletion of the water and release toxic substances, both of which are known to increase the possibility of death to some fish (MacKenthun and Herman, 1948). Furthermore, "blooms" of algae may cause the water to become supersaturated with oxygen to a point that could result in death to some fish. Woodbury (1942) observed mortalities of black crappies, bluegills, northern pike, walleye pike, white suckers, and carp when the water of Lake Waubesa, Wis., became highly supersaturated with oxygen up to about 32 p.p.m. as a result of a heavy algal "bloom" composed principally of *Chlamydomonas*.

The above changes, direct or indirect, will of course vary with the magnitude of temperature increase and will be important in accordance with the local water uses and recreational values. It is difficult to delineate accurately what the magnitude of these temperature increases will be in every environment treated with a monolayer because the magnitude of the increases will be influenced by such factors as climate, size and depth of the water mass, and by the extent and duration of the monolayer.

Indications at present are that temperature increases seldom will exceed 4° F. at the water surface for any length of time and the findings of Crow (1960) and those in this study appear to indicate that temperature increases will be slight, if detectable at all, at depths greater than 7 feet from the surface of the water. Furthermore, there is no indication to date of monolayers enhancing algae "blooms" or increasing temperatures of entire water masses above critical values for fish in environments that have been treated with monolayers.

Considering that many of the environments proposed for monomolecular-film treatment are normally of the warm-water fishery type and usually are comparatively large and deep with a fairly rapid exchange of water, the temperature increases resulting from monolayers applied to these waters probably will have little influence on the fishery. Adverse effects from temperature increases probably will only be a problem to the fishery in treated environments that are comparatively small and shallow with none or little exchange of water and that normally, before treatment, have water temperatures near the maximum critical temperature for fish species therein.

Retardation of gas diffusion.—The evidence indicates that close-packed, long-chain alcohol monolayers retard the passage of gases at the gas-water interface and constitute a diffusion barrier to the absorption of gases such as oxygen, carbon dioxide, and nitrous oxide by water (Blank, 1962). The permeabilities of monolayers to these gases were found by Blank (1962) to be of the same magnitude (about 10^{-3} cm/sec). Not only does a monolayer retard the absorption of gases by the water, but it also retards the passage of some gases from the water to the atmosphere.

Hayes (1959) applied an A 52 monolayer to half of a pond that was supersaturated with dissolved oxygen before treatment and found that the treated half after about 24 hours contained an average of 0.38 p.p.m. more dissolved oxygen than the untreated half. This same "buildup" phenomenon occurred in the treated pens at College Lake during 1961 and 1962 (table 3). Over approximately a 2-month period of monolayer coverage, the dissolved oxygen concentration averaged 0.7 p.p.m. higher in surface waters of A 52 treated pens. Although this buildup was only slight and did not appear to be accumulative, the reason for this was believed to be due to the experimental design of the pens in the lake. The pens, which were constructed primarily of polyethylene, were always surrounded by untreated lake water. Since it is known that some gases can diffuse through polyethylene, it was believed that the gases in the water of the untreated lake and the gases in the water of treated pens tended toward equilibrium, thus minimizing the influence of the monolayers.

Since monolayers retard the passage of oxygen both to and from the atmosphere, it is possible that monolayers could either lower or raise dissolved oxygen concentrations in the water. This raising or lowering, however, depends on various factors such as the saturation of dissolved oxygen in the water prior to monolayer treatment, the oxygen demand by the plants and animals in the water, and the processes under which oxygen is replenished to the water. Assuming that the oxygen demand by the plants and animals in the water remains relatively constant, undisturbed surface waters which are not saturated with oxygen prior to treatment theoretically should exhibit a slight depletion of oxygen during monolayer treatment because the plants and animals will be utilizing available oxygen while the monolayer will be retarding the diffusion of replaceable oxygen into the water. Hayes (1959) demonstrated this in the laboratory by using green sunfish, which created a relatively constant oxygen demand and which held the oxygen concentrations below saturation levels in untreated and A 52 treated aquaria. With these conditions, oxygen was diffusing from the atmosphere into the aquaria water and Hayes found that after 75 hours, the dissolved oxygen concentrations in five A 52 treated aquaria averaged 37.4 percent less than that of five untreated aquaria.

Despite the fact that monolayers can retard the diffusion of oxygen into the water, a significant depletion of oxygen probably will not occur in the surface waters of most of the large reservoirs proposed for treatment with monolayers because their dissolved oxygen concentrations normally are held very near or above saturation levels by wind-induced mixing. Such mixing usually replenishes much, if not all, of the dissolved oxygen that is being utilized by the plants and animals. Furthermore, the monolayer would tend to trap oxygen being released by plants during photosynthetic periods which consequently would tend to compensate oxygen depletion. The possibility of significant buildups of oxygen in these large reservoirs is also slight, again, because of wind-induced mixing which would tend to permit much of any excessive oxygen that is accumulating due to the monolayer to diffuse into the atmosphere.

Hayes (1959) concluded that the diffusion process is not a limiting factor in the aeration of surface waters of large reservoirs, and an effect on this factor by a hexadecanol monolayer may be dismissed as inconsequential at least so long as it remains small compared with the range of natural variation in oxygen demand. In addition, Blank (1962) has even gone so far as to say that the effect of monolayers on carbon dioxide and oxygen exchange at a reservoir surface (by diffusion processes) can be neglected.

It should be pointed out, however, that at times some aquatic environments normally experience concentrations of carbon dioxide or oxygen very near the maximal or minimal tolerance limits for the fish therein. Such conditions usually are found in environments which are small and shallow and which have little wind mixing. Slight changes in the concentrations of oxygen and carbon dioxide due to monolayers could result in fish losses in these environments, but these small environments are not of the type generally proposed for treatment with monolayers.

Effects to insects.—In discussing the effects of hexadecanol monolayers, Hayes (1959) mentioned that most adult aquatic insects of Coleoptera and Hemiptera appeared to be unaffected by the monolayer except for water striders which fell through and drowned. He also found that the emergences of mosquitoes, mayflies, and midges were hindered by the monolayer and concluded his study by saying that, overall, most biological effects of hexadecanol are small in magnitude and well within the range of natural variation, but, one effect, that of surface tension reduction is large, and members of the biota dependent upon support of the surface film at some stage in their life history may be adversely affected.

There can be little doubt from the evidence in the present study that Hayes (1959) was correct because adverse effects of monolayers have definitely been shown to occur to insects which utilize the surface tension of the water for support. All water striders drowned in monolayer-treated water in less than 7 minutes (table 16) and the emergence of mosquitoes was reduced between 80 and 100 percent depending on the particular material used to form the monolayer (table 13). In addition, the emergence of the midge, *Tendipes riparius,* was reduced between 41 and 88 percent in the laboratory, again, depending on the particular material tested. With two monolayer-forming materials tested in the field pens at College Lake, the overall reduction in emergence of the insects, of which 98 percent were midges, was 46.5 percent with A 52 and 30.9 percent with T.A. 1618 (table 11). The reductions to particular species ranged between 0 and 73.2 percent with A 52, and between 0 and 53.1 percent with T.A. 1618.

Of primary concern from a fish and wildlife point of view is the considerable reduction in emergence of some of the midge species because the midge insects are important both in the diets of fish and wildlife and in the biology of reservoirs. However, to evaluate properly these reductions due to monolayers as to the adversity they may cause to the insects themselves and subsequently to fish and wildlife, involves the consideration of certain factors which thus far have not been stressed.

First, the magnitude of the reductions to the emergence of these midges was determined under "ideal" conditions in both the laboratory and in the field studies. Total coverages of compressed monolayers, rarely a case in the field, were maintained over the entire period when emergence data were collected. The masonite-modified pens in College Lake eliminated wind as an influencing factor and Timblin, Florey and Garstka (1962) point out that with winds of 15–20 m.p.h., it appears impractical, if not impossible, to maintain any appreciable monolayer coverage at the present time. Winds of such and greater velocities frequently occur over the reservoirs in the west and, consequently, it becomes apparent that the magnitude of the reductions in emergence of the midges noted in this study probably are considerably greater than what would occur in many of the large, windblown reservoirs proposed for treatment with monolayers.

Other factors which must be considered are the general characteristics of the reservoirs proposed for treatment with respect to the species of midges present along with the time when the emergence of these species occurs in relation to when the monolayers will be on the surfaces of these reservoirs. Most storage reservoirs seldom have a well-developed littoral or sublittoral flora or fauna because of water level fluctuations and, consequently, the midge species therein are usually predominantly of the profundal type (species normally associated with waters below the light-controlled limit of plant growth). Brundin (1951) has noted that most of these profundal midges have their "peak" emergences and oviposit in direct relation to the spring and autumn circulations of the lake. Therefore, if one considers the fact that monolayers probably will be applied to the reservoirs when evaporation is greatest (July and August in temperate regions), any loss of the relatively few members of these profundal species which are emerging during this film-treated period should be of little consequence to the survival and carrying capacity of these species.

This phenomenon may also be true for some of the midge species normally associated with littoral zones of lakes if these species even occur in the reservoirs proposed for treatment with monolayers. Mundie (1957) has shown that some littoral midge species exhibit the "bimodal" pattern of emergence as described above for profundal species. However, it should also be noted that some of the midges which exhibit this "bimodal" emergence pattern may have both "peaks" or at least one "peak" coinciding with the July–August film-treated period. The emergence of these species, therefore, probably will be reduced by the monolayer but it is not known if these reductions will be great enough to influence adversely the carrying capacity of these species.

Unfortunately, little is known on how much the adults of a particular midge species can be reduced and still maintain the population at carrying capacity. In this regard, however, it may be well to refer to the general wastefulness of reproductive effort in nature and to the phenomenon found among most animals, that juvenile survival rather than the number of reproducing adults (reproductive capacity) determines carrying capacity. Here then, factors such as the availability of food, space, and predation which for the most part are unaffected by monolayers should be more influential in maintaining the carrying capacity of a species than the number of successful emergents, at least, so long as all emergents are not lost. Furthermore, it has already been pointed out that some midge species can replenish themselves without their adults emerging, mating and egg laying, and the fact that monolayers may adversely affect these processes will be of little consequence to these species.

Taking into consideration all of the above-mentioned factors regarding the insects together with the discussion of temperature increases, retardation of diffusion of gases by monolayers and wetting influences to waterfowl plumage, the long-chain alcohol materials tested in this study when in the quantity and manner proposed for use as monolayers, do not appear to constitute a limiting factor to the biota which is in or associated with most of the reservoirs now proposed for treatment. These reservoirs are characteristically large, windblown and fluctuating, all of which will tend to minimize the influences of these monolayers. However, since research is almost sure to improve the extent and duration of monolayers and since the future use of monolayers may encompass a variety of environments, the influences of monolayers should be reevaluated accordingly.

At present, it appears that a long-chain alcohol material which does not reduce surface tension greatly and which contains primarily nontoxic hexadecanol and octadecanol with little, if any, alcohols heavier than C 18, probably will have lesser influences on the biota. These characteristics should minimize the wetting influences of monolayers to insects and waterfowl plumage and also minimize the toxic influence that was suggested in this study for the mosquitoes.

Suggestions for Further Study

The present study suggests the need to answer the following questions by additional studies:

1. What would be the influences of total monolayer coverage to temperature and gas diffusion in small shallow environments which do not have water exchanges or buffering influences?
2. What are the details regarding the relationships midges have with surface tension while in contact with the water surface? Are all midge pupae buoyant while at the surface? Do some midge pupae have a relationship with surface tension to maintain hydrostatic balance as the mosquitoes have? What are the critical values of surface tension at which support and hydrostatic balance are lost? Are these values relatively constant or do they vary for various midge species?
3. Do the monolayer-forming materials get into the midge insect's body, and if so, by what means? Does "clogging" of the respiratory filaments occur in pupae having these structures?
4. Would greater amounts of alcohols heavier than C 18 in the monolayer result in toxic influences to the midges? What influence would the addition of algacides or bactericides to the monolayer have on the midge insects?
5. How much can the emergence of particular midge species be reduced and still maintain the population at carrying capacity?

Chapter V

SUMMARY

1. A study of some physical and biological effects of seven long-chain alcohol materials which contain primarily hexadecanol and octadecanol and which are used as monolayers to suppress water evaporation from reservoirs has been made at Colorado State University under contract with the U.S. Bureau of Reclamation. Aspects of this study were conducted during the period July 10, 1961, to January 1, 1964, and had as their purpose two objectives. The primary objective was to determine some of the influences long-chain alcohol monolayers have on the aquatic insects, particularly the biologically important midges of the family Tendipedidae. The secondary objective was to amass available knowledge regarding the known influences of monolayers together with those determined in the present study and evaluate these influences from a fish and wildlife point of view.

2. Investigations were conducted in the fishery and chemistry laboratories of Colorado State University, as well as the fishery laboratory of the Colorado Game, Fish, and Parks Department in Fort Collins, Colo. In addition, investigations concerned primarily with the emergence of insects were conducted in 12 polyethylene pens located in College Lake, near Fort Collins. Compressed monolayers of the seven materials were maintained in these investigations but only two materials, A 52 and T.A. 1618, were tested in the field pens. In both summers of the field studies, each pen was randomly assigned the treatment that it received. Only 3 of the 12 pens received the same treatment in both summers.

3. Increases of both water temperature and dissolved oxygen concentration occurred in all treated pens during these studies. The temperature of surface waters increased by an average of 0.5° F. in A 52 pens and by 0.4° F. in T.A. 1618 pens during about 2 months of monolayer coverage in each summer. There was a tendency for these increases to diminish with depth. During the film-treated period, the dissolved oxygen concentration in the surface waters increased by an average of 0.7 p.p.m. in A 52 pens and by an average of 0.6 p.p.m. in T.A. 1618 pens. No significant differences between the two materials were detected for either temperature increases or oxygen concentration increases. Increases such as were observed in this study have usually been found to be of greater magnitude in other studies. It was believed that the untreated lake water which surrounded the pens, buffered the water of the treated pens and was the reason for the minimal increases in temperature and oxygen noted in the treated pens during this study.

4. The emergence of insects from the pens was estimated in both years by using three floating surface traps in each pen. Such traps sampled a 5-percent area of each of the 12 pens. However, four tents, which completely capped entire pen areas, were used in 1962 to test the efficiency of the trap sampling. The trap sampling during 1962 was found to be inefficient (inaccurate) because the numbers of insects estimated by the traps to have emerged in the pens were not found to be proportionate to the numbers that the tent-trap total catches indicated had actually emerged. Since the traps were in the same location both years, the 1961 trap data were also believed inaccurate at least so far as the magnitude of the emergence was concerned. The most accurate estimates for the magnitude of the emergence were obtainable from the total-count data collected from the pens covered by the tents. Consequently, only the total-count data were used to estimate the effects of a monolayer despite only four tent-covered pens yielding total-count data at any given time. The effect of monolayers on the emergence was considered the difference between the numbers of emergents from untreated and treated pens expressed as a percentage difference. In most cases the numbers of particular species emerging from the pens were fewer in the treated pens and the percentage differences were therefore referred to as percentage reductions.

5. Approximately 98 percent of the insects which emerged in the pens at College Lake were midges of the family Tendipedidae. The overall percentage reduction in the emergence of these midges due to the monolayers was 46.5 percent with A 52 and 30.9 percent with T.A. 1618. Statistically, A 52 was found to be highly significantly more adverse in reducing the overall emergence of these insects. This, however, was not the case for all species since only 8 of the 15 species of midges were found to be significantly more reduced by A 52. The reductions to the species ranged between 0 and 73.2 percent with A 52 and between 0 and 53.1 percent with T.A. 1618.

6. Significant differences between the emergence of the sexes due to the monolayers were not detected in any of the 15 species of midges at College Lake.

7. Four midge species having pupae with horns or trumpets were reduced between 0 and 73.2 percent by the monolayers in the pens and it was found that the reductions of particular species were progressively greater as the sizes of these species decreased. Only one exception to this trend was found with the monolayers tested. Reductions to seven species having apneustic pupae with respiratory filaments were fairly consistent ranging between 35.7 and 49.4 percent with A 52 in the field. The emergence of one species having such pupae, *Tendipes riparius,* was reduced in the laboratory with A 52 by 54.4 percent. With six insect species having propneustic pupae, reductions in their emergents were almost undetectable in the pens. In fact, one species with pupae of this type, *Pentaneura monilis,* actually had emergences which were approximately 15 percent greater in the A 52 treated pens.

8. *Tendipes riparius* was the only midge species for which monolayer effects on emergence were investigated in the laboratory. The emergence of this species was reduced betwen 41.2 and 88.2 percent, depending on the particular material used to form the monolayer. Significant differences were noted between the magnitudes of these reductions for the seven materials tested. ST 1820 was significantly more adverse in reducing the emergence of this midge than all other materials except SPC. Significant differences were not found between the numbers of larvae surviving to pupae in untreated and monolayer-treated experiments and this indicated little, if any, adverse influence of the monolayers to these larvae. The losses were primarily to the pupae but a few adults were also lost when they stepped off the pupal skin before flying away from the monolayer-treated surface. Although not observed, the means by which some of the midges from the field pens could have been affected primarily as adults rather than as pupae were discussed.

9. Experiments on the rearing of *Aedes* mosquitoes from larvae to emerged adults in untreated and treated aquaria indicated that the effects of the monolayers were primarily to the pupae. Pupal mortality with the seven materials ranged between 69.4 and 96.7 percent, whereas larval mortality ranged between 12.5 and 60.0 percent. The overall reduction to the mosquito emergence ranged between 80 and 100 percent. Significant differences between the magnitudes of these reductions for the various materials were demonstrated and ST 1820, again, was found to be the most adverse. Statistical differences, however, could not be demonstrated between the magnitude of the reduction by this material and that of either A 54 or A 62.

10. Film-forming materials containing alcohols heavier than C 18 resulted in greater mortalities to both larvae and pupae of *Aedes* mosquitoes. There was a trend for greater pupal mortality with increased amounts of the C 20 alcohol in the materials. This trend was not found in the midge experiments.

11. The effects of the monolayers to mosquitoes and midges were believed to be the direct result of the monolayers reducing the surface tension of the water, but other possibilities were noted. These monolayers reduced the normal 72 dynes per centimeter surface tension of clean water to surface tensions ranging between 30.8 and 38.4 dynes per centimeter. Such reduced surface tensions were very close to or below the critical limit at which mosquito larvae and pupae are known to drown. The larvae drown because they cannot remain attached to the water surface while breathing and feeding and the pupae drown because they lose their hydrostatic balance and tip over, allowing their respiratory trumpets to become submerged while they are breathing at the water surface. Known details as to the utilization of surface tension by mosquitoes and midges were described and speculations were made as to how surface tension reductions could explain many of the trends noted in the midge emergence reductions.

12. Gerrid water striders were observed falling through all monolayer-treated waters and drowning in less than 7 minutes. With any given monolayer, smaller sized water striders drowned sooner than larger sized water striders. In addition, it was found that the more the surface tension was reduced by these monolayer materials the faster the water striders drowned.

13. None of the film-forming materials caused mortality to green sunfish maintained in laboratory aquaria on diets containing 50 percent of a particular material for a period of 105 days. The average weights of green sunfish fed the treated diets after 105 days of feeding did not differ much from that of green sunfish fed an untreated control diet. It was suggested that green sunfish may be able to metabolize much of the film-forming materials.

14. Although not quantitative, no obvious adverse effects to largemouth bass, green sunfish, redear sunfish, frogs, turtles, water snakes, muskrats, ducks, ducklings, or geese were noticed in the monolayer-treated pens in College Lake.

15. Mallard duck breast feathers were observed in the laboratory to take on water about twice as fast in monolayer-treated water than in untreated water. Feathers placed on the surfaces of treated water became wetted and sank in an average time ranging between 60 and 74 hours, whereas those placed on untreated water were wetted but were only about half submerged in this time period. This wetting influence was not considerd to be a problem to waterfowl in the field because of evaporation of the additional water while in flight and because of the preening behavior of waterfowl that would tend to restore water repelency to the plumage.

16. In the evaluation phase of this study many factors were taken into consideration. The general physical characteristics of the reservoirs and the type of biota usually found in the reservoirs now proposed for treatment with monolayers were briefly discussed. Wind velocities of 15–20 m.p.h., which are common at many of these reservoirs, were described to reduce considerably the extent and duration of the monolayers. It was also noted that wind-induced mixing of the surface waters of the reservoirs would tend to minimize both the temperature and diffusion-barrier influences of monolayers. Specifically regarding the insects, the "ideal" condition under which the reductions to emergence were determined both in the laboratory and in the field studies was described as not being similar to normal field conditions and therefore this influence of monolayers was magnified considerably in this study. Furthermore, the time when the "peak" emergence of many of the midge insects occurs was delineated as coinciding with times that monolayers may not even be on the reservoirs now proposed for treatment with monolayers.

17. It was concluded that the long-chain alcohol materials tested in this study, when in the quantities and manner proposed for use as monolayers for evaporation reduction, do not appear to constitute a limiting factor to the biota in or associated with most of the reservoirs now proposed for treatment. However, it was also mentioned that since research is almost sure to improve the extent and duration of monolayers and since the future use of monolayers may encompass a variety of environments, the influences of monolayers should be re-evaluated accordingly.

18. Characteristics of environments where monolayers may be a problem were briefly described. A long-chain alcohol material which contains primarily nontoxic hexadecanol and nontoxic octadecanol with little, if any, alcohols heavier than C 18, was noted as a material which probably would have lesser influences on the biota.

19. Recommendations were made regarding suggestions for future studies.

APPENDIX

APPENDIX A.—*Statistical Comparison of the Total Number of Insects Emerging in Untreated and Monolayer-Treated Pens in College Lake Between July 30 and September 10, 1962*

Date	A Untreated	B Treated T.A. 1618	 Adol 52	C Total A+B	D Expected untreated (0.5 of C)	E Expected treated (0.5 of C)	F Chi-square $\frac{(A-D)^2}{D}+\frac{(B-E)^2}{E}$
1962							
July 30–Aug. 8	2, 303	1, 881		4, 184	2, 092	2, 092	[1] 42. 56
July 30–Aug. 8	963		805	1, 768	884	884	[1] 14. 12
Aug. 9–Aug. 18	5, 138		2, 546	7, 684	3, 842	3, 842	[1] 874. 34
Aug. 9–Aug. 18	2, 703	1, 708		4, 411	2, 205. 5	2, 205. 5	[1] 224. 45
Aug. 19–Aug. 29	7, 932	5, 300		13, 232	6, 616	6, 616	[1] 523. 54
Aug. 19–Aug. 29	3, 860		1, 489	5, 349	2, 674. 5	2, 674. 5	[1] 1, 050. 97
Aug. 30–Sept. 10	4, 700		2, 997	7, 697	3, 848. 5	3, 848. 5	[1] 376. 80
Aug. 30–Sept. 10	1, 895	1, 358		3, 253	1, 626. 5	1, 626. 5	[1] 88. 65

[1] Highly significant difference with 1 degree of freedom.

APPENDIX B.—*Statistical Comparison of the Overall Reduction in the Total Number of Insects Emerging in Monolayer-Treated Pens in College Lake, 1962*

Treatment	A Untreated pens	B Treated pens	C (A+B)	D PR	D_1 Expected PR	A_1 Expected untreated	B_1 Expected treated	E Chi-square
TA 1618	14, 833	10, 247	25, 080	30. 92	38. 73	15, 551. 6	9, 528. 4	87. 40
Adol 52	14, 661	7, 837	22, 498	46. 54	38. 73	13, 950. 5	8, 547. 5	95. 25
Sum								[1] 182. 65

A, B, C=From table 9 grand totals.

D=The observed percentage reduction (overall reducing effect) determined from $\frac{A-B}{A}$ (100) for each treatment.

D_1=The expected percentage reduction if the reducing effects of the treatments are the same. Determined by averaging the D values.

A_1=Determined algebraically from the following: $A_1+B_1=C$; $\frac{A_1-B_1}{A_1}=0.3873$; solving then; $A_1=\frac{C}{1.6127}=\frac{25{,}080}{1.6127}$ $=15{,}551.6$ for the TA 1618 treatment. Changing the C value will give the A_1 for Adol 52 treatment.

B_1=By subtraction: $B_1=C-A_1=(25{,}080-15{,}551.6=9{,}528.4$ for the TA 1618 treatment).

$E=\frac{(A-A_1)^2}{A_1}+\frac{(B-B_1)^2}{B_1}$ for each treatment with their sum indicating the total chi-square with 1 degree of freedom for treatment differences.

[1] Highly significant difference.

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ABSTRACT

Studies conducted for approximately 3 years in laboratories and lake pens indicate that evaporation reduction monolayers are not a limiting factor to the biota of reservoirs. Because reservoirs considered for monolayer treatment are also used for recreational fishing and hunting, the influence on fish and wildlife of seven hexadecanol and octadecanol alcohols were evaluated, with particular emphasis on insect mortality. Emergence of Tendipedidae midges, constituting 98 percent of all emergent insects, was reduced 30.9 percent with T.A. 1618 and 46.5 percent with Adol 52, the two alcohols used in the lake pens. Reduction of mosquito emergence ranged between 80 and 100 percent. None of the alcohols proved toxic to fish, animals, reptiles, and waterfowl studied in laboratory aquaria or observed in the lake pens. Water temperature and dissolved oxygen increased in all treated pens. Although significantly adverse effects to insects have been noted in this study, it is believed the effects were magnified by maintaining ideal monolayers in the laboratory and lake pens. Winds normally present in Western reservoirs will reduce the efficiency of the monolayers and will minimize temperature and diffussion-barrier influences. In addition, monolayers normally will not be used during times of peak emergence of midges.

DESCRIPTORS—*evaporation control/*monomolecular films/*reservoir evaporation/biology/*ecology/recreation/fish and wildlife/alcohols/*hexadecanols/*octadecanol/insects/mosquitoes/toxicity/dissolved oxygen/temperature/ /wind/meteorology/ / diffusion/reservoirs/aquatic life.

IDENTIFIERS—biota/insect emergence/*midges/ water temperature.

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